

Rice Research

Recent Advances and Perspective

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Foreword

Rice cultivation is currently confronted with various challenges like depletion of natural resources, changing climate and biotic and abiotic stresses. A paradigm shift is required in rice research to realize the potential of technological advancement, policy and investment support. The research programmes need to be prioritized to address future challenges. Genomic research, precision farming, secondary agriculture, farm mechanization, energy use, market demand and technology diffusion are to be focused to make rice farming globally competitive.

I am happy to note that the ICAR-National Rice Research Institute (NRRI), Cuttack has brought out the publication in the form of a book entitled "Rice research: recent advances and perspective" during the Platinum Jubilee Celebrations of the Institute. This book also discusses the research activities in the field of genome editing, genomic-assisted breeding, grain quality, biotic and abiotic stress management, precision resource management, and recent tools for technology assessment. I hope, the scientific information available in this book would further strengthen our understanding for future rice research and provide suitable knowledge to different stakeholders for enhancement of rice productivity in the country.

(T. Mohapatra)

Date: 26th August 2021
Place: New Delhi-110 001



Preface

Rice is a staple food for more than half of the global population. It is grown under diverse ecologies spread over about 43 million hectares of India. Significant advancements have been made in rice on developing high yielding and pest/disease-resistant varieties as well as production technologies for different ecologies. However, new challenges have emerged for rice production mainly due to climate change, degradation of soil quality, less per capita water availability, low nutrient use efficiency and increased incidence of insects and diseases. Hence, emphasis is being given on developing climate resilient rice varieties having multiple stress-tolerance along with developing climate-smart production and protection technologies for ensuring food and nutritional security. Therefore, the current challenge is to enhance rice productivity while increasing the climate resilience by implementing advanced research findings.

This book is an attempt to document the scientific literature available on the advanced developments in rice science and suggest implementable research findings for the overall development of different stakeholders. The nine chapters of this book cover the latest research information capturing various aspects of rice research such as omics-based genomics selection, haplotype-based precision breeding, transgenic, CRISPR/Cas and modern biochemical analysis tools and technologies. These strategies have potential to develop climate resilient rice varieties for biotic and abiotic stress tolerance, and essentially derived varieties for nutritional security. Besides, other important aspects like rice mechanization, microbial intervention for nitrogen dynamics in rice soils, the precision techniques *i.e.* drones and sensors for managing the problems of insect-pest in rice, and advancements in assessing and maintaining the rice grain quality in terms of its digestibility have been extensively covered. Finally, a methodological perspective-based impact assessment of rice varieties and production technologies in farmers' field has been systemically presented.

Authors are extremely thankful to all who have provided help and support for finalizing the different chapters. The editors take this opportunity to express their gratitude to all the authors for developing the chapters in a comprehensive and time-bound manner. The authors believe that the outcome and recommendations of this book will be of immense help to the researchers, teachers, policy makers, planners, administrators, progressive farmers, development practitioners, academicians, civil society members and students of the rice sciences.

Editors'



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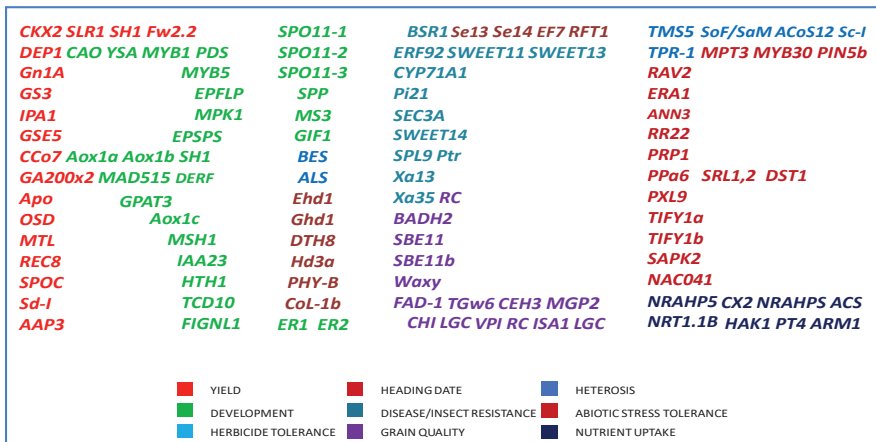
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RICE GENOME EDITING (CRISPR-CAS): INSIGHTS AND FUTURE THRUST

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GRAPHICAL ABSTRACT



SUMMARY

Genome editing through Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology provides innumerable opportunities for gene characterization, trait improvement, and novel traits exploration in rice. There are more than a hundred publications and ~130 genes that have been targeted through genome editing using CRISPR in rice. The present attempt categorized the rice CRISPR developments into two phases from 2013 to 2016 and 2017 to 2020 for understanding the insights and future research thrust in the utilization of genome editing technology in rice. The analysis showed rice CRISPR approaches so far have validated the already characterized major rice genes for yield, biotic, abiotic stresses, grain quality, and nutrient uptake etc. Further, CRISPR resulted in development of novel apomictic and haploid inducers in rice. Therefore, achievements in CRISPR technology have the potential to significantly enhance the genetic gain for yield in rice.



1. INTRODUCTION

Genome editing technology assists to edit the genome (sequence) of organisms using different tools. Recently developed genome editing tool namely Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/ Cas (CRISPR-associated protein) is becoming highly popular due to its simple cloning procedures and sequence specificity in editing. Rice is an important crop and also has smaller genome size of ~ 400 Mbp. Besides, a well-established transformation protocols especially in *Japonica* rice makes it a better choice for genome editing and crop improvement (Shan et al., 2014). In rice, more than a hundred genes are edited so far using CRISPR-Cas technology and it has provided valuable insights within these initial few years of research and applications employing genome editing. The present book chapter highlights the genes-targeted using CRISPR-Cas9 in rice and also provides a brief overview of the future considerations in utilizing the genome editing for rice improvement.

2. CRISPR-Cas9 AND ITS UTILIZATION IN RICE

2.1. CRISPR technique

The trait improvement in rice by using biotechnological tools relied mostly on the development of transgenic plants and its evaluation (Chen et al., 2009). Several traits namely biotic and abiotic stress tolerance has been improved using transgenic approaches in rice. Similarly, recently developed CRISPR technique has also been applied widely in rice now-a-days for functional characterization of genes and traits improvement. The initial knowledge on CRISPR dates back to late 1980s wherein unique repeats have been identified in the genome of several microbes for which function was unknown. Later, CRISPR repeats were functionally characterized to provide acquired resistance in bacteria against viruses (Barrangou et al., 2007). Further, Jinek et al. (2012) reported the mechanism of RNA guided DNA endonuclease activity by CRISPR-Cas combinations. Thus, these two components namely guide RNA having homology with the target genomic region and Cas proteins formed the basis for the widely used genome editing technology in multiple organisms.

2.2. Insights gained in rice genome editing

An attempt was made to categorize the number of genes edited in rice using CRISPR (Table 1) by the year of publications, number of genes reported per year, number of different traits targeted and different ecotypes *viz.*, *Indica* and *Japonica* used for genome editing. For the year-wise comparison, publications related to rice CRISPR was divided into two phase namely first phase (2013-2016) which is characterized by validation of the technology and second phase (2017-2020) for rice improvement and functional genomics aspects. This



attempt collected all the genes reported in rice in these two phases, however, inadvertently few articles might have been included in this analysis.

Table 1 List of genes targeted through CRISPR technique in rice

Sl No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
1	<i>Chlorophyllase (CAO)</i> <i>Lazy1</i>	Leaf blade, Tiller angle	<i>Japonica</i> cv. Kitaake	Pale green color	Miao et al., 2013
2	<i>Young seedling albino (YSA)</i> <i>ROC 5 and Stomal processing peptidase</i>	Albino			Feng et al., 2013
3	<i>OsMYB1, OsYSA, OsROC5, OsDERF1</i> <i>OsPDS, OsMSH1, OsMYB5, OsSPP</i> <i>OsPMS3, OsEPSP S</i>	Pigment synthesis, noncoding RNA synthesis, aromatic amino acid synthesis	<i>Japonica, Indica</i>	Chlorosis spot, loss of function leading to plant death	Zhang et al., 2014
4	<i>Bentazon Sensitive Lethal (BEL)</i>	Herbicide tolerance	<i>Japonica</i> cv. Nipponbare	Bantazon and Sulfonylurea tolerant	Xu et al., 2014
5	<i>AOX 1a, 1b, 1c</i> (Alternate respiratory pathway)	Chlorophyll development	<i>Japonica</i> cv. Nipponbare	-	Xu et al., 2015
6	<i>Dropping leaf (DP)</i>	Leaf morphology	-	Dropping of leaf, failed to formation of midrib	Ikeda et al., 2015
7	<i>SWEET13</i>	Bacterial blight disease resistance	<i>Indica</i> cv. Minghui 63	Increased BB resistance	Zhou et al., 2015
8	<i>Gn1a</i> <i>DEP1</i> <i>Gs3</i> <i>IPA1</i>	Yield	<i>Japonica</i> cv. Zhonghua 11	Enhanced grain number, dense erect panicle, semi-dwarf, larger grain size, grain with long awn, fewer/more tillers	Li et al., 2016
9	<i>OsERF922</i>	Blast resistance	<i>Japonica</i> cv. Kuiku 131	Increased blast resistance	Wang et al., 2016
10	<i>Acetolactate synthase (ALS)</i>	Herbicide tolerance	<i>Japonica</i> cv. Nipponbare	Bispyribac sodium tolerant	Sun et al., 2016

SI No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
11	<i>TMS5</i>	Male sterility	<i>Indica</i> , <i>Japonica</i>	Loss of function of <i>TMS5</i> , produced TGMS lines	Zhou et al., 2016
12	<i>OsRAV2</i>	Salt tolerance		Salt stress responsive	Duan et al., 2017
13	<i>EPFL9</i>	Developmental		Reduction in stomatal density	Yin et al., 2017
14	<i>OsNramp5</i>	Cadmium uptake	<i>Indica</i> cv. Huazhan, Longke 638s	Low cadmium accumulation	Tang et al., 2017
15	<i>DEP1</i>	Yield	<i>Indica</i> cv. IR58025b	Dense and erect panicles with reduced plant height	Wang et al., 2017
16	<i>MPK1</i>	Developmental	<i>Japonica</i> cv. Kitaake	Loss of function: abnormal embryo	Minckenberget al., 2017
17	<i>SBEII</i> <i>SBEIIB</i>	Starch content	<i>Japonica</i> cv. Kitaake	High amylose, resistant starch	Sun et al., 2017
18	<i>OsAnn3</i>	Cold tolerance		Loss of function: reduced cold tolerance	Shen et al., 2017
19	<i>OsHAK1</i>	Cesium uptake	<i>Japonica</i> cv. Nipponbare	Reduced cesium uptake	Nieves-Cordones et al., 2017
20	<i>TIFY1a</i> , <i>TIFY1b</i>	Cold tolerance	<i>Japonica</i> cv. Nipponbare	Enhanced cold tolerance	Huang et al., 2017
21	<i>GS3</i> <i>Gn1a</i>	Grain size Grain number		Longer grains, increased test weight, increased no. of grains	Shen et al., 2017
22	<i>OsSWEET11</i>	Grain filling	<i>Japonica</i> cv. Nipponbare	Loss of function: defective grain filling	Ma et al., 2017
23	<i>SAPK2</i>	Drought stress	<i>Japonica</i>	ABA-mediated seed dormancy, drought tolerance	Lou et al., 2017
24	<i>OsPT4</i>	Arsenate uptake	<i>Japonica</i> cv. Nipponbare	Increased/ reduces arsenic accumulation	Ye et al., 2017



Sl No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
25	<i>BSR1</i>	Broad spectrum disease resistance	<i>Japonica</i> cv. Nipponbare	Knock-out: Suppressed defense response	Kanda et al., 2017
26	<i>GIF1</i>	Developmental	<i>Japonica</i> cv. Nipponbare	Plant architecture	He et al., 2017
27	<i>HTH1</i>	Anther development	<i>Japonica</i>	Defective anther wall, aborted pollen	Xu et al., 2017
28	<i>SaF/SaM</i>	Male sterility	<i>Indica</i>	Heterosis	Xie et al., 2017
29	<i>ACos12</i>	Male sterility		Defective pollen wall	Zou et al., 2017
30	<i>Sc-i</i>	Male sterility	<i>Japonica</i>	Heterosis	Shen et al., 2017
31	<i>MADs15</i>	Developmental genes		Plant height	Song et al., 2017
32	<i>OsFIGNL1</i>	Anther development	<i>Indica</i>	Pollen abortion	Zhang et al., 2017
33	<i>OsARM1</i>	Arsenic stress	<i>Japonica</i>	Tolerant /sensitive to arsenic	Wang et al., 2017
34	<i>OsMADS3</i>	Flower development		Increased pistil number	Yasui et al., 2017
35	<i>GSE 5</i>	Grain size	<i>Japonica</i>	Variability in grain size	Duan et al., 2017
36	<i>TPR1</i>	Interspecific hybrid sterility	<i>Japonica</i>	Heterosis	Xie et al., 2017
37	<i>OsCOL16</i>	Heading date		Delayed flowering	Wu et al., 2017
38	<i>TCD10</i>	Chloroplast development	<i>Japonica</i>	Chloroplast development under cold stress	Wu et al., 2016
39	<i>Waxy</i>	Glutinous rice	<i>Japonica</i>	Enhanced amylose content	Zhang et al., 2018
40	<i>OsER1, OsER2</i>	Development	<i>Indica, Japonica</i>	Shortened plant height, reduced panicle size	Zhang et al., 2018
41	<i>Gn1a, DEPI</i>	Yield related traits	<i>japoinca</i> cv. Nipponbare	Genetic gain for yield	Huang et al., 2018
42	<i>Wx, TGW6</i>	Glutinous grain – CMS		Heterosis	Han et al., 2018
43	<i>FAD2-1</i>	Oil quality		Increased oleic acid content, improved fatty acid composition grain	Abe et al., 2018

SI No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
44	<i>NRT 1.1B</i>	Allelic replacement		Enhanced efficiency	Li et al., 2018
45	<i>SEC 3A</i>	Defense Response	<i>Japonica</i> cv. Kitaake	Increased salicylic acid	Ma et al., 2018
46	<i>CCd7</i>	Striogalactone biosynthesis	<i>Japonica</i> cv. Nipponbare	Increased tiller no. and reduced plant height	Butt et al., 2018
47	<i>Ptr</i>	Blast resistance	<i>Japonica</i> cv. Katy	Development of broad spectrum blast resistant	Zhao et al., 2018
48	<i>GPAT3</i>	Pollen function	<i>Indica</i> cv. ZH8015	Abnormal anther wall development and pollen formation	Sun et al., 2018
49	<i>AAP3</i>	Yield traits	<i>Indica, Japonica</i>	Increased tiller numbers, increased grain yield	Lu et al., 2018
50	<i>OsCHI</i>	Hull color		Brown pigmentation in the panicle at heading stage and golden pigmentation in the panicle at maturity	Que et al., 2018
51	<i>CEH 3</i> (<i>pCOUMAROYL ESTER 3HYDROXYLASE</i>)	Saccharification		Lignin content and enhanced biomass saccharification	Takeda et al., 2018
52	<i>CCx2</i>	Cadmium stress	<i>Japonica</i> cv. Nipponbare	Grain cadmium accumulation	Haoet al., 2018
53	<i>APO</i> <i>OSD</i> <i>MTL</i> <i>REC8</i>	Apomixis	<i>Japonica</i> cv. Yandao 8	Fixation of heterosis	Wang et al., 2018
54	<i>CYP71A1</i>	Serotonin biosynthesis	<i>Japonica</i>	Tolerance to BPH	Lu et al., 2018
55	<i>SPOC</i>	Photosynthesis efficiency	<i>Japonica</i> cv. Zhonghua11	Regulate leaf inclination	Chen et al., 2018
56	<i>RR22</i>	Salinity	<i>Japonica</i> cv. WPB106	Enhanced tolerance	Zhang et al., 2019
57	<i>IAA23</i>	Phenotypic plasticity	<i>Indica</i> cv. Kasalath, <i>Japonica</i> cv. Wuyunjing24	Reduced no. and length of the seedling and fresh weight of total roots	Jiang et al., 2019



SI No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
58	<i>RC</i>	Red rice	<i>Japonica</i> , <i>Indica</i>	Grain color	Zhu et al., 2019
59	<i>C-5 Hydroxylase</i>	Lignin content	<i>Japonica</i> cv. Nipponbare	Reduction in culm length, panicle weight and spikelet fertility	Takeda et al., 2019
60	<i>Sd-1</i>	Height	<i>Indica</i> cv. Kasalath, <i>Japonica</i> cv. Nipponbare	Reduced height	Hu et al., 2019
61	i) <i>Se13</i> ii) <i>PHYB</i> iii) <i>Se14</i> iv) <i>Hd3a</i> v) <i>Ej7</i> vi) <i>RFT1</i> vii) <i>Ehd 1</i> viii) <i>Ghd7</i> ix) <i>DTH8</i>	Heading and yield	<i>Japonica</i> cv. Sasanishiki	i) Fewer panicles ii) Decreased no. of grains per panicles iii) Smaller grain size iv) Decreased in panicle no. and setting rate v) Increased grain yield vi) Decreased grain no. per panicle vii) Higher grain no. per panicle viii) Decreased both panicle and grain nos. and yield reduced	Cui et al., 2019
62	<i>NRAHP5</i>	Cadmium accumulation	<i>Japonica</i> cv. Nanjing 46, Huaida 05	Reduced cadmium accumulation	Yang et al., 2019
63	<i>GA200X2</i>	Plant height	<i>Indica</i>	Reduced flag leaf length and plant height. Increased yield per plant	Han et al., 2019
64	<i>Semi rolled leaf 1,2</i>	Drought tolerance	Restorer rice lines (GXU16, GXU20 and GXU28)	Leaf rolling, increased panicle number, grain number per panicle and yield	Liao et al., 2019
65	<i>VPI</i>	Seed dormancy	<i>Japonica</i> cv. Dongjin	Fast seed germination in mutant lines	Jung et al., 2019

SI No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
66	<i>Waxy/GBSSI</i>	Endosperm starch	<i>Japonica</i> cv. Kur and Mushashimochi	More rounded granules with softer, less-defined edges of grain quality	Perez et al., 2019
67	<i>F3'H, DFR, LDOX</i>	Anthocyanin biosynthesis	<i>Japonica</i> cv. Heugseonchal, Sinmyungheugchal	Differential anthocyanin accumulation that showed other seeds	Jung et al., 2019
68	<i>qPE9-1/DEP 1</i>	Grain length and weight	<i>Japonica</i> cv. Nipponbare	Increased grain yield per panicle	Li et al., 2019
69	<i>ISA 1</i>	Starch formation	<i>Japonica</i> cv. Zhonghua	Increased grain length	Shufen et al., 2019
70	<i>OsASN 1</i>	Asparagine development		Tiller outgrowth and reduced plant height	Luo et al., 2019
71	<i>ACS</i>	Phosphate deficiency	<i>Japonica</i> cv. Nipponbare	Low phosphate tolerance and lateral root elongation	Lee et al., 2019
72	<i>PRP1</i>	Cold response	<i>Indica</i> cv. GXU2-44	Enhanced cold tolerance	Nawaz et al., 2019
73	<i>MS1</i>	Tapetum formation	<i>Indica</i> cv. Zhonghui 8015	Pollen development	Yang et al., 2019
74	<i>SPL9</i>	Antivirus response		Enhanced plant resistance	Yao et al., 2019
75	<i>MGD2</i>	Grain quality	<i>Japonica</i>	Shortened plant height, reduced panicle length, and fewer number of filled grains per panicles	Basnet et al., 2019
76	<i>NACO41</i>	Salt response	<i>Japonica</i> cv. Nipponbare	Salt sensitivity plant	Bo et al., 2019
77	<i>PPa6</i>	Salinity response	<i>Japonica</i> cv. Kitaake	Lowered alkaline tolerance and dwarf plant height	Wang et al., 2019
78	<i>Xa13</i>	Bacterial blight	<i>Indica</i> cv. Huanghuazhan <i>Japonica</i> cv. KY131	Enhanced bacterial blight resistance at seedling stage	Li et al., 2020



SI No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
79	<i>BADH 2</i>	Aroma traits		Improved aroma	Ashokkumar et al., 2020
80	<i>PIN5b</i> <i>GS3</i> <i>MYB 30</i>	Yield and cold tolerance	<i>Japonica</i> cv. Nipponbare	Increased in panicle length, enlarged grain size and enhanced cold tolerance	Zeng et al., 2020
81	<i>Fw2.2</i>	Tiller development	<i>Japonica</i> cv. Zhonghua 11	Increased in tiller number, leaf width and plant yield	Gao et al., 2020
82	<i>Pi21</i>	Blast resistance		Resistance to rice blast at seedling stage	Nawaz et al., 2020
83	<i>SPO11-1</i> <i>SPO11-2</i> <i>SPO11-4</i>	Meiosis	<i>Japonica</i> cv. Kitaake	Double strand breaks	Fayos et al., 2020
84	<i>SWEET14</i>	Bacterial blight	cv. Zhonghua 11	Enhanced bacterial blight resistance with yield penalty. Increased plant height	Zeng et al., 2020
85	<i>Cytochrome P450</i>	Yield and aroma	<i>Indica</i>	Increased grain width and yield	Usman et al., 2020
86	<i>DST1</i>	Drought and salinity tolerance	<i>Indica</i> cv. MTU1010	Improved drought and salt tolerance and grain yield	Santoshkumar et al., 2020
87	<i>Ghd 7</i>	Heading date traits		Early heading	Wang et al., 2020
88	<i>qSH1</i>	Shattering	<i>Indica</i>	Reduced seed shattering and increased grain yields	Sheng et al., 2020
89	<i>ERA1</i>	Drought tolerance	<i>Japonica</i> cv. <i>Nipponbare</i>	Increased primary root growth	Ogata et al., 2020
90	<i>LGC</i> <i>SBE11 b</i>	Starch content	<i>Japonica</i>	High resistance to starch	Guo et al., 2020
91	<i>SLR 1</i>	Plant height	<i>Japonica</i>	Slow growth rate, shriveled leaves and reduced plant height	Jung et al., 2020

Sl No.	Gene	Trait	ssp./ Cultivar	Phenotypic changes	Reference
92	<i>Ehd 1</i>	Heading date	<i>Japonica</i> cv. Nipponbare	Delayed heading date	Wu et al., 2020
93	<i>PYL9</i>	Drought tolerance and grain yield		Enhanced drought tolerance, 1000 grain weight, grain length, grain width and yield per panicle. Decreased flag leaf length and width	Usman et al., 2020
94	<i>OSMPT3</i>	Osmotic regulatory factor	<i>Japonica</i> cv. Kitaake	Enhanced tolerance to abiotic stress	Huang et al., 2020

The first analysis was done to compare the number of publications during the first and second phase of rice CRISPR. There were 14 and 81 publications during 2013-16 and 2017-2020, respectively. Further, mean number of publications in first phase was 3.5 and second phase was 20.25 (Fig. 1). This analysis showed during the last few years, at least 20 numbers of publications related to rice CRISPR was published every year. Especially, two publications were reported in the second rice CRISPR phase from India targeting *DST* and *BADH2* gene. This increasing trend in the publications related to rice CRISPR reflects the simple cloning and characterization protocols involved in genome editing techniques.

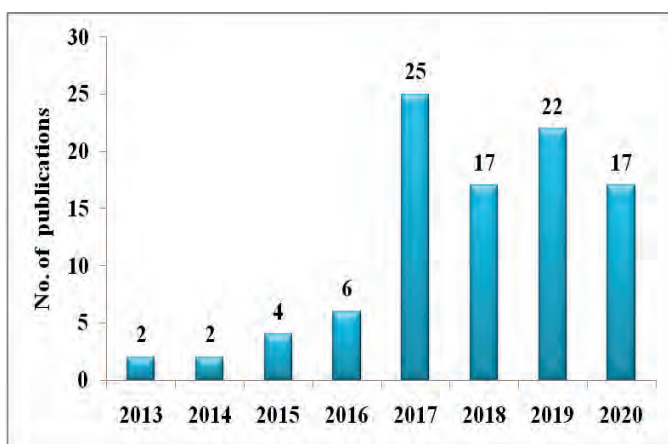


Fig. 1 Number of publications related to CRISPR-Cas9 in rice



The second analysis compared the number of genes targeted during the first (2013-2016) and second (2017-2020) rice CRISPR phase. The analysis showed ~ 129 rice genes were edited in both the phases and specifically 30 and 99 genes were reported in first and second phase, respectively. Further, genes related to rice development like chlorophyll synthesis, leaf morphology and major yield genes (*IPA1*, *DEP1*, *Gn1a*, and *GS3*) were published in first rice CRISPR phase and most of the rice traits including apomixis were reported in second rice CRISPR phase (Fig. 2). Additionally, number of genes edited in the second CRISPR phase was found to be 24.75 genes per year.

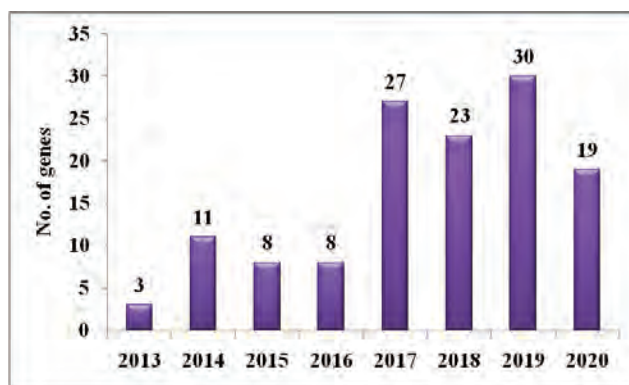


Fig. 2 Number of genes targeted in rice using CRISPR-Cas9 approach

In the third analysis, we compared the ecotype of rice genotypes used for genome editing of targeted genes. This analysis was done for the both rice CRISPR phase taken together *i.e.* from 2013 to 2020 as compared to previous analysis (Fig. 3). The analysis showed popular *Japonica* cultivars namely Nipponbare, Kitaake, Zhinghua 11 were mostly used for genome editing in rice. Also, *Indica* varieties namely IR64, MTU1010 and ASD16 were also used for the genome editing approaches. Further, aus ecotype Kasalath was also used to target the *IAA23* gene in rice. This finding reflects that the relatively higher transformation efficiency of *Japonica* cultivars might be the one of the major reason for more number of genes edited in *Japonica* background. Hence, utilization of genome editing for rice improvement requires proper standardization of transformation protocols in popular Indian rice varieties.

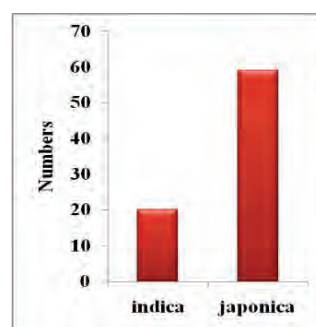


Fig. 3 Number of *Indica* and *Japonica* cultivars used for genome editing

The fourth analysis studied the number of traits targeted in rice during both the rice CRISPR phases. The analysis showed that ~ 25% of reports targeted rice development related genes for genome editing (Fig. 4). Especially, first CRISPR phase characterized by validation of CRISPR concepts in rice targeted many developmental related genes in rice. Further, ~17% of reports targeted yield genes for rice improvement. There were many previously characterized yield genes (*IAP1*, *DEP1*, *Gn1a*, *GS3*, *APO*, *AAP3*) regulating the ideal plant types, plant architecture, number of tillers and grain size in rice were validated by using CRISPR approaches. Also, development of apomictic rice was achieved targeting four genes namely *PAIR1*, *OSD1*, *REC8* and *SPO11* through CRISPR technology. Further, genes related to abiotic stress response, grain quality, and biotic stress response contributed to 15%, 14% and 11%, respectively of the total number of publications related to rice CRISPR. Noteworthy to mention was the durable resistance development against bacterial blight in rice targeting the promoter region of *SWEET11*, *SWEET13* and *SWEET14* genes in rice.

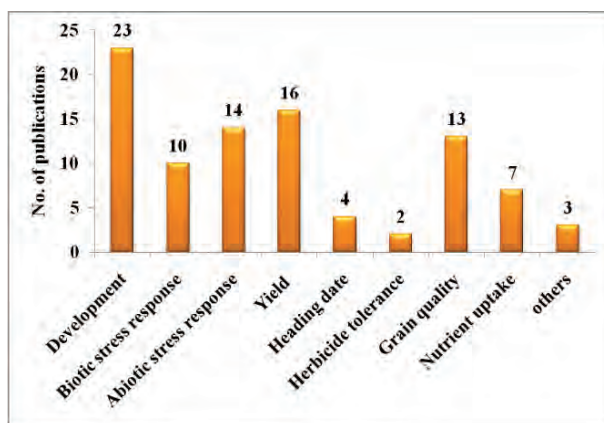


Fig. 4 Number of publications targeting different traits in rice using CRISPR-Cas9 approach

3. FUTURE CONSIDERATIONS

3.1. Genome-wide mutant libraries in rice

The recent annotation of rice genome, RGAP7 showed there are 55,986 genes of which 39,045 are protein coding (<http://rice.plantbiology.msu.edu/>; <http://rice.plantbiology.msu.edu/>). At present, approximately 2300 genes have been cloned and functionally characterized for multiple traits in rice (Li et al., 2018). Also, probable functions of ~8000 genes are identified through mapping, expression and other genetic approaches. The major prerequisite of



characterization of complete set of genes in rice is the development and availability of mutant genetic stocks. For example, in model plant Arabidopsis, T-DNA mutant stocks of almost all the genes were developed and being distributed to researchers all over the world (<https://www.arabidopsis.org/>). Recently in rice, Meng et al. (2017) and Lu et al. (2017) reported the construction of guide RNA and Cas9 vectors for the development of genome-wide mutant libraries in Zhonghua 11 cultivar of rice. Thus, availability of CRISPR mutant stocks for all the genes in rice will greatly facilitate the functional characterization of complete set of rice genes. In India, similar efforts have been initiated for the development of genome-wide CRISPR libraries in MTU1010 variety of rice.

3.2. Genome editing mediated haploid inducers in rice for speed breeding and genomic selection

Speed breeding is a highly sought technique now-a-days for varietal development and other genetic studies in crops (Watson et al., 2018). The major principle of speed breeding is to reduce the crop breeding cycles for the development of homozygous breeding lines or mapping population for genetic studies in rice. Genomic selection is used as major technique for addressing the genetic gain for yield in rice (Grenier et al., 2015). In both these techniques, rapid development of homozygous lines and its utilization could be achieved using doubled haploid strategies. Even though, *in vitro* androgenesis for doubled haploid development is successfully employed in *Indica* rice (Rout et al., 2016; Naik et al., 2017; Samantaray et al., 2020), development of haploid inducers targeting *MTL* and *DMP* genes (Zhong et al., 2020) in rice using genome editing technologies will immensely transform the breeding strategies for the utilization of speed breeding and genomic selection in rice.

3.3. Genome editing for indigenous landrace improvement

A rich diversity of rice landraces available in India are generally used as genetic resources for traits identifications (Umakanth et al., 2017). Further, many landraces are also popular for their nutritional and stress tolerance (Longvah and Prasad, 2020). The major advantage of landrace is their ability of local adaptation for biotic and abiotic factors. However, yield of these landraces are very low and could not result in profitable farming (Roy et al., 2015). The genetic improvement of Kalanamak for plant height using the *Sd1* alleles was recently reported through marker assisted recurrent breeding strategy (Srivastava et al., 2019). However, it is very difficult to retain the complete genome of recurrent parent due to linkage drag using recurrent breeding approach. In contrast, genome editing through CRISPR results in small insertions or deletions without altering the major traits of parent varieties. Thus,

landrace improvement through CRISPR targeting the plant height and number of tillers for enhancement of yield needs to be given more emphasis in future for the economic cultivation of rice landraces in India. Further, genetic diversity loss due to cultivation of improved varieties (Singh et al., 2016) can be stabilized by wide spread cultivation of locally adapted landraces improved through genome editing approaches.

3.4. Genome editing for varietal improvement

There are more than a thousand rice varieties are commercially approved for cultivation in India (Subba Rao et al., 2019). Further, very meagre genetic gain for yield was achieved in rice varieties in India (Muralidharan et al., 2019). Besides, genetic gain for drought tolerant varieties ranged between 0.68 and 1.9% in different ecologies (Kumar et al., 2021). However, there is a requirement to increase the genetic gain in rice up to 2% to meet the demand of the growing population in India. In this scenario, proper understanding of the contributing traits to yield of newly released varieties will assist in the selection of genes for yield improvement using CRISPR approaches. For example, DEP1 or IPA1 genes could be targeted for the newly released varieties having good tillering ability. Thus, increase in the number of spikelets and photosynthetic efficiency of the genome edited lines will be able to address the enhancement in genetic gain over parent variety for rice in India.

3.5. Novel alleles impact assessment

In nature, frequency of the alleles of a gene varies depending on the effect on alleles on the crop viability, selection pressure, and response to various biotic and abiotic stresses. For example, multiple alleles of the BPH9 gene was naturally selected in different regions of rice growing areas and provided resistance against brown plant hopper (Zhao et al., 2016). Similarly, genome editing technologies in rice results in the development of novel alleles of the targeted genes. Even though the edited novel alleles might bring the expected phenotype, additionally, it may affect the crop response to other factors both positively or negatively. Therefore, it is essential to thoroughly evaluate the associated effects of the novel alleles developed through CRISPR technology for achieving the genetic gain for yield in rice.

4. CONCLUSIONS

The ease of genome editing using CRISPR technology in rice provides multiple opportunities in functional genomics, genetics, and rice improvement. In the next ten years, whole genome CRISPR mutant libraries may be available in rice which will significantly hasten the knowledge and discovery, and enhance rice improvement program. Further, development of large scale transformation



protocols in popular Indian varieties, region specific varietal/landrace improvement through genome editing for genetic gain in yield, along with comprehensive evaluation of edited novel alleles will help the rice researchers and farming community.

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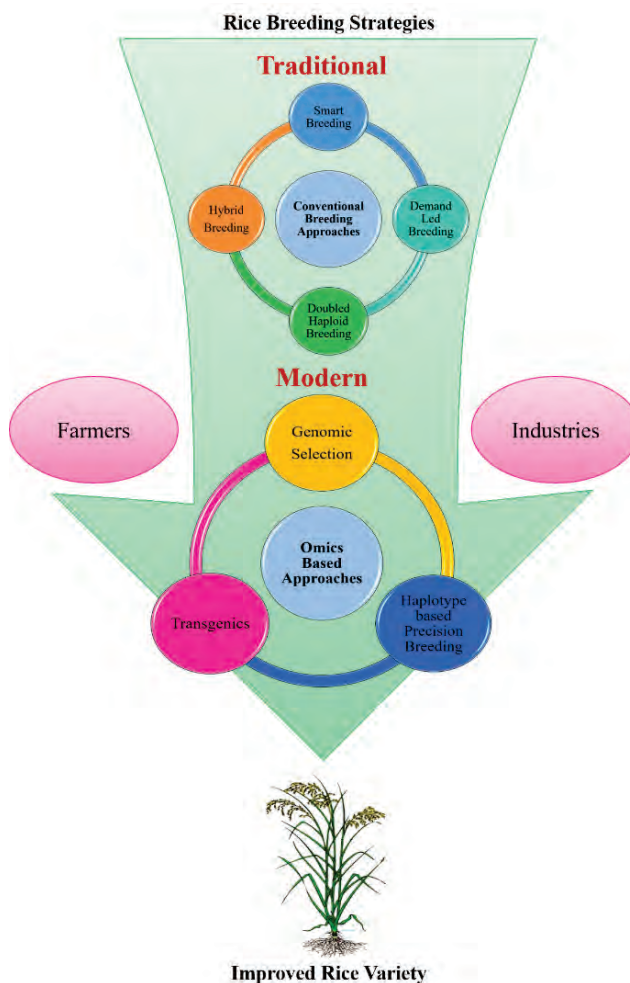
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ADVANCED BREEDING STRATEGIES FOR RICE IMPROVEMENT

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GRAPHICAL ABSTRACT





SUMMARY

Rice is directly linked to the state economy and livelihood in most of the Asian countries. Discovery of semi-dwarf trait has made revolutionary changes in rice farming across the world. The rice varieties like IR8, IR36 and IR64 developed through the traditional pedigree approach transformed rice cultivation. India has made commendable progress in rice research, released over 1300 high yielding varieties/hybrids, which led to >300% growth in rice production. However, the global climate change and change in consumers' food habits coupled with minimal rate of genetic gain through classical approaches have necessitated reorientation of breeding strategies to make this entity sustainable and substantial in future. The demand-led smart breeding strategies involve sizable breeding population, robust phenomics, precise data management system and advanced genomic interventions, which can ensure sustainable rice production.

1. INTRODUCTION

Rice (*Oryza sativa* L., $2n = 2x = 24$) is staple food crop for more than half of the world population with substantial acreage (167.13 mha) and production (782.0 million tons) (FAO, 2018). It is domesticated from its wild progenitor (*O. rufipogon*) during middle Miocene, 13–15 million years ago (Zou et al., 2013). In India, it is grown on ~ 44.5 mha land with total production of 172.5 million tons (paddy) and average productivity of 3.88 tons per hectare (FAOSTAT, 2018), which is directly linked to the livelihood and economy. According to the Food and Agriculture Organization, in next three decades 70% more food is required to feed over 9 billion people by the year 2050 (FAO, 2009). Present demographic (8.5 billion till 2030) and changing climatic scenarios make the situation challenging which needs further breeding attention to improve rice for yield and resilient traits (Godfray et al., 2010; Stein et al., 2018). Adoption of semi-dwarf rice genotype TN1 during sixties of 20th century has made revolutionary changes in rice farming. Subsequently, rice breeding programmes in India has made commendable progress and could release >1300 varieties (including 121 hybrids), and increase *per se* rice production (over 300%). However, stagnated rate of genetic gain (~1% per year) in most of the breeding strategies necessitates more efficient alternatives to make this entity sustainable (Peng et al., 2000). Furthermore, occurrence of frequent climatic adversity / extremes like salinity, drought, flooding, heat (Wassmann et al., 2009), high night temperatures (Peng et al., 2004), limit the rice production and expansion. These awaited challenges have ignited interest of researchers to replace their breeding goal and strategies (Cobb et al., 2019). Breeding rice with enhanced yield, quality and sustainability needs to be part of the overall strategy towards the food security. The demand for rice is going to be increased dramatically



which necessitates substantial yield improvements (Van et al., 2015) with limited acreage. It is noteworthy that rice breeding in majority of the global institutions (78%) has restricted to conventional approach (pedigree approach) which takes ~10-12 years to reach the products to the farmers (Lenaerts et al., 2018). Several rice varieties like IR8, IR36 and IR64 were developed through the traditional approach and revolutionized rice cultivation in Asia. However, recent success of technological interventions in breeding has sparked researchers for critical evaluation and redesigning of their respective breeding programs. Since increasing genetic gain for yield, quality and sustainability is the major goal, this process involved transforming breeding activities into more efficient, market-driven and product-oriented variety development. This chapter deals with rice research status and probable future breeding strategies to make rice crop more substantial and sustainable across the ecologies.

2. INSIGHT INTO RICE BREEDING

Breeding high yielding cultivars to meet food demand for ever-growing population remains prime goal in rice research (Xu et al., 2014). Significant changes have been made in the field of rice breeding science from phenotype based selection to today's molecular and genomics-based selection (Patra et al., 2020). Changing climate and advances in science has been the driver of rice breeding to meet the global demands. The breeding objective in rice has been shifted from developing high yielding varieties to achieve the nutritional security by breeding varieties/hybrids for appropriate nutrient contents coupled with high yield levels. Rice has been greatly transformed from its wild appearance to the present day form through the process of domestication, which happened to over the centuries (Tang et al., 2010). Over the past six decades, the developments of modern high yielding varieties associated with green revolution have almost doubled rice production. The highlight made by Shull (1948) on exploitation of heterosis in crop production is also utilized in rice with 15% higher yield than better pure line variety (Khan et al., 2015). Hybrid rice cultivation became ground reality in India with significant achievements in this area. With this boom in the rice production many countries achieved the self-sufficiency and some become exporters of rice. Cutting-edge research advances in breeding science shifted the focus of breeding from phenotype based selection towards utilization of genomic resources. Advent of molecular markers paved the path for changing paradigm of rice breeding by identification of genomic regions controlling several economic traits. Several genes associated with biotic stress, abiotic stress, quality characters and many physiological characters have been identified and characterized. Modern tools such as genomics, genetic engineering and molecular biology techniques are more precise and rapidly being deployed to boost the yield potentials of rice.

Substantial advances have been made in engineering for resistance to insects like stem borer, leaf hopper, brown plant hopper (BPH) and diseases like blast, sheath blight, bacterial leaf blight (BLB), rice tungro virus and nutritional enrichment by enhancing protein content and increasing β -carotene using novel genes. The advances made in androgenesis led to development of double haploid technology in rice for rapid achievement of homozygosity of a target locus (Sakina et al., 2020). The recent genome editing tools like CRISPR-Cas has also been deployed in rice research (Zafar et al., 2020).

Shift in the approach from solely phenotype-based selection to molecular or DNA marker, surrogate the selection difficulties to phenotype-based rice improvement. Trait linked DNA marker assisted selection (MAS) and its variants created new avenues in plant breeding to dissect genetic inheritance of many complex traits and finding genetic solutions for many problems. Several genes responsible for biotic and abiotic stress tolerance have been transferred into elite rice background through surrogate markers. Genes have been pyramided into improved varieties through molecular markers and released in India (Singh et al., 2001). Improvement in marker technology from hybridization-based markers (RFLP) to PCR based markers (AFLP, RAPD, SSR), and to the latest sequence-based marker (SNP) have made a significant impact on efficiency of MAS in rice breeding program. Recent and systematic advances in rice genomic approaches led to accelerated improvement and rapid genetic gain in rice (Xu et al., 2020). However, stagnated yield and genetic gain was not altered for several years with available breeding methods (Muralidharan et al., 2019). An improved version of marker assisted selection, Genomic Selection (GS) introduced by Meuwissen et al. (2001) promised to improve genetic gain per breeding cycle and achieve rapid success in breeding programs for climate resilience.

3. MODERN BREEDING STRATEGIES

3.1. *Smart breeding*

The accelerative product development with enhanced genetic gain for yield, quality and sustainability in rice needs robust and high throughput phenotyping facilities for effective and precise assessment of better performing breeding lines within population (Fahlgren et al., 2015). Intervention of advanced phenomics tools like sensor (unmanned air vehicle-UAV) based high resolution imagery system has elevating error free and volumetric data collection and management efficiently (Atkinson et al., 2019). High Throughput Phenotyping (HTP) allows the screening for plant architectural traits and early detection of desirable genotypes. It facilitates automated, repeatable measurements of



agronomic and physiological traits with great accuracy. HTP like RGB imaging, 3-D scanning, thermal and hyper spectral sensing and fluorescence-imaging have been successfully utilized to identify, quantify and monitor plant diseases (Shakoor et al., 2017). Integrating genomics tools like GWAS with HTP is found to be suitable in excavating desirable traits with enhanced crop breeding efficiency (Yang et al., 2014). Recently, Deep Learning (DL) strategy is extensively utilized to analyze and interpret big data, especially for advancing plant image analysis and environmental stress phenotyping (Singh et al., 2018). Electronic data collection encompasses speed, real-time data collection, more accuracy and digitization of all records for further analysis. Bar-coding, electronic data capture and digitization along with smart breeding components bring innovativeness, precision, efficiency in breeding activities and reduce error significantly. IRRI and HTPG have facilitated excellent support to the NARES in India for bar-coding, electronic data collection, use of plant breeding apps and digitization by utilizing hand-held electronic devices.

3.2. Demand- led breeding

The global climate and food habits dynamics necessitate reorientation of breeding strategies and goals. The changing climate and its impulsiveness has obligated breeding with inbuilt resilient. However, the demand for better quality, high protein and other micronutrients, substantial antioxidants, low GI, low phytate and pharmaceutically acceptable, high value rice is increasing to provide better nutrition, health benefits and higher income. The classical pedigree based varietal development process is slow (8-10 years), resource intensive, inefficient, which also cannot facilitate selection intensity for achieving the least genetic gain. Besides, despite of varietal abundance, replacement of old mega varieties e.g. Swarna, MTU1010, MTU1001, Lalat, Pooja, Ranjit, Satabdi and IR 64 remained a major challenge. To deal with these challenges, breeder needs strategic plan and breeding reorientation with specific product development and replacement strategies to deliver higher rate of genetic gain at a faster and cost effective way. Today's breeding objectives need to be defined envisaging the upcoming market trend and consumer's preference based on robust market research. Extensive understanding of various rice value chain stakeholders' (farmers, millers, dealers, distributors, exporters, retailers and consumers) preference is imperative to design future rice cultivars, and accordingly the breeding and product development and product replacement strategies. The process is a market oriented system where products are released in a continuous manner fitting to the specific needs of the clients. ICAR-NRRI in collaboration with IRRI-TRB and EiB platform of CGIAR has started demand-led rice breeding and developing the targeted "Product Profiles" for specific geography to replace an old rice mega variety / product. Under this strategy, we are working on need-based product profiling and modern breeding

strategies (population-based genomic selection and speed breeding approach) to explore substantial genetic gain and replacement of old rice varieties.

3.3. Doubled haploid breeding

Doubled Haploid (DH) breeding through anther culture has emerged as an exciting and powerful tool and a convenient alternative to conventional techniques for crop improvement in terms of yield and quality in a sustainable manner (Purwoko et al., 2010; López-Cristoffanini et al., 2018). DH approach shows several advantages like shortening breeding cycle by immediate fixation of homozygosity, offers high selection efficiency, widen genetic variability through the production of gametoclonal variants and earlier expression of desirable recessive genes suitable for breeding (Devaux and Pickering, 2005). Presently, more than 250 plant species and hybrids have been regenerated using anther culture technique, which include major cereals such as rice, wheat, maize, barley, and a range of other economically important trees, fruit crops and medicinal plants (Maluszynski et al., 2003). There are plenty of reports available explaining the successful application of DH through androgenesis in varietal development (Forster et al., 2007; Seguí-Simarro, 2008; Touraev et al., 2009; Germana, 2011).

An integrated MAS and doubled haploid approach to introgress desirable genes/QTLs conferring tolerance/resistance to major abiotic and biotic stresses along with improved yield and quality will help to combat the present situation (Kumar et al., 2018). Since marker-assisted/genomics-assisted breeding strategies have been instrumental in introgression of various major QTLs/genes into rice mega varieties (Pandey et al., 2015), DH approach coupled with MAS could certainly be useful in acceleration of the product development in achieving homozygosity within a short period of time. Therefore, keeping the present scenario of changing climate in mind, the novel approach of integrating DH technology with marker-assisted breeding could be envisaged for the development of high-yielding climate resilient rice varieties through stacking of multiple genes/QTLs (Fig.1). The developed lines could withstand a cascade of multiple biotic and abiotic stresses and assist in achieving global food security.

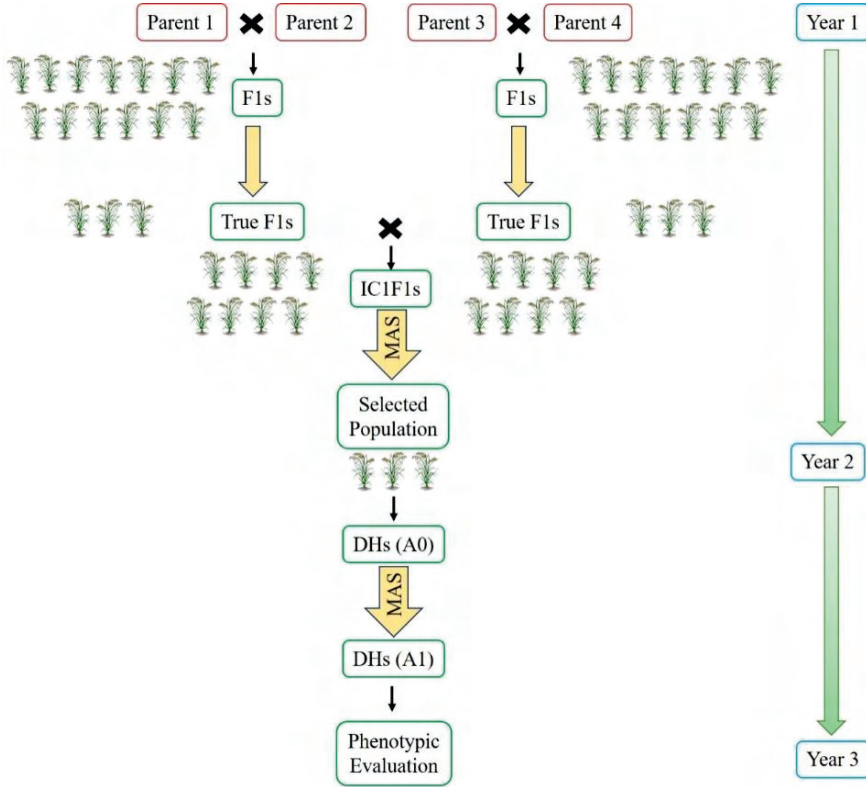


Fig. 1 Different donors carrying target trait used for generation of F1s and intermating of F1s to generate IC1F1s followed by generation of doubled haploids through anther culture for fixation desirable QTLs/genes combinations

3.4. Hybrid breeding

Hybrid technology exploits heterosis in crop plants for economic advantages. This phenomenon has benefited agriculture and fascinated geneticists for over 100 years for development of superior cultivar in many crops. Hybrids cover large acreage in many crops including rice and have fundamentally affected agricultural practices and the seed industry across the world (Rout et al., 2020). Hybrids in rice have ability to produce 20-30% more yield than HYVs (Virmani et al., 1981) and hybrid rice is commercialized in more than 40 countries with huge seed industry. China is a pioneer in the hybrid rice and realized the technology in rice during early 1970s. In India, hybrid rice technology was launched in systematic way by the Indian Council of Agricultural Research (ICAR) during 1989. So far, country has released a total 121 of rice hybrids (38 from public and 83 from private sectors) for different rice ecosystems (115-150

days), which covers ~30 lakhs hectares (6.8%) rice area. Moreover, country has also developed a very rich heterotic parental pools which includes >100 CMS and restorers in diversified genetic and cytoplasmic background. Since the CMS such as CRMS 31A, CRMS 32A, CRMS 8A, PMS10A, PMS 17A, APMS 6A, DR8A, PUSA 5A, PUSA6A, RTN 12A etc. are most suitable, possessing stability across the territories and ecosystems, these CMSs are being utilized in hybrid rice development.

In the beginning, hybrid rice programme was technically supported by the International Rice Research Institute (IRRI), Philippines and Food and Agriculture Organization (FAO), Rome and financially supported from United Nations Development Programme (UNDP), Mahyco Research Foundation, World Bank funded National Agricultural Technology Project (NATP) and IRRI/ADB projects on hybrid rice. Union government of India through various schemes like RKVY, BGREI etc. is promoting the up-scaling of this venture. This programme is further supported by ICAR - Consortium Research Platform on Hybrid Crops Hybrid Technology for Higher Productivity in Selected Field and Horticultural Crops and Indo-ASEAN group.

Indian rice hybrids are adaptive to tropics and subtropics, having unambiguous specificity to their adoptive zones (Table 1). Hybrids like CRHR 105, CRHR 106, 25P25, 27P31 are sustainable under high temperature regime. Besides, the varieties like US 382, Indam 200-17, US 312, DRRH3, JKRH 401 found to have high N-use efficiency which can be utilized for cultivation in poor soil condition. Medium early duration hybrids, PNPB 24, RH 1531, Arize Tej are suitable for drought situations. The low productivity problems in most fragile rice ecosystem *i.e.* coastal and shallow lowland which share ~32% of total rice area is addressed by introducing long duration, low-photo-insensitive hybrids, CRHR 32, Arize Dhani, CRHR 34, CRHR 102 and Sahyadri 5.

Table 1 State-wise suitable rice hybrids in India

State	Identified hybrids
UP	NDR-2, NDRH-3, KRH-2, PRH-10, PSD3, PSD 1, PHB 71, PA 6444, PRH-122, NUSD-3, Sahyadri-4, ARIZE PRIMA, PAC 835, PACH 837, US 312, 27P61, RH 1531, 27P63
Bihar	KRH-2, PRH-10, JRH-5., CRHR-32, US-314, VNR 2375 PLUS, HRI 169, JKRH 3333, PNPB-24, US-312, PAC 835, PACH 837, JKRH-401
Jharkhand	KRH-2, PRH-10, JRH-5, CRHR-32, US-314, VNR 2375 PLUS, HRI 169, JKRH 3333, PNPB-24, US-312, PAC 835, PACH 837, JKRH-401, BS 6444G



State	Identified hybrids
Chhattisgarh	Indira Sona, KRH-2, JRH-4 JRH-5, JRH-8, PRH-10, PAC 807, ANKUR 7434, KPH-371, KPH-199, 27P63
Punjab	KRH-2, PRH-10,
Gujrat	KRH-2, PRH-10, CRHR-32
Odisha	KRH-2, Ajay, Rajalaxmi, CR Dhan 701, Sahyadri-1, Sahyadri-2, Sahyadri-3, PAC835, JKRH-2000, PA6444, CRHR 32, Arize Dhani, PAC 837, 27P31, NK 16520, MEPH 126, 28P09, 28P67, 28P41, BIO 799, CNRH 102, 27P36
MP	JRH-4, JRH-5, JRH-8, Indira Sona, PRH-10, KRH-2
Maharashtra	Sahyadri-1, Sahyadri-2, Sahyadri-3, KRH-2, PRH-10, DRRH-2
AP	DRRH 1,2, KRH 2, Sahyadri 1, CNRH 3, US 305, RH 1531, 27P61
Assam	KRH 2, Ajay, Rajalaxmi, PA6444
Kerala	TNRH 1, KRH 2
Karnataka	KRH 2, Sahyadri 1, 2, 3
TN	TNRH 1, KRH 2
WB	KRH-2, Ajay, Rajalaxmi, Sahyadri-1, Sahyadri-2, Sahyadri-3

The ICAR-National Rice Research Institute (NRRI), Cuttack is pioneer in hybrid rice breeding in India and commercialized three popular hybrids viz., Ajay, Rajalaxmi and CR Dhan 701 for irrigated and shallow-lowland ecosystem. Besides, institute has developed several promising CMS lines possessing stable male sterility (WA, Kalinga-I and *O. perennis* etc. cytoplasmic background) along with maintainers and effective restorers. More than 45 CMS lines in diverse genetic and cytoplasmic background have been developed among which Sarasa A, Pusa 33A (WA), Annada A (WA), Kiran A (WA), Deepa A (WA), Manipuri A (WA), Moti A (WA), Krishna A (*O. perennis*), Krishna A (Kalinga I), Mirai (Kalinga I), Padmini A, PS 92A and Sahabghadhan A etc. are more prominent to be utilized in hybrid development. The medium duration CMSs like CRMS 31A (WA) and CRMS 32A (Kalinga-I) are prominently utilized for hybrid development at NRRI and elsewhere in the country. The CRMS 24A and CRMS 40A, developed under the nuclear background of Moti and Padmini are found suitable for late duration hybrid

breeding. Moreover, short duration CMSs such as CRMS 8A, CRMS 51A, CRMS 52A and CRMS 53A carrying drought tolerance traits are also being used for development of hybrids for drought-prone ecosystem.

In addition, hybrid rice seed production is found to be a profitable venture, provide additional income (Rs.75000-85000/ha net return) and employment opportunity to the 20.0 million rural population (requires 100-105 extra man days per ha area of HR seed production). Thus, this entity has great scope for improvement of livelihood of the nation (Verma et al., 2018).

4. OMICS-BASED APPROACHES IN RICE BREEDING

4.1. Genomic selection

New advancement in high-throughput DNA marker technology and data science gave birth to new tool in breeding *i.e.* genomic selection (GS), which estimates and utilizes true genetic values of individuals for selection purposes. For quantitative traits, genome-wide dense marker-based prediction of genetic values through genomic estimated breeding values allows accurate selection and improves genetic gain (Hickey et al., 2017). Even though application of GS in rice is limited compared to other crops, the precision and repeatability of GS results helped in achieving greater success in rice breeding from parental selection to varietal development. A GS program consists of training population (size and genetic structure), markers (number), relationship between training population (TP) and breeding population (BP) as components on which success of the programs depends. Accuracy of genomic predictions largely been affected by population type and size, trait heritability, marker type, genotyping method, statistical model, breeding platform, etc. (Xu et al., 2020). All these factors connected and relative to each other, consideration of these in a combined approach for better results is a concern (Grenier et al., 2015). The success of genomic selection mainly relies on the prediction accuracy of training population, which is also referred as degree of correlation between predicted GEBV and true genetic value. Sincere efforts have been made to improve rice yield and related traits using GS approach with different statistical models (Xu et al., 2014; Onogi et al., 2015; Spindel et al., 2015; Wang et al., 2017). Yabe et al. (2018) improved the grain filling ability by considering diverse panel of 128 lines using GBLUP model approach. Grain weight and grain number improvement have been made by adapting BLUP-HAT statistical model for estimating GEBVs and selection of appropriate genotypes in RIL population (Wang et al., 2020). Recently, focus has been made to improve the disease resistance using GS approach; maiden attempt to improve blast resistance in rice was made by Huang et al. (2019) in 161 African rice accessions and selected a resistance accession to use as donor in cultivar



development. Similar attempt has been made by Frouin et al. (2019) to improve low arsenic content in flag leaf in a panel of 225 diverse accessions using GBLUP and RKHS statistical models of prediction. Yanru Cui et al. (2020) applied GBUP approach to predict and select hybrids with high yield and grain quality traits from 1495 hybrids. Since, $G \times E$ plays major role in the expression of quantitative trait, Burgueño et al. (2012) reported extension of GBLUP to multi-environment trials to account GEI. In rice, Ben Hassen et al. (2018) implemented multi-environment genomic prediction to manage abiotic stress experiments. The extended GBLUP model (Lopez-Cruz et al., 2015) and the extended RKHS model (Cuevas et al. 2017) integrate environmental effects and inferred that models with multi-environment data delivered higher predictive accuracy than single-environment. Considering $G \times E$, developing GS models is still a challenge, and more compact research is required to untangle the effects of environment on different genotypes and reliably predict their plasticity.

Even though, the success of GS is evidenced by several reports in many crops, its implementation in rice is very limited. Understanding the basic principle and workflow of GS may encourage rice breeders to adapt it in the rice breeding programs. The sound knowledge on predictive accuracy and factors affecting this programme, application of different GS models under different breeding objectives is prerequisite before implementation in existing breeding programs. GS helps in more precise conversion of genotype value into phenotype compared to MAS. This idea may help to design ideal genotypes without any QTL mapping and pyramiding of QTLs. Integrating environmental variables to GS models will also account for higher levels of precision in genotype prediction. However, GS is not a solution to the drawbacks of MAS, but helps to upgrade MAS to the next phase in rice breeding programs. It will be more fruitful if GS is integrated into rice breeding programs in near future.

4.2. Haplotype-based precision breeding

With the development of molecular markers and mapping of quantitative trait loci (QTLs) for various traits in crops, a number of genomic-assisted breeding (GAB) approach like marker-assisted selection (MAS), marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) have been used for transfer of favourable allele(s) into elite breeding lines or high yield rice varieties. Recently, GS, which is based on Genomic-Estimated Breeding Values (GEBVs) is also becoming popular for crop improvement (Abbai et al., 2019; Pallavi et al., 2020). However, marker-assisted backcrossing have been extensively used for transfer of biotic, abiotic stresses tolerant QTLs/genes for improvement of popular rice varieties/elite lines (Joseph et al., 2004; Singh et al., 2016; Bhandari et al., 2019; Pradhan et al., 2019). Besides, a number of important genes related to agronomic traits have been cloned and

characterized (Li et al., 2018). Additionally, with the fast development of genome sequencing technologies such as third generation sequencing technology (Schadt et al., 2010; Niedringhaus et al., 2011; Eid et al., 2009) along with precise phenotyping has been popular for identification of marker-trait associations in crops (Li et al., 2014). Sequencing-based trait mapping is also getting immensely popular in identification of desirable traits using SNP markers covering whole genome with higher resolution which led to detection of a number of haplotypes for the traits of interest. Haplotype is a collection of SNPs or alleles located on the same gene that tend to be inherited together, whereas a haplotype with significantly higher than the average phenotypic performance in a group of individuals, in comparison to other haplotypes is called as a superior haplotype (Bevan et al., 2017). After identification of gene(s) for target trait, the elite lines/ germplasm could be assessed for the presence of haplotypes using Next Generation Sequencing (NGS) technology. Thereafter, all genotypes containing various set of haplotypes are phenotyped for target traits in identification of superior haplotype called haplo-pheno analysis (Sinha et al., 2020). Once superior haplotypes are identified then superior haplotype-based SNP markers are designed which could be utilized for transfer of superior alleles into target genotypes for trait improvement. Besides, haplotypes specific markers could also be used for screening of genotypes in identification of the presence of favourable allele(s) for trait of interest. Furthermore, the haplotyping based genotyping can help in identification of donors where the original donar is not available. Moreover, superior haplotype-based SNP markers can play a major role in screening of the existing germplasm for further utilization in rice breeding programme. Therefore, haplotypes-based precision breeding is very much important for improvement of rice with multiple traits to mitigate the problem of low productivity in the context of climate change scenarios.

4.3. Transgenics

The conventional breeding methods were very successful in rice improvement which was evident from the first green revolution during later-half of the 20th century. Subsequently, the developments in the field of biotechnology, specifically the ‘Transgenic Technology’ approach have enabled the rice researchers to break the crossing-barriers. Besides the direct application in rice improvement, this technology is being used for the characterization of both rice native genes (cis-genes) and transgenes. A significant step towards journey of trasngenics in rice started with the production of the transgenic rice with high-throughput transformation protocol (Hiei et al., 1994). Subsequently, the rice genome project, which led to the sequencing of the genome of japonica rice line, Nipponbare guided the spurt in the identification of many genes governing the important traits in rice. Similar was the result after the availability of



sequence information of genomes from different species of plants, and other organisms (Clement et al., 2017). The availability of genome sequence database and flexibility to genetic transformation consider rice as a model plant for monocots. In rice, transgenic approach has been utilized for the development of rice lines for disease, pest, abiotic stress and quality improvement. The list of transgenic rice developed for various traits is listed in Table 2. Besides, transgenics in rice was reviewed by Giri and Laxmi (2000), Clement et al. (2017), Mohammed et al. (2019) and Dong et al. (2019).

Table 2 Developed transgenic rice for improved stress tolerance and quality

Trait	Cultivar/variety	Transformation method	Gene transferred	References
<i>Herbicide resistance</i>				
	Yamahoushi	Electroporation	bar (Basta)	Toki et al., 1992
	IR-72	PEG	bar (Basta)	Datta et al., 1992
	Taipei-309	Particle Bombardment	bar (Basta)	Cao et al., 1992
	Radon	PEG	bar (Basta)	Rathore et al., 1993
	Yamahoushi	Electroporation	bar (Bialaphos)	Uchimiya et al., 1993
	Gulfmont, IR-72, Koshihikari	Particle Bombardment	bar (Glufosinate)	Oard et al., 1996
	Nipponbare, Kitaake	<i>Agrobacterium</i>	bar (Bialaphos)	Toki, 1997
	Lido, Carnaroli, Thaibonnet	Particle Bombardment	bar (Bialaphos)	Arencibia et al., 1998
	CR-5272, M-7, M-9, ITA-212, IR-64, KDML-105, Bas-370, M202, Jodan, Drew, Bengal, Cypress, Gulfmont	Particle Bombardment	bar (Basta)	Sudhakar et al., 1998
	Gulfmont, Koshihikari, Cypress, Bengal Lido, IR-72	Particle Bombardment	bar (PAT)	Kohli et al., 1999
	Nipponbare	Particle Bombardment	bar (Basta)	Kim et al., 1999
	Notohihkari	Silicon Carbide	bar (Bialaphos)	Matsushita et al., 1999
	Zhonghua 11	<i>Agrobacterium</i>	<i>glnA</i> (GS, Basta)	Cai et al., 2009
	Xiushui-110	<i>Agrobacterium</i>	G6 (EPSPS, (Glyphosate)	Te et al., 2011
	Zhonghua	<i>Agrobacterium</i>	<i>OsGSTL2</i>	Hu et al., 2014

Trait	Cultivar/variety	Transformation method	Gene transferred	References
	Swarna	<i>Agrobacterium</i>	AHAS (Bensulfuronmethyl, Glufosinate herbicides)	Fartyal et al., 2018a
	Swarna	<i>Agrobacterium</i>	<i>igrA</i> and <i>OsmEPS</i> (Glyphosate)	Fartyal et al., 2018b
	Xiushui134	<i>Agrobacterium</i>	AHAS(Sulfometuron-methyl)	Fang et al., 2020
<i>Biotic stress tolerance</i>				
Insect resistance (Bt)	Nipponbare	Electroporation	TCryIA(b)	Fujimoto et al., 1993
	IR-58	Particle Bombardment	Sg CryIA (b)	Wunn et al., 1996
	Taromolaii	Particle Bombardment	CryIA(b)	Ghareyazie et al., 1997
	Taipei-309	Particle Bombardment	CryIA (b)	Wu et al., 1997
	IR-64	Particle Bombardment	Sg CryIA (c)	Nayak et al., 1997
	Vaidehi, TCA-48	Particle Bombardment	CryIA (b)	Alam et al., 1998
	Bas-370, M-7	Particle Bombardment	CryIIA (Bt.)	Maqbool et al., 1998
	Nipponbare	<i>Agrobacterium</i>	SgCryIA (b) SgCryIA (c)	Cheng et al., 1998
	IR-72, IR-64, CBII, Taipei-309, IR-68899B, MH-63-63, IR-51500-AC11 Vaideh-1, IRR1-npt	Particle Bombardment	CryIA (b)	Datta et al., 1998
	IR-68899B-ml	Particle Bombardment	SgCryIA(b)	Alam et al., 1999
	Chaitanya and BPT5204	<i>Agrobacterium</i>	ASAL (BPH, GLH and WBPH)	Yarasi et al., 2008
	Tachisugata	<i>Agrobacterium</i>	DBI (BPH)	Yoshimura et al., 2012
	Ariete	<i>Agrobacterium</i>	<i>mpi-pci</i> (striped stem borer)	Quilis et al., 2014
MH86	<i>Agrobacterium</i>	<i>cry2A*</i>	Ling et al., 2016	
Protease inhibitor	Taipei-309	PEG	CpTi	Xu et al., 1996a
	Nipponbare	Electroporation	CC	Irie et al., 1996
	ITA212, IDSA6,	Particle	Oryza	Vain et al., 1998



Trait	Cultivar/variety	Transformation method	Gene transferred	References
Lectins	LAC 23, Im-S, WAB 56-104	Bombardment		
	Nagdongbyeo	PEG	SKTI	Lee et al., 1999
	Radon, Nortai, FX92, ASD16, M5, M12	Electroporation	GNA	Rao et al., 1998
Viral resistance	Bas-370, M-7	Particle Bombardment	CryIA(c) CryIIA, GNA	Maqbool and Christou, 1999
	Kinuhikari, Nipponbare	Electroporation	RSV-CP	Hayakawa et al., 1992
	CT-6241-17-1-5-1, Savana-6, Oryzica-1, cica8	Particle Bombardment	AsRHBV-NS4 AsRNA4	Lentini et al., 1996
	Taipei-309, Kinuhikari	Particle Bombardment	RTBV CP-1,3,4	Kloti et al., 1996
	Zhonghua-8, Zhonghua-10	Particle Bombardment	RDVS8	Zheng et al., 1997
	ITA 212, Bouake-189, BG-90-2	Particle Bombardment	RYMV PDR	Pinto et al., 1999
	TN1, Taipei-309	Particle Bombardment	RTSV-CP1, CP2, CP3	Sivamani et al., 1999
	Pusa Basmati-1	<i>Agrobacterium</i>	RTBV coat protein	Ganesan et al., 2009
Bacterial resistance (Bacterial blight)	Pusa Basmati-1	<i>Agrobacterium</i>	RSTV RNA	Verma et al., 2012
	Taipei-309	Particle Bombardment	<i>Xa-21</i>	Wang et al., 1996
	IR-64, IR-72 Minghuy-63, BG-90-2	Particle Bombardment	<i>Xa-21</i>	Zhang et al., 1998
	IR-72	Particle Bombardment	<i>Xa-21</i>	Tu et al., 1998
	Taipei0-309	<i>Agrobacterium</i>	<i>OsNPR1/NH1</i>	Yuan et al., 2007
	Mudanjiang 8	<i>Agrobacterium</i>	<i>Xa3/Xa26</i>	Li et al., 2012
	Zhonghua 11	<i>Agrobacterium</i>	<i>HPL3</i>	Tong et al., 2012
Fungal resistance	Dongjin	<i>Agrobacterium</i>	<i>OsTGA2</i>	Moon et al., 2018
	CB-II	PEG	Chitinase (CH11)	Lin et al., 1995
	Nipponbare	PEG	Stilbene Synthase, VstI	Stark-Lorengen et al., 1997
	Nipponbare	Particle Bombardment	Maize-RIP, b-32	Kim et al., 1999
	CB-II, IR-72, IR-51500	Particle Bombardment	TLP-D34	Datta et al., 1999
Nipponbare,	<i>Agrobacterium</i>	Rice class-I Cht-	Nishizawa et al.,	

Trait	Cultivar/variety	Transformation method	Gene transferred	References
	Koshihikari		2, Cht-3	1999
	Nipponbare	<i>Agrobacterium</i>	Chitinase C (Blast)	Itoh et al., 2003
	Nipponbare	<i>Agrobacterium</i>	<i>Gns1</i> (β -glucanase; blast)	Nishizawa et al., 2003
	Pusa Basmati1 (PB1)	<i>Agrobacterium</i>	<i>cht42</i> (Endochitinase - Sheath blight)	Shah et al., 2009
	Nipponbare	<i>Agrobacterium</i>	<i>Pi-d2</i> (Blast)	Chen et al., 2010
	Zhonghua 11	<i>Agrobacterium</i>	<i>OsMPK6</i>	Shen et al., 2010
	Taipei-309	Particle Bombardment	<i>Pi54</i> (Blast)	Rai et al., 2011
	Taipei-309	Particle Bombardment	<i>Pi54rh</i> (Blast)	Das et al., 2012
	Kitaake	<i>Agrobacterium</i>	<i>ACS2</i> (Blast, Sheath blight)	Helliwell et al., 2012
	Aichi Asahi	<i>Agrobacterium</i>	<i>OsGA20ox3</i> (Blast and bacterial blight)	Qin et al., 2013
	Ariete	<i>Agrobacterium</i>	<i>mpi-pci</i> (Blast)	Quilis et al., 2014
	Taipei-309, IET16310 (Pusa Sugandh-2)	Particle Bombardment	<i>Pi54of</i> (Blast)	Devanna et al., 2014
	Taipei 309	Particle Bombardment	<i>LOC_Os11g47510</i> (Chitinase, sheath blight)	Richa et al., 2017
Abiotic stress tolerance				
Water deficit and salt stress	Taipei-309	PEG	CpTi, HVA1	Xu et al., 1996a
	Nipponbare	Particle Bombardment	Barley HVA1	Xu et al., 1996b
	Donggin	<i>Agrobacterium</i>	<i>OsMAPK44</i>	Jeong et al., 2003
Salinity and heat	Sasanishiki	<i>Agrobacterium</i>	Choline monooxygenase	Shirasawa et al., 2006
ROS and salinity	Zhonghua No.11	<i>Agrobacterium</i>	<i>SsNHX1, AVP1</i>	Zhao et al., 2006
Chilling	Yamahoushi	<i>Agrobacterium</i>	<i>Arabidopsis</i>	Yokoi et al.,1998
Photosynthetic efficiency	Kitaake, Nipponbare	<i>Agrobacterium</i>	Maize C4 PEP	Ku et al.,1999
Cold	Xiushui 63	<i>Agrobacterium</i>	<i>OsPIP1;3</i>	Lian et al., 2004
Submergence	Liaogeng	<i>Agrobacterium</i>	<i>SUB1A</i>	Xu et al., 2006



Trait	Cultivar/variety	Transformation method	Gene transferred	References
Heat	Zhonghua 11	<i>Agrobacterium</i>	<i>OsSBPase</i>	Feng et al., 2007
Salt	Nipponbare	<i>Agrobacterium</i>	<i>katE</i>	Nagamiya et al., 2007
Drought, cold and salinity	Nakdong	<i>Agrobacterium</i>	<i>HvCBF4</i>	Oh et al., 2007
	Nipponbare	<i>Agrobacterium</i>	<i>OsDREB1F</i>	Wang et al., 2008
	Zhonghua 10	<i>Agrobacterium</i>	<i>OsNAC5</i>	Song et al., 2011
Salinity	PB1	<i>Agrobacterium</i>	<i>PgNHX1</i>	Verma et al., 2007
	KJT-3	<i>Agrobacterium</i>	<i>P5CSF129A</i>	Kumar et al., 2009
Drought	Zhonghua 11	<i>Agrobacterium</i>	<i>OsLea1-3</i>	Xiao et al., 2007
Aluminium toxicity	star1 (Mutant Koshihikar)	<i>Agrobacterium</i>	<i>STAR1, STAR2</i>	Huang et al., 2009a
Cold, drought and ROS tolerance	Zhonghua 11	<i>Agrobacterium</i>	<i>ZFP245</i>	Huang et al., 2009b
Heavy metal	Dongjin	<i>Agrobacterium</i>	<i>OsNAS3</i>	Lee et al., 2009
Cadmium toxicity	Anjana Dhan	<i>Agrobacterium</i>	<i>OsHMA3</i>	Ueno et al., 2010
	Tsukinohikari	<i>Agrobacterium</i>	<i>OsNRAMP1</i>	Takahashi et al., 2011
Phosphate deficiency	IR64 and Nipponbare	<i>Agrobacterium</i>	<i>OsPSTOL1</i>	Gamuyao et al., 2012
<i>Nutritional quality</i>				
Seed storage protein	Nipponbare	PEG	Bean β -phaseolin	Zheng et al., 1995
	Nipponbare	PEG	Pea Legumin	Sindhu et al., 1997
	Matsuyamamii	Electroporation	Soybean Glycinin	Katsube et al., 1999
Provitamin A	Taipei-309	Particle Bombardment	Daffodil Phytoene Synthase	Burkhardt et al., 1997
Iron	Kitaake	<i>Agrobacterium</i>	Soybean Ferritin	Goto et al., 1999
Vitamin B9	Nipponbare	<i>Agrobacterium</i>	<i>Atgtpchi, Atadcs</i>	Storozhenko et al., 2007
Protein	MH86	<i>Agrobacterium</i>	<i>AmA1</i>	Xu et al., 2017

5. CONCLUSIONS

Under changing global climatic scenarios, rice researchers are tasked with a huge challenge of developing rice varieties with enhanced yield for global food security. Improved rice varieties with inherent resistance/tolerance against biotic and abiotic stresses could help to achieve the goal of climate resilient

agriculture in future. The smart breeding strategy which involves sizable breeding population, robust phenomics facilities, efficient and big data management system along with advanced genomics interventions would be useful to enhance the sustainability of rice plants to changing climate. The proficient conservation of genetic resources is prerequisite for climate-smart breeding. Genetic-engineering is being exploited for the exploration of novel variation/alleles available in the distant genetic proximity in wild or related species. The increasing information on rice physiological and genetic mechanisms is also being utilized for generating new crop cultivars with the capability to tolerate multiple stresses. The technological improvements in phenotypic and genotypic analysis, along with the biotechnological and digital revolutions are paving the way for the development of climate-smart rice varieties.

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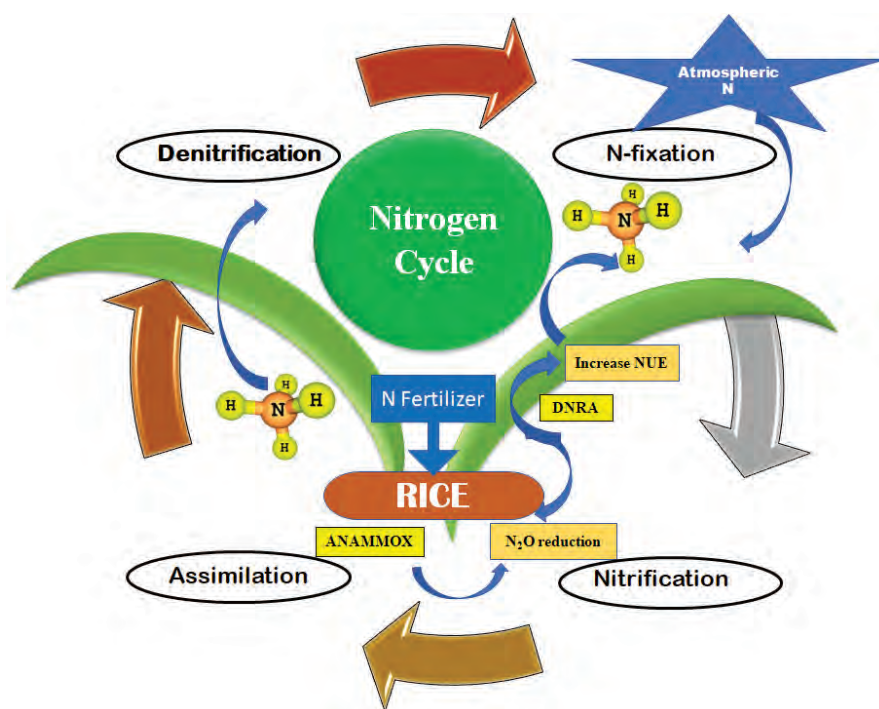
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NITROGEN BIOGEOCHEMICAL CYCLING IN RICE ECOSYSTEM: RECENT ADVANCES AND PROSPECTIVE

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GRAPHICAL ABSTRACT



SUMMARY

The earth's atmosphere contains nearly 78% nitrogen (N), which is a critical nutrient for the growth and production of agricultural crops including rice. N-fertilizers are commonly used to provide ammonium (NH_4^+), which is a preferred source of N. However, N-use efficiency is just 32-50% in rice, and more than half of the fertilizer is lost to the environment as a result of vicious biotic and abiotic N-transforming reactions. N-fixation, ammonification,



nitrification, and denitrification are major pathways in N-transformation in the rice ecosystem. Besides, two unseen pathways such as Dissimilatory Nitrate Reduction to Ammonium (DNRA) and Anaerobic Ammonium Oxidation (ANAMMOX) have been recently discovered in rice-based ecosystem. The contributions of these two pathways in rice paddy environments have been overlooked in the past and hence, recently many researchers initiated studies to understand the potentiality of these N-pathways in rice, particularly to boost N-use efficiency *vis à vis* reducing N-loss *via* nitrous oxide (N₂O), a potent green house gas. The present chapter is an attempt to highlight the new advancement in N-cycle research in rice, covering the most applicable pathways of microbial N-transformation including DNRA and ANAMMOX.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most cultivated grains on the planet (Ishii et al., 2011; Kumar et al., 2018). India is not only the second-largest producer of rice in the world (20% of global production) but also it is the largest consumer, with over 50% of India's population depending on rice for nourishment (Kumar et al., 2020). The cultivation of rice requires plenty of water for its growth, and paddy soils face multiple drying-wetting during rice-growing season, providing unique environments for soil microorganisms. Thus, paddy soils encounter the occurrence of different aerobic and anaerobic biochemical processes, including methanogenesis, sulfate reduction, Mn⁴⁺ and Fe³⁺ reduction, nitrification, nitrate respiration and denitrification (Kimura, 2004).

Nitrogen (N) cycle is one of the most essential biogeochemical cycles on the planet earth and consists of a huge variation of N-species and biochemical transformations (Pandey et al., 2019; Rahman et al., 2019; Shan et al., 2016). As a quintessential element, N is critical to rice crop productivity, however, many environmental issues, such as emission of greenhouse and ozone-depleting gases are linked to the N-fertilizers (Mohanty et al., 2020). The emissions of nitrous oxide (N₂O), a very potent greenhouse gas, continue to rise and more than two-thirds of which occur from bacterial and fungal denitrification and nitrification processes in soils, mostly as end result of the use of N-fertilizers (Kumar et al., 2021). Ecosystems and microbial populations are currently affected by the large amount of reactive N-compounds released to the environment, and microorganisms are playing a very important role in removing these pollutants as part of the biogeochemical cycles (Dobermann et al., 2003; Chaparro et al., 2013).

Microbes are the major players, responsible for N-transformation reaction in rice-ecosystem. N-transformation pathways in the rice ecosystem include N-fixation, ammonification, nitrification, and denitrification. In

addition, two previously unknown pathways in rice-based ecosystems have recently been discovered: dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (ANAMMOX) (Fig. 1). The importance of these two pathways in rice paddy environments has been ignored in the past, and as a result, several researchers have recently begun research to better understand the potential of these N-pathways in rice, especially in terms of increasing N-use efficiency and reducing N-loss, particularly nitrous oxide (N_2O). The current chapter highlights the new advancement in N-cycle research in rice, covering the most applicable pathways of microbial N-transformation. The chapter also addresses the unique transformations reactions in N-cycle such as DNRA and ANAMMOX at molecular, physiological and environmental levels, particularly in rice-ecosystems.

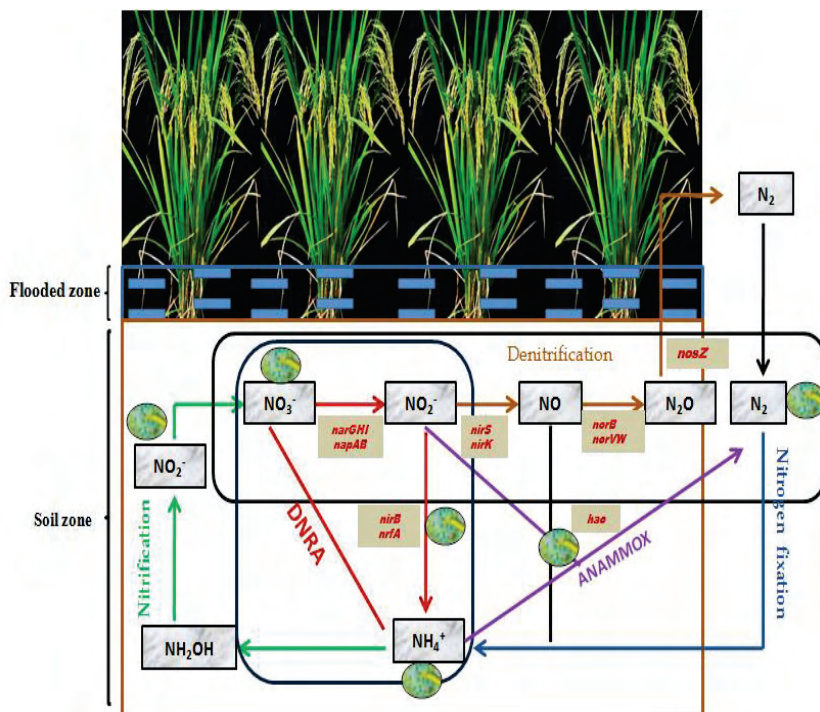


Fig. 1 Schematic diagram of biogeochemical nitrogen-transformation cycle in rice with special emphasis to Dissimilatory Nitrate Reduction to Ammonium (DNRA) and Anaerobic Ammonium oxidation (ANAMMOX) pathways



2. BACKGROUND OF BIOGEOCHEMICAL N-TRANSFORMATION IN RICE

Rice is the important staple food consumed by majority of the global population. Although the crop is cultivated under different water regimes, almost 75% of the world's rice supply comes from continuously flooded rice paddies (Choudhury and Kennedy, 2005; Pandey et al., 2019; Kumar et al., 2020). Unlike other arable crops, the rice plant has aerenchyma, longitudinal interconnection of gas spaces extending from leaves, stems and to the roots of the plant, which supply oxygen (O_2) to the roots for respiration and thus enables the survival of the rice plant in submerged conditions (Kirk, 2003; Kumar et al., 2020). The O_2 supplied to the root also leaks into the adjacent soil and creates oxic conditions around the root surface (Cao et al., 2011a, b, c; Pandey et al., 2019). In addition, the rice plant supplies a considerable amount of organic carbon through root exudates and decaying plant parts (Kraft et al., 2014), which accelerate the development of anaerobic conditions in the soil (Wu et al., 2011). Therefore, paddy soils are comprised of a complex set of oxic and partially oxic environments in the rhizosphere and strictly anoxic environment in the non-rhizosphere (bulk) soil (Yang et al., 2013). These complex set of environment (rhizosphere and bulk soils) in rice paddies favor simultaneous occurrence of several microbial N-transformation processes which have direct effects on the N-use efficiency of rice.

Nitrogen is one of the most important yield-limiting nutrients for arable crops including rice production (Cassman et al., 1998). Rice paddies are also one of the biggest fertilizer-N sinks in the world, which is similar to wheat and maize, although the area under rice is less than that under wheat and maize (He et al., 2012; FAO, 2018). However, fertilizer-N-use efficiency is generally below 40% in rice (Choudhury and Kennedy, 2005), which is mainly attributed to ammonia (NH_3) volatilization (10-25% of applied-N), and denitrification loss (~36% of applied-N) (Cassman et al., 1998; Li et al., 2010 a, b; Li et al., 2011 a, b, c; Jones et al., 2017). On the other hand, rice paddies which do not receive fertilizer-N have shown sustained yields (at moderate level) for hundreds, if not thousands of years (Cassman et al., 1995). Long-term (10-40 years) experimental trials conducted at several locations in different countries have shown that soil-maintained organic carbon and N-status, and sustained rice yields without fertilizer-N input (Bi et al., 2009; Sato et al., 2012; Wang et al., 2013). These and other long-term experiments have documented net N accretion in paddy soils which do not receive fertilizer-N (Cassman et al., 1998; Bi et al., 2009; Wang et al., 2014).

Microbes play an important role in N-transformation cycle in rice paddy soils. The major N-pathways such as N-fixation, ammonification,

nitrification and denitrification are basically operated in rice paddy soil. Among them N-fixation, ammonification and nitrification are the constructive process, whereas denitrification is a destructive process in rice-ecosystem as one third of applied N is lost through this process. Nitrifying microorganisms are autotrophic and use electrons from oxidation of ammonium and nitrite to decrease CO₂ and build biomass. Denitrifiers that consume natural organic substrates are heterotrophic, while those employ inorganic substrates may be autotrophic. Instead of performing denitrification, a few microbes hire a wonderful reductive pathway known as DNRA (Thamdrup and Dalsgaard, 2002; Mohan et al., 2004), this process has now-a-days been reported in different aquatic and terrestrial systems, suggesting that it can additionally be of greater common importance (Silver et al., 2001; Lam et al., 2007; Putz et al., 2018; Pandey et al., 2020). Besides the loss of N through denitrification, ANAMMOX is another process, which is responsible for the N-loss under lowland rice ecosystem. Therefore, two new microbial groups related to N-transformation (DNRA and ANAMMOX) in rice paddy have led to current advances in our understanding of N-cycle. Microbial processes occurring through these processes have a new set of organisms that make major contributions to rice ecosystem in N-transformation.

3. BUDGETARY AND NON-BUDGETARY PROCESSES OF N-CYCLE IN RICE

A combination of assimilatory and dissimilatory biological transformation processes mainly drive the N-transformation in rice paddy soil. In comparison to the contributions of microbial transformations, atmospheric N-fixation associated with lightning, as well as chemodenitrification (Tai and Dempsey, 2009), make only minor contributions (Canfield et al., 2005; Gruber and Galloway, 2008; Samarkin et al., 2010). The geological N-cycle *i.e.* the flux of N through the earth's crust driven by burial sediment and rock weathering, is another minor component (Berner, 2006), while weathering of N-rich bedrock can be a substantial source of N to terrestrial ecosystems (Morford et al., 2011). The ability of the N-cycle to control the supply of fixed-N to the biota is its primary ecological and evolutionary significance. Though, free nitrogen (N₂) makes up the majority of the earth's atmosphere, it is only available to N-fixing bacteria and archaea, which convert it to ammonium and use it. Other prokaryotes, as well as all eukaryotes, need fixed-N (also known as reactive or combined-N) in the form of nitrate, ammonium, or organic-N. Fixed-N in the biosphere accounts for less than 0.1% of the total N₂ pool, and it restricts primary development in both terrestrial and marine habitats (Vitousek and Howarth, 1991; Canfield et al., 2010). On a global scale, the balance between N-fixation and the recycling of fixed-N to N₂ through dissimilatory



transformations regulates the availability of fixed-N. Thus, the N-cycle is closely coupled to the carbon cycle, and its processes and their regulation are of fundamental importance in both modern and ancient ecosystems. Some of the N-transformation reactions are prevalent in rice-ecosystems which decide the budgetary and non-budgetary processes of N-cycle in rice.

3.1. Volatilization

The volatilization of ammonia (NH_3) is one of the important ways of N-loss from the rice fields and accounts for 10-60% of the applied-N (Xu et al., 2012). Researchers also observed that loss of N due to volatilization varied between 48-56% at N level of 53-80 kg ha⁻¹ in lowland rice. However, in another study, Lin De et al. (2007) reported that total NH_3 loss from transplanted rice by ammonia-volatilization during the entire rice growth stage ranged from 9.0-16.7% of the applied-N (Liao et al., 2015). Quantitative estimation of NH_3 emission from soil is, however, often characterized by large biases and uncertainties due to high degree of temporal and spatial variability. Soil physical condition, fertilizer, and water management practices followed, and micrometeorological factors such as wind speed, rainfall, and pH of flood water affect quantification of NH_3 emission.

3.2. Nitrification-denitrification

Coupling nitrification-denitrification processes at aerobic-anaerobic interface plays a key role in efflux of N from rice ecosystem accounting up to >40% loss from applied-N (Buresh et al., 1991). The magnitude may vary from negligible to 46% of the applied-N depending on the amount of urea application and crop establishment methods (Buresh et al., 1991). Isotopic analysis using ¹⁵N indicated denitrification loss of 5-10% for applied urea-N (Pandey et al., 2019). The removal of N through denitrification accounted for 38.9% and 9.9% applied in the drained-reflooded rice paddy soil and continuously flooded rice paddy soil, respectively. Denitrification is usually not explicitly accounted for N-budgets on the national level, but estimated indirectly from N-balances; Abichandani and Patnaik (1955) estimated 20-40% losses in India.

3.3. Leaching

Leaching loss of N from lowland rice fields accounts for 0.1-4.9% of the applied-N (Wen et al., 2019; Xu et al., 2012). Flooded rice fields of eastern India are characterized by low percolation rate and high ground water table; the leaching loss of N was measured to be less than 1% (Pathak et al., 2004; Ladha et al., 2005; Mohanty et al., 2020). However, N-mass balance result showed that loss of N inputs as outflow to the water (leaching and runoff) could be as high as 19% (Ladha et al., 2005).

4. ROLE OF EMERGING PATHWAYS INVOLVED IN N-TRANSFORMATION

The N-transformation cycle is a conversion between sequences of N-compounds through redox reactions in which microbes are predominant driving force (Jetten, 2008). Nitrogen is typically a key component that limits crop production. Therefore, many researchers have focused on the influencing factors of N-retention and loss in croplands (Stevens et al., 1998; Wang et al., 2015; Shan et al., 2016). Extensive usage of chemical fertilizers in agriculture are the sources of high N-loss in many densely populated countries and become an essential problem and have received top notch attention from both ecological and agronomic point of view (Zhao et al., 2009). Ammonium (NH_4^+) ion is the main form of chemical N-fertilizer in rice paddies which subsequently converted into nitrate (NO_3^-) by microbial nitrification reaction and these two species of N participate in the NO_3^- reduction process in wetland rice ecosystem. Research on the NO_3^- reduction process in rice paddies is critical for a proper understanding of the N-cycle and application of N-fertilizers. Previous research on dissimilatory NO_3^- reduction processes has mostly been limited to one or two independent processes (e.g., denitrification and anaerobic ammonium oxidation (ANAMMOX) or dissimilatory nitrate reduction to ammonium (DNRA)) (Yin et al., 2002; Zhu et al., 2011; Yang et al., 2015b), and only a few studies have focused on the simultaneous investigation of the dissimilatory NO_3^- reduction processes including denitrification, ANAMMOX, denitrifying anaerobic methane oxidation (DAMO), and DNRA. Denitrification (reduction of NO_3^- into N_2) was thought to be the main N-loss pathway until the discovery of ANAMMOX in which NH_4^+ is oxidized with NO_2^- and converted into N_2 under anaerobic conditions without N_2O emission (Jetten, 2001; Burgin and Hamilton, 2007; Hu et al., 2011). This process is widespread in marine sediments (Thamdrup and Dalsgaard, 2002), river estuaries (Risgaard-Petersen et al., 2004; Hou et al., 2013), lake sediments (Zhu et al., 2010), groundwater (Smith et al., 2015) and paddy field (Nie et al., 2015). To reduce N-loss and N_2O emissions from soil systems, various methods have been devised, including the use of nitrification inhibitors and slow-release fertilizers (Akiyama et al., 2010; Chen et al., 2010). Another recently suggested strategy for better soil-N management is to compete with the denitrification pathway through N-retaining DNRA pathway (Shan et al., 2016; Putz et al., 2018; Yoon et al., 2019; Friedl et al., 2018). The DNRA pathway is the reduction of $\text{NO}_3^-/\text{NO}_2^-$ to NH_4^+ catalyzed by the microorganisms equipped with cytochrome C_{552} nitrite reductases (encoded by *nrfA* genes) or NADH-dependent nitrite reductases (encoded by *nirB* genes) generally perceived as assimilatory nitrite reductases (Moreno and flores, 2007). Unlike N-loss caused by denitrification and ANAMMOX, DNRA contributes to N-retention in soils by supplying NH_4^+ for crop uptake and microbial immobilization. Positively charged NH_4^+ , as compared to NO_3^- and NO_2^- , tends to remain in soils and has a higher tendency to integrate into plant



biomass (Laima et al., 1999; Silver et al., 2001; Fitzhugh et al., 2003). Hence, the relative contributions of DNRA versus denitrification and ANAMMOX activities play important role in plant growth, N-balance and even climate change.

Denitrifying Anaerobic Methane Oxidation (DAMO) is a dissimilatory NO_3^- reduction mechanism in which NO_3^- or NO_2^- is reduced anaerobically by *Methylomirabilis* bacteria or *Methanoperedens* archaea using methane (CH_4) as an electron donor (Raghoebarsing et al., 2006; Ettwig et al., 2010; Haroon et al., 2013). This is a connection between the biogeochemical C and N-cycles that occurs in inland water and wetland ecosystems (Deutzmann and Schink, 2011; Hu et al., 2014; Zhu et al., 2018b). When estimating the global N-budget, the DAMO process was not taken into account. It is therefore important to begin investigating the contribution and function of DAMO processes in agricultural soil systems in order to improve the accuracy of N and C-cycle models. Denitrification, ANAMMOX, DAMO, and DNRA have a dynamic symbiosis and competition. However, there has been no analysis of the possible simultaneous occurrence of denitrification, ANAMMOX, DAMO, and DNRA, as well as their relative contributions to dissimilatory nitrate reduction in rice paddy soil, until now. Some of the studies related to emerging N-pathways are mentioned in Table 1.

Table 1 Case studies of emerging nitrogen-transformation pathways in rice ecosystem

Country	Type of experiment	Soil type/ Factors	Methodology	Significance	Sources
China	Field	Monolith; ^{15}N -enriched nitrogen application (0, 100 and 250 kg N ha^{-1})	N_2O flux, NH_3 volatilization and ^{15}N abundance in soil were measured	NH_3 volatilization, NO_3^- leaching and N_2O emission increased proportionally in accordance to the fertilizer increase.	Zhao et al., 2009
Australia	Field study	Very limited fertilizer-N (25–40 kg fertilizer-N ha^{-1} , as urea or compound fertilizer)	^{15}N isotope analysis	DNRA, denitrification and ANAMMOX are significant N-transformation pathways in rice paddies. These processes initiated within a week of flooding of rice paddies.	Pandey et al., 2016

Country	Type of experiment	Soil type/ Factors	Methodology	Significance	Sources
China	Field study	Nitrogen fertilizer regimes from 120-300 kg urea-N ha ⁻¹	Soil core incubation study-based on N ₂ /Ar technique, ¹⁵ N isotope analysis	First simultaneous investigation of the dissimilatory nitrate reduction processes in paddy soils, highlighting that ANAMMOX and DNRA play important roles in removing NO ₃ ⁻ .	Shan et al., 2016
Japan	Field study	Free-water-surface constructed wetland cultivated with wild rice (<i>Zizania latifolia</i>)	¹⁵ N isotope analysis and clone library analysis	The highest potential ANAMMOX activity observed in the upper layer of soil, and also found in the sites of submerged and vegetated, and immediately adjacent to the influent supply.	Waki et al., 2015
China	Field and rhizo bag experiment	Applied urea as 100 mg kg ⁻¹ dry soil	Catalyzed reporter deposition–fluorescence <i>in situ</i> hybridization, isotope-tracing technique, quantitative PCR assay and 16S rRNA gene clone libraries analysis	Rhizosphere ANAMMOX contributed to 31–41% whereas the non-rhizosphere ANAMMOX bacteria contributed to only 2–3% N ₂ production. Higher ANAMMOX bacterial cells were observed in the rhizosphere, which were twofold higher compared with the non-rhizosphere soil.	Nie et al., 2015
China	Pot experiment	Experiment with three treatment-no rice straw addition, 0.33% (w:w)straw addition and 0.66% (w:w) straw addition	Stable isotope tracer analysis and quantitative PCR assay	The rates of DNRA, abundance and transcription activity of <i>nrfA</i> gene positively correlated to soil C/N and C/NO ₃ ⁻ induced by straw application than the control or no incorporation.	Bai et al., 2020
Japan	Microcosm experiment	Grey lowland soil	¹⁵ N isotope analysis, clone library	DNRA and denitrification	Nojiri et al., 2020



Country	Type of experiment	Soil type/ Factors	Methodology	Significance	Sources
			analysis	occurred to a similar extent and appeared to be enhanced by a nitrate limitation relative to organic carbon. It reduces N-loss through denitrification and nitrate leaching and provides ammonium to rice roots in rice paddy fields.	
Australia	Glasshouse pot experiment and laboratory incubation study	Three different rice paddies (Finley, Jerilderie, Coree) in the Riverina region of New South Wales	¹⁵ N isotope analysis and clone library analysis	DNRA plays a significant positive role in paddy soil N-retention process, as it accounts for up to 55% of the total nitrate reduction, but this is reduced by N-application in rice field.	Pandey et al., 2018
Korea	Batch and chemostat incubation experiment	DNRA-catalyzing isolates with varying C to N ratios were applied	High-throughput DNRA phenotype screening technique	Both batch and chemostat incubations of DNRA producing isolates with excess organic electron donors produced NH ₄ ⁺ from reduction of NO ₃ ⁻ . But incubation with excess NO ₃ ⁻ showed in NO ₂ ⁻ build-up instead of N- NH ₄ ⁺ production, presumably due to high concentration of NO ₃ ⁻ presence.	Heo et al., 2020
China	Field experiment	The soil type is characterized by a mollic epipedon, an albic E-horizon, and a thick argillic	Real-time reverse-transcriptional quantitative PCR analysis	Ammonia oxidizing archaea and bacteria are playing an important role in N-transformation in agricultural soils in oxic or anoxic environment but ANAMMOX bacteria	Wang et al., 2014

Country	Type of experiment	Soil type/ Factors	Methodology	Significance	Sources
		horizon which is good for water holding but very poor for air flux vertically		contribute less extent to N- transformation in studied agricultural soils under anoxic condition.	
China	Mesocosm experiment in three rice production areas	Soil types: inceptisol, ultisol and oxisol, respectively.	¹⁵ N isotope analysis, clone library and RT-PCR analysis	ANAMMOX accounted for 0.4%-12.2% of the total N ₂ production, and correlated with soil pH and electric conductivity. ANAMMOX activity and abundance of <i>hzsB</i> genes found significantly lower in the surface soil than in the sub-surface soil layers.	Bai et al., 2015

4.1. DNRA in rice ecosystem

Rice cultivation has shown sustained production for hundreds, if not thousands, of years without N-fertilization (Stewart, 1995). Several long-term (up to 40 years) experimental trials in various locations have shown stable grain yields (up to 3 t ha⁻¹) and soil-N status in rice paddies without N fertilization (Stewart, 1995; Cassman et al., 1998; Dobermann et al., 2003; Bi et al., 2009). This means that unfertilized paddy systems contain N-cycling and efficient conservation strategies that promote rice N-nutrition and soil-N status. In unfertilized rice paddies, diazotrophs can supply more than 45 kg N ha⁻¹ (Bei et al., 2013), which is thought to be a contributing factor in the rice paddies sustained N-nutrition. However, it is unclear if N-fixation alone will preserve the N status of unfertilized rice paddies in areas where the denitrification process is highly favourable. In the oxic-rhizosphere, long-term unfertilized rice paddies have shown low but persistent NO₃⁻ production through nitrification process (Zhang et al., 2012; Wang et al., 2014). Denitrifiers may then reduce the produced NO₃⁻ to N₂O or N₂, resulting in N-loss (Arth et al., 1998). In marine and sediment environments, a close coupling between N-fixation and denitrification, resulted in negative feedback in the N-budget (Deutsch et al., 2007; Moore et al., 2009). Ammonia-oxidizing bacteria can actively use N-derived from N-fixation in unfertilized rice paddies, according to a recent report (Bei et al., 2019). Denitrifiers can then use NO₃⁻ formed by ammonia oxidation



to catalyse N-loss in rice paddies, which is aided by the supply of labile organic carbon (OC) provided by rice root exudates and decaying plant pieces (Kimura et al., 2004). This implies that without mechanisms to restrict N-loss through denitrification, N-fixation alone will not be able to preserve the N-status of paddy systems. DNRA, is catalyzed by the cytochrome c nitrite reductase encoded by the *nrfA* gene in bacteria (Kraft et al., 2011), which has been found to compete with denitrification for NO_3^- and minimize N-loss in forest (Silver et al., 2001) and temperate arable soils (Putz et al., 2018). Studies using bacterial cultures to investigate the function of DNRA have revealed that the process is dominant in NO_3^- -limiting conditions (Strohm et al., 2007; Van den Berg et al., 2015, 2016). Long-term N-fertilizer application raises NO_3^- content in paddy soils, while long-term unfertilized rice paddies have lower nitrification capacity and lower NO_3^- content (Wang et al., 2014; Wu et al., 2011; Yang et al., 2013). This low NO_3^- content, combined with the rice plant labile OC supply, will favour DNRA over denitrification. In long-term low N-fertilized rice paddies, it has been found that DNRA dominates over denitrification and the DNRA is positively related to the SOC/ NO_3^- ratio (Pandey et al., 2019).

4.1.1. Taxonomy and phylogenetic microbial players for DNRA

The DNRA process involves a wide range of microorganisms, including heterotrophic and chemolithoautotrophic microbes (Giblin et al., 2010; Pang and Ji, 2019; Rahman et al., 2019). Though biomarker genes for all DNRA-functioning microbes are still unavailable (Mohan et al., 2004), a PCR primer set targeting the *nrfA* gene, encodes the nitrite reductase that catalyses the second stage of the DNRA mechanism, has been established so far (Kraft et al., 2011; Welsh et al., 2014). The study based on this primer set suggested that the *nrfA* gene could be found in diverse groups of bacteria including Proteobacteria, Planctomycetes, Firmicutes, Bacteroides, Verrucomicrobia, Acidobacteria and Chloroflexi, and that community composition of *nrfA* gene greatly varied among different habitats (Welsh et al., 2014). Unlike the traditional assumptions, facultative and obligatory fermentative bacteria (*Clostridium*, *Bacillus*, and most Enterobacters) are now known to be primarily responsible for DNRA in soils (Caskey and Tiedje, 1979). Dissimilatory metal- and sulfur (S)-reducing bacteria such as *Thiobacillus* and *Desulfovibrio* spp. (Dalsgaard and Bak, 1994; Brunet and Garcia-Gil, 1996), and iron (Fe)-oxide reducing bacteria like *Geobacter metallireducens* (Finneran et al., 2002) have also been found to catalyze DNRA in different terrestrial ecosystems (Pandey et al., 2020). Recently, Polcyn and Podeszwa (2009) reported DNRA to occur in several Rhizobial species. *Arthrobacter*, being obligate aerobes are found abundantly in soils worldwide, and carry out DNRA when incubated anaerobically (Eschbach et al., 2003). Freitag et al. (1987) have reported eight obligate aerobic *Nitrobacter* strains, which act as NO_2^- oxidizers and execute

DNRA metabolism in anaerobic conditions. Now soil fungi, mostly belonging to the Ascomycota fungal phylum, have been reported to have the capability to perform DNRA (Zhou et al., 2002). Moreover, Zhou et al. (2002) also reported that denitrification and DNRA are alternatively expressed in a common soil fungus (*Fusarium oxysporum*) depending on the O₂ status. Sulfate reducing bacteria like *Desulfovibrio* (Dalsgaard and Bak, 1994), *Desulfobacterium* (Szewzyk and Pfennig, 1987), and *Desulfobulbus* (Brunet et al., 1990) are bacteria performing DNRA in rice paddy soils.

4.1.2. Genetic marker and regulation of DNRA

DNRA and denitrification are two processes metabolically compete with NO₃⁻ in soils under reducing conditions (Silver et al., 2001; Minick et al., 2016). The denitrification is catalyzed by four enzymes together with nitrate reductase (NarG), nitrite reductase (NirS/NirK), nitric oxide reductase (NorB) and nitrous oxide reductase (NosZ) and are encoded by using *narG*, *nirS/nirK*, *norB* and *nosZ* genes, respectively (Giles et al., 2012). The nitrate reductase enzyme is frequent in both DNRA and denitrification pathways (Wang et al., 2018). However, the prevalence of two different nitrite reductase enzymes, NirB and NrfA, are encoded by the *nirB* and *nrfA* genes, respectively, separates the pathway of DNRA from denitrification in bacteria (Kraft et al., 2011, 2014; Kim et al., 2017; Wang et al., 2018). The enzymes NirB and NrfA reduce NO₂⁻ to NH₄⁺ via the fermentative (heterotrophic) and respiratory DNRA pathway, respectively. The NirB is a nitrite reductase enzyme of the siroheme type and provides both assimilatory (Oliveira et al., 2013; Horchani et al., 2008) and dissimilatory reduction of NO₂⁻ (Clark, 1989; Wang et al., 2018). As a result the *nirB* gene is no longer considered as a molecular marker for DNRA.

Some studies have shown that hypoxic roots of rice, tomato and soybean assimilate NO₃⁻ by Nir-mediated fermentative nitrate-ammonification (Horchani et al., 2008; Horchani and Aschi-Smiti, 2010; Oliveira et al., 2013), as evident from reduced ethanol following application of NO₃⁻ (Oliveira et al., 2013). However, the *nrfA* gene is discovered in unique groups of microbes that perform DNRA along with Proteobacteria, Planctomycetes, Bacteroides and Firmicutes (Brunet and Garcia-Gil, 1996; Mohan et al., 2004; Smith et al., 2007; Giblin et al., 2010). Several studies traced out a positive and significant association between DNRA activity and the abundance of the *nrfA* gene in rice paddy ecosystems (Li et al., 2019; Li et al., 2020).

4.1.3. Environmental drivers for DNRA activity in rice paddy soil

Active DNRA has been found mostly in anoxic ecosystems including floodplains and terrestrial habitats (Gao et al., 2017; Jones et al., 2017). Some environmental factors, such as soil redox, salinity, carbon to nitrogen ratio



(C/N), and carbon to NO_3^- ratio (C/NO_3^-), can influence the process of DNRA (Giblin et al., 2010; Lu et al., 2013; Hardison et al., 2015; Minick et al., 2016; Robertson et al., 2016; Zhou et al., 2017). Among all the drivers, C/N and C/NO_3^- are considered to be the control elements that regulate the occurrence of DNRA and denitrification, as microbes involved in the two processes usually compete for available carbon sources as electron donors and for NO_3^- as an electron acceptor (Strohm et al., 2007; van den Berg et al., 2015, 2016). Denitrification was previously restricted primarily by NO_3^- supply, but DNRA was more sensitive to the C/NO_3^- ratio, as evident from recent field study in wetlands (Rahman et al., 2019). In rice soil ecosystems with wetland-like habitats, little is known about DNRA. Rice fields have ideal conditions for DNRA processes, such as low redox potential and anoxic conditions. In paddy soils fertilized with livestock waste, DNRA rates have been estimated to range from 3.06 to 10.40 mg N kg^{-1} dry soil d^{-1} (Lu et al., 2012). Similarly, Shan and colleagues (2016) revealed that the DNRA in 11 typical Chinese paddy soils ranged from 2.37 to 8.31 nmol N g^{-1} h^{-1} , which is closely related to the C/N ratio and extractable organic carbon to NO_3^- concentrations. In addition, rice fields are quite rich in carbon inventories due to low mineralization rate under waterlogged condition, and high organic matter input through fertilization, plant residues and root exudates (Guo and Lin, 2001; Liu et al., 2019; Pandey et al., 2019). Besides, crop residues such as straw incorporation are widely applied in rice paddy fields to enhance soil fertility. It has been suggested that retaining straw in rice paddy soil can significantly increase soil organic carbon stock and has positive effects on greenhouse gas emission and soil microbial community by changing soil physico-chemical properties and soil C/N ratio (Yan et al., 2013; Lu et al., 2015; Wang et al., 2018; Wu et al., 2018; Yao et al., 2018). Moreover, recent studies suggest that increased N-fertilizer application in rice paddy soils can reduce the abundance of beneficial DNRA microbes (Friedl et al., 2018; Pandey et al., 2019). All of these concerns suggest that DNRA is a significant NO_3^- reduction pathway in rice paddies, and that fertilization regimes, especially C and N input, can influence the behaviour and performance of associated microbial players.

4.2. ANAMMOX in rice ecosystem

In the conventional view of the N-cycle, NH_4^+ was considered as the end product of anaerobic degradation, and its transformation would require the presence of oxygen and NH_4^+ -oxidizing bacteria. Chemical data from oxygen-depleted oceanic waters and deep-sea sediments, on the other hand, revealed that NH_4^+ is oxidized anaerobically to N_2 in these conditions in the presence of NO_3^- and NO_2^- (Richards, 1965; Emerson et al., 1980). A microbial process termed as ANAMMOX that could account for these observations, was first reported on experimental wastewater treatment system (Mulder et al., 1995; van

de Graaf et al., 1997). Investigations of especially purified enrichment cultures revealed that the organisms belong to a wonderful clade within the bacterial phylum Planctomycetes and that NO_2^- is the oxidant for NH_4^+ in the lithotrophic ANAMMOX procedure from which the organisms could get energy (van de Graaf et al., 1997; Strous et al., 1999). The discovery of ANAMMOX is essential for N-cycling, and it also sheds light on the mechanisms that cause N-loss (Thamdrup and Dalsgaard, 2002; Dalsgaard et al., 2003; Kuypers et al., 2003).

Recently, ANAMMOX microorganism were observed to be widely distributed in agricultural fields including rice ecosystems (Humbert et al., 2010; Wang et al., 2012; Shen et al., 2013; Wang and Gu., 2013). Since oxygen inhibits ANAMMOX bacteria, anoxic environments are needed for its occurrence (Jetten et al., 2009). Hence, most of the previous studies focused on anoxic environments such as oceanic sediments and oxygen minimum zones (OMZs). It was also reported that in environments where these microbes exist, more than half of the N_2 produced is due to activities of these microbes (Thamdrup and Dalsgaard, 2002; Engstrom et al., 2005; Rich et al., 2008). Prevalence of ANAMMOX was later discovered in terrestrial ecosystems such as freshwater wetlands (Humbert et al., 2010; Zhu et al., 2011a, b; He et al., 2012). ANAMMOX bacteria are also responsible for N-loss from farmland soils that receive a large volume of N-fertilizer (Long et al., 2013; Shen et al., 2013). Rice paddy soils are often flooded, resembling freshwater wetlands in appearance.

The presence of ANAMMOX bacteria in rhizospheric soils contributes to higher N_2 production than bulk soils, particularly during the rice tillering growth stage (Nie et al., 2015). Root exudates have been suggested to play a critical role in shaping the microbial community in soils (Lugtenberg et al., 2002; Hartmann et al., 2008; Shi et al., 2013) which shifts the diversity and abundance of microorganisms during rice growth (Eller and Frenzel, 2001; Badri and Vivanco, 2009; Dianou et al., 2012; Chaparro et al., 2013). The observed ANAMMOX rates ($0.33\text{--}0.64 \text{ nmol N}_2 \text{ g}^{-1} \text{ h}^{-1}$) in the rhizosphere had been appreciably higher than that in the bulk soils ($0.08\text{--}0.26 \text{ nmol N}_2 \text{ g}^{-1} \text{ h}^{-1}$), indicating that rhizospheric ANAMMOX technique would possibly be an overlooked pathway for N-loss from rice paddy soils (Nie et al., 2015; Wang et al., 2015). Exposure to rhizosphere to oxygen, may produce nitrite for ANAMMOX bacteria, in several environments, inclusive of marine (Kuypers et al., 2005; Lam et al., 2007; Cao et al., 2011 a, b, c, d, 2012), freshwater (Zhu et al., 2010, 2011a; Wang et al., 2012a) and terrestrial soil (Zhu et al., 2011b). In oxygen-limited bulk soil, the place where nitrification is inhibited, denitrification may supply ANAMMOX with nitrite. Higher ANAMMOX rates had been also detected in the surface layer of different paddy soils (Zhu et al.,



2011b; Sato et al., 2012). The rhizosphere and surface layer of standing water are common oxic–anoxic interfaces in wetland ecosystems; therefore, the redox gradient in the rhizosphere is a hotspot for ANAMMOX activity. In waterlogged ecosystems, oxygen demand and NO_x-manufacturing may be considered to be the key elements in determining ANAMMOX activity. Thus, ANAMMOX is one of the imperative N-transformation pathways in rice paddy soils.

4.2.1. Taxonomy and phylogenetic microbial distribution of ANAMMOX

The ANAMMOX bacteria, a group of slow-growing lithotrophic microorganisms (van de Graaf et al., 1996) are responsible for removing inorganic-N from a wastewater treatment system (Mulder et al., 1959; vande Graaf et al., 1995; Jetten et al., 1997; Strous et al., 1997). ANAMMOX microorganism have been detected in a wider areas consisting of oxygen-minimum and oxygen-limited zones including rice ecosystems and responsible for major portion of N-loss. ANAMMOX process are also carried out by both ammonia-oxidizing bacteria and archaea in a wide array of environments (Koops et al., 2003; Strous and Jetten, 2004; Konneke et al., 2005; Li et al., 2010a, b; Cao et al., 2011a, b, c, d; 2012). ANAMMOX microorganism are autotrophic belonging to the order Planctomycetales (genera, *Candidatus*). There is an important distinct division of bacteria which has been also revealed by phylogenetic evaluation based on 16S rRNA genes (Schmid et al., 2005). Furthermore, ANAMMOX bacteria are considered to be habitat-specific with certain groups observed to be dominant in distinct environments (Kuypers et al., 2005; Humbert et al., 2010). For instance, *Candidatus scalindua* was once majorly discovered in marine ecosystems (Penton et al., 2006; Rich et al., 2008; Li et al., 2010), whilst *Candidatus brocadia* and *C. kuenenia* had been more predominant in agricultural soils (Shen et al., 2013; Bai et al., 2015). However, a recent study about the dominance of *C. calindua* in rice paddy soils in Northeast China, raised the question of a heterogeneous distribution of ANAMMOX bacteria in distinct soil types (Wang and Gu, 2013).

4.2.2. Genetic marker and regulation of ANAMMOX

The ANAMMOX microorganism couple NO₂⁻ reduction to NH₄⁺ oxidation, with nitric oxide (NO) and hydrazine as intermediates, subsequently producing N₂ and NO₃⁻ (Kartal et al., 2012; Van and Jetten, 2012; Katal et al., 2013). The NO, a key intermediate in ANAMMOX bacteria is frequently produced with the aid of the reduction of NO₂⁻ to NO. This response is catalyzed by two kinds of enzymes copper-containing (NirK) and cd1 (NirS) nitrite reductases (Vlaemick et al., 2011). However, ANAMMOX bacteria do not always possess the genes encoding these enzymes. Interestingly, the *nirK* gene has been detected in the

Candidatus sp., *Brocadia* sp. (Lawsen et al., 2017) and *Brocadia caroliniensis* (Park et al., 2017) genomes. However, neither *nirK* nor *nirS* genes have been detected in other *Brocadia* genomes. *Candidatus brocadiasinica* is one of the species with its whole genome sequenced and it lacks genes encoding NO forming nitrite reductase (i.e. *nirK* and *nirS*) (Oshiki et al., 2016). Functional hydrazine oxidoreductase (*hzo*) genes were identified as a particular molecular marker for quantifying and characterizing ANAMMOX bacteria (Harhangi et al., 2012), and the *hzoA* and *hzoB* genes have been reported in a few wetlands including rice and marine ecosystems (Humbert et al., 2010; Shen et al., 2013; Lipssewiers et al., 2014). The enzyme Hzo encoded by *hzo* gene is a multi-heme type protein abundantly produced by strain of KSU-1, an ANAMMOX bacterium (Shimamura et al., 2008). Hydrazine, an extremely toxic chemical compound, is an intermediate product from coupling oxidation of NH_4^+ and reduction of NO_2^- in the anammoxosome of that microorganism (Schalk et al., 2000). Hzo is one of the key enzymes concerned in ANAMMOX reaction as it can oxidize the unique ANAMMOX transformation intermediate hydrazine to N_2 (Shimamura et al., 2007; Quan et al., 2008). For classification purposes, *hzo* is a marker gene that is greater accurate and notably unique in classification and phylogeny compared to 16S rRNA gene (Li et al., 2010b). Although, detection of *hzo* gene was rarely suggested from environmental samples due to the low efficiency of well-known primer sets, several attempts have been made recently in improving the efficiency of amplifying *hzo* genes (Li et al., 2010a, b; 2011a, c).

4.2.3. Environmental drivers for ANAMMOX activity in rice paddy soil

Stable environmental conditions are thought to be a necessity for the habitat and enrichment of ANAMMOX microorganism (Dalsgaard et al., 2003; Humbert et al., 2010). The previous agricultural cultivation history coupled with recent practices would possibly be the predominant contributing factors limiting the range and distribution of ANAMMOX bacteria. Besides, the relative short land use from natural wetland to agricultural soil also ought to be viewed when interpreting the incidence of ANAMMOX microorganism (Koops et al., 2003). Another study confirmed that pesticides and fertilizers may have an immense effect on the ANAMMOX microorganism and their relative abundances of the total microbial neighborhood (Rahman et al., 2020). Successful amplification of ANAMMOX 16S rRNA and *hzo* genes suggests that flooding is the fundamental cause for the occurrence of ANAMMOX bacteria and fertilization could also alter N- transforming microorganisms in soil.



5. ASSESSMENT OF THE DNRA AND ANAMMOX IN RICE PADDY SOIL

The ^{15}N tracer, $^{15}\text{N}_2$ -uptake, acetylene reduction assay (ARA), and q-RT-PCR techniques were used to quantify N-fixation, DNRA, ANAMMOX, and denitrification from rice paddy soils. These techniques are very sensitive and highly reproducible. A very small amount of the heavy N isotope (^{15}N) is believed to be present in soils under normal environmental conditions (Kirkham and Bartholomew, 1954). With this in mind, Hart et al. (1994) and Kirkham and Bartholomew (1954) developed the ^{15}N -pool dilution process, in which ^{15}N is enriched in test soils and the ratio of ^{15}N to ^{14}N is measured using an Isotope Ratio Mass Spectrometer (IRMS). Kirkham and Bartholomew (1954) developed three assumptions for isotopic N-transformation studies using the pool dilution method: (i) ^{15}N and ^{14}N react similarly, implying that isotope discrimination is minimal; (ii) development and consumption rates remain constant during the incubation period (iii) ^{15}N is not recycled from the sink (NH_4^+) to the source pool (NO_3^-). These assumptions usually remain true for around 1- 2 days (Hart et al., 1994). Using KCl extracts of soils and a diffusion method described by Brooks et al. (1989) and updated by Stark and Hart (1997) to include the Teflon [(polytetrafluoroethylene (PTFE)) acid trap diffusion procedure, initial and final concentrations of enriched ^{15}N are calculated at T0 (initial time) and T1 (final time). The methodology for determining DNRA has been defined in detail by Silver et al. (2001) and Minick et al. (2016). The new Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) analysis was used to examine *in situ* ANAMMOX bacteria in flooded rice soils (Nie et al., 2015).

6. CONCLUSIONS

The N-cycle is a network of intermediate N-species transformations. Since N-cycling is often based around oxic-anoxic interfaces, interactions between aerobes and anaerobes are important research issue. Newly discovered processes in N-cycle, such as DNRA and ANAMMOX, are often and partly ignored when estimating N-budgets. A delicate balance between sources, primarily N-fixation, and removal through N_2 processing regulates the pool of fixed-N in rice. In low-N input rice paddies, microbial N-cycling processes have developed to efficiently add and maintain N within the system, allowing for minimal N-loss. Therefore, identification of several microbes in different rice ecologies can perform DNRA, an important aspect to maintain N-loss. The rhizosphere-driven DNRA and ANAMMOX processes have also been largely overlooked as a result of the differences in phylogenetic diversity of DNRA and ANAMMOX microbes as well as their metabolic footprints in rice ecosystems should be investigated.



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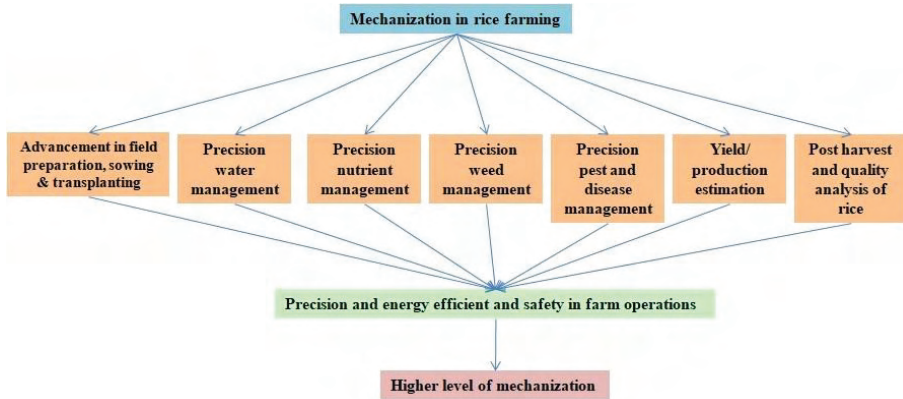
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RICE MECHANIZATION AND PRECISION FARM OPERATION IN INDIA AND FUTURE PROSPECTS

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GRAPHICAL ABSTRACT



SUMMARY

Mechanization and productivity of rice are having a proportional relationship in India. The precision farming tools having the potential to enhance the mechanization level in rice by precision, energy efficiency, and safety in farm operations through optimum field preparation, sowing and transplanting, nutrient management, weed management, pest and disease management, post-harvest, and quality of the final product. It ensures optimum resource utilization to make rice farming less energy-intensive, and environment-friendly. This chapter provides a status and usage of rice machinery and identifies the thrust areas of future research to enhance the mechanization and precision in rice farming.

1. INTRODUCTION

Rice is staple food for more than 50% of the population and for Asia this figure goes to 70%. Asian region is also referred as the ‘Rice Bowl’, producing around 90% of world’s rice. China and India together produce almost 50% of the global rice production. Rice is a staple food crop for more than 60% of Indians, which at present occupies around 44 M ha (22%) of cropped land in India. China, the top rice producer, contributes 21.5% of global rice production. India is the second largest producer of rice. The production and productivity of rice is on increasing trend mainly because of genetic improvement, agro-chemical practices, irrigation and farm mechanization. The use of various inputs, such as fertilizers, irrigation water, plant protection chemicals, use of machinery, electricity in rice production have increased tremendously paving the way for a significant progress in Indian agriculture in the past few decades. However, serious challenges have been thrown in past few years due to reduction in per capita land availability, stagnating net sown area, and climate change and land degradation. Therefore, rice farming in future has to be multi-functional and ecologically sustainable for delivering ecosystem goods and services as well as livelihoods to producers and societies. Hence rice farming at current and in future has to address local challenges of food, water and energy insecurity as well as issues related to climate change and degradation of natural resources. Mechanization in rice farming is having potentials to further transform rice farming by addressing these issues. This chapter covers the mechanization scope and status of precision in rice farming in India and, identification of future thrust areas of research.

2. LAND HOLDING AND MECHANIZATION

Farm mechanization plays a major role in increasing productivity, reducing drudgery by replacing manual labour and animals in agriculture, bringing rural prosperity. The overall farm mechanization in India is 40-55%, lagging behind other countries such as USA (95%), Western Europe (95%), Russia (80%), Brazil (75%) and China (57%) (Renpu, 2014). Small field size, smaller individual land holdings and socioeconomic conditions are some of the possible reasons for low productivity of rice. In India, 80% of farm holdings have less than two hectares land with 62% possessing less than half a hectare. Operational holding is decreasing and number of farmers under marginal and small categories is increasing. As per 2015-16 agricultural census, average size of land holding in India decreased to 1.08 ha from 2.28 ha in 1970-71. There is a linear relationship between level of farm mechanization and farm production. Farm mechanization contributes in saving seeds (15-20%), fertilizers (15-20%) increasing crop intensity (5-20%) and crop productivity by 10-15% (Guru et



al., 2019). Assam, Bihar, Jharkhand, Eastern UP, Chhattisgarh and Odisha have farm power availability less than 1.50 kW per ha and happens to be the low productivity states. The machine power use in Indian agriculture has increased from 1.08 kW per ha in 1995 to 2.0 kW per ha in 2016-17. The rapid growth of farm mechanization is evident from steady rise in the sale of tractors. Indian tractor industry is largest in the world accounting one third of the global product. Nevertheless, there has been wide variation in farm mechanization with respect to the geographical region and crops. Northern states such as Punjab, Haryana and western Uttar Pradesh have 70-80% mechanization in rice while it is 30-45% in southern and eastern states. The mechanization levels for different farm operations are 40%, 30% and 37% for tillage, seeding/planting and for irrigation. Rice being the dominant staple food crops, its mechanization and precision farm operations has potential to revolutionize the agriculture.

3. CONSTRAINTS IN RICE FARMING AND MECHANISATION

Rice farming is currently facing the challenges of decline or stagnation in productivity, depletion of natural resources, several economic and environmental problems, increasing cost of cultivation, fossil fuel burning, greenhouse gas emission, pollution of water bodies in rice production system.

Use of machines and precision in farm operation can play a big role in addressing the challenges in rice farming through its applications by bringing efficiency in field preparation, weed management, irrigation water management, fertilizer application, insect and pest control, crop harvest and post-harvest operations. Precision farming is a promising technology to identify, analyze and manage the spatial and temporal variability of different parameters like soil, water, nutrient, etc. within the field and ensures correct and timely application of inputs in required amounts with minimum negative impact on environment. Rice farms in developed countries are often large (sometimes 1000 ha or more) comprising of bigger fields, have already adopted precision farming technologies. In developing countries, such as India, fragmented land holding, rapid changes in the socio-economic condition have offered both challenges and opportunities for mechanization and application of precision farming technologies. Starting from manual tilling and bullock operated tillage operation. Indian agriculture has travelled a long tortuous path of farm mechanization. The status of rice mechanization in different states is given in Table 1. The operation wise mechanization in rice farming is discussed in detail in the succeeding sections.

Table 1 Farm implements/machines used for rice cultivation in India

State	Operation	Implements/machinery used
Andhra Pradesh	Land preparation	Tractor drawn (TD) Laser guided land leveler, TD rotavator, TD MB Plough, TD Cultivator, TD Disc harrow
	Sowing/Transplanting	TD zero-till drill, TD Inclined plate planter
	Weeding	Power weeder, TD Aero blast sprayer
	Harvesting / Threshing	Multicrop thresher, Flow through paddy thresher
Assam	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, AD Helical blade puddler
	Sowing/Transplanting	TD Seed-cum-fertilizer drill, Self-propelled transplanter/Manual transplanter
	Weeding	Manual tools, knapsack sprayer
	Harvesting / Threshing	Vertical Conveyor Reaper (Self Propelled), Flow through thresher, Hold on thresher, Axial flow thresher
Bihar	Land preparation	TD rotavator, TD cultivator, TD disc harrow
	Sowing/Transplanting	TD seed-cum-fertilizer drill, Self propelled 8 row riding type transplanter, Manual operated low land paddy drum seeder, TD Zero till seed cum fertilizer drill, TD incline plate multicrop planter, TD raised bed planter
	Weeding	Power weeder, Aero blast sprayer
	Harvesting / Threshing	Self propelled Vertical conveyor reaper (Walk behind type), S/P Reaper cum binder, Multicrop thresher, Flow through paddy thresher, Tractor operated straw combine
Chhattisgarh	Land preparation	TD rotavator, TD cultivator, TD disc harrow
	Sowing/Transplanting	TD seed-cum-fertilizer drill, Manual operated low land paddy drum seeder, TD Zero till seed cum fertilizer drill,
	Weeding	Cono-weeder, Power weeder
	Harvesting / Threshing	Self propelled Vertical conveyor reaper(Walk behind type),
Himachal Pradesh	Land preparation	TD Cultivator, TD Disc harrow, TD Rotavator
	Sowing/Transplanting	AD/Power tiller operated/TD ridger seeder cum seed cum fertilizer drill, Manual operated low land paddy drum seeder, TD Zero till seed cum fertilizer drill, TD incline plate multi-crop planter, TD raised bed planter
	Weeding	Power weeder
	Harvesting / Threshing	Self-propelled reaper binder, Self-propelled Vertical conveyor reaper (Walk behind type), Multicrop thresher



State	Operation	Implements/machinery used
Jharkhand	Land preparation	TD cultivator, TD Rotavator, AD Puddler
	Sowing/Transplanting	AD/Power tiller operated/TD seed cum fertilizer drill, Manual operated rice transplanter, Manually operated 4 row paddy transplanter, Manual low land paddy row seeder
	Weeding	Wheel hand hoe, Cono paddy weeder, Japanese paddy weeder
	Harvesting / Threshing	Self-propelled vertical conveyor reaper, Hold on paddy thresher
Karnataka	Land preparation	TD MB/Reversible plough, TD cultivator, TD disc harrow, TD Rotavator, Wet land puddler, TD Laser leveller
	Sowing/Transplanting	AD/Power tiller operated/TD seed cum fertilizer drill, Manual operated low land paddy drum seeder, Self propelled 8 row riding type transplanter, TD Zero till seed cum fertilizer drill, TD incline plate multi crop planter, TD raised bed planter
	Weeding	Wheel hand hoe, Power weeder, Cono weeder, Three row tractor operated rotary weeder
	Harvesting / Threshing	Self-propelled reaper binder, Self propelled Vertical conveyor reaper (Walk behind type), Multi-crop thresher, Straw baler
	Others	Self-propelled sprayer, TD aero blast Sprayer
Kerala	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, Wetland puddler
	Sowing/Transplanting	Manual operated low land paddy drum seeder, Self-propelled 8 row riding type transplanter
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder
	Harvesting / Threshing	Self propelled Vertical conveyor reaper (Walk behind type)
	Others	TD aero blast Sprayer
Manipur	Land preparation	PT rotary tiller
	Sowing/Transplanting	Low land rice seeder, AD/Power tiller operated seed cum fertilizer drill
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder
	Harvesting / Threshing	Wire- loop Thresher, Self propelled vertical conveyor reaper, Hold on paddy thresher
Odisha	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, Wetland puddler
	Sowing/Transplanting	AD/ PT operated/TD seed cum fertilizer drill, TD zero till drill, Manually operated low land rice seeder, Self propelled

State	Operation	Implements/machinery used
		rice transplanter, PT operated inclined plate planter, Tractor/Power tiller operated multi crop planter cum fertilizer drill
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder
	Harvesting / Threshing	Self-propelled vertical conveyer reaper, Combine harvester, Power operated axial flow paddy thresher
Punjab	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, Pulverizing roller attachment to cultivator, TD Laser leveller
	Sowing/Transplanting	TD seed cum fertilizer drill, Manual operated low land paddy drum seeder, Self-propelled 8 row riding type transplanter, TD Zero till seed cum fertilizer drill, TD incline plate multicrop planter, TD raised bed planter, TD Happy Seeder
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder, Three row tractor operated rotary weeder
	Harvesting / Threshing	Self propelled reaper binder, Self propelled Vertical conveyor reaper (Walk behind type), Tractor front mounted Vertical conveyor reaper, Multicrop thresher, TD straw combine, Self Propelled combines
	Others	Self propelled sprayer, TD sprayer, TD aero blast Sprayer, TD air assisted sprayer, Straw baler, Chaff cutter (manual and power operated)
Sikkim	Land preparation	PT rotary tiller
	Sowing/Transplanting	Low land rice seeder, AD/Power tiller operated seed cum fertilizer drill, Self propelled rice transplanter
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder
	Harvesting / Threshing	Wire- loop Thresher, Self propelled vertical conveyer reaper, Hold on paddy thresher
Tamil Nadu	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, Wetland puddler, MB plow, TD Laser leveller
	Sowing/Transplanting	AD/Power tiller operated/TD seed cum fertilizer drill, Manual operated low land paddy drum seeder, Self-propelled 8 row riding type transplanter, TD incline plate multi-crop planter, TD raised bed planter
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder, Three row tractor operated rotary weeder
	Harvesting / Threshing	Self-propelled reaper binder, Self propelled Vertical conveyor reaper (Walk behind type), Multi-crop thresher, SP combines, Straw baler
	Others	Self-propelled sprayer, Tractor operated aero blast Sprayer



State	Operation	Implements/machinery used
Uttar Pradesh	Land preparation	TD cultivator, TD disc harrow, TD Rotavator, Wetland puddler, TD Laser leveller
	Sowing/Transplanting	AD/Power tiller operated/TD seed cum fertilizer drill, Manual operated low land paddy drum seeder, Self propelled 8 row riding type transplanter, TD Zero till seed cum fertilizer drill, TD raised bed planter, TD Happy Seeder
	Weeding	Wheel hand hoe, Power weeder, Cono-weeder, Three row tractor operated rotary weeder
	Harvesting / Threshing	Self-propelled reaper binder, Self-propelled Vertical conveyor reaper (Walk behind type), Multi-crop thresher, TD straw combine, SP combines, straw baler, Chaff cutter
	Others	Self-propelled sprayer, TD aero blast Sprayer
West Bengal	Land preparation	TD cultivator, Wetland puddler
	Sowing/Transplanting	AD/Power tiller operated/TD seed cum fertilizer drill, Manually operated low land rice seeder, Self-propelled rice transplanter, Power tiller operated inclined plate planter
	Weeding	Cono-weeder, Hand hoe, Power weeder
	Harvesting / Threshing	Power operated axial flow paddy thresher, Self-propelled vertical conveyer reaper, Hold on thresher

Source: Din et al., 2014

4. MECHANIZATION IN CROP ESTABLISHMENT

Optimum field preparation is one of the most important steps in farm mechanization. It ensures uniform standing water, suppress the growth of weeds and improve the utilization efficiency of pesticides and fertilizers. Rice farming in India, mostly followed three methods of crop establishment such as (i) dry direct sowing (DDSR) (ii) wet direct sowing (WDSR) and (iii) transplanting. The DDSR refers to the direct sowing of rice seeds in the dry field. For field preparation, tillage operations are performed by bullock drawn implements or power tiller / tractor drawn implements. In WDSR, pre-germinated paddy seeds are either broadcasted or line sowed using drum seeder into well-puddled seedbeds having proper drainage facility. This method of sowing is more commonly used in irrigated areas. Field preparation for WDSR is done through primary and secondary tillage puddling followed by sowing of sprouted seeds. In transplanted rice, field preparation is similar to WDSR followed by transplanting of 3-4 weeks old seedlings at required spacing. Seedlings for transplanting are grown in a separate nursery bed. Paddy fields prepared with optimum tillage ensures maintaining adequate water depth in rice field for



longer period ensuring higher efficiency of all resources like water, nutrients, and herbicides. For proper leveling of rice field, laser land leveling is being widely adopted due to its multiple benefits. Laser levelling using water surface to measure the height of the scraper ensures uniform puddled soil that reduces conveyance loss at field level, enhancing the water and nutrient use efficiency. Transplanted paddy cultivation is considered to be better from crop management and productivity point of view.

Mechanized direct seeding of rice is a cost-effective and efficient approach for rice cultivation and it is gaining popularity among farmers mainly because of labour shortage and increasing costs of rice production. With the technological advancement in rice production system, adequate tools have been available, which helps to minimize the cost and time requirement. With varietal improvement in rice, precision sowing and transplanting of rice is required to realize their yield potentials and reduce the yield gaps. Precision seeders for sowing of rice crop are getting worldwide popularity due to their cost effectiveness and efficiency. Roller type metering unit in precision seeders is simple, low cost, and easy to adjust for varying speed of operation. Spiral grooved metering device enhance the uniform application of paddy seeds. Various types of precision seed drills were developed mostly for bold grain crops to get the optimum seed rate and proper line sowing for better crop establishment, higher yield and higher returns as compared to traditional systems. The different seeders developed for dry direct sowing have seed rate ranging from 25 to 30 kg ha⁻¹ for fluted roller type metering mechanism while seed rate ranged from 15 to 20 kg ha⁻¹ for inclined plate and cup type seed metering mechanism. For WDSR the lowest seed rate with precision in seed placement can be attained by using precision vacuum type metering unit.

On the other hand, automated farming robots for direct seeding of rice may be a viable option which can control the number of rice seeds per dropping, maintain the distance between the dropping points, as well as navigating automatically in the field. To get the optimum sowing rate for conventional and hybrid rice simultaneously, combined hole-type metering device including small and big type holes can be developed. The seedling emergence rates of the rice varieties sown by the precision rice hill-drop machine with horizontal profiling mechanism were 7.8 to 8.7% higher than that without horizontal profiling mechanism (Zhang et al., 2017). For WDSR, drum seeders are most commonly used. Flow rate of drum seeders need to be standardized to obtain the optimum seed rate under varying seed drum fill conditions. For operating the drum seeder, the orifices diameter on its circumference, optimum speed of rotation, forward speed of operation, forward speed of operation to peripheral speed of drum at an appropriate range need to be standardized for best performance.



Like laser land leveller, various rice transplanters are gaining popularity now-a-days. Manual transplanting involves drudgery and is highly labour intensive making it a costly affair, while mechanical transplanters involve relatively less drudgery and provide higher field capacity that ensure easy and timely transplanting of rice. However, the performance of transplanters depends on field conditions. Well puddled and levelled field are required for transplanting with no or minimum standing water (1-2 cm) on the surface; a higher standing water results missing/floating hills. Saleem et al. (2015) reported that mechanical transplanting of paddy with six row rice transplanter, resulted in the maximum yield of paddy (5.20 t ha^{-1}) when water depth was maintained at 2.5 cm instead of 7.5 cm. For better seedling establishment, the field needs to be uniformly puddled by using a mechanical transplanter and left at least for 24 hours to complete settling of the puddled soil to check the soil flow. However, if rotary puddler (single pass) or peg type puddlers (double pass) are used, field should be left for around 48 hours to get the ideal condition for transplanting (Behera et al., 2009). The float sinkage of transplanter usually decreased with increase in sedimentation period of the puddled soil, but draft requirement for operation is increased (Goel et al., 2008). Under such circumstances, 32 hours sedimentation period could be the best for minimum percentage of floating, mechanically damaged and missing hills. However, operation of mechanical transplanter without puddling, the soil was reported to give better crop yield as compared to puddled transplanted rice (Kamboj et al., 2013). It was also observed that different methods of field preparation had significant difference in energy requirement and it also affected the crop performance. Precision field operations not only reduce the energy requirement but also results in saving of inputs with higher yields.

Precision in transplanting with respect to depth of transplanting, spacing between the hills, and between the rows can be made using either manual or mechanical (walk type and riding type) transplanters. The mechanical transplanters are having higher field efficiency and reduce drudgery. In order to develop automated paddy transplanter, it is necessary to locate the position and direction of the vehicle in the programme. For highly precise transplanting operation, variable row automated rice transplanter can be developed by using a real-time kinematic global positioning system (RTKGPS) to locate the position and fibre optic gyro (FOG) sensors to measure the direction and inclination of the vehicle. By following the similar approaches, GPS guided transplanter have been developed and validated by many researchers. Monitoring of rice transplanter's navigation through row detection can be performed using a dual-spectral camera that enables capturing real-time images of the same location with reduced noise at water surface and clearly detects seedling rows. Recently on-the-go soil sensor with 6 row rice transplanter equipped with an ultrasonic

sensor to measure the topsoil depth accurately has been developed and validated (Morimoto and Hayashi, 2017).

To match the low seed rate of the hybrid varieties of rice as well as different methods of rice cultivation such as system of rice intensification (SRI), quantification of number of seedlings is having due importance. A seedling number counting device is fitted with high-speed rice transplanter to analyze the marginal signal count at various transplanting speeds. The seedling counting device first counts transplanted seedlings through signals then displays it on the board. An absolute rotary encoder was used to determine the angle of push point and transplanting claws stop position to get the synchronization between counter signal and transplanting claws. A logic analyser is used to process the encoder signal into a readable data form on a computer. A rice transplanter can also be modified and equipped with a ground-based integrated sensor and instrumentation system to measure real-time field conditions (Singh and Sharma, 2017). The integrated system could include multispectral camera and N-sensor for real time nitrogen application. The system interfaced with a differential global positioning system (DGPS) receiver will provide spatial coordinates for sensor readings and path preview with a control system for auto-regulation of rice transplanter (Zhou, 2014). This system improves working precision of transplanter by tracing the predetermined navigation in straight way, 90°curve and arc way. For this purpose, a navigational control system embedded in a path planning and preview control algorithm can be used. Application of robots and automation of farm operation is the need for the future farming. Agricultural robots could only be successful if they work under continuously changing agricultural environments with the similar quality of work achieved by current methods. Many researches demonstrated that agricultural robots are highly suitable in paddy fields.

5. WATER MANAGEMENT IN RICE

Rice crop is known to have high water requirement. Approximately 80% of the total irrigated fresh water resources in Asia are used by the rice crop alone. In India 45% of total irrigation water is used for rice cultivation. Farmers generally keep rice field continuously flooded from transplanting to physiological maturity of crop, which is not necessarily required. Globally, researchers have proved that after early vegetative phase, rice plant has the ability to extract water from the soil below root zone. Therefore, rice may be one of the most opportune crops where large volume of water saving may be possible.

Precision irrigation is the application of irrigation water in right amount, at right place, and at right time to save irrigation water without hampering the crop production and thereby maximizing the net profit. Besides,



micro irrigation, drip irrigation and other automated irrigation technologies, geographical information system (GIS), global positioning system (GPS), remote sensing (RS), and laser land leveling etc. were primarily employed for bringing precision in irrigating rice crops.

Flood irrigation is the most common practice of irrigation in rice which consumes a substantially higher volume of water for irrigating the crop. A large amount of water and nutrients are lost in seepage and deep percolation. Loss from deep percolation is estimated to be 50% and 80% in heavy texture (clayey soil) and light textured soil (loamy sand), respectively and can be reduced substantially by using precision tools. Precise irrigation in rice can be achieved by (i) Right time (ii) Right amount and (iii) Right method. Different techniques *viz.* alternate wetting and drying (AWD), SRI etc. have already been introduced for increasing water productivity (WP) in rice without compromising the yield. Besides sensor-based irrigation, use of GIS and GPS are also available for rice. Introduction of micro-irrigation technologies (drip, sprinkler), centre pivot irrigation and automated irrigation scheduling approaches are recently started for precise irrigation to rice.

Application of irrigation water through adoption of sensor technologies based on the site-specific needs is the newer dimension of precision irrigation in rice. Various types of sensor technologies have been deployed for correct identification of time of irrigation as per crop need. Initially, gypsum block sensor, time domain reflectometry (TDR), frequency domain reflectometry (FDR), neutron probe sensors, etc. were used for precision irrigation in rice. However, due to the higher cost and applicability limitations of these sensors, they are not much used in rice fields. Multi-sensor capacitance probe is being used in rice field for irrigation scheduling by monitoring varying parameters *viz.* ponded water level, soil moisture, temperature profile etc., but it is also costly. Tensiometer, which is comparatively a low-cost device, is widely used sensor for irrigation scheduling in rice. It measures the soil moisture potential in the range of 0 to -90 kPa and is effective in saving irrigation water and labour costs. It also requires correction for temperature, regular refilling with water and careful removal of air by pumping the air from the water column, also having the limitations in using it under water-logging conditions. Tensiometers only provide estimates of soil water potential at a point scale representing a small measurement volume. Hence, several measurements are needed to capture soil moisture regimes required in heterogeneous and spatially variable field soils. Granular matrix sensors are also getting popularity for precision irrigation scheduling in rice because of the advantages of low cost, no maintenance and easy to read output manually or through automation using battery-powered meters or data loggers. Soil moisture tension is a major component of the total soil water potential, and of the soil hydraulic gradient. Its combination with



hydraulic conductivity is used for calculation of soil water flow for plant roots, as per Darcy-Buckingham law (Gaudin and Rapanoelina, 2003).

In larger crop fields, there is variability in soil texture and soil moisture which needs to be accounted for precise application of irrigation water. Application of RS and GIS helps in estimation and forecasting of soil moisture and mapping in surface and crop root zone depth based on which decision on depth of water to be applied and irrigation scheduling is done. GIS-based water management interface like rice irrigation management information system (RIMIS) are able to improve irrigation efficiencies in larger rice cultivated areas by properly quantifying the time and amount of irrigation water to be applied in the field. Diffuse reflectance spectroscopy (DRS) is emerging as a rapid and non-destructive approach for measuring several soil properties and crop parameters. This tool has been widely used to monitor crop parameters from remote sensing platforms providing continuous spatial coverage over large areas.

In rice fields, the application efficiency of flood irrigation is merely 40 to 60%, largely due to high spatial and temporal variability of soil properties and uneven fields, which results in a significant amount of irrigation water losses (10-25%) at the farm (Choudhary et al., 2002). A properly designed surface irrigation system should be able to achieve application efficiency up to 70 to 85%. Laser land levelling is the technology which has been started with the aim to bring precision in water application under surface irrigation by making the fields uniformly levelled. Laser land levelling significantly improves uniform soil moisture distribution, seed germination, weed control, initial crop establishment and irrigation water productivity. Laser levelled rice field requires less standing water (3-4 cm) than flooded rice (5-6 cm) because of precise slope of 1-2% which allows uniform water distribution throughout the field. Laser levelled rice field has been found effective in giving higher yield ranging from 7-24%, saving the irrigation water by 12-21% and reduction in irrigation time by 47-69 h/ha per season for rice.

Drip and sprinkler irrigation are the improved precision irrigation water application technologies which improve water application efficiency resulting in increased crop water use efficiency and yield of rice. These irrigation technologies have the advantage of irrigation water saving along with increased yield of rice crop. Centre-pivot irrigation also called as central pivot irrigation or water-wheel and circle irrigation is an irrigation method which is also used for precision water application in a spatially varied field. This system comprised with pipe and sprinklers which gets powered by electric motor or hydraulic means, rotates around a centrally located pivot, and irrigate the crops. This irrigation method has been found effective in saving irrigation water in rice up to 67%, also increasing yield by 1.8 to 2.25 times (Arns, 1999).



Further precision in irrigation for rice crops can be achieved through automation of flood or micro irrigation system using sensor, internet technologies and artificial intelligence which saves the time of irrigation, also helps farmers to save irrigation water. Application efficiency can be improved by 80-90% through of sensor based drip and sprinkler irrigation system as against 40-45% in surface irrigation method. Automated centre pivot irrigation can also be used for applying irrigation water uniformly in the field as per spatial variability of soil moisture in the field.

6. PRECISION NUTRIENT MANAGEMENT IN RICE

The precision nutrient management refers to the science of using advanced, innovative, cutting edge, site-specific technologies which capture spatial and temporal variability of soil inherent nutrient supply and aims to enhance productivity, efficiency and profitability of agricultural production systems. Judicious nutrient management plays a vital role in any crop production including rice. The N, P and K are three major nutrients required for better growth of rice. Globally, most of the developing countries either overuse the fertilizers or apply fertilizers in lesser amount instead of applying at recommended dose. Traditional method of fertilizer application was preferred by the farmers and it does not supply the nutrients efficiently to the crop as per the need because of uncertainty arise due to spatial variability of nutrient availability in a large field. Improper time and rate of application of fertilizers is the major reason behind the low fertilizer use efficiency in rice. Overuse of chemical fertilizers is not only a wastage of money, but also leads to serious land degradation and environmental pollution which includes non-point source of pollution, greenhouse gas emissions, and even reduces the yield response to the applied fertilizer. Overuse of N and P fertilizers lead to environmental problems such as increased greenhouse gas emissions, eutrophication, and ground water pollution; and P fertilizers induce heavy metal and radioactive pollutant into the soil-water-plant continuum. On the contrary, proper application of NPK fertilizer can increase productivity level up to 2-3 times in rice.

Precision application of nutrients can help in reducing the cost of production and better yield of rice. P-availability is not a yield-limiting factor for rice. Similarly, due to high seasonal K inputs through irrigation water and release of non-exchangeable K, significant responses of rice to fertilizer K application are not observed in many rice growing areas. Hence, N-management in rice has become more vital, because N-deficiency in rice is the most common problem which has got a large economic significance as the crop growth and grain yields mostly depend upon the proper application of N-fertilizer in the field. Providing information on the spatial crop N-status is important for

precision nutrient management and hence determination of plant N-concentration by proximal or remote sensing techniques is much more helpful than the traditional destructive chemical analyses of plant samples involving high cost and time. One of the key factors in implementing precision nutrient management is the ability to provide timely information regarding spatial distribution of crop N-status within a field. From this perspective, site specific nutrient management strategy in rice in Asia specifically for N has been found effective in enhancing rice yield by 11%, and also increase the recovery efficiency by 31% to 40%, on an average (Dobermann et al., 2002). Precision application of fertilizers includes two steps, first to decide when to apply and how much to apply using precision tools, and second selection of precise application methods to apply the required amount of fertilizers in the crop. Commonly available approaches for deciding the precise amount and time of N-application in rice includes field diagnostic approaches through leaf colour by using leaf colour chart, leaf chlorophyll concentration transmittance using SPAD meter, crop light reflectance using ground based remote sensors like Crop Circle™ and Green Seeker® etc., and also by using GIS, RS and crop simulation modelling approaches.

Precision fertilizer application methods available so far for rice includes deep placement of fertilizer as briquettes which have been found effective in reducing nutrient losses (NPK), surface runoff of N and P, and emissions of nitrous oxide and nitric oxide to the environment, and also enhanced the rice yield. Application of soluble fertilizers through drip irrigation (fertigation) in rice helps in minimizing the losses of fertilizers besides increasing the yield. Precise rate, optimum time and proper methods for fertilizer application resulted in saving of fertilizers, apart from improving crop yield as compared to traditional nutrient management practices. Application of slow release N-fertilisers, also called as intelligent fertilizers which release the nutrients as per the plants requirements is also an important strategy for enhancing NUE (Trenkel, 2007).

Delineating homogenous management zones are also an important strategy for addressing the spatial and temporal variability in the nutrient supply. Various factors such as crop yield, soil data or crop conditions by adopting remote sensing techniques are used for delineation of management zones. These management zone maps can guide for application of site-specific input management through variable fertilizer technology rather than uniform application of nutrients (Tripathi et al., 2015, 2019).



7. PRECISION WEED MANAGEMENT IN RICE

Weeds competition with rice is higher during the initial stages of crop growth (2-6 weeks after planting). Controlling weeds during this period is very essential for realizing maximum crop yield. Weeding is mostly done manually, which is time consuming and labour-intensive; therefore, chemical weed control method is rapidly gaining ground in the country due to low operational costs and lesser operational time. However, the wastage of herbicide/pesticide due to its blanket application is becoming a major environmental concern. All these issues can be addressed by using precision agriculture principles in weed management. Precision weed management (PWM), can be simply stated as “right amount of inputs on the right target [weeds] at the right time”. Synchronous application of herbicide not only reduces the non-target impact on the environment but also reduces the input cost without decreasing weed control efficacy. The precision weed management is based on (1) computerized decision support systems (DSS), (2) identification and mapping of weed and crop plants and (3) variable rate herbicide application and (4) site specific weed management. For the development and application in all these aspects, quantitative understanding of weed population dynamics and crop-weed interactions is needed.

Models can be used to integrate the available quantitative knowledge to design preventive measures, to develop long-term and short-term strategies for weed management, to assist in decision making to determine if, when, where and how weeds should be controlled and to identify new opportunities for weed control. Zijlstra et al. (2011) suggested that an elementary DSS for a crop protection system could be based on simple monitoring tools and precision application technologies. Generic model of an innovative crop protection system consists of (1) monitoring step (macro-level and micro-level) and (2) decision steps before and during the crop growth period. The decision step invariably involves interpretation of data and identification of abnormalities.

Growth and production of species in mixtures is simulated by eco-physiological simulation models for crop-weed competition. This model is based on eco-physiological processes in plants and their response to the environment. These models help in improving insight into the crop-weed system and can be used for the development of simple predictive yield-loss models, threshold levels or the design of competitive crop plant types. Quantitative insight into the dynamics and spatial patterns of weed populations is essential for strategic weed management decisions for preventive measures and the identification of new opportunities for weed control. The models are essential due to the complexity of the process and the long-term character of weed population dynamics. Over the years, many DSS models such as CPOWeeds, DecidHerb, OptHerbClim, GestInf, MLHD, IPMIDSS, DoseKey and

WeedManager have been developed (Been et al., 2010) for managing weeds in cereals. Such DSS models can be developed and validated in rice.

Identification and mapping of various weeds are important for monitoring the strategies and their effectiveness for weed management to ascertain the chemical requirements for weed control. Maps are also used for studying the population dynamics and identifying the best models for prediction of weed diversity. Weed maps can be used in GPS based sprayers for navigation. The differentiation of different weeds only on the basis of spectral reflectance is difficult due to less variation in spectral reflectance of different weeds. Zwiggelaar (1998) also found that use of spectral information alone is difficult to discriminate between crops and weeds, therefore spatial geometric effects from the weeds and crops should be taken into account. Various factors determine whether weeds can or cannot be distinguished using remote sensing, such as, differences in the growth rates of weeds and crops, time of emergence, differences in vigour and differential stages of inflorescence and senescence (Menges et al., 1985). Some proximal sensors such as the WeedSeeker[®] system including some sensors which vary from simple colour detectors to complex machine vision systems are used for discriminating and identify populations of different weed.

Spatial and spectral resolutions are important factors for decoding the satellite images for weed discrimination. The IKONOS satellite provides 1m spatial resolution in panchromatic mode (450-900 nm) and 4 m resolution in multi-spectral mode. Other satellites are also available with varied spatial resolution such as SPOT (20m), Landsat TM (30m), IRS-1B (36m), AVHRR (1km). Multi-spectral airborne imaging systems, with inbuilt image processing systems with high spatial resolution with high temporal resolution may help in weed mapping.

Post-emergence applications of herbicide for weed control generally occur during intermediate crop growth stages before canopy closure. A variable rate herbicide application technology avoids application to undesired areas of the field crop and changes the application rate according to weed density. In conventional method, herbicide is uniformly applied over entire area; however, map-based weed management has the potential to reduce herbicide inputs in the field by skipping the area not infested with weeds. In map-based variable rate herbicide application adjusts the rate of application based on a prescription map, using a GPS receiver to identify the field position and the input concentration is changed as the applicator moves through the field. This is done by using UAV imagery, machine learning, remote sensing, video cameras on tractors, or manual counts to map weeds in rice paddies at early stages of the growing cycle, and then spraying the field at a uniformly low rate with a higher dosage of herbicide in areas with weed patches to support variable rate technologies for



site-specific weed management. To detect Gramineae weed in rice crop after 50 days of sowing, the combination of high resolution red–green–blue (RGB) image and low resolution multispectral (MS) image taken from a fixed-wing unmanned aerial vehicle (UAV) at 60 and 70 m altitude is used (Barrero and Perdomo, 2018).

In sensor-based approaches, application rate changes on the basis of pest stress or canopy characteristics that is identified by the difference on colour, shape, size, texture, reflectance, and temperatures of pests that is detected by different sensor types (colour cameras, photo-detectors, laser scanners, multispectral and hyperspectral cameras, thermal cameras, and ultrasonic sensors). The sensor input can also be used to control the direction and rate of chemical application. Weeds and other types of pest were detected by using colour images. The weed density and weed coverage area in the field is calculated based on digital images, using a fuzzy algorithm for planning site-specific application of herbicides. Use microcontroller and GPS for generating weed maps. These weed maps are needed to apply the right amount of herbicides where these are actually needed. A major step towards a practical solution for site-specific weed management is the development of precise and powerful sampling techniques for automatic and continuous detection of variation in crop and weed populations. Despite the additional costs of the weed sensing and application technology, site-specific weed control resulted in higher economic returns compared to conventional uniform applications even over periods of several years. It has been widely accepted that site-specific weed management can reduce weed control costs. However, the offline mapping process is time-consuming and a broader acceptance of site-specific weed management in practical agriculture will require an online system that combines weed species detection and herbicide application in one operation.

8. PEST AND DISEASES MANAGEMENT

Insect pests and diseases are usually unevenly distributed throughout the cropped area. However, the current analytical methods/ technology have limitations in making insect pest and disease management decisions that can be implemented uniformly in the field. Recently, spectroscopic methods involving proximal and remote sensing tools have been developed to map and analyze insects and disease's spatial distribution to a level where the uneven nature of insect populations and disease severity can be considered for the application of management tactics. The precision pests and diseases management is based on (1) forecasting of pests and diseases outbreak, (2) computerized decision support systems (DSS), (3) identification and mapping of pests and diseases in the crop, and (4) variable rate pesticides application. This precision pests and diseases control approach is also termed site-specific pest management it

includes a pest distribution scenario that helps in identifying the most suitable pest control tactics economically.

Infestation by insect pests and diseases is highly varied with the cropping seasons and location. Pesticides are the effective means to manage insect pests and diseases. Timely management of the disease outbreak or pest attack at an early stage is highly recommended and it serves as a strong management tool. A location or region-specific crop, and season-specific prediction model help in forewarning about these menaces. Forecast models are effective tools to early identify the pest attack and it is an alternative to calendar-based spray schedules. Since, insect pests and disease menaces are weather-dependent, weather-based prediction models enable the management of these pests. Weather parameters *i.e.* temperature, rainfall, and relative humidity, were tested for different pests and diseases attack. Among all weather parameters, the temperature is critical for insect pest growth, development, pest epidemics, the extent of damage caused to crops, and the overall crop yield. Insects are sensitive to temperature, the rate of development of insects' increases with the temperature rise.

Pest forecasting is mainly of two types based on the period of forecasting. i). short-term forecasting - based on one or two season's ii). long-term forecasting - based on the effect of weather parameters on the pest. Diverse modeling approaches *viz.*, Generalized regression neural networks (GRNN) and multiple regression (REG), back-propagation neural network (BPNN), support vector machine (SVM) have been followed to date for disease prediction in plant populations. In pest forecasting, several intrinsic attributes of the insects, and the determining environmental and host factors need to be considered. Most pest forecast models take into account the phenology of the herbivore and its host. Near real-time pest incidence data coupled with remote sensing and GIS tools facilitate early warning of impending pest build-up in a temporal and spatial perspective. Besides, the collection and analysis of weather data from pest-affected areas is an essential input for models. The practical application of model outputs is aided by decision support systems. Weather-based forecasting systems help in reducing the cost of production. These systems worked on DSS to optimizing the timing and frequency of application of control measures for minimizing crop loss and reducing the cost of plant protection. The database generation for the application of forecast models needed a lot of resources *viz.*, manpower, time, etc. Hence, the prioritization of crops is highly important before using the forecast model. Precision in the application of pesticide is achieved by forecast models by avoiding calendar spray schedule, e.g., instead of sprays at 7-14days intervals, it will apply the spray at right time, right place, and the right amount. Thus, precision pest management may bring down the number of chemical pesticide sprays to provide economic and environmental



benefits. Finally, the system of the forecast should enable to take an economically acceptable action, an integral part of the IPM package while growers should be capable and flexible enough to take due advantage of a pest forewarning.

Several workers have analyzed the influence of weather factors on the brown plant hopper (BPH) population and observed temperature, humidity, and rainfall to be important ones. In general, pest-weather relations have been analyzed through empirical models, which behave in a location-specific manner. A forewarning model for rice leaf folder was developed based on peaks of its light trap catches weather parameters such as maximum temperature, morning relative humidity, evening relative humidity, and SSH, wherein these four weather factors together could explain 99% variability in leaf folder light trap peaks in Punjab. Weather analysis suggested that besides other factors, hotter and drier conditions during June and July in 2012 might have played a role in the leaf folder outbreak (Singh et al., 2015).

For rice crop, many simulation models have been developed to understand, predict, and manage diseases. All major environmental and cultural factors are taken into consideration in model simulation to predict disease incidence more precisely. The model is region-specific and it may or may not be widely adaptable. Besides, due to structural complexities and temporal and spatial restrictions of their input requirements, it is difficult to link these models to other applications such as GIS and global climate model (GCM)-generated climate data at various temporal and spatial resolutions. To consider as many factors affecting disease development as possible, many rice disease models need cultivation-related information such as the rice cultivar, transplanting date, and even daily trapped airborne fungal spore numbers as input variables. A broad range of weather variables are also used for the modeling, including air temperature, relative humidity, rainfall, solar radiation, wind speed, and others.

Rice blast is the most destructive rice disease causing enormous losses every year. The most frequent climatic variables used are air temperature, followed by relative humidity and rainfall. The key pathogenesis factors, for instance, leaf wetness, nitrogen fertilization, and varietal resistance showed restricted incorporation for the development of these models. Currently, five forecasting systems are operational in Japan, Korea, and India. Manibhushan Rao and Krishnan (1991) developed the EPIBLA (EPIdemiology of BLAst) model using multiple regression equations based on maximum temperature and maximum RH for simulation of leaf blast incidence. Yoshino (1979) in Japan developed a model that determined *Pyricularia oryzae* infection periods, evaluating weather conditions every hour, producing hourly results that indicated if the conditions would result in successful infections.



The Yoshino model is still used as part of three forecasting systems: a commercial system developed in Austria and the models published by Kang et al. (2010). In India, Manibhushan Rao and Krishnan (1991) developed EPIBLA (EPIde miology of BLAst) simulated incidence of blast and made 7 days forecasts of disease progression in many parts of India. Calvero and Teng (1992) in the Philippines developed BLASTSIM.2 model for the blast. Kang et al., (2010) developed a forecasting model describing an online information system for plant diseases based on weather data. Savary et al. (2012) in Korea developed EPIRICE, a generic model for plant diseases which was coupled with GIS.

Computer-based DSSs have the potential to be important tools in the decision-making process for farmers in pest management and their advisers (Ritchie, 1995). Several DSS systems were developed for agricultural management, but there has been limited research on real-time estimation of damage inflicted by insect pests and diseases in rice. DSS for insect pests of rice and cotton-based cropping systems has been developed by Central Research Institution for Dryland Agriculture, India. National Institute of Agricultural Extension Management (MANAGE), India developed an expert system to diagnose pests for rice crop and suggest preventive/ curative measures. The rice crop doctor illustrates the use of expert systems broadly in the area of agriculture and more specifically in the area of rice production through the development of a prototype, taking into consideration a few major pests and some deficiency problems limiting rice yield.

To ensure timely management of insect and pest infestation it is essential to take up timely management measures. The real-time rice crop pest and disease detection in large-scale farming for their effective management has remained a challenging task. Hyperspectral remote sensing is a powerful tool that is being used for detecting stresses caused due to insect pests and diseases in the crop. The interaction of electromagnetic radiation with plants varies with the wavelength of the radiation. The same plant leaves may exhibit significantly different reflectance depending on the level of health and or vigor. Healthy and vigorously growing plant leaves will generally have (i) low reflectance at visible wavelengths owing to strong absorption by photoactive pigments (chlorophylls, anthocyanins, carotenoids), (ii) high reflectance in the near-infrared because of multiple scattering at the air-cell interfaces in the leaf's internal tissue, (iii) low reflectance in wide wavebands in the short-wave infrared range, because of absorption by water, proteins, and other carbon constituents. The incidence and severity of pests and diseases can be monitored according to the differences in spectral characteristics between healthy and stressed plants.

Infestation of rice insect pests such as yellow stem borer (YSB) (*Scirpophaga incertulas*), brown planthopper (BPH) (*Nilaparvata lugens*), leaf



folder (*Cnaphalocrocis medinalis*), and diseases like a blast, bacterial blight, sheath blight, false smut is the most notable risks in rice yield in tropical areas, especially in Asia. To use visible and infrared images to detect stress in rice crop caused by these biotic stresses, several proximal or remote sensing techniques have been developed. High spectral resolution remote sensing imagery with more bands and narrower bandwidth is required to diagnose crop disease stress. The crop affected by insects gives different tonal variation in imageries than a normal crop. Normal crops give red, bright red, and dark red with a smooth texture but pest-affected areas give pink, yellow, and yellow pinkish-red color with irregular shape and rough texture.

Having established evidence for the technical feasibility of automated crop protection, it is time to look at how solutions are being designed and implemented in practice. The system is developed with an information flow from aggregate DSS sending spraying decisions to a task controller with GPS, which in turn sends spraying instructions to the computerized sprayer; through an information flow, the opposite way completed spraying operations are stored in the DSS. The detailed description about precision crop protection technologies are provided in Chapter-5.

9. YIELD/PRODUCTION ESTIMATION

Accurate assessment of rice yields before and/or after harvest not only improves the storage, transportation and marketing linkage but also help in framing policies beforehand which enhances the profitability and is vital for ensuring food security. This requires monitoring and warning systems using spatial information of area under cultivation and its production. The data based on the existing methods such as crop cutting experiments, regression models with rainfall and previous yield data as independent parameters and surveyed sampling is fraught with risk of reliability. These methods have drawbacks such as (i) time-consuming, subjective, costly, and labour-intensive; and (ii) the outcomes are usually made available to the government and public after several months of the harvesting of the crop; thus becomes less efficient for on field management as well as food security purposes. In addition to this, the survey data based on rice yield mapping gets updated at a slower rate. It has been demonstrated that the spatial estimate of rice production can be made 2-3 months prior to actual harvesting of the crop, thus enabling inclusion of the management decision in the yield prediction process.

The use of remote sensing techniques that obtains surface information spatially and periodically in wide area can make forecasting of rice yield economically feasible. These forecasting techniques can broadly be divided into optical and microwave-based sensing. In optical sensing, pre-harvest rice yield

estimation can be done because of the fact that some of the spectral bands shows variations to the vegetative conditions of the rice. Many vegetative indices such as NDVI (Normalized Difference Vegetation index), RVI (Ratio Vegetation Index), DVI (Difference Vegetation Index), IPVI (Infrared Percentage Vegetation Index), SAVI (Soil-Adjusted Vegetation Index), Vegetation Condition Index (VCI), Vegetation Health Index (VHI), Temperature Condition Index (TCI), and Green Normalized Difference Vegetation Index (GNDVI) have been developed based on this concept to estimate the rice yield before actual on-field harvesting. Besides these, Radiation Use Efficiency (RUE), Harvest Index (HI), Absorbed Photo Synthetically Active Radiation (APAR), Leaf Area Index (LAI), Rice Canopy Reflectance can also act as good indicators for predicting rice yield.

However, crop yields are affected by a complex combination of dynamic interactions between biotic, soil and climatic factors. The temporal instability of delineated management zones is problematic when characterizing yield-soil relationships and making management decisions. The relationship between the plant parameters and the yield estimation of paddy crop was carried out in many studies. Positive correlations have been reported with LAI, plant conditions and Vegetative Index (VI). Globally, many researchers have been using remote sensing techniques to study the production of rice using different sensors. This estimation has been carried out from the images of Landsat, NOAA AVHRR, MODIS, SPOT, ALOS, AVNIR etc.

In order to predict the yield, spectral information obtained from the MODIS images was transformed into the NDVI. It was found that there existed a significant relationship between EVI/NDVI-based estimated yields and the observed yield statistics (p -value < 0.001) (Son et al., 2014). The correlation coefficient (R^2) of EVI based models was found to be better than NDVI-derived models with root mean square error (RMSE) and mean absolute error (MAE). The NDVI values are also having correlations with chlorophyll content. Increase in NDVI indicates a high chlorophyll content which fills carbohydrates to the rice grain. In another study by Peng (2013), a synergy was shown among the remote sensing techniques, meteorological and crop growth models.

The images of SPOT which includes a wide range of reflective spectral bands and their derivatives which take the form of several vegetation indices such as NDVI, RVIDVI, IPVI, SAVI, VHI etc. have been widely used for assessing the yield. Satellite imagery, synergistic models from LAI and other vegetation indices obtained under normal agricultural practices could be used for improving the efficiency of forecasting rice yield.

Although the mapping and forecasting methods gives higher accuracy and precision, the challenges mostly arise because of data acquisition and



development of appropriate statistical methods. It also encounters with the difficulty of producing cloud-free remote sensing conditions which is utmost necessary during the entire cropping year. Besides, the empirical models developed using satellite imagery or data are restricted to certain environmental conditions which may vary the aerial data. Taking into account the availability of satellite data, the development of image classification methods must be effective, efficient, and easy to implement so that it provides a good fit in mapping rice areas and forecasting its production.

10. POST-HARVEST AND QUALITY ANALYSIS OF RICE

The lack of proper grading and assessment of grain quality amounts to loss of more than a few million dollars every year, cutting into the profits of farmers and the agriculture industry. Rice grain quality is of great importance for human beings as it directly impacts the human health. Hence, there is a need to measure the quality of grain by identifying adulteration or non-quality elements. Also, the quality of rice after all the post-harvesting operations has to be assessed in order to guarantee its quality in the international market as export requirements seek absolute rice dimensions and stringent percentage requirement of the broken and undesirable items. The quality of rice is generally based on parameters like colour, shape, size, internal quality, appearance and no. of broken kernels. Texture and moisture content are also considered as important quality indicators of rice grains. The grade and price of different rice varieties are largely decided by its commercial value, eating quality, genetic characteristics, agronomic characteristics and the quality.

Conventional methods for the quality assessment of cereals and grains are always laborious, destructive and time consuming. For assessing the grain quality, usually manually-operated micrometer is used to measure the geometrical features such as length, width and area of rice kernels. The manual classification methods that are used in the industries are not subjective and efficient. It takes long time, complicated process and also gives eye fatigue even for the people who are trained to perform these tasks resulting in uncertainty in results. So, an automated rapid measurement system would be appropriate for identifying the grain type and classify it in a non-contact manner. However, this is not possible without the aid of such type of precision instruments and human expertise.

Precision techniques like computer vision, image processing with artificial neural network, flatbed scanning, Multi-class Support Vector Machine (SVM) as classifiers are used in the classification and quality evaluation of rice grains accurately. Machine vision allows automatic monitoring of the object in question based on the image captured in the camera fitted in the machine. This

system is additionally equipped with a sensor that can process the image and automatically take action based on the image captured. On the other hand, Artificial Neural Network (ANN) is a complex computing system which mimics the biological neurons, a framework for many different machine learning algorithms which can work together and process complex data inputs. It has higher flexibility to model systems accurately and with ease, whereas SVM uses the underlying concept of structural risk minimization.

11. MACHINE LEARNING AND ARTIFICIAL INTELLIGENCE

The mechanization of agriculture is needed to reduce production costs; enhance the product quality; environment protection; enhance safety and comfort to farm workers; timeliness in farm operation; by managing the farm operation in a better and precise manner. To meet the desired level of mechanization, India has to adopt new and innovative precision agriculture tools. Machine learning and AI help in getting much higher precision in farming which enabling farmers to increase the effectiveness of farm-related decisions. Starting from field preparation to harvesting, and post-harvesting operations, precision farming tools having their role to enhance the production level. Chat bots are virtual assistant for automation of the communication between different users or information providers. Chat bots can be used to facilitate the information flow between farmers, government stakeholders, manufacturers and markets. This technology in future can be helpful for assisting farmers with answers to their questions, giving advice and recommendations on specific farm problems particularly in emergency conditions (Mostaço et al., 2018). Unmanned aerial vehicles (UAV) capture images and collect data from the field. In coming years, UAVs fitted with various need based sensors and more widely accessible gadgets will be used in assessing the soil and crop condition and helping farmers to make informed decisions. Robotic agriculture is also an anticipated future in which driverless tractors performing all the farming operations, autonomously. The different sensors, GPS and radar technology fixed to these driverless tractors will be able to assess the spatial variability of soil nutrients and apply the need based fertilizer or pesticides. Driverless vehicle technology has been the adaptation across a wide array of technological firms. The automated systems having historical weather data accessibility will perform the automated irrigation in the fields by utilizing real time machine learning to constantly maintain desired soil conditions in order to increase average yields. The advancement in machine learning and artificial intelligence in agriculture will create new avenues of enterprising farming and pressures on an already strained workforce will be reduced.



12. CONCLUSIONS

There is a tremendous scope for transforming the conventional agriculture through optimum resource utilization in environmental friendly way by using precision farming technologies. In India, precision rice farming can be adopted to enhance yield with reduced input and environmental footprint. Precision farming technologies are required to be customized involving one or more PF technologies for multiple agricultural operations such as field preparation, crop establishment, nutrient and water management, crop protection, harvest and post-harvest etc. to get maximum benefit out of their adoption. Among the modern technologies evolved to minimize human labour in agriculture, precision farming technologies is spreading fast not only in developed countries, but also in the developing countries. These set of technologies create a balance between the traditional knowledge and machine algorithm. Increased use of the computers, GPS, GIS, machine learning, laser etc. help to decrease the injudicious over-application of agricultural inputs, and thereby increase agricultural production and profit of the system making agriculture more attractive to youth. Some key points identified to enhance the mechanization of rice are custom hiring model of farm implements for rice based farming system, mechanical transplanter with mat type nursery, mechanical weeders, zero tillage seed drill, straw management machinery (straw chopper, happy seeder, baler etc.) and converting rice-fallow cropping system into rice-pulses cropping system by using improved conservation agriculture (CA) machinery. Variable fertilizer, pesticide applicator, UAV, Robotics coupled with decision support system, machine learning, AI and automation in communication among the stakeholders are some future prospects of rice farming.

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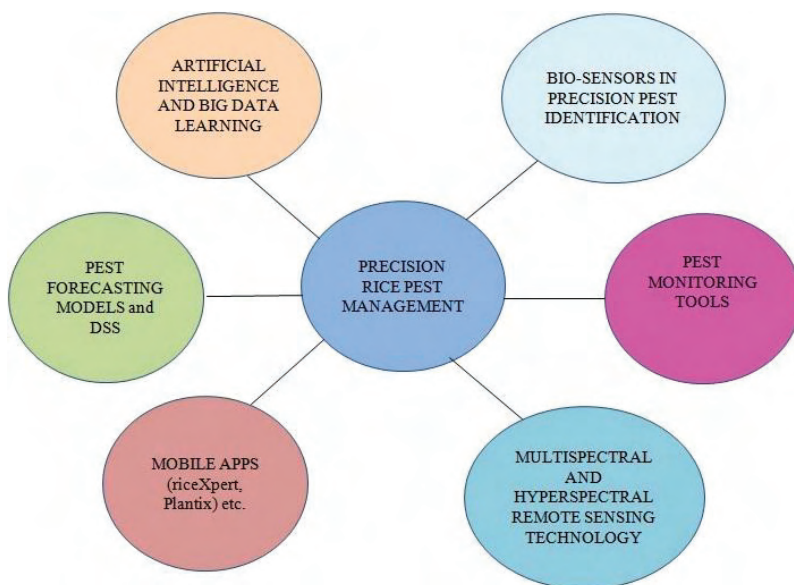
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PRECISION RICE PEST MANAGEMENT: TOMORROW'S TECHNOLOGY FOR TODAY'S FARMER

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GRAPHICAL ABSTRACT



SUMMARY

Biotic stresses are often time and space heterogeneous in agriculture. In modern large-scale farming, heterogeneity needs selective application of agricultural inputs including pesticides. The heterogeneity is often ignored largely due to lack of appropriate technologies. Precision pest management is a low-input, site-specific management technique that uses a variable rate operating system to minimize usage of chemical pesticides. Traditional rice-field spraying methods pose a range of issues and challenges for farmers, including farmer movement while spraying and spray particle drift, which endangers farmers' health. Pesticides are often distributed uniformly in the field, which can lead to over and under-dosing, which is inefficient, uneconomical, and potentially



detrimental to the ecosystem in both cases. Emerging technologies such as Geographic Information Systems (GIS), the Global Positioning System (GPS), Variable Rate Technology (VRT), and Remote Sensing (RS) are gaining popularity in today's high-tech precision agricultural systems. The GIS enables users to simulate multiple data values for the same geo-referenced location as a graphical representation. VRT techniques may be used to execute management decisions in a more comprehensive "micro-managed" manner when GIS is combined with GPS and RS. This technology has the potential to reduce agronomic crop production costs as well as environmental hazards.

1. INTRODUCTION

Global agriculture is facing many challenges like climate change (Change in temperature, CO₂ concentration, erratic rain fall, soil salinity and alkalinity), increased incidence of insect pests, diseases and weeds which ultimately leads to low production and productivity (Oerke, 2006). The pest and diseases cause both direct and indirect losses, and the direct losses in terms of yield accounts for 20-40% roughly (Teng, 1987; Oerke et al., 1996; Oerke, 2006). Major diseases like rice blast, bacterial blight, false smut, bakane; insect pests such as stem borer, brown planthopper, swarming caterpillar continue to cause significant damage and many minor insect pests and diseases are emerging as a major problem (Laha et al., 2017; Mohapatra et al., 2008, 2016 and 2018c). Thus, there is an urgent need to address these problems on a war footing basis to achieve food security. In many regions of the world, the emerging insect pests and diseases increase quickly over a short period of time with high rate of infection (Daszak et al., 1999). In some other cases, the major diseases cause epidemics in a particular season. Apart from this, new invasive pests and diseases may enter new areas through seed/planting materials and other modes; finally causing serious damage in new areas (Table 1).

Table 1 Outbreak of insect pests and diseases in rice in India

SI No.	Insect pest/disease	Intensity of damage	Year of damage
1.	Brown spot disease	Severe	1942-43
2.	Bacterial Blight	Severe	1963
3.	Blast	Severe	2006-07
4.	Neck blast	Severe	2006-07
5.	Stem borer and Leaf folder	Moderate	2006-08
6.	Stem rot	Severe	2006-07
7.	Bacterial blight, Brown spot, BPH,	Moderate	2008-09

Sl No.	Insect pest/disease	Intensity of damage	Year of damage
	Bacterial leaf streak		
8.	BPH and WBPH	Moderate to severe	2008-09
9.	Army worm	Moderate	2009-10
10.	Brown planthopper	Severe	2017-18

Invasive species also pose potential threats to global agricultural production but the magnitude of damage and distribution of the threats between and among the countries is still undefined (Painiet al., 2016). It is estimated that USA bears a loss of approximately 40 billion US\$ per year due to invasive insects and pathogens in crops (Pimentel et al., 2005). Almost 1300 known invasive insects and plant pathogens invade 124 countries of the world and are potential threats to the other countries in near future. Biggest agricultural producers such as USA, China and India are the potential countries with high risk of invasion of these alien species. Since, these countries export food and planting materials to their trade partners, they serve as a source of these invasive insect pests/pathogens (Table 2). Therefore, these invasive pests/pathogens also possess a severe damage to food security of the trading countries.

Worldwide estimated loss due to insects, weeds, and diseases are 10.2%, 12.2% and 14.10%, respectively which accounts for an overall loss of 36.50%. The estimated crop losses caused by arthropods, pathogen, and weeds have increased from 34.9% in 1965 to 42.1% in late 1990s. The losses are increasing year by year and in alarming state for some of the specific pests. In many of the developing countries like India, the farmers face many constraints in crop production. The yield losses in India due to insect pests range from 15-25%, which is approximately 15-23.3 billion US\$ in annual monetary value. In 2017, farmers in most of the states in India suffered heavy losses in crop yields due to an erratic monsoon and multiple pest attacks. Some examples have been given in Table 2. The annual and currently ongoing locust infestation over Africa, West Asia and South Asia is perhaps the most dangerous of all insect infestations.

Table 2 Recent insect pest invasions to India

Name of the pest/pathogen	Scientific name	Crop	Reference
Fall armyworm	<i>Spodoptera frugiperda</i> (J E Smith)	Maize	Sharanabasappa et al., 2018
Western flower thrip	<i>Frankliniella occidentalis</i> (Pergande)	Fruits and vegetables	Tyagi and Kumar, 2015
Eucalyptus gall	<i>Leptocybe invasia</i> Fisher	Eucalyptus	Jacob et al., 2015



Name of the pest/ pathogen	Scientific name	Crop	Reference
wasp	and La Salle		
Tomato leaf minor	<i>Tuta absoluta</i> (Meyrick)	Tomato	Sridhar et al., 2014
Papaya mealybug	<i>Paracoccus marginatus</i> (William Granara de Willink)	Papaya, cotton and Mulberry	Muniyappan et al., 2008
Coconut eriophyid mite	<i>Aceria guerrenonis</i>	Coconut	Sathiamma et al., 1998

Even with current levels of crop productivity and stagnation in yields for some crops, India can still meet its domestic needs by 2030 if losses due to pest damage in terms of quantity are avoided (Savary et al., 2000). The agriculture sector continues to be the backbone of global economy. Furthermore, it is necessary to meet the ever-increasing demand for food products that has arisen as a result of the world's rapid population growth (Sinha et al., 2019). Crop conservation is a critical component of farm management. It helps in coping with major threats and costly pesticides. Pesticide use is estimated to be about two million tons per year worldwide, with Europe accounting for 45%, the United States for 25%, and the rest of the world for 25%. Pesticides used indiscriminately increase pathogen and pest resistance, reduces soil biodiversity, and kills beneficial soil microbes; they induce pesticide bio-magnification, decrease pollinator populations, and destroy natural habitats of farmer friends like insect predators and birds (Tilman et al., 2002). To minimize significant production and economic losses, reliable detection and identification of insect pests and diseases in the early stages of crop production is highly desirable. The main aim of crop pest diagnosis is to assess if a plant is healthy and, if not so, what is causing the infestation. However, assessing the physical, chemical, and biological changes in crop plants during the asymptomatic stages as a result of insect pests, diseases, and abiotic stress is a current agricultural challenge.

The accurate detection and identification often rely on crop protection scientists through manual inspection of the crop for diagnostic symptoms of damage that is already visible. This method of plant health monitoring is both time consuming and demanding, depending on the type of crop and the size of the crop area—which for many commercial crops is often very large. The biotic and abiotic stresses exhibiting clearly noticeable symptoms, which often manifest at the middle to late stages of infection, are often used for manual detection. As a result, the difficulty of completing the mission in a timely and cost-effective manner is the challenge. To overcome the challenges of



traditional methods of pest management, precision farming techniques have been proved to be successful. According to a study conducted by the Tamil Nadu Precision Farming Project (TNPF), precision farming has resulted in an 80% increase in tomato yield and a 34% increase in brinjal yield. In tomato and brinjal cultivation, gross margins increased by 165 and 67%, respectively (Chouraddi et al., 2011). With the application of information technology, RS, GPS, GIS, and the progress of these tools in agriculture, precision pest management has been increasingly gaining attentions worldwide to attain a sustainable agricultural need (Mondal et al., 2011; Natikar et al., 2016).

With a view to understand the recent advances in pest management, this chapter is focused on the use of new technologies including GIS, Variable Rate Technology (VRT), RS, artificial intelligence, pest forecasting, Decision Support System (DSS) and disruptive technologies like mobile applications in pest management which can be applied in a more precise "micro-managed" manner. Pest control input costs, as well as crop and environmental damage, could be minimised with this technology.

2. ADVANCES IN PEST MANAGEMENT

In crop production, precision agriculture methodologies are used for site-specific fertiliser or pesticide application, automated guidance of agricultural vehicles, product traceability, on-farm testing, and production system management. Precision insect pest and disease control is a challenging task in precision agriculture, but it has the potential to reduce pesticide costs and environmental effects. Digital resources have revolutionized the way farmers obtain information, identify and manage insect pests and diseases. Non-destructive methods are one of the most practical ways to track crop health conditions in real time. As a result, efficient and cost-effective methods are preferred because the measurements do not require sample planning or repeated procedures. Preventing insect pests and pathogens during the preliminary stage helps with plant health control and yield optimization without relying on pesticides. The development of advanced precision technology, such as computer-aided systems for tracking plant stresses, has boosted these techniques.

With the advancement of precision pest management, farmers have opportunity to follow the "toolbox" approach for pest control and disease identification. Farmers have more choices than ever before with today's toolbox. Combining multiple alternatives increases their effectiveness and decreases the use of pesticides by farmers. Pest management technologies have increased in size and efficacy over the last century, enabling farmers to produce more food with less environmental impact.



3. INSECT PEST AND DISEASE IDENTIFICATION

3.1. Biosensors in precision pest identification

Farmers can detect insect pests and pathogens on their crops using a variety of sensors. These sensors range from the simplest to the most complex. Some of the most common sensor types are:

3.1.1. Low-power image sensor

A wireless autonomous monitoring system based on a low-cost image sensor is the low-power image sensor. The wireless sensor, which is mounted in a single trap, takes images of the trap contents on a regular basis and sends them to a control station. The number of pests found at each trap is then calculated using the images sent. Accordingly, a farmer can schedule the crop protection measure, and target those specific areas in the field based on the insect population number. Farmers use this sensor to track vast areas while using very little resources. Low-cost image sensors have various advantages in farm output. Some of them include (i) a substantial reduction in pest monitoring costs (ii) no need for human interference in the field (iii) suitability for both small and large areas (iv) low maintenance costs (v) real-time insect pest monitoring. Honey bee activity is monitored by counting the honey bees entering and leaving the beehive (Ngo et al., 2019). At the beehive entrance, images are continuously collected, and the honey bees in the images are segmented and detected using the background subtraction process. An integrated Kalman filter and Hungarian algorithm tracking system were used to track honey bees. In contrast to manual counting, the tracking algorithm was used to calculate the incoming and outgoing operation of individual honey bees with an automated counting accuracy of $93.9 \pm 1.1\%$.

3.1.2. Acoustic sensor

An acoustic sensor is an insect pest detection sensor that monitors the insect pests' noise levels. In the field, wireless sensor nodes are attached to a base station. A sensor transmits the information to the control room computer when the pest's noise level reaches the threshold, which then accurately indicates the infestation location. These sensors can detect an infestation at an early stage, minimizing crop damage significantly. This is an excellent tool for tracking wide field areas while using very little resources.

Sensors for measuring the Leaf Area Index (LAI) may also be used to monitor the presence of insect pests. Leaf destruction is caused by insect pest feeding. Plants lose chlorophyll as a result of this. This results in a reduced total leaf area and, as a result, a reduction in the plant's photosynthetic ability. The

sensor will detect an insect attack early on by measuring the leaf area index and alerting farmers to take appropriate action.

3.2. Early pest and disease detection

Direct detection approaches are predominantly laboratory-based disease detection techniques. PCR, Immune Fluorescence (IF), Fluorescence In-Situ Hybridization (FISH), Enzyme-Linked Immune Sorbent Assay (ELISA), Flow Cytometry (FCM), and Gas Chromatography-Mass Spectrometry are the most popular (GC-MS). Although providing accurate data, these methods can't be used for on-field disease detection. In contrast to direct methods, indirect methods are used in the field. Indirect method sensors can detect biotic and abiotic stresses in crops, based on plant stress and levels of plant volatility. These are optical sensors that can predict plant diseases using thermography, fluorescence imaging, and hyperspectral techniques.

3.3. Thermography method

Thermal remote sensing technology (thermography) is a non-destructive technique for determining the thermal properties of any material of interest. Thermal remote sensing operates on the concept of transforming invisible object radiation patterns into visible images, which are referred to as thermal images. Portable, handheld or thermal sensors, in combination with optical systems mounted on an aeroplane or satellite, may be used to obtain these images. Thermography may be used for close or far sensing, ranging from microscopes to ground-based devices to airborne sensors. The non-invasive, non-contact, and non-destructive aspect of infrared thermal imaging makes it ideal for determining the temperature distribution of any material in a short period of time (Vadivambal and Jayas, 2011). Thermography sensors measure the temperature variations between the plant leaves and the canopy. Infrared radiation emitted from the plant surface is captured by the sensor. Because of the reduced transpiration, the plant surface temperature will rise if there is a pathogen infection. The sensor can detect the presence of disease-based on temperature changes. Thermography sensors can detect disease-related changes before symptoms occur. Because of its high sensitivity to changes in environmental conditions during measurements, precision disease control is reduced. Thermography system can't tell about the kind and type of infection.

3.4. Fluorescence method

The fluorescence method uses sensors to measure the fluorescence of chlorophyll on the leaves, as well as incident light and changes in fluorescence parameters. It detects pathogen presence by measuring changes in chlorophyll



and photosynthetic activity. While fluorescence measurement allows for sensitive detection of photosynthesis abnormalities, its practical application in the field is limited. Lidar can be used in studies of pest insect migration and movement, including studies of their behavior near pheromone traps and in pheromone-treated fields.

3.5. Gas chromatography method

This is a non-optical sensor that detects crop disease by assessing the volatile chemical compounds emitted by infected plants. Plant pathogens release volatile organic compounds (VOCs) that are specific to each pathogen class. The same thing happens when a plant is stressed due to mechanical damage. Gas chromatography sensors will reliably distinguish the form and extent of infection in this case. The only drawback of this approach is that it needs longer sampling of pre-collected volatile organic compounds before data analysis, which significantly restricts its on-field use.

The ability to detect and monitor insect infestations in stored grains in real-time is critical for taking the appropriate insect control measures. Traditional methods such as visual inspection, sieving, insect traps, insect fragment inspection, and floatation have all been used to detect insects in grain. The majority of these approaches have one or more flaws, such as being arbitrary, destructive, unreliable, time-consuming, and unable to detect internal insect infestations. Furthermore, they are incapable of detecting low-level internal infestations and have not demonstrated the capability of automated inspection. Lampson et al. (2014) created a portable system that uses carbon black–polymer composite sensors to draw volatiles from pests or pest-damaged goods and quantify the change in resistance for each sensor. The system successfully sampled pest and plant volatiles, and these volatiles were detected, indicating that using an electronic nose to detect pests and/or pest damage is a viable option.

The electronic nose (E-nose) is a great instrument for odour analysis. It is an instrument that incorporates electronic chemical gas sensors with partial specificity and efficient pattern analysis techniques for the identification, recognition, or quantification of volatile compounds. For over 20 years, it has been commonly used in the food and flavour industries (Rajendran and Steve, 2005). Persaud and Dodd (1982) were the first to develop the E-nose, which was designed to imitate the human olfactory system's discrimination. An array of chemical sensors to detect odour above the sample with varying selectivity was designed (Persaud and Dodd, 1982; Mart et al., 2005; Rock et al., 2008).

The electronic nose, as a bionic olfactory device, is a complex system with many gas sensors to obtain the knowledge "fingerprint" of the target's

volatile compounds. When it comes to detecting insects, electronic noses are simple to use and are unaffected by the cover of stored grain. Electronic noses can overcome all of the drawbacks of other detection methods, such as manual detection, acoustic signature detection, image recognition, and spectrum detection, and are more suitable for insect identification in storage environments.

3.6. Multispectral and hyperspectral remote sensing technology

The term "remote sensing" refers to data collection from a distance. Data sensors may be simple hand-held instruments, aircraft-mounted sensors, or satellite-based sensors. Remotely sensed data can be used to assess crop health. The remote sensing technology utilizes sensors to monitor plant diseases in two ways:

- i. Imaging approaches which include RGB camera, multispectral imaging, thermal imaging, hyper spectral imaging and fluorescence imaging.
- ii. VIS and IR spectroscopy, as well as fluorescence spectroscopy, are examples of non-imaging techniques.

Some of the other imaging techniques such as terahertz spectroscopy, X-ray-imaging can also be used to detect plant diseases. But, these techniques are not cost-effective (Gogoi et al., 2018). In overhead photos, plant stress related to moisture, nutrients, compaction, crop diseases, and other plant health issues is often visible. Near-infrared images captured by electronic cameras are strongly associated with healthy plant tissue. Satellite data is being increased, thanks to new image sensors with high spectral resolution. Remote sensing can expose in-season variability that affects crop yield and can be used to make timely management decisions that boost current crop profitability. Hyperspectral remote sensing can discriminate and assess the damage by various insect pests like rice leaf folder which was examined by Adhikari et al. (2020) and found that the most sensitive regions were identified in the visible and the NIR regions. Some of the prominent diseases identified using remote sensing has been given below in Table 3.

Table 3 Plant diseases detected using different optical sensors

Sl. No	Sensor	Crop	Diseases	Reference
1	Spectral sensors	Wheat	Yellow rust head blight	Moshou et al., 2004 Bravo et al., 2003
		Tomato	Late blight	Wang et al., 2008



Sl. No	Sensor	Crop	Diseases	Reference
		Tulip	Tulip breaking	Polder et al., 2014
2	Thermal sensors	Sugar beet	<i>Cercospora</i> leaf spot	Chaerle et al., 2007
		Apple	Scab	Oerke et al., 2011
3	Fluorescence imaging	Bean	Bacterial blight	Rousseau et al., 2013
		Wheat	Leaf rust	Burling et al., 2010
4	RGB	Cotton	Angular leaf spot	Camargo and Smith, 2009

The effect of pests and diseases are unevenly distributed throughout an agricultural area. Despite this experience, analytical approaches and technologies have restricted the ability of insect pest and disease management systems to make field decisions. Recently, methods and technologies for spatially mapping and analyzing insects and diseases have advanced to the point that the uneven existence of insect populations and disease intensity can be used for management tactics. Site-specific pest management is the term given to this modern approach to pest control. Pest control techniques are only used when pest density is economically high within a sector using site-specific pest management, which uses spatial knowledge about pest distribution (Park et al., 2007). Early detection of insect pest infestation is an essential step to take up timely management measure. The real time pest and disease detection in large-scale farming of rice crop for pest management is a difficult task; to overcome this hyper spectral remote sensing is a powerful tool for detecting stresses caused due to insect pests and diseases in green vegetation at the leaf and canopy levels (Kobayashi et al., 2001; Qin et al., 2003; Prasanna Kumar et al., 2014).

The wavelength of electromagnetic radiation has an effect on how it interacts with plants. Depending on the degree of health and/or vigour, the same plant leaves can have different significant reflectance (West et al., 2003). In general, healthy and vigorously developing plant leaves will have (i) low reflectance at visible wavelengths due to strong absorption by photoactive pigments (chlorophylls, anthocyanins and carotenoids) and (ii) high reflectance in the near infrared due to multiple scattering at the leaf's internal tissue's air-cell interfaces (iii) water, proteins, and other carbon constituents absorb light, resulting in low reflectance in small wavebands in the short-wave infrared. The difference in spectral characteristics between healthy and stressed plants can be used to monitor the occurrence and severity of pests and diseases.

For the first time, the attempt was made to use the aerial photography to study and monitor cereal diseases especially black stem rust caused by *Puccinia tritici*, yellow dwarf of oat by Colwell in 1956 at university of California and the results were published in Hilgardia in 1956 (Colwell, 1956). He used panchromatic infrared and colour films (Aerial Ektachrome). Similarly, Qin and Zhang (2005) used four airborne remote sensing images for the detection of sheath blight of rice caused by *Rhizoctonia solani* in USA. The remote sensing images were acquired by ADAR (Airborne Data Acquisition and Registration) system 5500. The captured images had four bands with different colours 1. Blue: Band-1 (450-540 nm), 2. Green: Band-2 (530-600nm), 3. Red: Band-3(610-680 nm) and 4. Near infrared Band: Band-4 (780-1000nm). Three methods were used for comparison of acquired data with different field disease index i) Direct band digital number (DN) Value, ii) Ratio indices (RI), and iii) Standard difference indices (SDI). Results shown that, RI and SDI had the potential to serve as indicators of RS for sheath blight disease identification and monitoring (Qin and Zhang, 2005). In India, the first attempt to use the remote sensing technique was experimentally used by Prof. Pisharoty in Kerala on coconut root-wilt diseases in early 1970s. For studying root wilt of coconut disease, he took aerial photographs with “Hasselblad Camera” using helicopter in seven different combinations at two different altitudes of 500 and 1000 feet. The colour ektachrome infrared film, ordinary black and white film, and black and white panchromatic plus X with three separate filters in red, blue, and green bands were used in these photographic exposures (Gogoi et al., 2018). Multispectral sensors were the first spectral sensors, followed by hyperspectral sensors, and finally ultra-spectral sensors. Over the covered spectral range (350–2500 nm), these technological complex devices provide a wealth of knowledge. Modern instruments can have a spectral resolution of less than 1 nm. Non-imaging sensors average reflectance over a region based on the sensor's field of view and the mixed data from various objects makes it difficult to make significant inferences about the reflectance sources/objects (Steiner et al., 2008). Spectral range for detecting some of the plant diseases are mentioned in Table 4.

Table 4 Optimum spectral range for detecting plant diseases

Sl No	Crop	Diseases	Statistical methods	Optimum spectral range	Reference
1.	Tomato	Late blight	Monitoring noise fraction transformation and spectral angle mapping-based classification	700-750 nm, 750-930 nm, 950-1030 nm and 1040-1130 nm	Zhang et al., 2005
2.	Grapefruit	Citrus canker	Principal component analysis	553, 677, 718 and 858 nm	Qin et al., 2009



Rice insect pests like the Yellow Stem Borer (YSB) (*Scirpophaga incertulas*), Brown Planthopper (BPH) (*Nilaparvata lugens*), and leaf folder (*Cnaphalocrocis medinalis*), as well as diseases like blast, bacterial blight, sheath blight, and false smut, are the most notable risks in rice yield in tropical areas, especially in Asia. Several remote sensing techniques have been developed to use visible and infrared images to detect stress in rice production caused by these biotic stresses. To diagnose crop disease stress, high spectral resolution remote sensing imagery with more bands and narrower bandwidth is needed (Qin et al., 2003). The crop affected by insect gives different tonal variation in imageries than normal crop. Pest-affected areas produce pink, purple, and yellow pinkish red colour with irregular form and rough texture, while normal crops produce red, bright red, and dark red with smooth texture (Koshal, 2010). Sensors that use the hyperspectral method test plant health using a broad range of wavelengths between 350 and 2500 nm. They monitor the changes in reflectance that occur as a result of the biophysical and biochemical changes that occur as a result of infection. Hyperspectral cameras gather data in three dimensions, with spatial data on the X and Y-axes and spectral data on the Z-axis, resulting in more precise and reliable information about plant health. Hyperspectral sensors are used to detect crop pests and diseases early, enabling farmers to protect their crops quickly and effectively. Hyperspectral imaging is becoming more popular in the field of fruit protection and quality control. Quin et al. (2009) devised a hyperspectral imaging method for detecting citrus canker lesions. Zhao et al. (2012) used hyperspectral imaging data to measure cabbage caterpillar infestation of oilseed rape. According to Mewes et al. (2011), the entire spectrum from 350 to 2500 nm is not needed to detect fungal infections in crops. Since most of the studies conducted have used non-imaging hyper-spectroscopy. Hyperspectral imaging based on spectral details of disease symptoms has been restricted. Hillnhütter et al. (2011b) successfully differentiated symptoms caused by *H. schachtii* and *Rhizoctonia solani* on sugar beet using field spectral images. Bravo et al. (2003) used field spectral images for an early detection of yellow rust infecting wheat. Hyperspectral imaging is becoming more popular in the field of fruit protection and quality control. Some of the bands used to discriminate some insect pests/diseases are mentioned in Table 5.

Table 5 Identification of optimum bands/indices for rice pests and diseases using hyperspectral remote sensing

Insect Pests/ Diseases	Platform	Optimum bands (in nm)/ indices/ technique used	Reference
Leaf folder	Ground based	716, 720, 733, 762, 766, 814, 896 nm	Adhikari et al., 2020
Rice stem borer	Ground based	570-700 nm, BPNN	Fan et al., 2017

Bacterial leaf blight	Ground based	943 and 1039 nm, MLR	Yang, 2010
Brown spot	Ground based	(R702/R718), (R692/R530), (R692/R732)	Liu et al., 2008
Brown Plant hopper	Ground based	426, 1450 nm, linear correlation intensity analysis	Yang et al., 2007
Panicle blast	Ground based and air borne	485, 675 nm; (R470/R570); (R520/R675); (R570/R675); (R550/R970); (R725/R900)	Kobayashi et al., 2001

MLR: Multiple linear regression; BPNN: Back-propagation neural network

Unmanned aerial vehicles or UAVs (also known as drones), have become one of the most well-known and fascinating inventions in the world, with people using them in a variety of fields, including agriculture. Farmers want correct and up-to-date crop health information at all times. Sensors are normally mounted on an unmanned aerial vehicle to track a wide field area. UAVs can also help with scouting. Drones are taking advantage of rapid innovation in the field of sensor production, even though they are still in the early adopter phase among farmers. From macro to micro, large-scale to small-scale farms, UAVs are a natural progression. Drones, at their most basic level, allow farmers to get a bird's-eye view of their crops, enabling them to spot slight changes that “crop scouts” on the ground might miss. Farmers can view crop changes that are otherwise invisible to the human eye using UAVs fitted with special sensors that capture multispectral Neutral Density Vegetation Index (NDVI) and infrared (IR) images. This aerial data can also be used to expedite the time-consuming process of conducting crop inventories and yield estimates. Similarly, Garcia et al. (2013) used UAV sensor and aircraft-based sensors to detect citrus Huang Long Bing (HLB) or citrus greening disease (yellow dragon disease) caused by *Candidatus liberobacter* sp. a fastidious bacterium. The sensors yielded different spatial resolution (5.45 cm and 0.5m per pixel). A single engine fixed aircraft was used to take hyperspectral images with Advanced Imaging Spectrometer for Application (AISA) such as hyperspectral imaging sensor, EAGLE VNIR (397-998 nm). The observations recorded by them suggested that, the infected citrus trees reflected higher amount of light in VIS region than healthy trees. Bravo et al. (2003) used VNIR hyperspectral imaging for early detection of wheat yellow rust disease caused by *Puccinia striiformis tritici*. Drones can be equipped with modern cameras and imaging equipment to detect possible crop damage. Another technology, known as LIDAR, uses light and RADAR to create high-resolution maps of fields, which can help farmers better understand the risk of damage from drought, pests, disease, and weeds.



3.7. Artificial intelligence and big data machine learning

One of the most important fields of Artificial Intelligence is machine learning. It's a branch of computer science that's used to construct algorithms with self-learning properties, *i.e.* learning that's performed by the system itself, hence the name 'Machine Learning'. It is one of the most rapidly expanding areas within the field of 'Artificial Intelligence' (AI). For a machine to exhibit intelligence, it has to interpret and analyze the input given by the developer. This is what the machine learning algorithms do. Big data comprises of enormous volume, velocity and variety of data generated from sensors and real-time platforms in a smart farming system (Wolfert et al., 2017). Machine learning is becoming increasingly common, and it provides a variety of solutions for dealing with big data challenges. To provide useful information on pest and disease, machine learning algorithms effectively take into account all possible causes, historic data, as well as satellite/sensor data of fields. A pattern recognition branch of machine learning was used to detect disease using images of crop leaves (ex. Plantix mentioned in Table 6). Patil et al. (2011) detected plant diseases using machine-learning methods that achieved, speed and accuracy. Corrales et al. (2015) developed supervised learning algorithms for crops like corn, rice, coffee, mango, peanut, and tomato for detection of pests and diseases.

Weeds, plant diseases, and insect pests in crops must be detected early and accurately for effective crop defence. These are related to the creation of non-invasive, high-resolution optical sensors, as well as data analysis methods that can handle the resolution, scale, and complexity of the signals produced by these sensors. The primary aim of using machine learning methods in precision crop protection is to detect variability or heterogeneity within crop stands caused by biotic stresses using sensor data (such as diseases or weeds). By learning the model structure directly from training data, machine learning methods may analyse high-dimensional data with unknown statistical characteristics for precision crop safety. The use of remote and near-infrared optical data to detect and track weeds and plant diseases in agricultural crops have been widely investigated (Mahlein et al., 2012). Study conducted by Bekker et al. (2019), on spatial distribution of fruit fly, *Ceratitits capitata* trap catches which was classified and later to identify its main geographical drivers. Data from trap catches was gathered from an area-wide integrated pest management programme and categorized into statistically relevant hot and cold spots (HCSs). Monthly and seasonal long-term HCSs were identified by combining trap data from four consecutive fruiting seasons. Using variable importance lists generated by the random forest (RF) machine learning (ML) algorithm, the key geographic drivers of the HCSs were established. Efficient

applications include early detection of plant diseases based on spectral features and weed detection based on shape descriptors using supervised or unsupervised learning methods. The most popular methods for data analysis are machine learning methods such as artificial neural networks (ANN) or support vector machines (SVM) (Zhang et al., 2003; Moshou et al., 2004; Rumpf et al., 2010). Moshou et al. (2004) used artificial neural networks to distinguish stable wheat plants from diseased wheat plants. Rumpf et al. (2010) used SVMs to identify sugar beet foliar diseases in the pre-symptomatic stage. Quin et al. (2009) used hyperspectral imaging sensors to distinguish between healthy citrus fruits, canker diseased citrus fruits, and damaged citrus fruits. Healthy and infected plants with *Cercospora* leaf spot, powdery mildew, and sugar beet rust had separate important regions spectra (Mahlein et al., 2010). Using SVIs, Delalieux et al. (2007) detected apple scab at various stages of disease development caused by *Venturia inaequalis*. Steddom et al. (2005) used SVIs to measure various sugar beet indices and compared them to disease severity as visually assessed by plant pathologists.

Plant disease detection and forecasting in the field is the foundation for long-term and reliable plant conservation. Machine learning is used to analyse various types of sensor data in this context. Moshou et al. (2004) discovered how to use machine learning to distinguish biotic stress in plants. They automatically detected yellow rust in wheat on the basis of reflectance measurements using neural networks (NNs). Using NNs, Wang et al. (2008) were able to predict *Phytophthora infestans* infections on tomato plants by looking at related hyperspectral signatures. When the first signs of diseased tomato plants became apparent, they were able to identify them. Wu et al. (2008) demonstrated that using PCA before NN classification and hyperspectral signatures as features, early detection of *Botrytis cinerea* on eggplant leaves is possible. The researchers were able to accurately identify the first small symptoms of grey mould on eggplant leaves in their sample. Liu et al. (2010) used PCA and subsequent NN classification to detect rice glume blight disease in rice panicles, distinguishing between stable, light, moderate, and severe infection levels. However, this study was performed under controlled conditions and they stated that it is probably not applicable for monitoring the health status of rice under field conditions. One of the first research using SVMs to demonstrate early detection of plant diseases before apparent symptoms emerged was reported by Rumpf et al. (2010). For automatic classification of relevant sugar beet diseases, nine spectral vegetation indices linked to physiological parameters were used as features.



4. PEST AND DISEASES MONITORING AND MANAGEMENT

4.1. *Scouting and monitoring*

Because of various crop insect pests and diseases, a farmer must closely track crops from the early stages. They are usually the most serious threat to crop production. Early pest detection can minimize yield loss by up to 20-40% depending on the crop type and growth stage. As a result, farmers must devote their entire attention to crop monitoring. Farmers must be extremely cautious from the start in order to obtain the full quantity and quality of product. Latest technologies can help farmers track crops effectively and potentially remotely detect destructive insects/pests and avoid their related disease at an early stage, lowering production costs and improving quantity and quality of produce (Sarika and Wagh, 2014). Farmers can now monitor their crops more easily and accurately using various instruments, such as sensors, rather than walking down the field. Insect pests and diseases make crop defence more difficult. As a result, new sensors for precision farming are being developed all the time. Pest detection sensors, for example, detect the presence of disease and insect pests on crops using modern technology. The sensors essentially provide real-time data from the field.

4.2. *Monitoring tools for pest management*

The foundation of an Integrated Pest Management (IPM) programme is monitoring the number of insect pests and disease incidence. Monitoring is used to get a better understanding of insect pest, mite, and beneficial insect behaviour in crop, as well as to determine the magnitude of disease and weed outbreaks. To track crops, understand how the weather affects pest pressure, and follow relevant weather forecasts, it's critical to hire experienced crop scouts. Monitoring involves determining the number of insect pests, mites, and beneficial insects in a crop, as well as the occurrence of diseases, weeds, and damage caused by any invertebrate or vertebrate pests. Recording this data, as well as any control measures taken, allows for a clearer understanding of crop management practices over time. Previous monitoring results can be referred to, and the impact and efficacy of control decisions can be calculated.

Pheromone traps, yellow sticky traps, blue sticky traps, leaf wetness sensors, pest forecasting models, disease prediction models, and insect growth models are among the resources available to help crop scouts and growers track pests and beneficial insects, as well as predict pest pressure. These methods can assist in determining the range and behaviour of pests and beneficial insects in your crop. The data obtained from these traps may be used to monitor flights or peak activity times. This information will aid in deciding the amount of crop



scouting that is needed. It's critical to check traps more regularly during peak production times (<https://ausveg.com.au>).

4.3. Geographic information system

Each crop necessitates a unique degree of attention and care; no two crops grow in the same way. Farmers must be able to pinpoint which crops are suffering from drought, insect pressure, or a lack of nitrogen. Rather than using a one-size-fits-all solution, GPS provides growers with the precise location of a plant in need. Geographic information systems (GIS) are computer hardware and software that create maps based on feature attributes and position data. Geographically referenced data may be viewed in a GIS, giving understanding a visual perspective. In addition to data storage and display, the GIS can be used to assess current and alternative management strategies by integrating and manipulating data layers to create a management scenario analysis. In precision pest management programmes, GIS and GPS are currently used for variable rate pesticide application, but the comparatively less-used methods of remote sensing and spatial analyses may be of additional use in integrated pest management practices.

4.4. Artificial neural networks

Artificial neural networks (ANNs) are one of the important components of artificial intelligence. It has been suggested that ANN could be used as possible methods for biological objects identification usually along with other statistical tools, such as classification trees or principal component analysis. An optimum architecture, established and supervised by taxonomic experts uses morphometric characteristics which transforms through multilayer system processing (artificially intelligent apparatus) into a metadata. It led to development of and successful identification of thrips (Thysanoptera) and could be better used in pest monitoring (Fedor et al., 2009).

4.5. GPS in crop scouting

GPS is an extremely useful tool when it comes to the advancement of crop scouting in precision agriculture. Crop scouting has always relied on farmers remembering and tracking where they scouted, but with the use of GPS, farmers can now provide a detailed recording of where they scouted down to one foot. Using this precise position data, they can make notes and have reliable positions where pests, low soil temperature, or moisture are located. Farmers may also use the accuracy of GPS to efficiently address threats in their fields.

Many different pieces of technology now include GPS, which allows farmers to scout their fields much more quickly and accurately. Different applications available for tablets or smartphones are an example of these



technologies. Farmers may use these apps to not only mark their exact position in a field, but also to take field notes, compare notes from previous years, and more. These apps will show a farmer where specific target areas of issue are on an aerial photo of their field, as well as assist farmers in making potential decisions based on previous crop issues.

4.6. UAV in crop scouting

The growing use of UAVs for pesticide application has been observed in East Asian countries such as Japan, South Korea, and China, with promising results. This UAV-based agrochemical application technology is viewed as a high-efficiency alternative to traditional manual spray operations, as well as a low-cost alternative to traditional manned aerial application. UAVs, also known as drones or Remotely Piloted Aircraft (RPA) systems, are gradually being studied and used for pesticide applications nowadays. The rapid growth of Asian agricultural UAVs is largely due to its benefits, which include: (1) UAVs do not need a dedicated airport or navigation station, and can land on the edge of cultivated land, the highway, or the top of a truck, lowering airline and agricultural and forestry costs; (2) a UAV with a short turning radius could hover and turn around flexibly in the air; (3) a UAV with a high rate of climb could travel vertically and have good super low flight performance; (4) a UAV with a low rate of no-load flight and fuel and liquid loading on the ground of the working area could minimize invalid working time; (5) As compared to conventional manned aircraft, UAVs are ideal for operating in rugged terrain and small plots with high efficiency; (6) high automaticity, less flight crew, low labour rate, and easy to use and maintain (Xiongkui et al., 2017). In terms of spray impact, (1) UAVs have the same high working efficiency and spray output as helicopters; (2) the downwash flow produced by rotors can minimize droplet drift, and the up-flow generated by the downwash flow allows droplets to crash into the reverse side of plant leaves. The UAV can change velocity flexibly, accelerate from 0 to normal speed directly, and get better droplet coverage at low speeds (<https://www.farms.com>).

4.7. Green data center and mobile applications in pest management

Smartphones have made high-definition video and images available to almost all, resulting in an endless stream of new data that must be processed and archived for potential use. This has far-reaching implications for data centre operational costs as well as the climate. Currently, millions of servers are in use on a daily basis all over the globe. While these are often upgraded and managed, they are not always used. These unused servers are expected to cost billions of dollars per year. This may be a significant loss of resources as well as precious energy. A data collection, management, and dissemination repository with technologies designed to maximize energy efficiency while minimizing

environmental impact. Advanced technology and techniques are used in the design and operation of a green data center. Green storage is the process of reducing a data center's carbon footprint and costs by using a range of "clean energy" storage technologies and goods. Data analytics and other digital methods are assisting farmers in making decisions about their activities, and they are also assisting them in finding ways to use less of our planet's limited resources. Another important aspect of digital services is to provide timely access to information for taking appropriate decision in pest management by the farmers. With this in mind, numerous options for transmitting information to farmers in a timely and cost-effective manner have been investigated.

It is clear that information and communication technologies (ICTs) have the potential to allow farmers' access to and exchange of information. Mobile phones are becoming more common among ICTs, which is changing the agricultural communication process. New services and apps have emerged as a result of the advent of mobile phones. In the crop protection sector, these include proper identification of insect pests, diseases and other biotic stresses etc.

Farmers and other stakeholders have benefited from the advancement of new services and applications in agriculture as a result of the advent of cell phones. Services that began with sporadic messages have progressed to multimodal and multimedia advisory distribution, as well as m-agriculture apps for smartphones. These programmes bridge the knowledge and coordination gap between farmers and extension staff while also providing farmers with a bargaining position (Saravanan and Bhattacharjee, 2014). Farmers can benefit from timely access to information on new crops, inputs such as seed, fertilisers, equipment, price information, weather, pests and diseases, and nutrient management to support activities ranging from production to marketing. According to the Telecom Regulatory Authority of India, the number of telephone subscribers as of 31 July 2017 was 1210.71 million (1186.79 million wireless and 23.92 million fixed land line telephones) (TRAI, 2017). As of July 2017, the tele-density had reached 93.88%. However, there is a significant difference in tele-density between urban and rural areas, with 173.21 and 57.45, respectively. India has the world's fastest-growing smartphone market, according to IDC, with 27.5 million smartphones sold in the second quarter of 2016, up 17% from the previous quarter. According to the Ericsson Mobility Report from June 2016, mobile subscriptions are projected to hit 1.4 billion by 2021 (CNBC, 2016). Different mobile apps for identification and classification of diseases such as blast, brown spot, Bacterial Leaf Blight (BLB), sheath blight, false smut, root knot nematode and white tip nematode in paddy has been developed (Rajmohan et al., 2018). Detailed information of such apps has been listed below (Table 6-9).

**Table 6 Details of mobile apps developed for disease and pest identification**

Name of the app	Available from	Operating system	Cost	Account needed	Description
Ag PhD Corn Diseases	<i>Ag PhD</i>	Android / iOS	Free	No	Disease identification and diagnostic guide for corn. Content includes disease description, symptoms, and similar diseases, conditions that favor a disease, and suggested management options. Save common diseases for a farm.
Ag PhD Soybean Disease	<i>Ag PhD</i>	Android / iOS	Free	No	A guide to soybean diseases and diagnosing the disease. Same features as the corn disease app.
Ag PHD Field Pest	<i>Ag PhD</i>	Android / iOS	Free	No	A guide to assist for identifying pests. Browse weeds by name or by image plus provides agronomic information and guidance. Save common pests for a farm.
IPM Toolkit	<i>University of Wisconsin integrated pest management tools</i>	Android / iOS	Free	No	This app allows reading news articles, view videos, download publications, and access pictures which will aid in adapting Integrated Pest Management (IPM) practices in agricultural operation.
PestXpert	<i>Bayer</i>	Android / iOS	Free	No	Provides professional pest controllers assistance in the identification and treatment of insect pests. Insect pests that are not easily identified can be photographed and uploaded for personalized identification within 2 working days.
Plantix	<i>PEAT GmbH</i>	Android / iOS	Free	Yes	Diagnostic tool for fruits, vegetable and field crops. Capture images of damaged crops and receive instant diagnosis on plant problems. Regional treatment suggestions provided.

Table 7 Details of apps developed for different equipment

Name of the app	Available from	Operating system	Cost	Account needed	Description
Tank Mix Calculator	<i>TapLogic, LLC</i>	Android / iOS	Free	No	Quickly generate tank mixes based on product and spray characteristics. Contains over 14,000 chemicals along with the ability to input your own.
Mix My Sprayer	<i>Clemson University</i>	Android / iOS	Free	No	Tank mix calculator for specific spray applications. Search by category, products, or codes. Calculated mix and user settings can be saved.
Tank Mix Calculator	<i>DuPont</i>	iOS	Free	No	Calculates the ratio and amount of product needed to treat a field specifying product amount based on tank size and amount of carrier required.
Spray Guide	<i>Precision Laboratories Inc.</i>	iOS	Free	No	Provides mix guide feature to plan tank mixture based on products. Maintain field and spray logs. Provides a database of over 1,300 crop protection products.

Table 8 Details of apps developed for sprayer selection and calibration

Name of the app	Available from	Operating system	Cost	Account needed	Description
TeeJetSpraySelect	TeeJet Technologies	iOS	Free	No	Provides a listing of suggested tips for a specific spray operation including drop size information.
Spray Tips Guide	<i>Ag PhD</i>	Android / iOS	Free	No	Tip selection tool based on equipment and spray operation. Select by chemical company or product name to obtain suggestions based on product label.
Sprayer Calibration Calculator	<i>University of Illinois</i>	iOS	Free	No	Helps calibrate a sprayer (aerial and ground-based including turf and



Name of the app	Available from	Operating system	Cost	Account needed	Description
					boomless setups). Determines flow rate, how to split rate among various orifices, and computes pressure. Calculates max and min ground speeds.

Table 9 Details of apps developed for different scouting technologies

Name of the app	Available from	Operating system	Cost	Account needed	Description
Ag PHD Scout	Ag PhD	Android / iOS	Yes	Yes	Record pests, diseases, and weeds when scouting. Able to collect images, create GPS boundaries, and diagnose field issues while geo-tagging problem areas. Syncs with desktop application.
Agra Scout	<i>Neucadia</i>	Android / iOS	No	Yes	Supports crop scouting. Geo-tagged pins for tracking weeds, pests, and crop diseases. Capture images and record comments that can be shared with others.
NDSU Pest Management	<i>NDSU Pest Management</i>	Android / iOS	Free	No	Combines information from the North Dakota State University Weed Guide, Disease Management Guide, and Insect Management Guide that is grouped by crop and pest. Provides users with control options to select additional information for further review.
Pesticide and Field Records	<i>Iowa State University</i>	Android / iOS	Free	No	Record and maintain pesticide applications, link to specific field locations using satellite mapping, and keep other important crop production information with. Use the convenient product search option to quickly identify EPA product registration numbers and restricted use products.

5. PEST AND DISEASE FORECASTING

Insect pests and diseases attack at different times and in different ways across regions, seasons, and individual crops within an area. Pesticides are the only viable way to control insect pests and diseases in the absence of safe, desirable, and diverse sources of resistance. Early detection of an imminent disease outbreak or pest attack is a powerful management method. The creation of region-specific, crop-specific, and pest-specific prediction models will help to forewarn about these threats. Forecast models can be used instead of calendar spray schedules to add need-based precision to spraying. Since insect pests and disease threats are weather-dependent, weather-based prediction models can help with pest management (Kumar et al., 2016). Many insect pests and disease studies have tested and published on weather variables such as temperature, rainfall, and relative humidity (Olatinwo et al., 2011). In most cases, a warm environment is essential for insect pest growth, production, pest epidemics, the extent of crop damage, and overall crop yield. Since insects are temperature sensitive, they usually react to higher temperatures, which speeds up development and decreases the time between generations.

5.1. Pest forecasting

Pest forecasting are mainly two types based on period of forecasting.

- a. Short term forecasting - based on one or two seasons.
- b. Long term forecasting - based on effect of weather parameters on pest.

Diverse modelling approaches *viz.* Generalized Regression Neural Networks (GRNN) and Multiple Regression (REG), Back-Propagation Neural Network (BPNN), Support Vector Machine (SVM) have been followed to date for disease prediction in plant populations. In pest forecasting, several intrinsic attributes of the insects and the determining environmental and host factors need to be considered. Most pest forecast models take into account the phenology of the herbivore and its host. Near real-time pest incidence data coupled with remote sensing and GIS tools facilitate early warning of impending pest build-up in a temporal and spatial perspective. In addition, collection and analysis of weather data from pest-affected areas is an essential input for models. The practical application of model outputs is aided by decision support systems. Weather-based forecasting systems lower production costs by optimizing the timing and frequency of control measures to minimize crop loss and lower plant security costs. The development of forecast models takes a lot of resources, such as manpower, time, and money. As a result, it's critical that such resources are restricted to a single significant crop. The candidate pest (both insect-pest and disease) should be erratic, and its incidence, magnitude, and development



should be affected by weather factors and thus vary accordingly; it should also cause economically significant yield losses over a wide region, and the availability of timely and accurate forecasts may help mitigate the risks associated with its occurrence through the use of effective management strategies. Forecast models offer an alternative to calendar spray schedules for bringing need-based precision, such as spraying at precise times exactly when and where the pest is expected to occur or has just begun to reduce input costs instead of spraying at 7-14days intervals. As a result, precision pest management can reduce the number of chemical pesticide sprays, resulting in cost and environmental savings. Lastly, as part of the IPM toolkit, the forecasting system should allow growers to take economically viable action while also ensuring that growers are competent and flexible enough to fully exploit pest forewarning. A forecasting model is a set of formulae, rules, or algorithms based on the biology of a particular pathogen or insect pest, as well as the host, and normal crop management practices.

5.2. Insect pests prediction model

The DYMEX model was used to investigate the relationship between climatic variables and changes in organism populations in a given area. DYMEX is divided into two sections: Builder (which consists of different modules that work together to construct models) and Simulator (to run the model that has been built in the builder). The model DYMEX was able to forecast the impact of climate on the dynamics of the YSB population and generate a monthly or seasonal trend pattern, with R^2 values of 0.74 (calibration) and 0.88 (validation) respectively.

Several workers have analyzed influence of weather factors on BPH population and observed temperature, humidity and rainfall to be important ones (Yadav et al., 2010; Win et al., 2011). In general, pest weather relations have been analysed through empirical models, which behave in location- specific manner (Chander and Singh, 2010). Based on the peaks of its light trap catches weather parameters such as maximum temperature, morning relative humidity, evening relative humidity, and SSH, a forewarning model for rice leaf folder was created, and these four weather factors together could explain 99% variability in leaf folder light trap peaks in Punjab. According to weather research, hotter and drier conditions in June and July of 2012 may have played a role in the leaf folder outbreak, among other things (Singh et al., 2015).

5.3. Diseases prediction model

To better understand, predict, and manage rice diseases, a number of simulation models have been created (Hashimoto et al., 1984; Teng and Savary, 1992). In

order to predict the disease occurrence more accurately, models usually aim to include both major environmental and cultural variables in the model simulation. As a result, a model that has been successfully established may not be widely adaptable to other areas with very different cultivars, cultural traditions, and ecosystems. Furthermore, linking these models to other applications such as GIS and GCM-generated climate data at various temporal and spatial resolutions is difficult due to structural complexities and temporal and spatial constraints of their input requirements. Many rice disease models need cultivation-related information as input variables, such as rice cultivar, transplanting date, and even daily trapped airborne fungal spore numbers, in order to consider as many factors influencing disease development as possible. The modelling incorporates a wide variety of weather variables, including air temperature, relative humidity, rainfall, solar radiation, wind speed, and others. In addition, some of the models were only validated for one or a few field-level locations, restricting their applicability to particular regions.

Rice blast is the most destructive rice disease causing enormous losses every year. The most frequent climatic variables used are air temperature, followed by relative humidity and rainfall. The key pathogenesis factors, for instance leaf wetness, nitrogen fertilization and varietal resistance showed restricted incorporation for the development of these models. Currently, five forecasting systems are operational in Japan, Korea and India. EPIBLA (Epidemiology of blast) model using multiple regression equations based on maximum temperature and maximum RH for simulation of leaf blast incidence. Machine learning techniques model for forecasting rice blast in India. Six weather variables were selected *viz.*, temperature, RH, rainfall and rainy days per week. Blast is mostly preferred by particular air and soil temperatures, RH, hours of continuous leaf wetness (LW), degree of light intensity and duration and timing of dark periods, all of which have been considered as very crucial for development of the disease. A model was developed in Japan that determined *P. oryzae* infection periods, evaluating weather conditions every hour, producing hourly results that indicated if the conditions would result in successful infections. The model consisted of two parts of which the first comprised three favourable conditions for successful conidium penetration and hence successful infections.

The second part calculated the number of "infection hours," or the hours during which the first part's three criteria are met. In order to measure the daily infection alert hours, the model's infection hours for each day were accumulated for 1 day (DIWH). The DIWH was divided into four risk prone levels: 1) zero risk, $DIWH = 0$ h; 2) low risk, $1 \text{ h} \leq DIWH < 3 \text{ h}$; 3) intermediate risk, $3 \text{ h} \leq DIWH < 6 \text{ h}$; and 4) high risk, $DIWH \geq 6 \text{ h}$. The Yoshino model is still used as part of three forecasting systems: a commercial system developed



in Austria. In India EPIBLA (Epidemiology of blast) was developed which simulated incidence of blast and made 7 days forecasts of disease progression in many parts of India. Calvero and Teng (1992) in the Philippines developed BLASTSIM.2 model for blast. Kang et al. (2010) developed a forecasting model describing an online information system for plant diseases based on weather data. Savary et al. (2012) in Korea developed EPIRICE, a generic model for plant diseases which was coupled with GIS.

5.4. Artificial Intelligence in pest and disease forecasting

Basically, artificial intelligence began during 1956 with a speculation that it can be used to describe every aspect of learning so precisely that a machine could be made to mimic it. Artificial intelligence in agriculture has giant potential for improving the effectiveness and sustainability. In the present day global food situation, scientists have been looking for innovative approaches to control insect pests of crops by developing integrated production systems involving various technologies like biotechnology, genetically modified organisms, newer pesticides etc.

With the introduction of data mining, the field of agriculture, as well as pest management, has become more concentrated. Tripathy et al. (2011) have used the Rapid Association Rule Mining and Gaussian Naive Bayes to develop and created a real-time decision-making method that uses a data mining technique and a wireless sensor network to predict pests. Through this, they were able to predict the time of occurrence of pests based on various pieces of information from the wireless sensor network. Wang and Ma (2011) contrasted the SVM (Support Vector Machine) and the Regression Method for predicting wheat stripe rust disease, one of China's most serious diseases. Successful algorithm of predicting outbreak of olive fruit fly using machine learning algorithm was developed by Kalamatianos et al. (2017). They used a variety of techniques to predict the next olive fruit fly trap measurement, taking into account previous trap measurements as well as an attribute known as the Degree Day model, which serves as a correlation model between the growth of a pest's population and temperature. Using machine learning algorithms, the model was effective in predicting future olive fruit fly trap measurements and, as a result, knowing olive fruit fly infestations/outbreaks.

Sensor systems, such as multi-spectral, hyperspectral, and chlorophyll fluorescence, have provided high resolution data concerning precision agriculture in recent years, resulting in remarkable results in crop protection and pest control (Mahlein et al., 2012). Progress in sensor technology, together with developments in computer science and GIS, opens up new possibilities for precision agriculture and lays the groundwork for early weed and disease

detection (Lee et al., 2010; Moshou et al., 2004; Rumpf et al., 2010). Advanced machine learning approaches, such as k-means, SVMs, and NNs, require less prior knowledge and are applicable to a broader range of tasks since they implicitly derive the underlying distributions and model assumptions from training data. A brief literature overview on the application of machine learning methods for the detection of pest and disease within the current process of digital innovations are given below in Table 10.

Table 10 Machine learning algorithms used in precision pest and disease management

Sl. No.	Application/ Model	Significance	Reference
1.	RIM (Ryegrass Integrated Management)	Model-based DSS for testing the biological and economic performance of strategies to control ryegrass <i>Lolium rigidum</i>	Lacoste and Powles, 2015
2.	GOSSYM COMAX	GOSSYM (GOSSYPiums Mulator) is a computer program that simulates growth and yield of cotton (<i>Gossypium hirsutum</i>) in the field GOSSYM along with the COMAX (Crop Management expert) system which interprets the results of GOSSYM,	Whisler et al., 1986; Lemmon, 1986
3.	riceXpert	Developed for the farmers to provide advisories on real-time basis about insect pests, nutrients, weeds, nematodes and disease-related problems, rice varieties for different ecologies	Mohapatra et al., 2018e
4.	Rice Crop Doctor	The MANAGE has developed an expert system to diagnose pests and diseases for rice crop	Prasad and Babu, 2006
5.	RICEsmart	Ontology-based expert system which will intended to help the farmers, extension workers, researchers and students and provides and efficient and solving common problems related to rice	Yadav et al., 2014
6.	e-SAP	Electronic solutions against agricultural pests (e-SAP). It is a dedicated system that effectively integrates mobile communications, tablet-based technologies and cloud solutions to bring different players of the agricultural ecosystem	eSAP: https://www.ua-sraichur.edu.in/index.php/en/research/e-sap



6. DECISION SUPPORT SYSTEM

A decision support system is a software-based collaborative system that assists decision-makers in identifying and solving problems and making decisions. A decision support system is a computer programme that includes expert information about a specific problem domain and can solve problems at a level that is equal to or greater than that of a human expert. DSSs are knowledge-based systems that serve as an integrated software-based framework to assist decision makers in compiling valuable information from a mixture of raw data, records, personal knowledge, and business models in order to identify and solve problems and make decisions. For farmers and their advisors, computer-based DSSs have the potential to be useful resources in the pest management decision-making process. There have been many DSS systems built for agricultural management, but the majority of them deal with issues like irrigation scheduling and water requirements. There has been limited research on decision support systems for real-time estimation of damage inflicted by insect pests and diseases in rice.

DSS for insect pests of rice and cotton-based cropping systems has been developed by Central Research Institution for Dryland Agriculture, India (CRIDA) (Patel and Kadam, 2016). National Institute of Agricultural Extension Management (MANAGE), India developed an expert system to diagnose pests for rice crop and suggest preventive/ curative measures. The rice crop doctor demonstrates the use of expert systems in agriculture in general, and in rice production in particular, through the creation of a prototype that takes into account a few major pests and deficiency problems that restrict rice yield. Expert systems to prepare and schedule in-season management activities for crops and other resources are known as management decision support systems,' and they are one of the most awaited applications of artificial intelligence technology in agriculture. DSSs are computer software tools that help people make decisions. A well-designed DSS aids decision makers in identifying, making decisions, and solving problems by providing valuable knowledge from raw data. DSSs can range in complexity from a simple data processing method to a computerized expert system. DSS provides decision support for pest and disease detection using artificial intelligence methods and knowledge engineering approaches (Mansingh et al., 2007). Farmers may benefit greatly from ICT-based DSSs in terms of gaining access to relevant knowledge/information and experts. The use of DDSs allows farmers to make timely and accurate pest management decisions when they are needed. ICT offers a diverse set of DSS tools; the following are a few examples that are widely used in IPM in India and around the world.



DECIPESTS, a decision-support system for pest management in sugarcane: three separate modules make up the framework. The first module covers the biology of the major pathogens, pests, and weeds that influence apple development. The second module consists of a visual key that identifies the main disease symptoms, insect damage, and weeds. Finally, a control recommendation module assists users in making management decisions. According to the assessment results, 92% of the students correctly identified the insect, which was quite satisfactory from both a realistic and educational standpoint (Mondino and Gonzalez-Andujar, 2019).

riceXpert app: A case study

The ICAR-National Rice Research Institute (NRRI), Cuttack has created a multilingual (English, Hindi, Odia, and Assamese) mobile app called *riceXpert* to bring the new rice technologies to farmers in real-time. This app is designed and built for both *Android* and *iOS* platforms, and it will work in an online system. Farmers can diagnose insect pests, diseases, nematodes, weeds, nutrient shortages, and toxicities in real time using this app. Among the features are rice varieties, agricultural implements, news, expert consultation through the e-advisory services module, weather information, and a personalized *Pest Solution* and *Fertilizer Calculator*. Farmers and farm women can use this app as a diagnostic tool in their rice fields, and they can also submit personalized queries *via* text, picture, or speech, which will be answered by a panel of experts in real time, and they will receive a fast solution along with recommendations *via* SMS (Mohapatra et al., 2017a; 2017b). The app is also a very valuable tool for rice crop researchers, scientists, students, and village staff, and it is used in 171 countries around the world. More than 43,000 people have downloaded *riceXpert*, covering India (73%), the Philippines (7%), Pakistan (4%), Nigeria (2%), Bangladesh (1%), and other countries (13%). More than 1500 questions about rice insect pests, diseases, weeds, varieties, farm implements, nutrient toxicity/deficiency, and other aspects of rice have been received from Indian users *via* the app's e-rice advisory module, which covers almost all rice-growing states in India. The questions are answered by a panel of experts, and the responses are sent to users *via* SMS.

In this app, a knowledge-based DSS module is also provided. Farmers may use this module to measure the amount of pesticide recommended for their affected area based on their rice pest, disease and weed problems. A prescription will be created automatically for their pest issue, which can be downloaded and printed (Mohapatra et al., 2018a, 2018b, 2018c, 2018d, 2019).

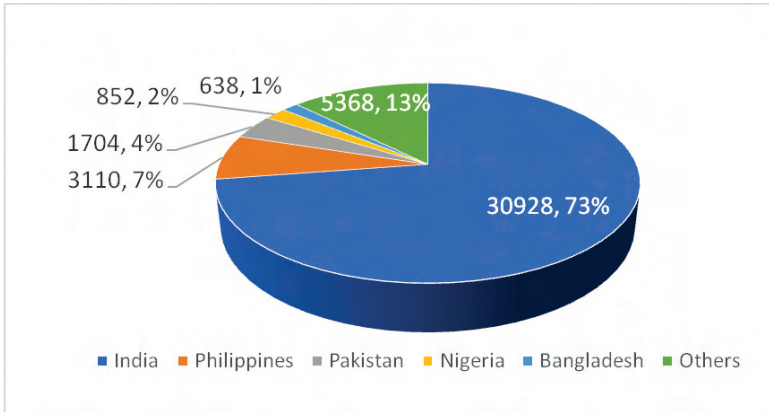


Fig. 1 Percentage of riceXpert users globally (as on 31st January 2021)

7. FUTURE OF PRECISION PEST MANAGEMENT

In addition to the ones mentioned, there are many other sensors that can be used to detect crop diseases and insect pests using electrical, chemical, electrochemical, optical, magnetic, or vibrational signals. Farm technology, on the other hand, is rapidly evolving. New sensors are being built for the round the clock monitoring and to help with early pest detection using bio-recognition elements like DNA/RNA, antibodies, and enzymes. Future technological advances, such as networked wireless sensors, meteorological sensors, and nano-sensors for recording plant health status, are expected to provide farmers with site-specific data on crop health conditions dispersed across fields. The use of high-powered optical sensors to detect, identify, and quantify pathogens on plants can result in an objective and automated evaluation of pests in the field. In the near future, ambient intelligence (automated systems that use sensors and artificial intelligence to predict the needs of crops and respond accordingly to monitor the primary occurrence of pests without the need of human interference) may be available.

8. CONCLUSIONS

Remote sensing approaches vary in many respects, including data collection, product accuracy, and applicability. According to the current research, a particular sensor and data processing method should be chosen for specific applications. Unlike vicarious calibration, pre-flight calibration methods for precision agriculture data acquisition do not necessitate putting reference targets on the ground in advance and synchronously calculating their reflectance. With the increased usability of UAVs and lower camera costs, multispectral and

hyperspectral remote sensing are gaining more attention; however, there are still many issues in precision agriculture that need further research. Finally, usability (e.g., technical skills and cost), application versatility, and data quality should be considered when designing UAV with multispectral and hyperspectral remote sensing technology for precision pest management.

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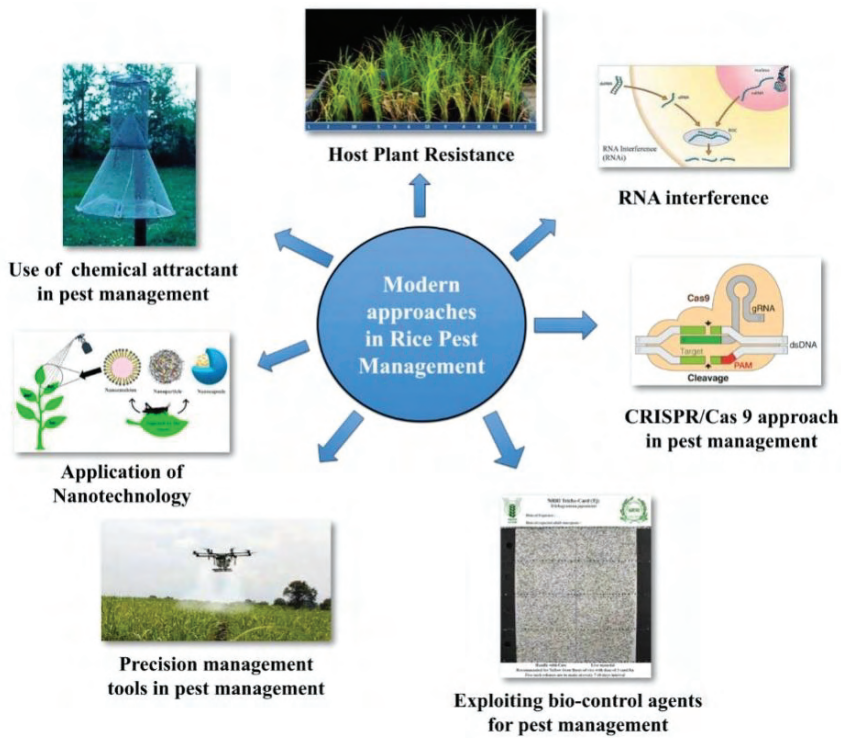
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RICE PEST MANAGEMENT USING MODERN TECHNIQUES AND NOVEL APPROACHES

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GRAPHICAL ABSTRACT



SUMMARY

Rice is the staple food crop in Asia, and the improvement in its productivity is necessary to meet the demand of growing population. However, pests are one of the major obstacles in rice farming. Damages caused by pests in rice are intensifying and have become more complex. To tackle the situation, new



innovations are being developed. This chapter discusses the recent advances in pest management and its implication in future rice production. Novelty in the field of host plant resistance including RNAi, genome editing techniques, the role of nanotechnology, advances in biological control, natural chemicals and info-chemical-based pest management are discussed in this chapter.

1. INTRODUCTION

Rice pests are major constraints for increasing its productivity under all ecosystems. Pest population changes both spatially and temporally over the period of time in rice. There are more than 100 insect species which feed on rice but few of these are considered to be the major pests. The numbers of major pests are increasing day by day. Seven out of 20 major problems in rice cultivations are related to rice pests (Herdt, 1991). At present number of major insect-pests are 20 and major diseases are 10 based on the information generated from All India Coordinated Project on Rice (AICRP) during 1965 to 2017 (Jena et al., 2018). The major concern of the farmers is frequent occurrence of pests with increased severity in recent years. The average yield losses in rice had been estimated to vary between 21-51%, out of which 10-15% yield losses occurred due to the infestation of insect pests. Among the different insect-pests, stem borer, plant hoppers, gall midge, leaf folder accounted for 30, 20, 15, and 10% of the crop losses especially at national level (Krishnaiah and Varma, 2014).

When compared to other crops, it was observed that around 15-25% potential of rice production was lost due to different pest infestation in south Asian countries (Dhaliwal et al., 2010) mainly due to the favourable climatic conditions for pests. It compels farmers to use a major chunk of pesticides to prevent/recover from pest attack. In India, Central Insecticide Board and Registration Committee (CIBRC) has recommended more than 90 pesticides or combination product to tackle wide range of pest problems. The large number of pesticide usage related to the benefits achieved through direct crop returns.

Despite the beneficial effects, there is genuine concern over the use of pesticides and its impact on non-target organisms especially human being. This is because small amounts of pesticide residues may remain in the crops, either resulted from the direct use of pesticides on the crops or environmental contamination. In India, problems resulting from unregulated and uncontrolled usage are quite alarming. There are reports that less than 1% of applied pesticides reach to target species and more than 99% of applied pesticides reach to non-target environment. Runoff and wind may cause non-point pesticide pollution and affecting other species. It is relevant to know the concentrations of



pesticide present in different matrices and if there is certain scope to avoid the pesticide contamination.

Search for viable and cost effective alternative strategies are required for sustainable pest management. Several technologies are being developed and available for managing these biotic stresses. Presently technologies available for managing rice pests aim to provide ecologically sound management programmes with sustainable use of renewable natural resources which can be classified under below mentioned broad categories. Based on economic and social consequences the limitations/ bottlenecks for implementation of different pest management practices, the following objectives are formulated:

- Recent technologies in Host Plant Resistance along with RNAi and CRISPR/Cas approach for pest management
- Advances in biological control and precision management tools to manage target pest
- Significance of nanotechnology and chemistry of natural chemicals (botanicals, semiochemicals, info chemicals, etc.) in plant protection

2. HOST PLANT RESISTANCE

Host Plant Resistance (HPR) plays an major role in insect pest and disease management in crop plants. HPR is the backbone of integrated pest management (IPM) in order to increase the production and productivity to meet ever growing human population (Godfray et al., 2010). Resistant crops can provide an effective and economical way for management of insect pests and diseases. Understanding the genetic diversity of insect pests and pathogen population requires continued effort in introducing new resistance gene/QTL sources against a virulent biotype. The important consideration for the effective utility and longevity of host plant resistance is the knowledge of the nature and degree of variability of genotypes in pest/pathogen population. How rapidly can gene frequencies in insect/pathogen population be expected to change so that we can understand dominance of virulent, resistant breaking biotypes/races? How should the gene frequencies in the population be selected and managed to preserve the utility of HPR? This is being discussed in details.

2.1. Exploration and deployment of new sources of resistance from wild and cultivated species

Wild species possess most important sources of HPR genes/QTLs to insect pest and diseases. These wild relatives have gene pools which possess considerable amount of resistance. Genotypes possessing high level of resistance to insect

pest and diseases are vital source for wide-hybridization program to upsurge the level of resistance to these biotic stresses. The wild relatives pose a challenge in breeding work because of problem of incompatibly particularly among the divergent taxa or genotypes, and the association of resistance with various undesirable traits. In general, resistant cultivar development program avoids wild species as parental lines because of the difficulty in transfer of desirable genes by genetic recombination. These introgressed wild parents result in inferior yield, poor grain quality and undesirable plant architecture (Tanksley and McCouch, 1997). Recent studies showed that, some wild relatives have genetic components which provide genetic gains in terms of improved agronomic performance along with pest resistance.

2.2. Application of molecular markers in host plant resistance against insect pest and pathogens

Numerous techniques have been developed and successfully employed over the past decades to characterize genetic diversity in a population, detect DNA sequence polymorphism, genome sequencing and fingerprinting, locating genes/QTLs on a chromosome, fine mapping, population genetics studies, diagnostics and plant breeding. Different types of molecular markers deployed to construct high-density genetic linkage maps for rice (Sharma et al., 2009). In general, six generations are required to transfer the pest/pathogen resistant traits from source to high-yielding cultivars in traditional breeding approaches. But, molecular marker technology had reduced the time and space of resistance breeding. Difficulties in transfer of traits from wild sources are greatly solved by using molecular markers (Sharma et al., 2009). Near isogenic lines (NILs), recombinant inbred lines (RILs), F2 and backcross populations along with doubled haploids technologies can be used in gene mapping programs of rice (Mohan et al., 1997; Sharma et al., 2009). DNA markers like Random Amplified Polymorphic DNA (RAPD), Restriction Fragmented Length Polymorphism (RFLP), Amplified Fragmented Length Polymorphism (AFLPs), Simple Sequence Repeats/microsatellites (SSRs), Sequence Tagged sites (STSs), and Single Nucleotide Polymorphisms (SNPs) are commonly used for molecular characterization of genotypes (Botstein et al., 1980; Williams et al., 1991; Vos et al., 1995). These markers have been successfully utilized in identification of genes/QTLs resistance to stem borers (Mohan et al., 1994; Jain et al., 2004), green leaf hoppers (Wang et al., 2004), brown plant hopper (Sharma et al., 2002; Jena et al., 2010), gall midge (Du et al., 2020), bacterial blight, sheath blight (Molla et al., 2020), blast and several other insects and diseases of rice (Table 1 and 2).



Table 1 List of insect pest resistance genes in rice with donor and linked marker details

Insect Pest	R-gene/QTL	Donor cultivars	#Chromosome	Linked marker	References
Brown Planthopper	39 Genes (<i>BPH1-39</i>) Bph1, bph2, BPH3, BPH6, bph7, BPH9, BPH10, BPH14, BPH15, BPH18, BPH21, BPH26, bph29, Bph32	Mudgo, ASD7, RathuHeenati, Swarnalata, T12, Pokkali, IR65482-4-136-2-2, B5, IR65482-7-216-1-2, IR71033-121-15, ADR52, <i>Oryza rufipogon</i> , PTB33	3, 4, 6, 12	RG634, RG457, RM7102, RM463, RM589, RM16994, RM119, RM5341, RM3448-RM313, RM463, RM260, RG457, SM1, G1318, RM261, S16, RM6869, R10289S, RM222, RM244, RM309, BYL8-BID2, RM19291-RM8072	Du et al., 2020; Kumar et al., 2020
White-backed brown planthopper (WBPH)	<i>WBPH1</i> , <i>WBPH2</i> , <i>WBPH3</i> , <i>wbph4</i> , <i>WBPH5</i> , <i>WBPH6</i> , <i>WBPH7</i> , <i>WBPH8</i> , <i>wbph9(t)</i> , <i>wbph10(t)</i> , <i>wbph11(t)</i> , <i>WBPH12(t)</i>	Nagina 22, ARC10239, ADR52, Podiwi A8, N'Diang Marie, Guiyigu, B5 (<i>O. officinalis</i>), Sinna Sivappu	3, 4, 6, 7, 11, 12	RZ667, RM167, R1925-G1318, R288-S11182, RM589-RM539, SSR12-17.2-RM28487, Rm3643-rm1223, RM16592-RM16649	Du et al., 2020
	<i>Ovc</i> , <i>qOVA-1-3</i> , <i>qOVA-4</i> , <i>qOVA-5-1</i> , <i>qOVA-5-2</i> , <i>qWPH2</i> , <i>qWBPH5</i> , <i>qWBPH9</i> , <i>qWL6</i> , <i>qWBPH3.2</i> , <i>qWBPH11</i>	Asominori, <i>O. rufipogon</i> , Chunjiang 06, IR54751	1, 2, 3, 4, 5, 6, 9, 11, 12	R2373-C946, XNpb346-C112, R1854, XNpb251-R3313, C1268, RM1285-RM555, RM3870-RZ70, RG451-RM245, M3-M5, InDel3-23-InDel3-26, DJ53973-SNP56	Du et al., 2020
Green leafhopper (GLH)	<i>GLH1</i> , <i>GLH2</i> , <i>GLH3</i> , <i>glh4</i> , <i>GLH5</i> , <i>GLH6</i> , <i>GLH7</i> , <i>glh8</i> , <i>GLH9</i> , <i>glh10</i> , <i>GLH11</i> , <i>GLH12</i> , <i>GLH13</i> , <i>GLH14</i>	Pankahari 203, ASD7, IR8, PTB8, ASD8, IR28, IR36, MaddaniKaruppan, DV85, IR20965-11-3-3, ARC10313, Asmaita, ARC11554	3, 5, 6, 8, 11	NA	Du et al., 2020
Leaf folder (LF)	<i>qRLF-1</i> , <i>qRLF-2</i> , <i>qRLF-3</i> , <i>qRLF-4</i> , <i>qRLF-8</i> ,	Taichung Native 1, Chuanjiang 06,	1, 2, 3, 4, 8	RM3412-RM6716, RM207-RM48, RM1022-RM7, RM3276-RM255, RM72-RM331	Rao et al., 2010

Insect Pest	R-gene/QTL	Donor cultivars	#Chromosome	Linked marker	References
Gall Midge (GM)	<i>GM1, GM2, gm3, GM4, GM5, GM6, GM7, GM8, GM9, GM10, GM11</i>	W1263, Phalguna, RP2068-18-3-5, Abhaya, ARC5984, Duokang 1, RP2333-156-8, Aganni, Madhuri-Line9, BG 380-2, CR57-MR1523	4, 7, 8, 9, 12	RM444-RM219, RM241-RM317, RM17480-gm3SSR4, RM22551-RM22562, RM101-RM309, RG214-RG476, F8LB-SA598, RM22685-RM22709, RM28574-RM28706	Himabindu et al., 2010; Du et al., 2020

Table 2 Genes/QTLs transferred in rice for different diseases using molecular markers

Disease	Pathogen	Gene/QTL	Marker(s)	Donor parent	Recurrent parent	Reference
Blast	<i>Pyricularia oryzae</i>	<i>Pi-ta</i>	RFLP	Pai-kan-tao	CO-39	Yu et al., 1991
		<i>Pi-5 (t)</i>	RFLP	Moroberken	CO-39, 281F7	Wang et al., 1994
		<i>Pi54</i>	SSR	Tetep	Improved samba mahsuri	Singh et al., 2012b
		<i>Piz-5 and Pi54</i>	SSR	C101A51 and Tetep	PRR78	Singh et al., 2012a
		<i>Pia, Pib</i>	SNP	Seolak	Chunheong	Kwon et al., 2008
		<i>Pish</i>	SSR	IRBLsh-S(CO)	CO-39	Koide et al., 2010
		<i>Pi1 and Pi2</i>	SSR	BL122 and CBB23	Rongfeng B	Fu et al., 2012
		<i>Pi-1 and Piz-5</i>	SSR	CO-39	PRR-78	Gouda et al., 2013
		<i>Pita, Pi1, Pi54, Pi5, Pib, Pi9, Pi2</i>	SSR	DHMASQ164-2a, IRBLz5-CA, IRBLb-B, IRBL5-M, and IRBL9-W	PB-1	Khanna et al., 2015
		<i>Pi-d(t)</i>	SSR	Digu	G46B	Chen et al., 2000
		<i>Pi-b</i>	SSR	BL-1	G46B	Chen et al., 2000
		<i>Pi-ta2</i>	SSR	Pi-4	G46B	Chen et al., 2000
		<i>Pia, Pib</i>	SNP	Seolak, Daeseong, Bongkwang	Chucheong	Kwon et al., 2008
<i>Pib, Piz-5,</i>	SSR	BL1, C101A51	Lijiang x	Telebanco-Yanoria et		



Disease	Pathogen	Gene/QTL	Marker(s)	Donor parent	Recurrent parent	Reference
		<i>Pi9, Pi3, Pi, Pik-s, Pik</i>			intuanheigu	al., 2010
		<i>Pizt, Pi2, Pigm, Pi40, Pi9</i>	SSR	Toride1, C101A51, Gumei, IR655482, 75-1-127, Fukunshiki	Yangdao 6 (93-11)	Wu et al., 2016
		<i>Pi2</i>	SSR	RPBio Patho-1	RPHR-1005	Kumar et al., 2016
		<i>Pi40</i>	CAPS DNA	IR83260-1-1-1-5-B-3-1-2-B	Osmanick-97 and Halibey	Man et al., 2016
		<i>Pi1, Pi2, Pi46</i>		BPT 5204	Intan	Hegde and Prashanthi, 2016
Bacterial leaf blight	<i>Xanthomonas oryzae pv oryzae</i>	<i>Xa-1, xa-3</i>	RFLP			Yoshimura et al., 1992
		<i>Xa-4, Xa21</i>	SSR, STS	WH-421	Siputeh	Luo et al., 2014
		<i>Xa21, xa13, xa-5</i>	SSR	RP-Bio-226	Dubraj and Safri 17	Dnyaneshwar et al., 2018
		<i>Xa-38</i>	Gene specific	PR114	Improved samba mahsuri	Yugander et al., 2018
		<i>xa13, Xa-21</i>	Gene based	-	CO-43	Krishnakumar and Kumaravadiv, 2018
		<i>Xa21</i>	STS	RP-Bio-Patho-2	DRR17B	Balachiranjeevi et al., 2018
		<i>Xa38</i>	Gene linked	SPS 97 and Pusa 1460	PB-1121 and PB-6	Ellur et al., 2016a
		<i>Xa23</i>	Indel	HBQ810	Guangzhan 63-4S (GZ63-4S)	Jiang et al., 2015
		<i>xa5, xa13, xa21</i>	STS	CRMAS 2232-85	Jalamagna	Pradhan et al., 2016
		<i>Xa21, Xa27</i>	STS,SSR	Wan Hui 21 and IRBB27	9311	Luo et al., 2017
		<i>Xa21, xa13, xa5</i>	STS	IRBB-60	CSR-30	Baliyan et al., 2018
		<i>Xa21, Xa33</i>	Gene specific	Improved samba mahsuri (ISM), Samba Mahsuri (FBR-1-15EM)	DRR17B	Balachiranjeevi et al., 2018
<i>Xa21</i>	RFLP	IRBB-21	LT2	Nguyen et al., 2018		

Disease	Pathogen	Gene/QTL	Marker(s)	Donor parent	Recurrent parent	Reference
Sheath blight	<i>Rhizoctonia solani</i>	<i>qSB-9Tq</i>	Indel	TeQing	Japonica cultivars (9 lines)	Zuo et al., 2008
		<i>qSB9-2, qSB12-1</i>	SSR	TeQing X Lemont backcross introgression lines TIL:567, TIL:615, TIL:642	Lemont	Wang et al., 2012
		<i>qSBR11-1</i>	SSR	Tetep	Improved PB-1	Singh et al., 2012b
		<i>qSB-7, qSB-9</i>	SSR, Indel	TeQing	WLJ1	Chen et al., 2014
		<i>qSBR11-1, qSBR11-2, qSBR7-1,</i>	SSR	TeQing	Pusa 6B	Singh et al., 2015
Panicle Blast	<i>Magnaporthe oryzae</i>	<i>Pb1</i>	CAPS	StNo.1	Koshiakari	Sugiura et al., 2004
		<i>Pb-1</i>	SSR	Hwayeong	Ilmi	Lee et al., 2015
Rice Stripe virus disease	<i>Rice stripe virus</i>	<i>Strvbi</i>	Gene linked	StNo.1	Koshiakari	Sugiura et al., 2004
		<i>Strvbi</i>	STS	B5	Shengdao 15, Shengdao 16 and Xudao3.	Xu, 2013
Bakanae	<i>Fusarium fujikuroi</i>	<i>qBK-1</i>	InDel	Shingwang	<i>Ilpum</i>	Lee et al., 2019
		<i>qBK1.2, qBK1.3, qBK1.1</i>	SSR	Pusa 1342	PB1121	Fiyaz et al., 2016
		<i>qB1</i>	SSR	Chunjiang06		Yang et al., 2006.
		<i>qFjR1, qBK1, qBK1.1</i>	SSR	Nampyeong		Ji et al., 2017
False smut	<i>Ustilaginoid-ea virens</i>	<i>qFsr8-1</i>	SSR	MR183-2	08R2394	Han et al., 2020
		<i>qRFSr-5.1, qRFSr-5.2</i>	SSR	IR-28	HXZ	Andargie et al., 2018



2.3. Gene pyramiding for durable and multiple pest and disease resistance

Gene pyramiding is a powerful tool for integrating multiple disease-resistant genes in a serious manner by repetitive backcrossing and selection (Sharma et al., 2009). The use of multiple resistance genes into an elite variety represents an ideal means for achieving the sustained control of insects in rice. Molecular breeding approaches helped to develop resistance genotypes against BPH alone or in combination with other biotic stress. Up to now, 38 BPH resistant genes have been explored in various rice chromosomes of cultivated and wild rice species (Anant et al., 2021; Akanksha et al., 2020), from them, seventeen genes have been fine mapped and characterized using advanced molecular techniques for insect-pests management (Anant et al., 2021; Akanksha et al., 2020; Du et al., 2020); and these resistant genes may be introduced either single, double or more by pyramiding which are given in Table 3. It is noteworthy that the maximum resistant genes were located as gene clusters and present on the locus of 3, 4, 6 and 12 chromosomes.

Gene pyramiding has been effectively utilized in rice diseases such as blast, where three blast tolerant prime genes, *Pita*, *Piz-5*, and *Pi-1*, have been successfully mapped on chromosomes 12, 6, and 11, respectively using RFLP markers (Hittalmani et al., 2000). Marker aided selection (MAS) was used to pyramid five bacterial blight resistant genes, *Xa1*, *Xa3*, *Xa4*, *Xa5*, and *Xa10* (Yoshimura et al., 1995). Likewise, using PCR-based markers and RFLP, the four bacterial blight tolerant genes *Xa4*, *Xa5*, *Xa13*, and *Xa 21* have been pyramided (Huand et al., 1997).

Table 3 BPH resistant varieties developed by incorporating resistant genes

S.N.	Genes pyramided	Donor variety	Recipient variety	Trait	Country	References
1	<i>Bph1+Bph2</i>	'Tsukushibare' (TSU) and 'Norin-PL4' (PL4)	Tsukushibare	BPH resistance	Japan	Sharma et al., 2004
2	<i>Bph6 + Bph9</i>	93-11/Pokkali	93-11	BPH resistance	China	Wang et al., 2017
3	<i>Bph6+Bph12</i>	93-11 and B14	93-11, Nipponbare	BPH resistance	China	Qiu et al., 2012
4	<i>Bph3+Bph27</i>	Japonica * Indica Ningjing3 (NJ3) * 93-11	93-11, Ningjing3	BPH resistance	China	Liu et al., 2015

S.N.	Genes pyramided	Donor variety	Recipient variety	Trait	Country	References
5	<i>Bph14+Bph15</i>	Minghui 63 and Shanyou 63	Minghui 63, Shanyou63	BPH resistance	China	Hu et al., 2012
6	<i>Bph14+Bph15</i>	Huahui938 and B5	Huahui938	BPH resistance	China	Wang et al., 2015
7	<i>Bph14+Bph15</i>	BR4831, HF106 and C602	Huang-Hua-Zhan	BPH resistance	China	Han et al., 2018
8	<i>Bph3 + Bph14 + Bph15</i>	B5 and RathuHeenati (RH; Acc. no. 11730)	Hemeizhan	BPH resistance	China	Hu et al., 2016
9	<i>Bph14 + Bph15+Bph18</i>	Minghui 63 from B5 and IR65482-7-216-1-2	93-11	Bacterial blight and BPH resistance	China	Hu et al., 2013
10	<i>Xa21 + Bph14 + Bph15</i>	Y58S/Hua15 and B5	Yuehui 9113	BPH and bacterial blight resistance	China	He et al., 2019
11	<i>Pita + Pi1 + Pi2 + xa5 + Xa23 + Bph3</i>	Shuhui 162, Zhongzu 14, CBB23, HN88 and RathuHeenathi	HN 88	BPH, bacterial blight and blast resistance	China	Ji et al., 2016; Yang et al., 2016
12	<i>Qbph12 + Sub1</i>	Abhaya and IR49830	KDML 105	BPH resistance and submergence	Thailand	Korinsak et al., 2016
13	<i>Bph14 + Bph15 + Stv-bⁱ</i>	B5	Shengdao 15, Shengdao 16, Xudao 3	BPH and rice stripe disease resistance	China	Xu, 2013
14	<i>Bph6 + Bph9 + Gn8.1 + Rf3+Rf4 + Rf5+ Rf6</i>	L1880 Shuhui527 Luoyang-6 Luoyang-9	93-11	BPH resistance, Big panicle and fertility restoration gene	China	Fan et al., 2017



2.4 Genetic transformation

Recombinant DNA technology has brought a revolution in resistant breeding program by engineering genes for promising applications. Novel insecticidal traits have also been identified, mapped and effectively incorporated into crop plants to confer insect resistance. Insecticidal genes derived through genetic transformation and conventional/MAS can be combined to achieve long-term insect pest resistance (Dhillon and Sharma, 2012).

3. RNAi FOR PEST MANAGEMENT

Crop damage and loss by insects each year costs billions of dollars, and the dependence on synthetic chemical insecticides for their management created unintended consequences like insect resistance, non-target effects on beneficial insects and environment pollution (Kumar et al., 2019). Thus, the top priority for researchers is to search for more target-specific pest management strategies and one such is the RNA interference (RNAi) (Fig. 1). RNAi is an emerging technology involves silencing important genes in insects or plants that features as a promising alternative strategy to synthetic chemical insecticides which ensues selective killing of targeted species (Joga et al., 2016). RNAi enables the down-regulation of gene expression through post-transcriptional gene silencing mechanism using artificial RNA molecules (Whangbo and Hunter, 2008) and was initially experimented in *Caenorhabditis elegans* by Fire et al. (1998). RNAi on the ground of small RNA biogenesis and the Argonaute protein can be majorly categorised into three pathways viz., microRNA (miRNA), small interfering RNA (siRNA) and piwi-interacting RNA pathways. In the last two decades, RNAi has been come out as an crucial molecular tool to silence key gene transcripts in many of insects comprising orders such as Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Baum et al., 2007; Zhao et al., 2008; Walshe et al., 2009; Upadhyay et al., 2011; Burand and Hunter, 2013). Owing to its specificity, RNAi is used as a new strategy for the management of a large number of pest species (Gu and Knipple, 2013). RNAi technology encompasses a suite of tools showing an extensive range of possible applications in genetic studies and in agriculture by protecting beneficial insects against viruses and parasites (Hunter et al., 2010; Zotti and Smagghe, 2015), by pest resistance management (Zhu et al., 2014) and plant management against insect species (Huvenne and Smagghe, 2010; Joga et al., 2016; San Miguel and Scott, 2016; Andrade and Hunter, 2016; Andrade and Hunter, 2017), plant pathogens and nematodes in a wide array of crops (Zotti et al., 2017).

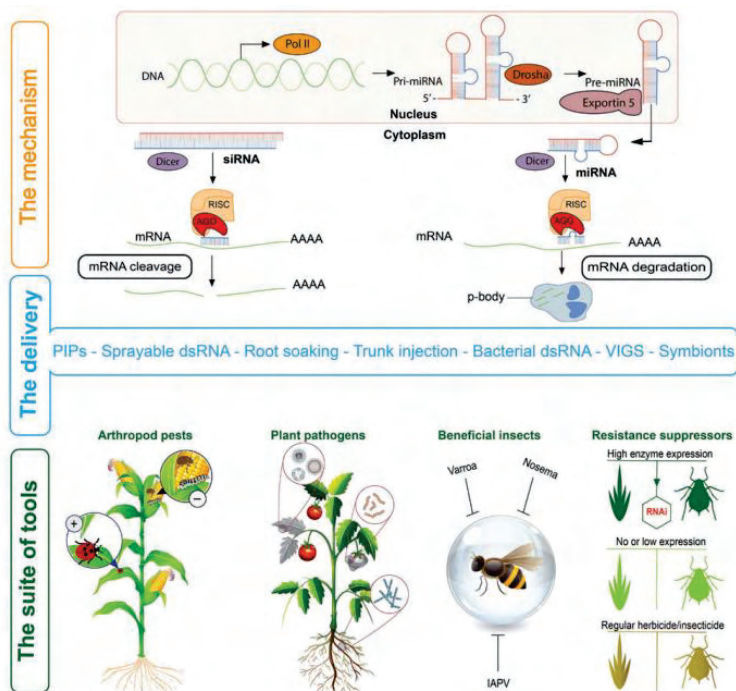


Fig. 1 Application of RNAi technique in rice insect pest control

RNA silencing is basically a homology-based process which is triggered by using double-stranded RNA (dsRNA) leading to the suppression of gene expression. This molecular tool was firstly discovered in plants and was predicted to be a part of function of a defence mechanism against viruses. RNA silencing works at three different levels in plants *viz.*, (i) cytoplasmic silencing by dsRNA results in cleavage of mRNA and is technically called as post-translational gene silencing (PTGS); (ii) micro-RNAs (miRNAs) silences the endogenous mRNAs which base-pairs to specific mRNAs thus negatively regulating gene expression resulting in either RNA cleavage or arrest of protein translation; (iii) transcriptional gene silencing (TGS) which involves RNA silencing associated with sequence-specific methylation of DNA thus consequent transcription suppression. The specific RNAs are targeted and degraded in RNAi mechanism. The long dsRNA is cleaved into small (21-25 nucleotides) fragments basically by a complex called Dicer (RNase II family), and these small RNAs produced are then incorporated into a complex “RNA-induced silencing complex” (RISC). Then the RISC is targeted to degrade single-stranded RNA (ssRNA) in a sequence-specific manner; specificity being provided by the sequence of the small RNAs.



3.1. RNAi for insect-pest management

Yu et al. (2014) reported that the ecdysone receptor (EcR) is a potential target for RNAi-based control of rice BPH. Shen et al. (2021) found the presence of three ferritin genes viz., ferritin 1 Heavy Chain (*NIFer1*), ferritin 2 Light Chain (*NIFer2*) and soma ferritin (*Nlsoma-Fer*) in rice BPH. They observed that injection of double-stranded RNA interfered with of *Nlsoma-Fer* expression resulted in < 14% mortality in BPH whereas knockdown of other two genes *NIFer1* or *NIFer2* showed retarded growth and 100% mortality in young nymphs and their down regulation in the newly emerged female adults shown undeveloped ovaries and extremely low fecundity and a zero hatching rate due to inhibited oocyte growth. Thus, it shows *NIFer1* and *NIFer2* were essential for development and reproduction of brown planthopper and seems to be a potential tool for RNAi based control of the most notorious pest of rice crop (Table 4).

Table 4 Application of RNAi in rice insect pest control

Family and species	Target genes	Gene location and function	Life stage of insect	RNAi delivery approach	dsRNA length (bp)	mRNA silencing (%) and duration	RNAi duration and effects	Reference
Cicadellidae Green rice leafhopper <i>Nephotettix cincticeps</i>	Salivary protein <i>NcSP75</i>	Salivary gland, Role in phloem ingestion	3 rd instar	Injection	469 bp	0.2-0.5%, 8 days	90% mortality, 24 days	Matsumoto and Hattori, 2018
	Laccase-2 gene <i>NcLac2</i>	Cuticle and epidermis, Cuticle tanning	5 th instar	Injection	401 bp	17.-23.8%, 2 days	60% mortality, 14 days	Matsumoto and Hattori, 2016
	Peptidoglycan recognition protein gene <i>NcPGRP12</i>	Hemolymph, Role in immune response	4 th , 5 th instar, Adult	Injection	481 bp	Lower expression level, 4 days	80-100% mortality, 10 days	Tomizawa and Noda, 2013
Delphacidae Small brown planthopper <i>Laodelphax striatellus</i>	Halloween gene <i>disembodied</i> <i>Lsdib</i>	Gut, Role in ecdysteroidogenesis	3 rd instar	Feeding	406 bp	18.8-56.7%, 5 days	15-29% mortality, 5 days, Impaired development	Wan et al., 2014
	Core RNAi gene <i>Ago2</i>	Midgut, Role in RNAi	3 rd instar	Injection	600 bp	70%, 6 days	-	Xu et al., 2012
Delphacidae Brown planthopper <i>Nilaparvata lugens</i>	<i>CYP4G76</i> , <i>CYP4G115</i>	Cuticle, role in biosynthesis of cuticular hydrocarbons	3 rd instar	Injection	435 bp, 472 bp	67%, 79% 4 days	70-80% mortality, 24 hours	Wang et al., 2019
	Eggshell-associated protein, <i>NIChP38</i> ,	Follicular cells, Ovulation	Adult	Injection	403 bp	92-95%, 3-5 days	Decreased fecundity, 9 days	Lou et al., 2019

Family and species	Target genes	Gene location and function	Life stage of insect	RNAi delivery approach	dsRNA length (bp)	mRNA silencing (%) and duration	RNAi duration and effects	Reference
	Chemosensory protein <i>NlugCSP8</i>	Wings, role in perception	3 rd instar	Injection	390 bp	86%, 7 days	56% mortality, 7 days	Waris et al., 2018
	Trypsin <i>Nltry</i> , Carboxypeptidase <i>Nlcar</i>	Gut, role in digestion	3 rd instar	Transgenic Rice	825 bp 625 bp	61-73%, 4 days 42-43%, 2 days	No significant mortality	Zha et al., 2011
	Trehalase	In cell, role in chitin synthesis	5 th instar	Injection	451 bp 321 bp 440 bp	Lower expression level, 72 hours	15-32% mortality, 3 days	Zhao et al., 2016
	V-ATPase-E <i>ds1-21E01</i> , <i>ds2-21E01</i> , <i>ds3-21E01</i>	GI tract, Ion transporter protein	2 nd instar	Ingestion	276 bp, 250 bp, 498 bp	41%, 55%, 48%, 10 days	12-17% mortality, 10 days	Li et al., 2011
	Calcium-binding protein Calmodulin/ <i>NlCaM1</i> , <i>NlCaM2</i>	Salivary gland, Role in insect development	3 rd instar, 5 th instar	Injection	200-671bp	27-71%, 4 days	50-92% mortality, Impaired molting, infertility, 10 days	Wang et al., 2018
Delphacidae White-backed planthopper <i>Sogatella furcifera</i>	Calreticulin, Cathepsin-B, <i>Nlβ2</i>	Whole body, gut, CNS	3 rd instar	Injection	675 bp, 585 bp, 574 bp	2-45%, 8 days	20-43% mortality, 3 days	Liu et al., 2010
	Halloween gene <i>disembodied</i> <i>Sfdib</i>	Midgut, Role in ecdysteroidogenesis	1 st instar	Feeding	458 bp	36-66%, 6 days	15.3-21.5% mortality, 6 days	Wan et al., 2014
	Ryanodine receptor gene	Muscle, brain, Transport of Calcium	2 nd instar	Feeding	336 bp, 451 bp	78-82%, 2 days	Decrease susceptibility to Chlorantranilprole	Yang et al., 2014

Zheng et al. (2020) developed transgenic rice line ‘*csu260*’ for the striped stem borer (SSB)-resistance by expressing *csu*-novel-miR260, endogenous miRNA of SSB through miRNA expression technology. In SSB, *csu*-novel-miR260 negatively regulates ecdysteroid biosynthesis. The larval mortality at 35 days after feeding in *csu260-16* and *csu260-18* engineered rice fed SSB larvae was 55.6% and 53.3%, respectively, whereas it was only 20% in control larvae. It was also observed that the SSB pupation was delayed by 4 days when SSB larvae consecutively fed with transgenic rice expressing the SSB novel miRNA candidate *csu*-novel-miR15 (*csu-15* rice) (Jiang et al., 2016).



3.2. RNAi for rice disease management

In rice plants, silencing of *OsDCL1* gene exhibited increased resistance to the rice blast pathogen, *Magnaporthe oryzae*, in a non-race specific manner (Zhang et al., 2015). RNA-dependent RNA polymerase (RdRp) genes were considered to have the vital roles in gene silencing and conferring resistance to plant pathogens. Inactivation of rice *RdRp6* gene showed increased susceptibility to rice necrosis mosaic virus, *Xanthomonas oryzae* pv. *oryzae* and *M. oryzae* (Wagh et al., 2016). Nakayashiki and colleagues (2005) have developed the protocol for silencing the *mpg1* gene and polyketide synthase-like genes. A gene, *mpg1* is a hydrophobin gene which is vital for pathogenicity particularly for adhesion and trigger for the development of appressorium. Tiwari et al. (2017) for the first time demonstrated the host delivered RNA interference (HD-RNAi) technology to silence the *Rhizoctonia solani* pathogenicity MAP kinase 1 (*PMK1*) homologues genes *RPMK1-1* and *RPMK1-2* using a hybrid RNAi construct. The results revealed that transgenic rice lines showed delayed sheath blight symptoms and reduced disease as compared to non-transformed controls.

4. CRISPR/CAS APPROACH FOR PEST MANAGEMENT

Plant diseases are largely managed by pesticides. Though pesticides showed a significant result in controlling pest, negative environmental impact and pesticide resistance are the major concerns (Damalas and Eleftherohorinos, 2011). Therefore, development of inherent genetic and durable resistance to diseases is considered as a sustainable approach for ensuring food security. Recently, genome editing technology; Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/ Cas (CRISPR-associated protein) is becoming highly popular because of its, precision and efficient editing for better understanding of biological function of genes in crop plants (Ishino et al., 2018). Moreover, CRISPR-Cas is more robust in terms of complete gene knockdown in comparison to RNAi which induces partial gene silencing (Shelake et al., 2019) and greater specificity (Brandt and Barrangou, 2019). The plant disease management utilizing CRISPR/Cas approach is an emerging tool for plant disease management (Munoz et al., 2019; Prabhukarthikeyan et al., 2020). The rice blast disease causing fungus *M. oryzae* is one among the top fungal pathogens affecting the food crops (Susan et al., 2019). The pathogen causes heavy yield losses worldwide to rice growers. Study showed that RNAi silencing of *ethylene responsive factors* (*OsERF922*) increased the rice blast resistance. In support of this finding, Wang et al. (2016) used CRISPR/Cas9 to knockdown *OsERF922* in rice. The seedlings of edited plants showed reduced blast symptoms as compared to wild plants. Further, significant differences between mutants and wild plants in the agronomic traits were observed (Wang et al., 2016). In addition, the expression of *ERF922* was up-regulated both

during abiotic stresses and infection by *M. oryzae* (Cao et al., 2006). Gene mutation of *OsSEC3A* through CRISPR/Cas induced the expression of defense related genes and results in enhanced salicylic acids and resistance to *M. oryzae* (Ma et al., 2018). Editing of *OsMPK5* and *Pi21* genes enhanced resistance to rice blast disease (Xie and Yang, 2013; Li et al., 2019). Management of plant diseases caused by bacteria is quite difficult due to their nature of fast multiplication and high diversity. The rice bacterial pathogen *X. oryzae* pv. *oryzae* (*Xoo*) causes bacterial leaf blight (BLB) disease and is one of the major pathogens of rice and causes yield losses up to 50% - 100% (Mew et al., 2019). The BLB pathogen translocates transcription activator-like effector (TALE) proteins into host cells via type III secretion system and induces the expression of susceptibility factor genes (S genes) in plants (Boch and Bonas, 2010; Doyle et al., 2013). The susceptibility gene *OsSWEET13* was induced by *Xoo* effector protein (PthXo2) and results in bacterial blight disease. The CRISPR/ Cas based mutation of *OsSWEET13* gene resulted in increased resistance to *Xoo* infection. Likewise, another TALE protein is called *AvrXa7* which targets *OsSWEET14* in rice, manipulation *OsSWEET14* restricted the binding of *AvrXa7* and finally enhanced the BLB resistance (Li et al., 2012). The mutation in the *SWEET11*, *SWEET13* and *SWEET14* resulted in a broad-spectrum resistance to BLB pathogen (Oliva et al., 2019). Plant viruses are obligate parasites which necessarily need the host cells for multiplication. Management of viral diseases is not possible by chemicals. Therefore, CRISPR/Cas is a major alternate strategy for control of viral diseases by targeting susceptibility genes or viral genome (*coat protein* gene, *replication* gene, intergenic region and nanonucleotide sequence). Among the viruses, Geminiviridae is the largest single-stranded DNA virus family infecting crop plants and causing drastic yield losses worldwide. Macovei et al. (2018) targeted *translation initiation factor 4 gamma gene (eIF4G)* in rice IR64 (susceptible cultivar) and developed tungro disease resistance [caused by *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV)].

In rice, the use of CRISPR/Cas tool was observed for knocking out of cytochrome *P450* gene, *CYP71A1* which encodes tryptamine 5-hydroxylase resulted in elevated SA levels and reduced serotonin levels in rice plants thus conferring enhanced resistance against brown planthopper, the most notorious pest of rice (Lu et al., 2018). Gilles et al. (2015) used CRISPR/Cas tool and tested the efficiency of knock out and knock in of E-cadherin gene; EGFP and found that E-cadherin gene brings about defects in dorsal closure which is consistent with RNAi-induced phenotypes. Homology-directed knock-in of marker transgenes was observed in nearly 14% of injected individuals and in the next generation, it was observed in 6% of the injected individuals. Since a limited information is available on the genome editing application using



CRISPR/CAS the use of this tool in rice insect pest management holds a bright prospective.

5. ADVANCES IN BIOLOGICAL CONTROL

It is high time to shift from chemical management of pests to other means such as biological pest management using potential biocontrol agents. Biological control of pests is the ecological way of pest management practice expedient in almost all agro-ecological situations. To manage insect pests effectively as well as sustainably, strategies like ecological engineering are currently being demanded. Landscapes can be a favorable factor for biological control; it has been observed that the effects of landscape on pests and natural enemies decide the biocontrol potential. There are hundreds of biocontrol agents used for the control of the insect pests of the rice ecosystems and their timely use and maintenance in the field would keep the pest population below the Economic Threshold Level (ETL) and would help in the sustainable production of rice (Table 5). These natural enemies are of three types-

- Parasitoids- These are the parasitic insects that parasitize the insect's pests and multiply on/in them; they do not kill the host insect instantly but the pest mortality would occur later on
- Predators -These are the insects that directly feed upon the prey insects and kill it
- Pathogens- These are microbial agents, which cause disease in insect pests

Table 5 Major parasitoids and predators of insect pests of rice

Natural enemy category	Natural enemy	Target pest
I. Parasitoids		
1. Egg parasitoids	<i>Trichogramma japonicum</i> Ashmead	Yellow stem borer
	<i>Trichogramma chilonis</i> Ishii	Leaf folder, case worm, yellow stem borer etc.
	<i>Tetrastichuss choenobii</i> Ferriere	Yellow stem borer and white stem borer.
	<i>Telenomus rowani</i> Gahan	Yellow stem borer and white stem borer.
	<i>Gonatocerus</i> spp. Nees ab Esenbeck	Planthoppers and leafhoppers.
	<i>Anagrus</i> spp. (Girault and Haliday)	Planthoppers and leafhoppers.

Natural enemy category	Natural enemy	Target pest
	<i>Oligosita</i> spp.	Planthoppers and leafhoppers..
	<i>Copidosomopsisna coleiae</i> Eady	Leaf folder
2. Larval parasitoids	<i>Amauromorpha accepta</i> Tosquinet	Yellow stem borer and white stem borer
	<i>Stenobracon nicevillei</i> Bingham	Leaf folder
	<i>Cotesia flavipes</i> Cameron	Stem borers and semi-loopers
	<i>Elasmus</i> sp.	Leaf folder
	<i>Haplogonatopus</i> sp. <i>Pseudogonatopus</i> spp.	Planthoppers and leafhoppers
	<i>Bracon</i> sp.	Yellow stem borer and rice hispa.
3. Larval and pupal parasitoids	<i>Xanthopimpla flavolineata</i> Cameron	Stem borers
	<i>Brachymerialasus</i> Walker <i>B. excarinata</i> Gahan	Leaf folder, Rice skipper, and green horned caterpillar.
	<i>Opius</i> sp.	Rice whorl maggot
II. Predators		
4. Coccinellid beetles	Ladybird beetles, <i>Micraspis hirashimai</i> Sasaji	Hoppers, small larvae and exposed eggs of Lepidoptera
	<i>Harmonia octamaculata</i> Fabricius	Hoppers, small larvae and exposed eggs
5. Carabid beetle	Ground beetle, <i>Ophioneanigro fasciata</i> Schmidt-Göbel	Planthoppers and leaf folders
6. Rove beetle	Rove beetle, <i>Paederus fuscipes</i> Curtis	Planthoppers and leaf hoppers, eggs and small moths
7. Spiders	Wolf spider, <i>Pardosa psuedoannulata</i> Bosenberg and Strand	Stem borers and leaf folder moths, whorl maggot flies, planthoppers, leafhoppers
	Lynx spider <i>Oxyopes javanus</i> Thorell	Stem borers and leaf folder moths, whorl maggot flies, planthoppers, leafhoppers
	Long-jawed spider <i>Tetragnatha maxillosa</i> Thorell	Stem borers and leaf folder moths, whorl maggot flies, planthoppers, leafhoppers
	Orb spider <i>Argiope catenulate</i> Doleschall	Borer moths, adults of different pests like grasshoppers

(Prakash et al., 2014; Fahad et al., 2015)



5.1. Important characteristics of biocontrol agents

All the biocontrol agents in the ecosystem do not necessarily cause significant pest suppression. From their establishment in the field until the mortality of the pest killing, biocontrol agents should possess certain characteristics to compete and outperform in the field. It is reported that effective biocontrol agents exhibit the following characteristics.

- Narrow host range - It is desired that biocontrol agents should be more host-specific so that other non-targets are unaffected
- Climatic adaptability - An effective biocontrol agent will always have the ability to survive weather extremities in a new habitat
- Synchrony with host (Prey) life cycle - Activities of host/prey and predator/parasitoid must be synchronized for greater biocontrol efficiency
- High reproductive potential - Higher the reproductive potential with large numbers of offsprings, the higher is the pest suppression. Generally, during each generation of the pest, parasitoids complete more than one generation
- Efficient searching ability - Effective biocontrol agents should locate their host or prey even during situations when the pest populations are low
- Short handling time -The biocontrol agents are expected to parasitize/attack to consume the prey/host quickly so that they can access and attack other pests
- Survival at low host (Prey) density - The biocontrol agents must have the capacity to complete their life cycle and reproduce even when the pest population is low

5.2. Biocontrol agents for sustainable rice production

The desire to achieve sustainable production of rice enforces the use of different control measures other than chemical control out of which biological control is highly convincing from the food and environmental security points of view. One of the strong bases for sustainable rice pest management is ‘conservation biocontrol’ which means conserving natural enemies locally. But to manage the pests using natural enemies, there are some prerequisites, which include their availability, conservation, augmentation, maintenance and release. Though all are aware of the positive aspects of biocontrol, it is still not widely adopted as compared to the use of chemical insecticides. It is the responsibility of the agricultural professionals not only to create awareness amongst the farming community regarding its use but also to produce momentum in the industrial production and marketing of biocontrol agents so that they are available at the

farmers' doorstep. As several of these beneficial agents belong to the insect groups, we should be careful in choosing the right agents which would kill only the target pests. We should be very cautious while opting for other management practices *viz.*, chemical control because they would be harmful to the beneficial insects of our interests. Hence, rice insect pest management is dependant not only on insect pest and natural enemy interactions but also on several biotic and abiotic factors. Biological and chemical control of insect pests in rice is most commonly used pest management methods explored. The full cascade from natural enemies to economic performance including the list is depicted. Strength of a cascade is very much dependent on landscape composition influencing specially the natural enemies and insect pests. It has been found that landscapes with higher proportion and diversity resulted in more natural enemies and subsequently higher rice yields.

5.3. Microbial agents in pest and disease management

The microorganisms like fungi, bacteria, virus, nematodes and protozoans directly or their active ingredients indirectly can be used as active agents of biological control (Table 6). Each of these microbes have specific mode of action to antagonize and kill specific pests. Other than microbial biocontrol agents, some of the plant-based products such as neem and karanja are also used in biological control (Kalra and Khanuja, 2007). Beneficial microbial agents induce sustainable change in the plant, resulting in a increased tolerance to subsequent infection by a pathogen called as Induced Systemic Resistance (ISR).

Qualities of a novel biological control agent:

- The level of pathogen suppression should be high
- Should adopt and multiply in wide environmental conditions
- Should live longer in soil or plant hosts
- Should not be pathogenic to plants or animals
- Should be a good competitor with pathogenic microbes
- Should be suitable for long-term storage
- Should be compatible with agrochemicals to some extent
- Its application should result in increased production and productivity with reduced pest/disease incidence

**Table 6 Important rice diseases and biological control agents**

Sl. No.	Disease	Causal organism	Biocontrol agents
1	Blast	<i>Magnaporthe oryzae</i>	<i>Pseudomonas fluorescens</i> , <i>Trichoderma harzianum</i> , <i>Bacillus subtilis</i>
2	Brown spot	<i>Bipolaris oryzae</i>	<i>Pseudomonas</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Bacillus</i> spp., <i>Bacillus subtilis</i> ,
3	Sheath blight	<i>Rhizoctonia solani</i>	<i>P. fluorescens</i> , <i>P. putida</i> , <i>Bacillus subtilis</i> , <i>B. laterosporus</i> , <i>B. pumilus</i> , <i>Serratia marcescens</i> , <i>Pseudomonas</i> spp., <i>B. amyloliquefaciense</i> , <i>T. harzianum</i> , <i>T. atroviridae</i> , <i>T. eranaceum</i> .
4	Sheath rot	<i>Sarocladium oryzae</i>	<i>P. fluorescens</i> , <i>P. arugenosa</i> , <i>B. subtilis</i> , <i>B. pumilus</i> .
5	False smut	<i>Ustiloginoidea virens</i>	<i>T. harzianum</i> .
6	Bakanae	<i>Fusarium fujikuroi</i>	<i>B. amyloliquefaciense</i> , <i>B. subtilis</i> , <i>P. fluorescens</i> .
7	Bacterial leaf blight	<i>Xanthomonas oryzae</i> pv <i>oryzae</i>	<i>P. fluorescens</i> , <i>P. putida</i> , <i>Bacillus</i> spp.
8	Grain discoloration		<i>B. amyloliquefaciense</i> , <i>B. subtilis</i> , <i>P. fluorescens</i> , <i>T. harzianum</i> , <i>T. atroviridae</i> .
9	Tungro	<i>Rice tungro virus</i>	<i>P. fluorescens</i>
10	Early seedling blight	<i>Sclerotium rolfsii</i>	<i>T. harzianum</i> , <i>T. atroviridae</i> .

Source: Vasudevan et al. (2002); Swain et al. (2018); Raghu et al. (2020).

6. PRECISION MANAGEMENT TOOLS

Indian agriculture has been tremendously benefited by scientific research and adoption of modern tools and techniques by farmers. Damages from insect pests could often have negative ripple effects. Effective pest management strongly relies on the utilization of appropriate strategies and tools. Digital agriculture accompanied relevant tools will enable decision-making that could help in integrated pest management. Previously, Integrated Pest Management (IPM) mostly concentrated on ecological features. But in recent times, it mostly depends on modern agricultural technology (Dara, 2019). Precision agriculture tools would help farmers to analyze the several key factors affecting plant health on a spatial-temporal scale (Table 7). Following are some of the tools which are gaining importance in recent days' pest management.

Table 7 Important precision tools and techniques used in pest management

Sl. No.	Application/model	Significance	Authors
1.	Ryegrass Integrated Management (RIM)	For testing the biological and economic performance of strategies to control ryegrass <i>Lolium rigidum</i>	Lacoste and Powles, 2015
2.	SOYBUG	To provide advice on the control of soybean insect pests in the Florida	Beck et al., 1989
3.	COTFLEX	Expert system that addresses these decisions on a total crop management basis	Stone et al., 1986
4.	GOSSYM COMAX	It simulates growth and yield of cotton (<i>Gossypium hirsutum</i>) in the field along with the COMAX (Crop Management expert) system which interprets the results of GOSSYM,	Whisler et al., 1986; Baker et al., 1983; Lemmon, 1986
5.	CALEX/Cotton	Expert system developed in California for cotton production and management	Plant et al., 1992
6.	RiceXpert	Developed for the rice farmers to provide real time information on insect pests, nutrients, weeds, nematodes and disease-related problems, varieties for different ecologies	Nayak et al., 2016
7.	Rice Crop Doctor	The MANAGE has developed an expert system to diagnose pests and diseases for rice crop	Prasad and Vinaya, 2006
8.	RICESmart	Ontology based expert system which is intended to help the farmers, extension workers, researchers and students for solving common problems related to rice	Yadav et al., 2014
9.	e-SAP	Electronic solutions against agricultural pests (e-SAP). It is a dedicated system that effectively integrates mobile communications, tablet-based technologies and cloud solutions to bring different players of the agricultural ecosystem	eSAP: https://www.uasr.aichur.edu.in/index.php/en/research/e-sap

6.1. Unmanned aerial vehicle (Drone)

Unmanned Aerial Vehicles (UAVs) or drones or Remotely-Piloted Aircraft (RPA), are revolutionizing the area of precision agriculture. Concerning pest management, because of relatively low cost and ease of deployment drones are replacing manned platforms rapidly in many circumstances (Colomina and Molina, 2014). Compared to conventional remote sensing, drone-based remote sensing ensue multiple advantages. Drones are very much useful particularly in spot or precise spraying in a massive landscape. Recently drone-based remote



sensing is mostly used for assessment in real-time and other activities of various applications. These drones help in reduction of the working hours, stability enhancement, accuracy measurement and productivity. These are very cheap as compared to other agricultural machineries and are very easy to handle or operate. These UAVs have opened a completely new trend in agriculture sector which includes pesticides and fertilizer prospecting and spraying, seed sowing, weed identification, assessment of fertility, mapping and forecasting of crops. The pesticides application at lower spray volume and lower flight altitude has become very popular in rice and other crops (He, 2018; Meng et al., 2020).

Drones carrying high resolution cameras can be utilized in many crop protection areas compared to conventional pest management. Drones can fly in rugged and difficult terrains to capture images of insect pests or symptoms, which helps not only in identification but also facilitate precise application of pesticides where human movement sometimes is difficult (Maslekar et al., 2020).

6.1.1. Advantages of drones for insect pest management

- It helps in preventing heatstroke incidents and poisoning
- With the help of drones around 50 to 100 acres of crop field can be sprayed per day which is 30 times more efficient than the conventional pesticide spraying
- It checks water and soil pollution as it sprays pesticides with fixed orientation and fixed position
- It can save 30% of pesticides and it is more cost effective than the traditional method of spraying
- It is very user friendly, easy to maintain and use
- Accidental poisoning of people from spraying can be avoided
- It provides more efficient application of pesticide sprays in areas which are not accessible like water logged area, rice fields, steeply sloping areas
- As the UAVs can be integrated with GPS location, it can be employed in precision agriculture

Without much doubt, drones can render efficient and precise pest management, especially in developing countries with few bottlenecks that farmers would face. There are policy issues that are inhibiting farmers to adopt drone for pest management. Currently, in India, UAV's are not officially permitted by Government of India and as such there are no pesticide formulations available for the same. However, the initial drone costs are bit

high; flying will be for short time and operations across different habitats and weather conditions resulting in uncertainty.

Despite many applications of drones, pesticide application is one of the main uses. The application of the pesticides is inevitable for pest management and better crop yields. The manual spraying of pesticides is quite difficult and dangerous to health because of direct contact with the person applying. It has been also realized that manual spraying results in the non-uniform spraying and poor efficacy of pest control. In order to overcome this, applying pesticide efficiently, the drone has emerged as an excellent plant protector that is able to fly on and spray farmland autonomously. Drones have the advantages of highest operational efficiency (Hilz and Vermeer, 2013). Hence, standardized drone operations in rice pest management will be able to improve the pest control efficiency, to save the labour requirement, to cover more area per unit time, etc. Recently, Government of India has made adhoc recommendation for spraying of insecticides using drones for dessert locust. But for other cases, the drone spraying is not recommended in India. The Directorate of Plant Protection, Quarantine & Storage, Faridabad has issued 'Standard Operating Procedures (SOP)' on aerial spraying using drone for control of Desert Locust on 18 May 2020. Also, the Ministry of Civil Aviation issued Draft Notification on 'The Unmanned Aircraft System Rules, 2020. There have to be some preparedness in terms of robust and pragmatic science-based research. In order to effectively use the drone for pest management, it needs to be standardized for many parameters. Among them, formulation, characterizing spray effects (droplet, drift etc.) (Huang and Thomson, 2011). Hence, studies should concentrate generating scientific information specific to locality, cropping system and weather conditions for the future use of drones for pesticide application in rice crop.

6.2. Artificial intelligence in pest management

a. Pest monitoring: Artificial Neural Networks (ANN) is one of the important components of artificial intelligence. It has been suggested that ANN could be used as possible methods for identification of biological objects (MacLeod, 2008) along with other statistical tools, such as classification phylogenetic trees or principal component analysis. Architecture developed by taxonomic experts uses morphometric characteristics which are transformed to an artificially intelligent apparatus i.e. multilayer system processing to a metadata. It leads to the successful identification of Thrips (Thysanoptera) and could be better used in pest monitoring (Fedor et al., 2009).

b. Pest Prediction: With the beginning of data mining field, several agricultural branches with pest management are focused. Tripathy et al. (2011) have developed decision making system in real-time mode by which insect pests can



be identified by means of wireless sensor network and data mining for which they used the Rapid Association Rule Mining and Gaussian Naive Bayes. Through this system they could be able to predict when insect pests appear, based on wireless sensor network information. Wang and Ma (2011) have compared the regression method and support vector machine and to predict disease in China *i.e.* wheat stripe rust.

6.3. Big data and machine learning in crop protection

Machine learning is branch of computer science and one of the major areas of Artificial Intelligence used to build algorithms which further exhibit self-learning. In order to exhibit intelligence by machine, machine has to retrieve process and analyze the input given by the developer. Big data comprises of huge volume, velocity and variety of data received as inputs generated from real-time platforms and various other sensors (Wolfert et al., 2017; Chen and Zhang, 2014). Machine learning is swiftly becoming trendy and offers plethora of avenues and helps in addressing the various challenges in big data.

Algorithms of machine learning gives due consideration to all possible factors like satellite/sensor data including the historic data to provide valuable information on insect pests and diseases. Using a pattern recognition branch of machine learning, a disease detection of crop has been implemented in India (ex. Plantix). Corrales et al. (2015) developed supervised learning algorithms for crops like corn, rice, coffee, mango, peanut, and tomato for detection of pests and diseases.

6.4. ICT based decision support system in pest management

Use of expert decision systems are one of the major anticipated applications of artificial intelligence and are popularly known as 'management decision support systems'. In a simpler term, Decision-Support Systems (DSSs) are actually computer software tools that support decision-making activities. A well designed DSS helps decision makers in obtaining useful information from raw data to identify, make decisions and solve problems. DSSs could be simple as a data processing tool or as complex as an expert decision system. DSSs basically rely on artificial intelligence techniques to provide decision support for insect pest and disease diagnosis (Mansingh et al., 2007; Lopez-Morales et al., 2008). Application of DDSs will help the farmers in timely and proper pest management decisions.

7. NANOTECHNOLOGY IN PLANT PROTECTION

The nanoparticles with distinctive properties can play a critical role in agriculture and especially in crop protection. In the last few years, there is a jump in research on nanotechnology in agriculture and it could be gauged from number of patents filed and papers published in this field. The potential of nanotechnology in agriculture is many-folds. The nano-based kits can treat plant diseases or nano-sensors can detect crop-pests, and nano-pesticides can be used for effective and environment friendly pest management. Compared to other fields, the definition of the term “nano” in pesticides is not so clearly defined. Generally, nanotechnology in agriculture does not strictly follow the size dimension (<100nm in any one dimension) but product saving properties associated with the small size come under the purview of nanotechnology.

Pesticide applications using nanotechnology can reduce the dose, improve the plant uptake and could be delivered in desired target. This will reduce the total requirement of pesticides which not only save the cost but also will be environmental benign. The reduction of pesticide requirements is related to smaller size of nano-pesticides with high surface area, which will lead to higher absorption. As such, nanotechnology is frequently portrayed as environmental benign (Kah, 2015; Liu and Lal, 2015). One group of nano-pesticides for pest management deals with inorganic nano-materials like nano-silica, nano-copper, nano-silver, nano-zinc, and nano-aluminum etc. having the potential to kill pests (Kah and Hofmann, 2014; Adak et al., 2020). Use of nano-metal pesticides might not have great potential as plant protection chemicals whereas pesticide formulation using nanotechnology can change the future of pesticide formulation technology. Recent importance in organic-based nano-materials as adjuvants of formulation is gaining the momentum. Amphiphilic polymers based nano-formulations of different pesticides namely carbofuran, imidacloprid, thiamethoxam, etc. are being developed which have greater residual life with sustained efficacy (Shakil et al., 2010; Adak et al., 2012). Nano-emulsions of pesticides including botanicals could have higher efficacy and targeted activity (Adak et al., 2019). Nano-emulsions of glyphosate, acephate, eucalyptus oil, and neem oil have improved efficacy as compared to conventional formulations (Anjali et al., 2012; Jiang et al., 2012). Another field of formulation is nano-composites, where volatile chemicals like essential oil, pheromones are trapped for slow release (Abreu et al., 2012; Bhagat et al., 2013). Nano-pesticide formulations can improve the solubility of poorly soluble molecules and protect from harsh environment and prevent losses through the sustained release of active ingredients.

Despite significant advances in nanotechnology applications techniques in agriculture, there are few challenges which needs to be considered more efficiently. More research is required to develop hybrid carriers for



pesticides and fertilizers delivery following the principles of green chemistry and environmental sustainability to maximize their efficiency. The present technology is at laboratory scale and non-cost effective. So, technologies are to be developed at industrial level to reduce the cost of preparation. Risk and life-cycle assessment of nano-pesticides should be checked and proper regulations about the use of nanomaterials should be enforced.

8. NATURAL CHEMICALS IN PLANT PROTECTION

8.1. *Botanicals*

To minimize the hazards caused to biosphere due to synthetic pesticide application, replacing them with botanical pesticides is the best alternative as they possess variety of inherent properties *viz.*, toxicity towards target pests, repellency activity, antifeedant properties and growth regulatory actions against insect pests of important field crops. Secondary metabolites produced by botanicals disrupt fundamental physiological or biochemical process of insect metabolism. Some of the botanicals (neem, pyrethrum, mahua, tobacco, bael, sweet flag and karanj etc.) have already reached the status as prospective pesticides of botanical origin which are used in managing the rice field insect pests and pests of storage niche (Prakash et al., 1989; Prakash and Rao, 1997). When compared to synthetic pesticides botanical pesticides have several advantages as listed below:

- Generally, botanicals possess little mammalian toxicity, for which there is least or no health hazards observed in non-target fauna of the ecosystem
- Eco-friendly option due least residues and environmental pollution
- When used in natural forms, there is no risk of resistance development (except synthetic pyrethroids)
- Non-target organisms are spared
- No adverse impact on germination, seed viability, seed vigour and quality of cooked food from the grains treated with plant origin pesticides
- Botanical insecticides are cost effective and helps to reduce the expenses of plant protection ultimately reduces the costof cultivation
- Due to their natural occurrence in abundant quantities, easily available for pest management or they can be prepared by farmers themselves

In paddy ecosystems, botanicals are not found to be effective against stem borers, since their developmental stages and damage to stem are confined within the stem. Hence, they escape from the insecticidal effects of potential botanicals. Against gundhi bug, a number of botanicals are found to protect developing rice grains *viz.*, spraying neem oil emulsion, extract of orange seed and lemon grass leaf extract, oil emulsion spray of neem, seed extract of orange and leaf extract of lemon grass (Gupta et al., 1990). Neem seed kernel extracts showed juvenile hormone mimic response when sprayed along with 0.16% teepol inhibited larval development of leaf folder (Schmutterer et al., 1983). Similarly, it also reduced the population of WBPH, when spray was initiated in rice crop (Mohan and Gopalan, 1990). However, soaking the rice seedlings roots under neem seed kernel extract decreased the WBPH incidence. Spraying neem oil @ 1% reduced the leaf folder incidence (Mohan et al., 1991). Similarly, deoiled neem cake application as soil amendment @ 1.5 q/ha followed by spraying neem oil every 10 days intervals showed reduced incidence of leaf folder (Krishnaiah and Kalode, 1990) and neem oil @ 1% sprayed along with teepol reduced the infestation of WBPH (Sontakke, 1993) and against BPH showed antifeedant property (Saxena et al., 1984). Reduced emergence of WBPH was observed under 5% neem cake extract spray treatment (Ramraju and Sundarababu, 1989).

Apart from neem, oil extracted from seeds of *Madhuca indica* (mahuwa) and *Pongamia glabra* (karanj) when sprayed @ 1%, showed reduced leaf folder incidence under paddy ecosystem (Narasimhan and Mariappan, 1988). Custard apple seed oil when applied @ 1% on rice plants hindered the leaf folder and GLH population, which indirectly checked rice tungro virus spread (Narasimhan and Mariappan, 1988).

Future emphasis on utility of plant based pesticides under plant protection programmes should be addressed through rigorous search for flora having biopesticidal features against major pests. Chemical characterization of active components present in the plant extracts, its identification, isolation and evaluation under lab and field should be done comprehensively. Commercialization should be done for biologically effective active components and economically feasible synthesis/ extraction is to be identified to achieve pest management through integrated approach.

8.2. Plant essential oils

The odoriferous volatile oils extracted from plant origin are termed as plant essential oils and they are available in very minute quantities in different plant parts *viz.*, flowers, leaves, bark, roots and fruits. Plant essential oils are secreted or excreted byproducts which are available during some plant metabolic activities, which may occur also as odorless compounds named as glycosides,



produced in more than 17,500 plants of aromatic species belong to very few families *viz.*, Asteraceae, Lauraceae, Lamiaceae and Myrtaceae from tropical countries and Mediterranean regions. There will be liberation of essential oil from secretory cells, cavities, epidermal cells, secretory cells and channels. An enzymatic reaction leads to change in glycoside chemical nature on maceration of plant tissues. Compositions of essential oils (EOs) are highly assorted across different sources of plant species. The constituents of EOs belong primarily toward phytochemical groups: a) terpenoids (of less molecular weight sesquiterpenes and monoterpenes) and b) phenyl propanoids to a minor extent. EOs were obtained in large or sufficient quantities from different extraction methods *viz.*, hydro-distillation, dry distillation, steam distillation and through mechanical freeze pressing the plants. Nowadays some of modern methods of extraction which are widely used include extraction by supercritical fluid and microwave-assisted process.

In general, EOs are non-phytotoxic compounds and potentially successful against numerous insect pests and diseases. Therefore, they can be used as a natural therapy to protect the crop from different biotic stresses. In recent years, more scope is there for the development of safer eco-friendly pesticides from products of plant origin *viz.*, essential oils and botanical extracts. Recently, work on nano formulations of these oils is gaining importance due to targeted pesticide delivery and avoiding the conventional pesticides use non judiciously and indiscriminately. This smart delivery of any chemical is essential to reduce dosage of active ingredients with enhanced efficiency and to overcome pesticide loss to achieve least negative impact on the ecosystem.

Apart from enhanced stability, specificity, permeability and solubility their slow releasing properties are assets to the nano formulated plant based pesticides. There are different types of nano formulations that can be used for agrochemicals like nano emulsions, nano encapsulations, nano suspensions and others which are intended to make them potent. The technique enables greater control over the circumstances depending on the need and even under specific abiotic stress conditions. They offer in-built control panels to switch the liberation and succeeding temporal pesticide availability or persistence over long period of time and their design or properties enables them to tackle natural processes such as microbial degradation, photolytic and hydrolytic degradation, leaching and evaporation when compared to conventional pesticides. The risk assessment of nano-encapsulated pesticides depends on the conditions such a way they are handled in workplace, their persistence and the magnitude of sources. Several approaches have been undertaken to develop nanoformulations of agrochemicals, however, research on biosafety, molecular interaction of soil, plant and environment is inadequate.

8.3. Infochemicals or semiochemicals

Infochemicals play an important responsibility in the biology and behavior of several insect species. Hence, it is the thrust area of increased research attention and gaining lot of importance in chemical ecology. An understanding of role of infochemicals in tri-trophic interactions will be used for the development and enhancement of environmentally benign tools and technologies that are alternatives to synthetic pesticides. Chemical information will be considered as pivotal factor which mediate behavioural and ecological communication between host plants and insect pests. The lessons learnt regarding chemicals mediating communication between living organisms, either from different species (allelochemicals) or, within the same species (pheromones) form the basement to the field of research in chemical ecology.

The word semiochemicals was originated from the word “semeon” which means signal or sign in greek language. Insects are using semiochemicals for various purposes *viz.*, locating the mate, to search food source or host, to evade competition, to escape from natural enemies attack and to conquer natural defence systems posed by their hosts. Based on carbon chain length semiochemicals have diverse molecular weights and their chemical characterization is intricate, since they are biologically energetic at the very little concentration in surrounding environment. Utilization of semiochemicals for insect pest control has been known to mankind since from 100 years ago. To tackle the important insect pests of field crops, the sex pheromones are widely used particularly for the Lepidopteran pests, similarly aggregation pheromones are used for Coleopterans. Infochemical/semiochemical based pest management techniques are mentioned below.

8.3.1. Attract and kill

As the name indicates the technique simply use semiochemical or an attractant or to lure or attract an insect to the source having killing agent which may be sterilant, pathogen or insecticide, hence, the technique is named as attract and sterilize, attract and infect or attract and kill, respectively. Through this technique target is achieved by diminished fitness and fecundity in target insect or disabling the pest by causing a disease which finally results in killing the target insect ultimately leading to reduction of the insect population (Mafrá-Neto et al., 2014).

8.3.2. Mating disruption

One of the most widely used technique in pest management approach which is based on semiochemicals, wherein population reduction is achieved by manipulating insect behavior. When the pheromone mimics of synthetic origin



are released into surrounding environment the capacity of males in locating natural pheromone cloud released by females will get disrupted. The mating process of some insects involves transferring some peptides which trigger the oviposition behaviour in females. There will be delay in mating process which may affect the fitness in females and also imparts negative influence on their ability of selecting suitable oviposition spot, hence, female insects will have to face risky/unsafe time for mating and reproduction (Mafra-Neto et al., 2014).

The extensively studied area under behavioural manipulation in pest management is mating disruption, wherein males are not permitted from searching suitable females (Roitberg, 2007). Under simulated storage studies successful mating disruption technique has been established for Angoumois grain moth, *Sitotroga cerealella* wherein, female moths emit a volatile pheromone and detected from far away by males having numerous sensilla that are containing neurons for olfactory reception which are specific in perceiving components present in sex pheromones to locate the sexually receptive female (Schlamp et al., 2006).

The position of female sex pheromone gland and ultrastructure of ovipositor and antennal sensilla of Angoumois moth was reported by Ma et al. (2017). The pheromone X-lure was found useful only for monitoring *S. cerealella* (Aker and Ali, 2015). Sex pheromone traps are the suitable tools to monitor the adult male moth population of yellow stem borer in paddy wherein, we can detect the pest occurrence, level of infestation, easily identify the cause by observing moth catches and to estimate the actual field population density of the pest. Through this pheromone traps we can know the ETL level of pest and decision can be taken in advance to check the spread of insect pest. Apart from pest management, in biological control programs also host searching ability of biocontrol agents can be improved by using kairomones in mass production and release protocols.

8.3.3. Mass trapping

One of the pheromone based technology which is frequently used directly for suppression of insect pest population. There will be employment of sufficiently high number of pheromone traps which eliminate sufficiently high number of adults within the population, simultaneously reduce larval damage during the subsequent season. The techniques of mass trapping and mating disruption with sex pheromones or releasing mock pheromones in the atmosphere will interrupt mating process as a result of reduced encounters between calling females and males which ultimately lure the males towards the trap thus reducing the pest population (Chen et al., 2013; Weinzierl et al., 2012). For the management of stem borers application of pheromone technology provides a tool for crop protection to farmers, which is one of the user and eco-friendly approach.

8.4 Repellents or push pull technique

Any substance that avoids or inhibits or deters insects from searching, ovipositing and feeding on an attractive and a suitable host substrate is considered as repellent (Mafra-Neto et al., 2014). To counter pests of forest trees and field crops numerous semiochemicals having repellent effect are also available. The other semiochemical dependent pest management options viz., mating disruption, mass trapping or the push-pull strategy, attract and kill requires comparatively exhaustive knowledge about chemistry involved in tri-trophic interactions (Cook et al., 2007). However, due to lack of suitable formulations for the delivery, the availability of effective and cheap pest management options and regulatory obstacles during registration made their practical application limited (Isman, 2016).

9. CONCLUSIONS

The present rice breeding research should focus on the identification of donors of novel pest resistant genes/QTLs, and integrate them into development of pest resistant rice varieties. Besides, the deployment of multiple resistant genes in a single variety should be the focus area of research. There is also a need to focus on the BPH, false smut and sheath blight by using genome editing technique and its incorporation in rice resistance against these pests. Pest monitoring and detection are the key to manage crop pests and among the different techniques, molecular diagnosis and sensors can play a critical role in future. Application of nanotechnology in agriculture is in nascent stage, it could help in controlled release of pesticide and its delivery in specific target location. New biocontrol agents, chemical elicitors and infochemicals have already started producing results in small scale and can be employed in large scale pest management.

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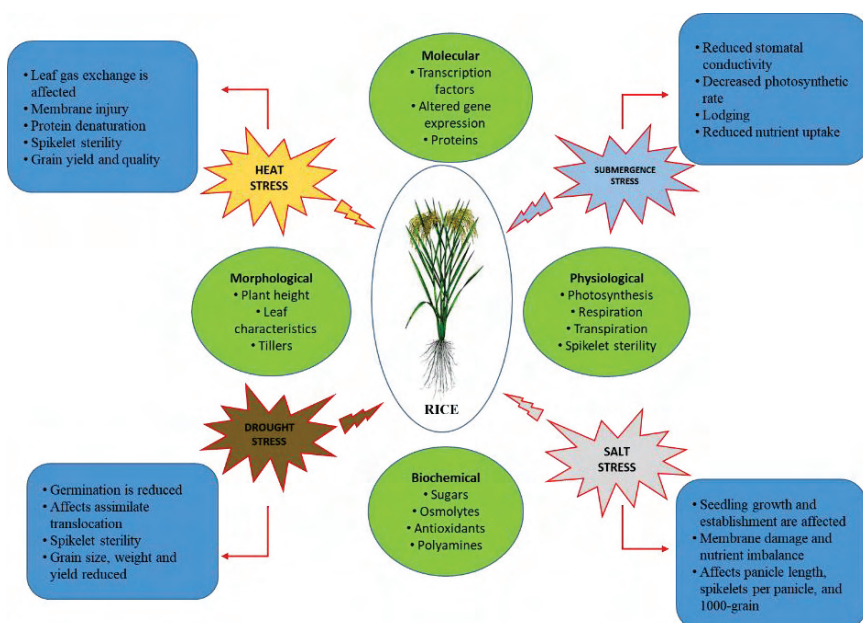
ABIOTIC STRESS PHYSIOLOGY OF RICE: AN UPDATED OVERVIEW IN THE ERA OF CLIMATE CHANGE

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GRAPHICAL ABSTRACT



SUMMARY

Abiotic stresses pose a serious threat to rice production globally. Under the changing climatic scenarios, the poor and marginal farmers, particularly from rainfed agro-ecologies (rainfed upland, shallow lowland and lowland) are compelled to bear a frequent heavy toil due to uncertain weather events. Among different abiotic stresses, rice crop is vulnerable to severe drought, excess water in terms of complete or partial submergence and anaerobic germination, salinity, high and low temperature, along with low light stress. Identification of unique germplasm/ landraces and understanding the underlying tolerance mechanism in them is of immense importance in the present context.

Characterization of these unique genotypes for morpho-physiological and key molecular traits are required to identify secondary traits contributing to stress tolerance and enhanced yield under unfavourable growing conditions. Hence, identification of suitable donor and secondary/putative traits are the need of the hour to develop climate resilient rice cultivars.

1. INTRODUCTION

Past few decades' have drawn serious attention of researchers and policy-makers globally due to the vulnerability to climate change, influenced by natural disasters and hazards, along with threat to food security. The mean surface air temperature of our planet is likely to increase by 1.8 to 4.0 °C by 2100 due to anthropogenic activities contributing to climate change (IPCC, 2007). There is an increase in the average global temperature of land and ocean surface to the tune of 0.85 °C during the period of 1880 and 2012, with a projected decadal increase of at least 0.2 °C from now onward. Rice (*Oryza sativa* L.) is one of the important cereal crops of the world. It is a staple food for nearly 50% the world's population (Carriger and Vallee, 2007). In fact, the backbone of global food security is dependent on rice production in Asia, where 90% of the world's rice is produced and consumed under both irrigated and rainfed agro-ecosystems. Due to its wide geographic distribution extending from 50°N to 35°S, rice production is projected to be highly vulnerable to future climatic changes. Rice crop is sensitive to different abiotic stresses to a varying extent, which is likely to be exacerbated with climate change scenarios. Extreme weather events such as high temperatures coinciding with critical developmental stages, floods causing complete or partial submergence, soil salinization due to intrusion of seawater and most importantly unprecedented drought spells are highly detrimental for rice production, particularly in rainfed ecosystems.

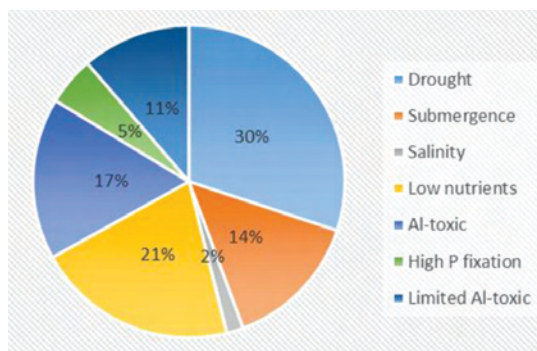
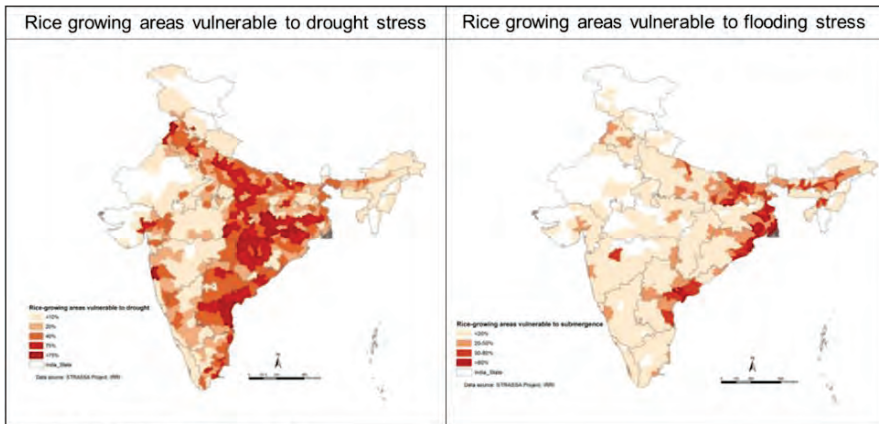


Fig. 1 Relative distribution of rice growing areas in Asia vulnerable to different abiotic stresses (FAOSTAT 2013). Database available at <http://faostat.fao.org/site/339/default.aspx>.



The changing climatic conditions make the rice crop vulnerable to moderate to severe drought stress, germination stage oxygen deficiency (GSOD) or submergence stress depending upon the timing of the natural events, and increase in salinity level, low light stress situation due to heavy down pouring or prolonged cloudy weather conditions, and heat stress due to increase in temperature during dry spells mostly in the grain filling stage. Frequent occurrence of such climatic extremities in many parts of the Indian subcontinent and other parts of globe has been indicated in recent reports. Secondary traits contributing towards tolerance to a particular stress or combination of stresses can help achieve yield improvements in such stressful environments. Land races with broad genetic bases and wider adaptability, combined with tolerance to various stresses serve as useful donors for different abiotic stress tolerance. Hence, in the present scenario, identification of suitable donor and secondary/putative traits for developing high yielding varieties through conventional or molecular approaches with an added advantage of understanding stress tolerance mechanism is of paramount importance. In this chapter, we have discussed recent advancements in (i) the impact of different abiotic stresses on growth and physiology of rice crop, (ii) the adaptive and tolerance response shown by rice to these abiotic stresses, and (iii) the possible management options to combat against these stresses.



Source: STRASSA Project Report, IRRI

Fig. 2 Vulnerability map of rainfed rice growing areas of India to drought and flooding stress.

2. IMPACT OF ABIOTIC STRESSES

Grown under diverse range of ecosystems, rice production is prone to different environmental stresses like drought, salinity, submergence, cold as well as high temperature and low light. With changing climatic scenarios, rice cultivation especially in the rain-fed ecology (rainfed upland, shallow lowland and lowland) is facing multi-faceted problems. The common cellular disorders caused by various abiotic stresses and common plant responses counteracting adverse effects of these abiotic stresses are given in Fig. 3.

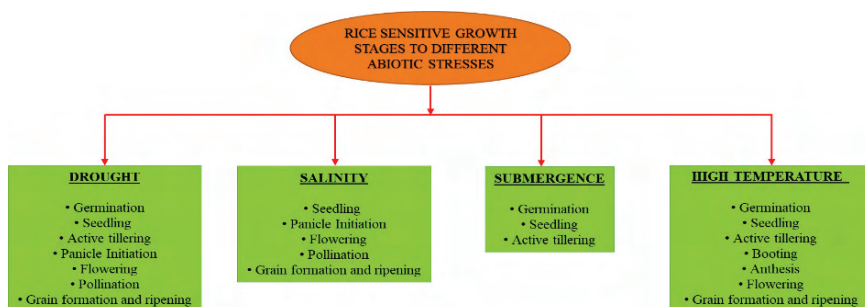


Fig. 3 Sensitivity of rice crop to different abiotic stresses at different growth stages.

2.1. Drought

Rice is very much susceptible to soil moisture stress during the reproductive stage, resulting in a significant reduction in grain yield (Palanog et al., 2014). Drought stress related symptoms appear in plants when the root cells experience a scarcity of water or when the overall leaf transpiration rate is very high compared to the normal conditions. Drought stress severely hampers the growth and developmental process, which ultimately leads to loss in production/ yield. The magnitude of yield loss depends on several factors, among them growth stage of rice and duration of stress are two important factors for deciding severity of drought stress. Severe drought stress at the vegetative stage can cause up to 20% yield loss while a mild drought stress at the flowering stage in rice resulted 28% yield loss (Kumar et al., 2014). Severe drought stress at flowering stage recorded a yield loss ranging from 53-92% (Lafitte et al., 2004; Lafitte et al., 2007). Rainfed rice growing regions of Eastern India experience moderate to severe drought irregularities during the south-west monsoon. With >17 million hectares of rainfed areas, Eastern India and few adjoining regions of Nepal are considered the largest drought-affected regions in Asia (Huke and Huke, 1997). In order to improve water uptake, plants under drought stress divert assimilates towards the roots thereby disturbing the assimilates balance



(Leport et al., 2006). Earlier reports showed that, the rate of photosynthesis and the sucrose concentration in leaves regulates the export of assimilates from source to sink (Komor, 2000). Drought stress affects the overall process of carbon dioxide fixation (photosynthesis) and hampers the net production of sucrose. Net amount of sucrose that has been produced in photosynthetic cells decreases, which eventually hamper the overall process of exportation from source (leaf) to sink (root or seed) (Kim et al., 2000), thereby limiting the net productivity of the plant. To meet the present and future global food security identification of drought-resistant crops that have been adopted to growth under dry weather conditions and also able to maintain a constant production (Barnabas et al., 2008).

2.2. Submergence

Among several different kinds of environmental constraints, flash flooding or submergence stress, particularly in low-lying rice growing ecologies is one of the major catastrophic events that detrimentally affects plant survival and crop yield. Globally, complete submergence due to flooding and associated loss is most prevalent in the low-lying rice growing areas of the world. It was reported that out of the 22-million-hectare of rice crop affected by flash flooding, half of which is in Eastern India (Dar et al., 2017). Although rice is a water loving crop and requires a considerable amount of water for their normal growth and development, the over-abundance of water in case of submergence or water-logging is destructive for rice. Globally, water stagnation due to floods was the reason for almost 66% of all harm and misfortunes to crops during the period of 2006 and 2016, with an estimated loss in billions of dollars (FAO, 2017).

Submergence is a form of flooding stress. It is further be differentiated into two types- complete submergence and partial submergence. In the case of complete submergence, entire plant canopy is fully dipped inside water for 1-2 weeks, whereas, in case of partial submergence, a portion of plant canopy or shoot terminal is present above the water level. Broadly, plants employ two different strategies to cope up with the anoxia that arises due to submerged conditions. The first is known as quiescence or low-oxygen quiescence syndrome, where plants exhibit a limited growth and elongation during the submergence stress (Colmer and Voesenek, 2009) and the second is an escape strategy, also called low-oxygen escape syndrome, where the plants show faster elongation of inter-node to come out of water as quickly as possible (Bailey-Serres and Voesenek, 2009). In quiescence strategy, plants store their carbohydrate reserve by restricting their inter-nodal growth. However, the plants remain alive under submergence and resume its growth by utilizing the energy from the reserved starch once the standing water recedes (Colmer and Voesenek, 2009).

2.3. Water-logging or stagnant flooding

Based on the depth of the water, soil flooding can be classified in two different categories- submergence and water-logging. Water-logging or stagnant flooding stress is defined as a condition, where a part of the plant canopy remains under water for a prolonged period of time. Setter and Water (2003) reported that water-logging substantially limits the rates of exchange of oxygen and other gases in different parts of plants. Therefore, the movement of oxygen from the air to different submerged portions of plant tissues (Lee et al., 2011) are hampered, creating an abnormal situation for the plant, which is popularly known as hypoxia (less than <21% O₂ in water) (Sasidharan et al., 2017). In case of stagnant flooding the diffusivity of oxygen in water is approximately 10,000 times slower compared to the rate present in air. Similarly, the flux of O₂ into soils is approximately 320,000 times less compared to normal soil, as instead of gases, here, water fills the pores that are present in soil (Colmer and Flowers, 2008). Therefore, it is said that the prime factor that is present behind water-logging stress is slow diffusion of oxygen in the different portions of plants. The low availability of O₂ under water-logging is further amplified/extended by the rapid consumption of it by the soil born microorganisms. Reports showed that, in some cases, O₂ deficiency responses in soil occur within a few hours of water-logging. In addition to the O₂ deficiency, Setter et al. (2009) showed that the stress responses further facilitated the production of toxic substances such as Fe²⁺, Mn²⁺ and H₂S by reduction of redox potential and causes severe damage to plants under water-logged conditions. Hence, growth and development of almost all cultivated crop is severely affected by water-logging stress. Rice being adapted to semi-aquatic ecology is reported to have a comparatively less effects of prolonged flooding stress leading to partial submergence of plant canopy, however, enough genotypic variation in water-logging tolerance is found in rice (Nishiuchi et al., 2012).

2.4. Germination stage oxygen deficiency

Submergence or soil flooding during the process of germination imposes germination stage oxygen deficiency (GSOD). The problem of GSOD is of immense importance in rain-fed ecologies and direct seeded rice, where sudden heavy rainfall just after direct sowing of rice creates havoc in germination, seedling emergence and initial crop establishment. Few ricegenotypes have developed a unique adaptive mechanism to germinate and extend its coleoptile (shoot portion) under water by sacrificing normal growth of the coleorhiza, even under complete anoxic condition (Magneschi and Perata, 2009) - a phenomenon popularly known as GSOD or anaerobic germination (AG) (Ray et al., 2016). It has been reported that, in general, rice coleoptile which is present under water can elongate at the rate of 1 mm h⁻¹ to reach the atmosphere or emerge out of



from the water level. At cellular level, it is facilitated by the rapid elongation of basal cells (up to 200 μm in 12 h), immediately after emerging from embryo (Narsai et al., 2015). Though rice has adaptations to grow under different aquatic habitat, its anaerobic germination potential (AGP) or survival ability varies greatly among different rice genotypes and landraces, which ultimately provide an edge / platform to a few genotypes that, perform better under oxygen deficient conditions over others. There are a few rice genotypes which can efficiently cope with the hypoxic/anoxic conditions in case of complete submergence of the seeds just after sowing (Vijayan et al., 2018).

2.5. Salinity

Soil salinization/ alkalization is a world-wide problem for the agriculture, affecting nearly 6% of the total land mass, as a result of natural accumulation and deposition over long periods of time. Besides, secondary salinization in agricultural soils occurs due to improper agricultural practices. Based on the global reports, 2% of all dry land is becoming salinized, and more than 20% of irrigated soils are affected. Faulty irrigation practices, due to the use of poor-quality irrigation water containing substantial amounts of dissolved sodium chloride or other neutral salts increase the soil salinity (Tester and Davenport, 2003). In general, rice genotypes show considerable variability for salt-tolerance. Rice is classified as a salt sensitive crop with a threshold salinity tolerance level of only 3 dS m^{-1} . Wide genotypic variation in salinity responsiveness was reported among different species of rice and their wild relatives (Menguer et al., 2017). Rice crop is reported to be relatively tolerant to salinity stress during germination, active tillering and towards maturity, but sensitive during early seedling and reproductive stages, where an additional concentration of as little as 50 mM NaCl to the soil can reduce rice yield significantly (Zeng et al., 2003). In general, salinity stress is associated with two different types of stresses on plants: initially it creates an osmotic stress, later it is accompanied by ionic stress. The genetic basis behind tolerance to osmotic stress has been poorly investigated and majority of work in the field of salinity is focused to identify the genetic basis of tolerance that is associated to ionic stress (Roy et al., 2014). Research findings indicate that members of *Indica* are good Na^+ excluders and K^+ maintainers, absorb high amounts of K^+ in cells, therefore, able to maintains a low Na^+/K^+ ratio in the shoot portion (Lee et al., 2003).

2.6. High temperature

The impact of heat stress on rice grain quality and quantity depends on the stage of crop development. Heat stress in rice affects the anthesis and grain filling stages. Even one-degree rise in temperature above the optimum temperature

results in 7-10% yield loss in rice (Fahad et al., 2017). Exposure of rice plant to 41 °C for 4 hours at flowering stage causes irreversible damage resulting in complete spikelet sterility. During the months of April and May in Eastern India, due to early sunrise, temperatures rise above 35 °C during anthesis affecting pollination in rice. The post-anthesis warming at night time has adverse effects on leaf net photosynthesis and increased night respiration rates. Heat-induced spikelet sterility at flowering reduced the grain yield. Temperatures above a critical point of 35 °C at the flowering time inhibited anther dehiscence, resulted lower pollen shed on a stigma, and leading to incomplete fertilization (Jagadish et al., 2010). Under heat stress, due to increased rate of transpiration, water loss is more during day time, which eventually affecting certain important physiological processes in plants. Furthermore, studies identified that a continuous exposure to heat stress decreases the mass and growth of roots, also hampers the water and nutrient transportation process in different parts of plants (Wahid et al., 2007; Huang et al., 2012).

2.7. Low light

Light is the immense source of energy and also an important environmental factor for plant growth, development and metabolism that regulates photosynthesis process and photo-morphogenesis (Müller-Xing et al., 2014; Zhu et al., 2017). However, plants frequently get exposed to different intensities of light like excess-light (EL) or low light (LL), causing stress to plants. These stressful conditions exert negative impacts on photosynthetic activity and eventually hamper the plant growth and yield (Nishiyama and Murata 2014). It has been reported that different intensities of light significantly hampered several physiological and metabolic processes including photosynthesis, antioxidant production and carbon-nitrogen fixation, which ultimately affect different important agronomic traits of plants (Apel and Hirt, 2004; Wang et al., 2013). Despite several reports of negative effects of light stress on plant growth and development, light stress is the one of the most uncharacterized and less studied abiotic stresses in plants. However, there is a recent increase in advocacy for deciphering the significance of light stress and how EL and LL affect plants development (Banerjee and Roy Choudhury, 2016; Zhu et al., 2017).

The low incidence of solar radiation coupled with fluctuating light during the wet season is one of the major constraints for realizing the low productivity in Eastern and North-Eastern India. Light being a crucial factor for the plant development, stress experienced by the plants under low irradiance results in an increased leaf length and width, increased leaf area, increased time period for growth, decreased differentiation of panicle and reduced grain yield



(Murchie et al., 2005). Lower rates of photosynthesis (due to low irradiance per unit leaf area) are accompanied by the reduction in the thickness of mesophyll and number of cells mm^{-2} in leaves. But surprisingly the total chlorophyll content, especially chlorophyll-b was higher under low light. Low light stress negatively influences the stomatal conductance. The decreased rubisco activity accompanied by subsequent increase in the intracellular carbon dioxide concentrations is also observed under low light intensity.

3. TOLERANCE MECHANISM

3.1. Drought

Propensity of the plant to grow, develop and produce a good economic yield under water stress is referred to as drought tolerance (Nezhadahmadi et al., 2013). Tolerance to drought in plants are maintained by complex traits, which is a combined function of various morphological, biochemical and molecular characters. To facilitate the selection or development of drought tolerant rice varieties, screening of different rice genotypes is necessary, along with this, further understanding of the various mechanisms that govern the yield of rice under water stress. Plants generally utilize several mechanisms to withstand drought stress, which are categorized as the escape, avoidance, or tolerance response to drought stress. Escape is a classical adaptive mechanism that characterized by a state of dormancy or necrosis caused by programmed death of cellular tissues, with the exception of those that are required for regeneration or regrowth upon a change of season or alleviation of stress such as renewal of available water after prolonged drought conditions.

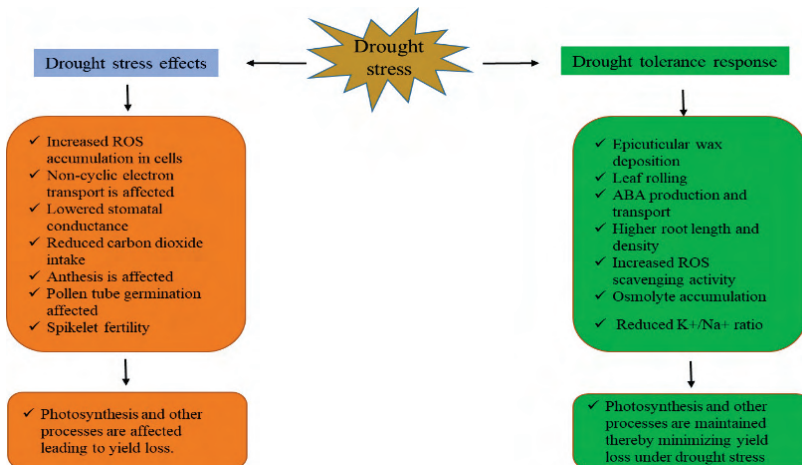


Fig. 4 Effects of drought stress on rice and physiological responses to cope up with the stress.

Similarly, drought avoidance is an adaptive strategy of rice, that involves a group of mechanisms including deep rooting or leaf curling to prevent cellular dehydration process under drought stress. Several morphological parameters play important role in maintenance of turgor, initiation of leaf rolling, induction of epicuticular wax deposition on the leaf surface, deep and coarse root with greater xylem vessel radii and lower axial resistance to water flux are indicators of drought stress tolerance. Besides, stomatal function or exchange, photosynthesis, photosystem activity, net chlorophyll content and translocation of solutes from source to sink is also important for a prolonged period of drought stress. Photosynthetic ability has been regarded as important indicator of the growth of plants, because of their direct link to net productivity. Drought causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration of thylakoid membranes. Chlorophyll pigment played an important role in photosynthesis, and chlorophyll stability index, which is a measure of integrity of membrane of the pigments found to correlate with drought tolerance. Apart from metabolic processes, hormonal activity under drought stress is also important. Previous studies involving ABA (Abscisic acid) showed that ABA act as a signaling molecule, and an efficient ABA signaling contributes to drought tolerance. In addition, at molecular level a large number of genes in rice are up- or down-regulated by drought stress, which not only enhances the plant survival in drought conditions but also improves the crop productivity.

3.2. Submergence

Among the cultivated plants, rice is the most efficient crop which is capable of surviving under complete submergence for a considerable period of time. There are different mechanisms to cope up with submergence stress. In case of complete submergence, reduced growth and elongation or in short quiescence under submergence was found to be an efficient strategy to withstand complete submergence stress. Only few rice genotypes processing a *SUB1* QTL in their genome can follow this process. Reduced elongation of internode under flash flood condition (complete submergence for 1-2 weeks) is crucial for survival as the elongating plants often tend to lodge as soon as the water subsides (Vergera et al., 2014). Therefore, elongation of internodes under submergence can be crucial for identifying susceptible/ tolerance rice lines. Also, as elongation of internodes is energy dependent process, rate of starch degradation is also decisive for identifying tolerance. Also, content of non-structural carbohydrates, which is important for providing energy and for the maintenance of key metabolic processes in cells, before and after submergence, is important for determining susceptible and tolerant genotypes. Besides, there is also important for regeneration and recovery of seedlings after the water recedes (Sarkar, 1998; Das et al., 2001). Underwater, maintenance of chloroplast integrity is another



vital factor for plant survival. Rapid change in chlorophyll fluorescence traits are also observed in sensitive genotypes under submergence stress. Thus, chlorophyll fluorescence (ChlF) trait could be possible selection criteria for identification of submergence tolerance genotypes in rice (Panda et al., 2007, 2008).

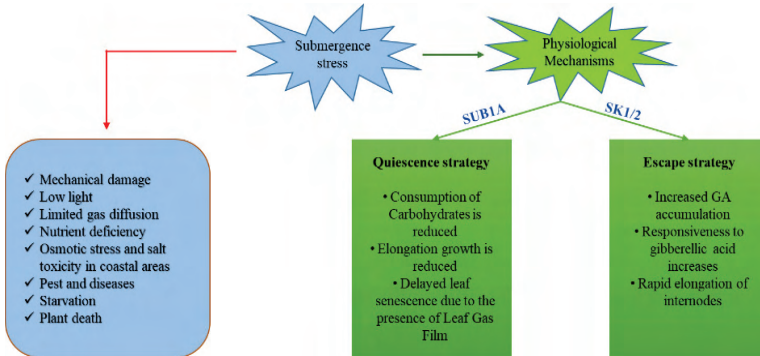


Fig. 5 Effects of submergence on rice physiology and tolerance mechanisms.

Besides these well studied adaptive and/or tolerance strategies, amount of leaf gas film thickness that is present in the surfaces of rice leaves is also important to decide plant survival under stress. It is also beneficial for rice and helps to exchange gases, when plants are dipped into water (Pedersen et al., 2009). Among many cereal leaves studied for the presence of such leaf gas film in adaxial and abaxial leaf surfaces, rice was found to possess the thicker leaf gas film, which may well be associated with its unique ability to withstand complete submergence for fairly long period of time among all the cereal crops. Recently, Chakraborty et al. (2021) showed that thickness of these leaf gas films were influenced by the presence of active *SUB1* gene in the rice genetic background.

The higher in-built activities of antioxidant enzymes in tolerant cultivars are reported to be useful physiological adaptation strategy to hypoxic condition. It is beneficial and helpful to maintain the functionality of the enzymes under hypoxic/ anoxic condition. A mechanistic understanding of molecular regulation underlying submergence tolerance and escape survival of plants has been studied through functional validation and characterization of key genes that are responsible for acclimation to submergence stress in rice. Modified ethylene signaling through *SUB1* gene action imparts submergence tolerance. Submergence stress facilitates the biosynthesis and entrapment of ethylene in tissues, which stimulates the induction of *SUB1A-1* gene and promotes its synthesis. Later, *SUB1A* activity limits the plant elongation process via suppression of ethylene production in feed-back inhibition manner, which

finally blocks the production of gibberellic acids and checked the overall elongation process of internodes under submergence.

3.3. *Water-logging or stagnant flooding*

Aerenchyma formation is a basic phenomenon of lowland rice and also an important adaptive strategy to withstand stagnant flooding, which allows unhindered transport of O₂ from well-aerated shoots to submerged roots (Jackson and Armstrong, 1999). Along with the formation of aerenchyma, lowland rice showed a limited diffusion of O₂ from root apex. This process was facilitated by forming a specialized barrier like structure on the root that popularly known as barrier to radial O₂ loss (ROL). This specialized structure was helpful to minimize the overall O₂ loss from root apex to the surrounding environment. Furthermore, this specialized structure or barrier is also helpful to check the movement of soil-derived toxins (i.e., reduced metal ions) and gases (e.g. methane, CO₂, and ethylene) into the roots (Greenway et al., 2006) that are inimical for the root cell. It has been reported that, this trait was associated with both upland and lowland rice species and helpful to withstand water-logging conditions (Colmer, 2003b).

Stagnant flooding may last for several months, where O₂ deficiency causes severe energy depletion in plants. In general, rice plants escape from the flooding to survive under semi-deep or deep-waterflood conditions. To achieve this, deep-water rice rapidly and substantially elongate their internodes to avoid complete immersion of plant canopy in water. Remarkably, some deep-water rice cultivars can increase their height by up to 25 cm day⁻¹ (Vergara et al., 1976). This rapid elongation allows the leaf tips to extend above the water surface and enable the rice plants to efficiently photosynthesize and exchange gases for respiration (Bailey-Serres and Voesenek, 2008). The process of elongation was regulated by different activities of hormones. Among different hormones, ethylene plays a master regulator role in elongation. Initially, ethylene biosynthesis is activated, and it accumulates in cells, later, accumulated ethylene further regulates the production of GA and ABA contents. As internodes elongation is promoted by GA or repressed by ABA, the overall GA/ABA ratio in cell is important to regulate the process of elongation (Kende et al., 1998; Sauter, 2000). Based on the reports of Steffens et al. (2011) aerenchyma formation in different tissues was enhanced by a gaseous hormone, ethylene. Additionally, Hattori et al. (2009) identified two important candidate genes *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) that are responsible for internodes elongation and present in deep-water rice. Introgression of *SK1* or *SK2* in non-deep-water rice showed enhanced growth of internodes, and validated that the *SK* gene members are the key players behind escape strategy of rice under deep-water.



3.4. Germination stage oxygen deficiency

Anaerobic mode of respiration usually yields much less energy as compared to the aerobic mode of respiration. As normal electron transport process, including energy production (ATP) was hampered due to scarcity of oxygen; in case of anaerobic germination the energy requirement is largely fulfilled by glycolysis followed by alcoholic fermentation (Guglielminetti et al., 1995, 2001; Hwang et al., 1999). Based on the transcriptomic data, it is observed that, under anaerobic mode of germination, starch and glucose metabolism enzymes related genes are majorly up-regulated (Lasanthi-Kudahettige et al., 2007; Hussain et al., 2016; Narsai et al., 2017). Therefore, based on these reports, it is predicted that, activity and up-regulation of the enzymes related to starch degradation is crucial for identifying susceptibility and tolerance. Previous reports showed that, starch degrading enzymes like α -amylase, aldolase and sucrose synthase are up-regulated in GSOD and specifically in case of tolerant cultivars. Susceptible plants are unable to up-regulate these enzymes and die due to scarcity of energy. (Ismail et al., 2009; Miro and Ismail, 2013). It is also reported that a higher *RAmy 3D* gene expression (Ismail et al. 2009; Takahashi et al. 2014) as well as greater up-regulation of rice cytosolic hexokinase (*OsHXX7*; Kim et al., 2016) was observed in case of GSOD stress. Similar study by Kretzschmar et al. (2015) identified a major gene *OsTPP7* that is related to trehalose breakdown pathway, which helps to maintain a balance between trehalose and starch, therefore, starch breakdown process and energy production is not hampered in case of GSOD stress. Mapping QTLs imparting high anaerobic germination potential (AGP) has been initiated (Angaji et al., 2010; Baltazar et al., 2014; Kretzschmar et al., 2015) and one of the identified QTL, *qAG-9-2* has been fine-mapped to identify *OsTPP7* gene, which encodes trehalose-6-phosphate phosphatase that are involved in starch mobilization process during germination (Kretzschmar et al., 2015). Recent studies showed effective operation of anaerobic respiration and nitrogen metabolism in tolerant rice genotypes which led to more energy efficient metabolic system under oxygen limiting GSOD condition, and resulted in better ROS handling and cellular pH maintenance (Vijayan et al., 2018).

3.5. Salinity

Under salinity stress, initially osmotic stress becomes more predominant, whereas with time when more and more salts are absorbed, ionic effect plays the leading role. Therefore, salt stress creates a cumulative response of both osmotic and ionic stress and adaptation to both is required to combat salt stress (Reddy et al., 2017). With time injury symptoms due to salinity are visible, susceptible cultivars die early compared to tolerant cultivars in a salty environment (Singh et al., 2014, Pradhan et al., 2019). To deal with this

adversity, plants generally restrict the movements of ions such Na^+ or Cl^- ions to the growing meristematic tissues and young photosynthetic organs that are crucial for plant survival. Tolerant rice cultivars like *Pokkali* either absorbs low levels of Na^+ or restricts the movement of Na^+ to the photosynthetically active portions in comparison to K^+ and thereby maintains low $\text{Na}^+:\text{K}^+$ ratio in shoot / leaf, and protects the vital tissues (Yeo and Flowers 1984; Yeo et al., 1990; Cuinet et al., 2011; Singh and Sarkar 2014; Kobayashi et al., 2017). High Na^+ in the cell cytoplasm impairs several physiological and biochemical courses of action (Chenet et al., 2014; Silva et al., 2015; Bendalyet et al., 2016), which restricts plant growth. Recently, Chakraborty et al. (2020) showed that a combination of both ion exclusion and tissue tolerance strategies can effectively balance the salt-tolerance strategies in rice, which could potentially minimize the energy cost of salt-tolerance.

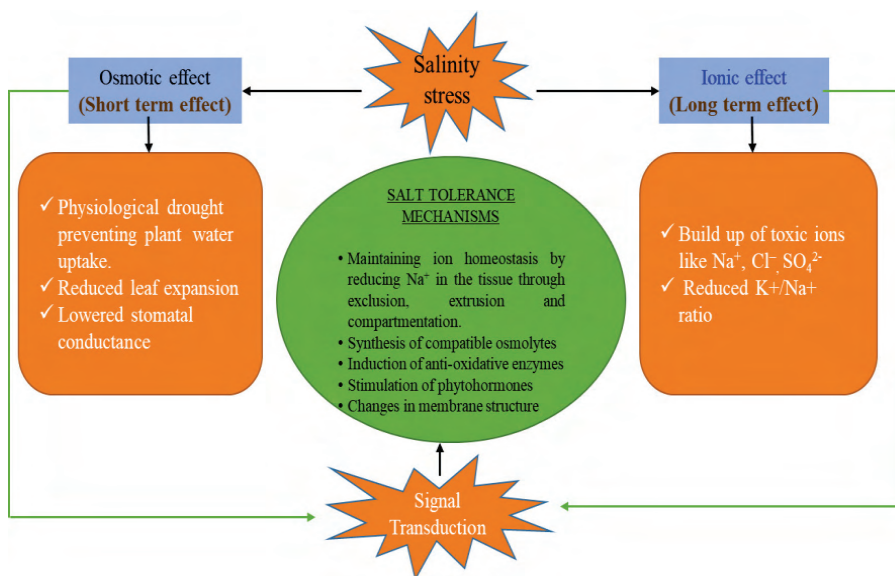


Fig. 6 Effects of salinity in rice plants and tolerance mechanisms.

Molecular studies of the transporters showed that different group of transporters are involved in reducing overall Na^+ accumulation in shoots and their sub-cellular compartmentalization in the vacuoles. Among them, the high affinity potassium transporter (HKT), salt overly sensitive (SOS) and Na^+/H^+ exchanger (NHX) gene families are the key players for salinity tolerance and important to maintain the cellular homeostasis (Mickelbart et al., 2015). HKT members are important for maintaining the overall tissue concentration of Na^+



and K^+ . *OsHKT1;5* was identified as the functional gene of *Saltol* QTL, the major quantitative trait locus (QTL) for salt accumulation in *O. sativa* genotypes (Ren et al., 2005). Reports showed that, *OsHKT1;5* is a plasma membrane localized Na^+/K^+ channel that allows selective transport of potassium to the upper portion of cells. It effectively effluxes Na^+ from the xylem and transport it to adjacent parenchyma cells, therefore minimizing the upper flow of Na^+ (Hauser and Horie, 2010). Robust screening effort including several *O. sativa* cultivars, landraces and *O. glaberrima* (AA genome) genotypes showed that overall concentration of sodium in leaf blades is a determining factor to identify susceptibility/ tolerance. *OsHKT1;5* gene was identified as a major determinant for tolerance at early vegetative stage; the more active the efflux transporter, the more tolerance was maintained by the plant, which directs the Na^+ exclusion from the transpiration stream and less amount of Na^+ is translocated to leaves (Platten et al., 2013). The scenario was quite complex in case of reproductive stage. Recently, Chakraborty et al. (2019) showed that selective transport of K^+ over Na^+ is an important factor for governing reproductive stage salt tolerance in rice. Besides, overall tissue sodium concentration in flag leaves is also important and crucial for deciding susceptibility and tolerance at reproductive stage. Different Na^+ , K^+ and proton transporters of the family SOS, NHX, HKT, V-ATPase and V-PPase was found to be important here and its over-expression ultimately decides the plant fate in case of reproductive stage salt tolerance.

3.6. High temperature

Rice is highly sensitive to heat stress particularly at flowering and post-flowering stages. Exposure to short periods of heat stress coinciding with flowering has resulted in significant yield losses reported in India, China and Japan. During anthesis heat stress leads to irreversible reduction in spikelet fertility, mainly by affecting sensitive physiological processes such as anther dehiscence, pollination, and early fertilization events. To minimize heat stress related damage, plants generally adopt three mechanisms *viz.*, heat escape, heat avoidance and heat tolerance. Heat escape generally deals with time of day of flowering, especially early morning flowering, heat avoidance strategy mainly applies transpiration cooling process for maintaining a convenient canopy temperature and heat tolerance strategy generally deals with resilient reproductive processes (Jagadish et al., 2010). Based on the reports, early – morning flowering genotypes which showed a better anther dehiscence and flower opening ability at a cooler time of day are preferably more desirable candidates for heat tolerance and heat escape (Satake and Yoshida, 1978). Later, Jumiatus et al. (2016) showed that rice cultivars those are able to maintain a low leaf temperature and well-exerted panicle under high temperature possibly perform better under high temperature stress.

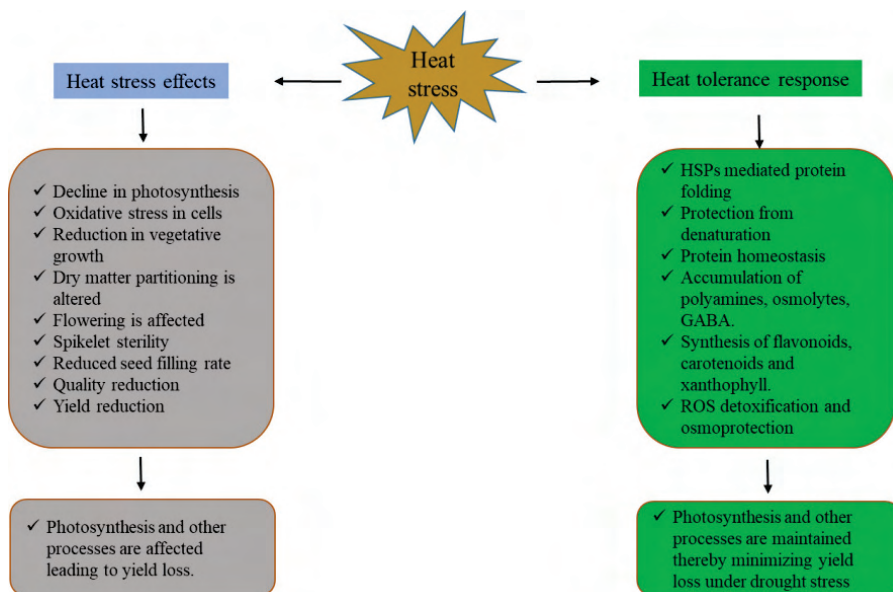


Fig. 7 Effects of heat stress on rice and physiological responses to cope up with the stress.

Apart from the heat resistance mechanisms, to limit the excess heat generation process in leaves, several plants maintain a thick cuticular wall out of the leaf surfaces that limits excess absorption of solar radiation. Altering leaf orientation under changing temperature by turning the leaf blades away from light and change their orientation by rolling in such a way that they seem to be parallel with falling sun rays thereby protecting leaves from heat stress (Hasanuzzaman et al., 2013). High temperature stress tolerant plants maintain homeostasis by physiological alterations like osmolyte accumulation resulting in increased water uptake and retention in the cell and stomatal regulation, and enhanced photosynthesis. Morphological alterations like reduced cell size, closure of stomata, greater xylem vessels and leaf rolling are also important adaptations to heat stress tolerance. Biochemical alterations like reduced generation of reactive oxygen species due to enhanced antioxidants (SOD, CAT, POD), activity and modifications in membrane by increasing unsaturated fatty acids alters membrane fluidity under temperature stress to re-establish homeostasis, and to protect and repair damaged proteins and membranes (Vinocur and Altman, 2005). At molecular level, upregulation of stress responsive genes, changes in proteomics, transcriptomics and metabolomics have shown to impart tolerance to heat stress. At cell level, activity of ion transporters, LEA family proteins, production of osmolytes, antioxidant defense



related enzyme activities, and transcriptional control of the genes that involved in this processes are essentially required to neutralize the stress effects (Rodríguez et al., 2005). Significant genotypic variation has been documented in rice for heat stress induced spikelet sterility (Khan et al., 2019).

Nowadays, combined effect of drought and heat is a common type of abiotic stress that negatively affects production in rice. Combined drought and heat stress during flowering and grain filling affect both yield and quality of the produce. Stress occurring at flowering stage affects yield and its components more significantly while combined stress during grain filling had a stronger negative impact on quality, primarily chalkiness of the grain. Combined stress during grain filling had significant impact on the overall chalkiness or the proportion of grains with >75% chalk compared with flowering stage stress. Combined drought and heat stress during flowering resulted in the reduction of plant height and biomass.

3.7. Low light stress

The exhaustive research carried out to understand the mechanisms of low light tolerance in rice exhibited two kinds of mechanism when they encounter low light (LL) stress such as (i) shade avoidance and (ii) shade tolerance. Primarily the phytochrome photoreceptors sense the reduction in the R:FR ratio, which may occur either due to the neighbouring vegetation, actual shade, future shade or reduced PAR, and induce a suite of traits to grow towards the light. Collectively this is known as shade avoidance response (SAS). Shade tolerance is exhibited by species from forest under stories that cannot outgrow the surrounding trees and adopt tolerance responses (Gommers et al., 2012). The various physiological and morphological characteristics of plants to withstand low light are depicted in Fig. 8.

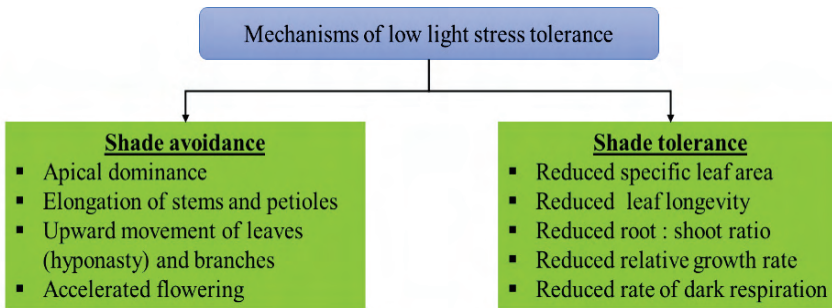


Fig. 8 Mechanisms of low light stress tolerance in rice.

4. OMICS APPROACHES: ABIOTIC STRESS TOLERANCE

‘Omics’, the modern-day scientific buzz word can be defined as the sciences of evaluating the functions of different biological information and their interaction in different clusters of life. It encompasses the studies at the gene level such as genomics and epigenomics; mRNA or transcript level such as transcriptomics; at functional protein level known as proteomics or may be at the metabolite level known as metabolomics and lipidomics or may the interactions (interactomics) and phenotype (phenomics) level (Langridge and Fleury, 2011). Different omics-based approaches for deciphering abiotic stress tolerance mechanisms in rice plants have been summarized in Fig. 9.

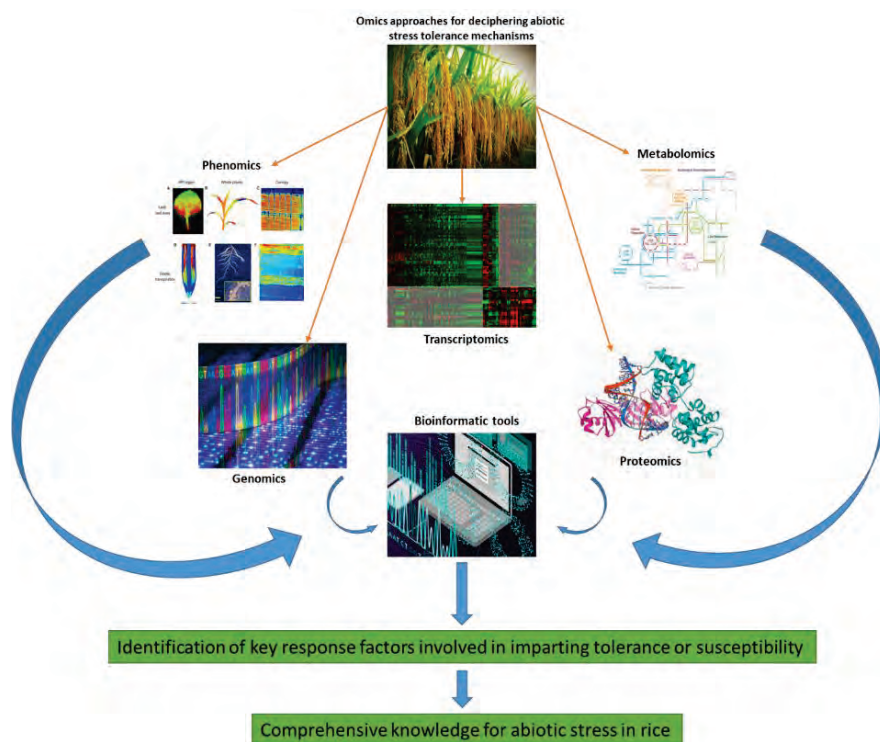


Fig. 9 Omics-based approaches for deciphering abiotic stress tolerance mechanisms in rice plants.

Omics approaches are inter-dependent and over-lapping. The amalgamation of the huge data generated through omics approaches is very



much important to derive a logical conclusion to ascertain the major cell or tissue specific response-cascades differing in two or more experimental conditions arising out of abiotic stress tolerance studies. Genomic studies can give an overall view of the most dominant responsive genes in tolerant varieties. The expression pattern and the complex networks of mRNAs which have an important role in the functioning of tolerant varieties under stress conditions are revealed through transcriptomics. Discovery of the major proteins participating in the defence systems of rice plants can be done with help of a key approach called proteomics (Zou et al., 2011). Metabolomics exhibitions crucial secondary metabolites of tolerant varieties for combating abiotic stress. Phenomics approaches are helpful to understand the relation between phenotypic traits and genomic data. Bioinformatics tools integrate all the data obtained from the “omics” sciences and leads to the accumulation of comprehensive knowledge about tolerance mechanisms to abiotic stresses in rice.

5. STRESS TOLERANCE IN RICE: POSSIBLE STRATEGIES

In order to successfully combat the adverse effect of abiotic stresses, both genetic improvement and agronomic/ physiological management strategies are important. The development of drought-tolerant varieties will greatly enhance and stabilize rice productivity in drought-prone areas. Introgression of yield QTLs *qDTY1.1*, *qDTY2.1* and *qDTY3.1* that is effective under drought stress into genetic background of popular rice varieties helps in mitigating yield losses due to drought stress. Breeding for plants with less root length density in shallow layers of soil, and high root length density in medium- and deep-layers, have been considered an efficient water management strategy (Wasson et al., 2012). Application of P (50% more than the recommended dose), Fe (30 kg ha⁻¹), and Si (200 kg ha⁻¹) under water deficit stress condition resulted in a significant increase in grain yield by 7, 9, and 12%, respectively (Kumar et al., 2019). Application of silicon improves the spikelet number, spikelet fertility, and grain weight and enhances yield.

Similarly, introgression of Saltol QTL and *SKC1* (*OsHKT1;5*) through gene pyramiding had yielded considerable success in developing salinity tolerant rice cultivars. Now, breeders are trying to combine *SUB1* and *Saltol* QTLs in the same genomic background of rice for increasing the toleranceto multiple abiotic stresses like salinity and submergence. Genetic variability could be utilized to screen for abiotic stress tolerant genotypes and could be utilized for developing tolerant and well-adapted rice cultivars. Genotypic variation exists in rice cultivars for anthesis time. Early flowering could be considered as

an escape mechanism for avoiding elevated temperature stress (Julia et al., 2012). Early morning flowering is a useful trait that could be used to reduce spikelet sterility by advancing the time of flowering in the early morning when the air temperature is cooler (Yoshida et al., 1981). Adopting a late or early maturing cultivar is one of the approaches to escape high temperature during grain filling (Ohe et al., 2007, Krishnan et al., 2011). Growth regulators can also be employed to minimize adverse effects of heat stress in rice. The foliar application of salicylic acid (0.1 mM) induced high-temperature tolerance and ameliorated the effect of heat stress in rice (Chandrakala et al., 2013). Some growth regulators, such as methyl jasmonate, which advances the flowering time to early morning, could be used to mitigate heat stress (Kobayasi et al., 2009). The application of CaCl_2 (10 mM) was shown to ameliorate the heat stress-induced reduction in gas exchange, PSII efficiency, photosynthetic water-use efficiency, spikelet fertility, and leaf chlorophyll content in rice (Chandrakala et al., 2013). Screening and identification of genotypes for higher non-structural carbohydrate pool, higher biomass and stable photosynthesis under high temperature could serve as genetic donors for reducing temperature-induced yield loss.

5. CONCLUSIONS

Harvesting the higher production with minimum losses under abiotic stress situation is essential in the context of changing climatic scenarios. It is also a challenge for the researchers to understand the adaptive mechanism and tailor rice genotypes for optimum performance from limited natural resources. The rainfed rice cultivation in the country is highly affected by the effects of climate change. To combat the stress, it is essential to integrate crop physiology, molecular genetics and breeding approaches to dissect complex abiotic stress tolerance traits, and develop the next generation crops which can withstand the adverse climate and ensure food security. The popular high yielding rice varieties should be stacked with multiple stress tolerant gene(s)/QTLs for making them climate resilient. Thus, mega varieties with multiple tolerant genes for submergence, anaerobic germination, yield QTLs under drought, and seed dormancy need to be deployed to make them truly climate resilient.

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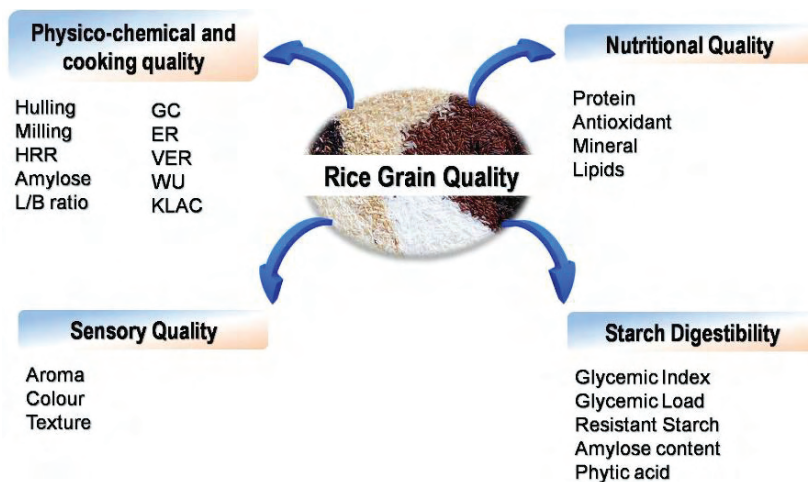
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RICE GRAIN QUALITY AND STARCH DIGESTIBILITY: DESIRED TRAITS FOR EVALUATING RICE VARIETIES

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GRAPHICAL ABSTRACT



SUMMARY

Rice grain quality is one of the most important factors for the evaluation of a variety from the perspective of farmers, millers, and consumers. It includes physico-chemical, nutritional and sensory qualities. The consumers in India and other South-East Asian countries prefer medium to high Amylose Content (AC) rice due to their non-stickiness property of boiled rice but in many Asian countries, waxy rice (having low AC) is mainly preferred. The colour of rice grain may be white, purple, red and black; which are mainly associated with pericarp or bran layers of the whole grain. The bran contains most of the phytochemicals like oils, antioxidants, minerals, proteins, vitamins and crude fibers in higher concentration than white endosperm. Pigmented rice is generally rich in nutritional compounds as compared to white one but those are unpopular among the farmers because of many undesirable physiological and agronomic characteristics. Sensory qualities like colour, texture, pasting properties, and aroma are essential for consumers as per their preference and



acceptance. As rice grains mainly consist of starch, hydrolyzed by digestive enzymes and converted into glucose which is the major energy source for various metabolic functions. After fulfilling the energy requirement of body, extra calories from starch are stored as fats or glycogen for later use. Therefore, overeating rice, combined with sedentary lifestyle potentially leads to some health issues, such as type-II diabetes, obesity and colon diseases in long-term particularly in Asian countries. Various biochemical factors affect the starch digestibility and Glycemic Index (GI) of a food or its products. These factors are resistant starch, AC and phytic acid which lowers the starch digestibility by various ways and are negatively correlated with GI value. Now-a-days quality assessment of rice grain in terms of its nutritional as well as physico-chemical parameters is gaining prime importance gradually.

1. INTRODUCTION

Rice is the most important staple crop and is a primary source of energy and nutrition for about half of the world's population. It is the most important cereal crop with respect to food security for common people. Due to increasing world population and shrinking farm land holdings area, there is an urgent need to increase the grain productivity. Therefore, increasing grain yield has become the primary objective for many rice-breeding programs. Globally, India is the second highest rice producer (about 120 million ton/year) and able to meet the demand of rice for the entire population. But along with yield, improvement of rice quality has now become a foremost consideration for rice consumers and millers. Quality is defined as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs" (ISO, 1986). The concept of grain quality covers many physico-chemical properties such as grain shape and size, milling and cooking properties, amylose content (AC), stickiness, chalkiness, texture, colour, gelatinization characters, and various other nutritional properties (Singh et al., 2005). Thus, rice grain quality generally includes three main classes (i) physico-chemical and cooking quality (ii) sensory quality, and (iii) nutritional quality. Grain quality and its assessment are very important to consumers, millers and rice breeders who are engaged in varietal improvement programme related to new features such as high nutritional quality, high yield potential, highly resistant to abiotic or biotic stresses, higher water use efficiency etc.

Rice being the staple food crop for half of the global population constitutes a vital source of dietary carbohydrates and energy. It is also an excellent source of starch, which makes up major part of rice grain. Starch is hydrolyzed by digestive enzymes and converted to glucose in the digestive tract which is directly used by cells to produce energy for their metabolic functions. Hence, degree of digestibility of starch has attracted significant nutritional



interest. Several studies in genetically divergent populations indicate that white rice consumption is correlated with increased risk for development of type-2 diabetes independent of ethnicity (Sun et al., 2010). White rice has been reported to adversely affect metabolic health where brown rice may play protective role. Thus, replacing white rice with brown rice has been recommended to counter the adverse metabolic consequences related with refined and processed rice consumption (Dixit et al., 2011). However, brown rice consumption is very low compare to white rice, and also earlier one consider inferior to white rice because of its unappealing taste and texture, longer cooking time along with shorter shelf-life (Zhang et al., 2010). Most of the commonly consumed rice have high Glycemic Index (GI) and Glycemic Load (GL) irrespective of whether they are brown or polished (Brand-Miller et al., 2003a; Lin et al., 2010). While primary prevention of type-2 diabetes through more appropriate food choices may be the promising strategy, behavioral change that is sustained with meaningful magnitude is very difficult to achieve practically, especially for the short term period. Lowering the GI of staple foods like rice seems to be more effective in promoting general public health, especially for communities in which rice consumption accounts for a large share of dietary portion and cultural preferences for consumption of white rice. In view of increasing public awareness regarding quality rice and sedentary lifestyle's related diseases, this chapter provides glimpse on grain quality and factors indicating GI and starch digestibility of rice.

2. PHYSICO-CHEMICAL AND COOKING QUALITY

Physico-chemical quality of rice include milling parameters *viz.*, hulling%, milling% and Head Rice Recovery (HRR)%, whereas cooking quality include Elongation Ratio (ER), Water Uptake (WU), Volume Expansion Ratio (VER), Alkali Spreading Value (ASV), Gel Consistency (GC), Gelatinization Temperature (GT) and Kernel Length After Cooking (KLAC). In case of chemical quality, it only includes AC. Milling quality of rice is very important for the farmers and millers because good milling quality rice (>60%) is profitable. Milling quality is dependent upon genotypes and affected by changes in starch, proteins, lipids and interactions among them under environmental stresses (Chen et al., 2012). Jing et al. (2016) reported that HRR was reduced (-23.4%) with elevated CO₂ condition (200 μmol mol⁻¹ throughout rice growth season). Many researchers found that HRR and milling% were drastically reduced by high temperature during grain filling period. Grain chalkiness also reduces the HRR%. Hulling, milling and HRR% can be determined after milling to remove the outer husk and bran layers through huller and miller machine at 12-13% moisture content of the grain.

Cooking quality of rice is mainly determined by the content as well as composition of starch and storage protein present in the endosperm, of which two kinds of starch (amylose and amylopectin) are the main factors. The AC of the grain above 25% is considered as hard or non sticky, 20-25% AC is designated as medium, whereas 10-20 % and 1-10% AC are called as soft and waxy rice, respectively. The protein content of the grain is also involved in determining the cooking quality as starch-protein interactions can impede starch gelatinization and disintegration of the proteins structure during cooking which can increase viscosity of the rice grain. GC is a test of grain quality that is aimed to distinguish the varietal difference between high AC and the waxy classes of rice. GC of the samples are categorised into: hard ≤ 40 mm, medium 41-60 mm and soft > 60 mm (Graham, 2002). GT is mainly used as an indicator of the cooking time (Cuevas et al., 2010), and three methods are being used to measure it: (i) based on the pasting temperature from a amylograph (Dang and Bason, 2014), (ii) visually assessment of the digested rice grains in an alkaline solution and aqueous KOH, and (iii) obtaining the temperature peaks through differential scanning calorimetry. The GT classes are either based on the temperature at which the crystalline forms of starch irreversibly melt or based on ASV of the polished grain. Depending upon temperature, rice varieties are classified into three major classes: high (> 74 °C), intermediate (70-74 °C), and low (< 70 °C) (Graham, 2002). Based on alkali spreading, on the other hand, there are four GT classes: high (2), intermediate-high (3), intermediate (4-5), and low (6-7) (Graham, 2002). Minerals content in rice grain are also related to cooking quality. It was found that mineral element have correlations with amino acid and protein content of the grain. GC is significantly correlated with K, Mn and Cu contents of the grain and AC is significantly associated with Na, Mg, K, Mn and Cu contents. The ASV has a positive association with Mg, Ca and Mg contents (Jiang et al., 2007) (Table 1).

Table 1 Physico-chemical quality of some rice varieties

Varieties	Hulling %	Milling %	HRR %	KL (mm)	KB (mm)	L/B	ASV	WU/100g	ER	VER	KLAC (mm)	Grain Type	Moisture %	Amylose (%)
Rajalaxmi	79.50	67.00	54.00	6.30	1.91	3.30	6.00	197.50	1.63	3.50	10.30	LS	12.64	16.28
Samali	78.50	69.00	64.00	6.34	1.96	3.23	4.00	130.00	1.70	3.75	10.80	LS	12.38	20.70
Sarala	79.50	64.00	59.50	5.11	1.80	2.85	4.00	180.00	1.88	4.00	9.60	SB	12.85	21.64
Ketakijoha	79.50	69.00	63.00	5.55	1.76	3.15	4.00	95.00	1.86	4.00	10.30	SS	12.61	18.86
Moti	78.50	67.00	58.50	6.11	2.10	2.92	4.00	165.00	1.80	3.75	11.00	LB	12.64	21.83
Uday	79.50	68.00	65.50	5.32	2.35	2.26	4.00	147.50	1.75	3.75	9.30	SB	12.80	20.55
Tapaswani	78.50	70.00	67.00	5.35	2.03	2.64	4.00	85.00	1.51	3.75	8.10	MS	12.57	19.31
Reeta	78.50	67.00	55.50	6.07	2.20	2.77	4.00	105.00	1.80	3.75	10.90	LB	12.84	17.85
Saket-4	78.50	68.50	65.50	6.00	1.87	3.22	4.00	115.00	1.57	3.75	9.40	LS	12.99	18.79

HRR: Head rice recovery; KL: Kernel length; KB: Kernel breadth; L/B: Length/Breadth ratio; ASV: Alkali spreading value; WV: Water uptake; ER: Elongation ratio; VER: Volume expansion ratio; KLAC: Kernel length after cooking



3. SENSORY QUALITY OF RICE GRAIN

Sensory quality of rice includes the attributes or traits of the grain/processed grains, which are generally recognised by our sensory organs. It includes aroma, texture profile of the grain and products, colour, viscosity of rice flour and physical impurities. Textural profile analysis of rice grain and its various products includes hardness, fracturability, adhesiveness, cohesiveness, chewiness, gumminess, resilience and springiness. The textural properties varied significantly according to the genotypes and processing techniques of the grains. It is reported that hardness arises from different arrangement of starch granules in different products, as evident from Scanning Electron Micrograph (SEM). The colour of the grains and different products can be determined through a machine “colourimeter” and it is quantifiable through the parameters L^* (whiteness), a^* (redness), b^* (yellowness) and ΔE (Colour difference of the sample from reference) = $\sqrt{L^{*2} + a^{*2} + b^{*2}}$. In rice, aroma is one of the major sensory quality for consumer preference. The odor-active volatile compounds (2-acetyl-1-pyrroline, guaiacol, hexanal, isovaleric acid, valeric acid, enantiic acid, 2- methyl naphthalene, 2-nonenal, octanal, myristic acid, 1-octen-3-ol, 2-heptanone and heptanal) contribute the most in different rice types (Yang et al., 2006). The rice aroma can be determined qualitatively and quantitatively through GC-MS-MS or electronic nose. The Indian rice varieties like basmati are well recognised for its typical aroma. However, there are many short and medium grain rice varieties or landraces, which also contain these aroma compounds. These varieties or landraces like Govindabhog, Badshabhog, Kalajeera, Chinikamini, Ketakijiha, Geetanjali etc. are being cultivated in tropical and subtropical climate.

Pasting properties of rice flour is very important with respect to product making quality. There are different pasting parameters like:

(a) The *pasting temperature* is the minimum temperature required to cook a given powdered sample. Higher amylose types generally show higher pasting temperatures on RVA (Rapid ViscoAnalyzer).

(b) The *peak viscosity* occurs at the equilibrium point between swelling and polymer leaching (viscosity to increase), rupture and polymer alignment (viscosity to decrease). It signifies the water-binding capacity of the starch or flour mixture. It is mainly correlated with the final product quality. The temperature and time corresponding to the peak viscosity are referred to as the *peak temperature and peak time*.

(c) During the holding period of a test, the sample remains in a constant temperature (generally 95 °C) and mechanical shear stress. So the granules undergo further disruption while the amylose molecules continue to leach out into solution and undergo further alignment. This period is usually accompanied

by the reduction of viscosity, which consequently reaches a minimum value. This viscosity is known as the *trough viscosity*.

(d) After cooling the mixture, re-association between starch molecules especially amylose, occurs to a greater or lesser degree. Given a sufficient concentration of starch, this usually causes the formation of a gel. The viscosity will normally increase and stabilize at a *final viscosity*.

(e) The phase of the pasting curve between the trough and final viscosity is commonly referred as setback region. The re-association between starch molecules during cooling is commonly known as *setback viscosity*.

Alpha-amylase is an endogenous enzyme that breaks the α -1,4 glucosidic bonds on a nearly random basis, rapidly decreasing the size of the starch molecule and thereby reducing the viscosity of a starch solution or slurry. The viscosity measurements such as RVA graph and falling number have been widely used to measure this enzyme activity.

4. NUTRITIONAL QUALITY OF RICE GRAIN

Nutritional quality of rice includes the bioactive molecules which provide us the nutrition and protect us from different diseases. Many macro- and micro-molecules like-proteins, carbohydrates, lipids, minerals, antioxidants, vitamins and fibers are responsible for providing us a balanced nutrition and healthy body. Rice contains 3-15% protein in brown rice but after polishing, it is reduced up to 10 to 15%. Rice bran constitutes 5-8% of the total rice grain and serves as important source of hypoallergenic proteins (14-16%), dietetic fibers and functional compounds such as oryzanol and tocotrienols. Based on solubility, rice protein is divided into four components *viz.*, glutelin, albumin, globulin and prolamin. Among these different fractions, glutelin, which is found mostly in endosperm localised protein body-II formed the major fraction (Ogawa et al., 1987). The mean value of different protein fractions decreased in the order: glutelins (7.02 - 8.05 g/100g FW) > globulins (2.87 - 3.78 g/100g FW) > prolamins (2.03 -3.02 g/100g FW) > albumins (0.27 - 0.43 g/100g FW). Yang et al. (2014) reported that the total soluble sugar content of 35 rice cultivars varied from 1.2 to 2.6% while starch content varied from 70 to 90% depending upon the genotypes. The whole lipid of rice is mainly concentrated into the bran layers. Rice bran is the upper thin layers of white endosperm of rice grain. It can be obtained as a bi-product of rice milling and contains 14 to 15% crude oil. Oleic acid (C18:1) is found at the highest levels in the rice bran oil, followed by linoleic acid (C18:2) and palmitic acid (C16:0). It also contains substantial amount of PUFA (Poly Unsaturated Fatty Acids), which is beneficial for the health of heart. The whole rice grain contains a substantial amount of antioxidants like oryzanol, phenolics, vitamins and different antioxidative



enzymes *viz.*, peroxidase, catalase, superoxide dismutase, glutathione peroxidase etc.

Chemically, γ -oryzanol is the esters of ferulic acid with phyosterols and triterpene alcohols, generally referred as sterylferulates. The γ -oryzanol biosynthesis initiates with acetyl-CoA and form iso-pentenyl diphosphate (IPP) *via* mevalonic acid through some enzymatic steps. Previous study reported that γ -oryzanol content of 196 Korean landraces ranged from 9.8 to 55.9 mg/100 g and a mean was 27.2 mg/100 g (Lee et al., 2013). Bergman and Xu (2003) indicated that genotype and environment had a significant role in controlling γ -oryzanol level per grain weight. However, cooking generally decrease the γ -oryzanol content of the grain with a few exception. γ -Oryzanol content of pigmented rice was higher (44.73mg/ml rice extract) than non-pigmented (0.0 to 2.5 mg/ml rice extract) genotypes.

Vitamin E is indispensable for reproduction and prevents diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflammation and neurologic disorders. Vitamin E has an additional positive effect on autoimmune disease by decreasing pro-inflammatory cytokines, lipid mediators and has important role in rheumatoid arthritis patients. Prolonged vitamin E deficiency leads to peripheral neuropathy, muscle weakness and ophthalmoplegia, which are irreversible. Among the children with chronic cholestasis, 50 to 70% are vitamin E deficient, even when consuming standard supplements of vitamin E. α -Tocopherol is the most bioactive form of vitamin E followed in order by β , γ and δ -tocopherol. In rice, vitamin E homologs varied according to the genotypes and post harvest processing. In Brazil, 32 different samples of dehulled brown rice (*Indica* and *Japonica*) were analyzed for tocopherol and tocotrianol content and observed that total vitamin E levels varied largely at 10.4–32.5 mg/kg. In, *Japonica* subspecies, the average concentrations of different tocols from high to low were α -tocopherol (10.0 mg/kg), α -tocotrianol (7.0 mg/kg), γ - tocotrianol (5.8 mg/kg), and γ - tocopherol (1.4 mg/kg). In *Indica* rice, the most abundant homolog was γ -tocotrianol (7.8 mg/kg), followed by α - tocopherol (4.8 mg/kg), then α -tocotrianol (2.3 mg/kg), and γ -tocopherol (1.3 mg/kg) (Heinemann et al., 2008).

Micronutrients (Zn, Mn, Cu and Fe) can be found in every cell where they play important roles in maintaining normal metabolic functions, proper fluid balance, blood pressure regulation, nerve transmission and immune system. Minerals act as a co-factor of different enzymes activity in our body. K and Mg were the most abundant minerals in whole rice grains, accounting for about 60% and 30% of total minerals, respectively. Mn and Zn showed higher content in black rice than in most of non-pigmented and red rice samples. Among all the minerals, phosphorus content (2062.1 to 2529.7 mg/kg) in pigmented brown rice (*Chak-hao* Angamba, brown rice; *Chak-hao* Poireiton,

purple rice and *Chak-haoAmubi*, black rice), was the highest, followed by potassium (1546.8 to 1843.6 mg/kg), sulphur (743.5 to 976.1 mg/kg), Mg (377.2 to 387.6 mg/kg), calcium (77.6 to 136.2 mg/kg), iron (47.2 to 88.8 mg/kg), and Zn (34.9 to 53.9 mg/kg), while Cu content (27.5 to 33.4 mg/kg) was the lowest. However, polishing led to significant declines (0.75 to 2.50 times) in the mineral contents of the pigmented rice (Reddy et al., 2017).

In plants, most of the phenolics originate from the phenylpropanoid and the phenylpropanoid acetate pathways. Both the phenylpropanoid and phenylpropanoid acetate pathways share the first three biochemical steps, the core phenylpropanoid pathway. These steps start with an amino acid L-phenylalanine as a precursor and end with the formation of p-coumaroyl CoA, a reactive compound that can participate in further reactions. Among the phenolics, there are different types depending upon the chemical structure-phenolic acids, flavonoids, anthocyanin and proanthocyanin. Rice endosperm cell walls is reported to contain 12 g/kg of esterified cinnamic acids, which comprises of 9 g/kg ferulic acid, 2.5 g/kg p-coumaric acid and 0.5 g/kg diferulic esters (Clifford, 1999). Previous study reported that the pigmented rice samples had higher content of phenolic compounds than non-pigmented samples. In addition, the brown rice was rich in different phenolic compounds (0.05 to 7.69 mg/100 g) than the polished rice (0.02 to 1.12 mg/100 g) with the predominance of p-coumaric acid, ferulic acid, gallic acid and protocatechuic acid (Vichapong et al., 2010).

5. QUALITY ANALYSIS OF RICE GRAIN

Hulling, *milling* and *HRR* percentage of rough rice are determined on the weight basis with the help of hulling, milling machine and broken grain separator (for HRR). The *Length* and *Breadth* of the milled grain is estimated using digital scale. *ASV* of polished white grains is determined through KOH (1.7%) solution. *WU* of the grain can be determined by cooking of polished grain (2 g) with 10 ml of distilled water. The value is determined by subtracting the volume (ml) of water in the sample from blank and expressed in terms of water absorbed (in ml) per 100 g rice grain. *KLAC* is measured by a scale and expressed in mm. *VER* is measured as the ratio of increase volume after cooking to the volume before cooking. *Elongation Ratio (EC)* is the ratio of kernel length after cooking to the kernel length before cooking. *AC* can be assayed through spectrophotometer along with the chemicals [1N NaOH, 1N acetic acid, ethanol and Iodine solution (1g iodine and 10g KI are dissolved in water and make upto 500 ml)]. *GC* of rice flour (100 mg) is measured using 0.2 ml ethanol containing 0.03% thymol blue and 2 ml 0.2 N KOH solutions. *The Optimum Cooking Time (OCT)* is the time required to cook the grains when 90% of the total grains no longer showed an opaque center. It is generally two minutes



more than minimum cooking time (Singh et al., 2005). The *Texture Profile (TP)* analysis of grains is performed through “Stable Micro Systems texture analyzer” (Jaiboonet al., 2016). The *colour* of grain samples at different state of processing were determined by CIE colour scales L^* , a^* and b^* using Hunter lab digital colourimeter (Model D25M, Hunter Associates Laboratory, Reston, VA), where L^* indicates the degree of lightness or darkness of the sample extended from 0 (black) to 100 (white), a^* and b^* indicates degree of redness (+a) to greenness (-a), whereas b^* indicates the degree of yellowness (+b) to blueness (-b), respectively. The *Pasting Properties (PP)* of rice flour is evaluated by an instrument “Rapid Visco Analyzer” taking 3.5 to 4 g finely powdered (100 mesh size) grain sample. The *aroma content* of the rice grain can be assayed quantitatively and qualitatively through GC-MS-MS or electronic nose with liquid or head space injection mode. *Total protein* content is measured by kjeldhal method. The total protein is equal to N_2 content \times 5.95. In case of soluble protein, it is measured by Lowry or Bradford methods with the help of spectrophotometer. For *storage protein fractionation*, the rice sample is treated sequentially with water (for Albumin), 5% NaCl (for Globulin), 70% ethanol (for prolamin) and 0.2 M sodium borate buffer (pH 10) containing 0.5% sodium dodecyl sulfate (SDS) and 0.6% β -mercaptoethanol (for glutelin). γ -*Oryzanol* and *vitamin-E* are extracted according to Chen and Bergman (2005), by taking 5 ml of HPLC-grade isopropanol. The separation of γ -oryzanol and vitamin-E is done by HPLC with PDA and fluorescence detector, respectively, with the help of RP-18 column. The mobile phase contains 35% acetonitrile, 55% methanol and 10% isopropanol. *Phytic acid* content of grains can be estimated using the PA (phytate)/total phosphorus kit from Megazyme (Megazyme, Ireland) (Kumar et al., 2017). *Anthocyanin* content in the samples can be determined using the method described by Fuleki and Francis (1968) with acidified organic solvent (95% methanol: 1.5 N HCl 85:15, v: v; PH-1) and spectrophotometer. *Total flavonoid content* is determined spectrophotometrically according to Eberhardt et al. (2000) by taking 5% $NaNO_2$, the sample (ethanolic extract diluted with 1 ml of water), 10% $AlCl_3$ and 1M NaOH solution. *Total phenolics content* of grains is determined spectrophotometrically through folineciocaltue reagent (Zilic et al., 2011) but fractionation of phenolic acid can be performed by HPLC-PDA system with C18 RP column. For fractionation, the sample is extracted with 50% methanol (free) or acidic (1% HCl) methanol (bound + free). For different *antioxidant activity* assay, there are many methods adopted by the researchers. Among all the methods, DPPH, FRAP, and ABTS are very important for assessing the antioxidative potential of rice grain.

6. STARCH DIGESTIBILITY OF RICE GRAIN

Rice is the largely single food source of carbohydrate and energy. Being a starchy cereal food, ~90% of a milled rice kernel is starch (dry weight basis). Rice grain quality depends mainly on its physico-chemical properties. Starch is composed by two glucan molecules: amylose and amylopectin. Amylose is mainly a linear polymer chain of α -1,4 linked glucose units, whereas amylopectin is a highly branched molecule with linear chains of α -1,4 links connected by α -1,6 branch linkages. Rice starch is digested by the two major enzymes: α -amylase and amyloglucosidase (AMG). α -Amylase hydrolyzes oversized starch polymers and breakdown them into short glucose polymers (maltose), whereas AMG catalyzes maltose into glucose monomers. During hydrolysis process, glucose is removed from the non-reducing end of amylose and amylopectin (Fig. 1).

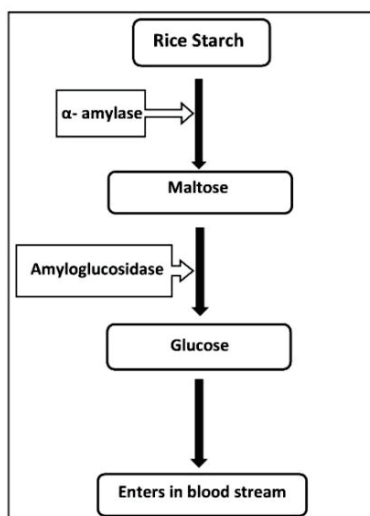


Fig. 1 Rice starch digestion to glucose by enzymes: α -amylase and amyloglucosidase.

7. PARAMETERS OF DIGESTIBILITY

7.1. Glycemic index

Rice is one of the most important cereal crops and is a staple food in many Asian countries. However, it is generally considered as a high GI food compared to other starchy foods. The GI of rice has been reported in the range from 54 to 121 (Srikaeo and Sangkhiaw, 2014). Rice starch, which is normally



hydrolyzed in the digestive tract by digestive enzymes followed by conversion into glucose and enters into blood stream to produce energy for various metabolic functions. However, when there is less energy requirement for cells, excess starch calories are stored in the body as fats or glycogen for later use. Therefore, long-term overeating high GI foods coupled with sedentary lifestyle or lack of proper exercise could potentially lead to some serious health problems, such as type-2 diabetes, obesity and other complications (Zhao et al., 2016). GI is defined as the incremental area under blood glucose response curve created after the ingestion of a 50g carbohydrate portion of a test food and expressed as a percent of response to equivalent amount of carbohydrate from a standard food taken by the same subject (Barclay et al., 2008; Brand-Miller et al., 2003b). GI values evaluate the grade of carbohydrates/carbohydrate rich foods from the perspective of their sugar and starch content to determine how they affect blood sugar levels after consumption. The GI has been used to classify foods based on their blood glucose raising potential (Augustin et al., 2015). Thus, there are three kinds of ratings for foods *viz.*, low GI foods (GI value 55 or less), medium GI foods (GI value of 56-69) and high GI foods (GI 70 or more) (Brand-Miller et al., 2003a). The importance of developing a simple protocol to identify foods with low GI can't be overemphasized as it provides a standardized measure of carbohydrates based on their potential as an energy source. The information on GI value of foods may help to choose the right ones so as to reduce the risk of type-2 diabetes, cardiovascular diseases, metabolic syndrome, chronic kidney disease, stroke, depression, gall stones formation, neural tube defects, uterine fibroids formation, breast, colon, prostate and pancreas cancers, to mention just a few (Kumar et al., 2021). There are some issues with the use of the GI as an absolute value of foods; most foods are not consumed in the volume needed to elicit the measured response and more often than no foods are eaten in combination which can change the GI value for each food in a meal (Grant and Wolever, 2011). Nevertheless, the importance of GI measurement remains relevant. The GI can be measured either using *in vivo* or *in vitro* methods. However, the *in vivo* method has long been the preferable method of choice, their complications including management of human volunteers and vulnerability to myriads of internal as well as external influences resulting in non reproducibility of results and lack of precision and in most of the cases (Barclay et al., 2008). The *in vitro* method for GI estimation is quick, precise and free from variability resulted from the individual metabolic differences. It is also suitable to screen large number of genotypes for starch digestibility (Kumar et al., 2020).

7.2. Glycemic load

The glycemic response of food upon ingestion depends not only on the GI but also on the total amount of carbohydrates consumed. The GL along with GI are

the important parameters majorly used to classify the carbohydrate rich foods. Postprandial blood glucose response is not only influenced by food GI value but also by the amount of carbohydrate taken per serving. GL is a function of both the amount of carbohydrate consumed and GI. The definition of GL is "the average GI of individual food multiplied by the amount of carbohydrate (in %) present in the individual food consumed". The higher the GL value of food, the higher is the postprandial blood glucose response (Salari-Moghaddam et al., 2019). For example, the carrot having high GI value, but low GL, in contrast, the starchy food such as rice and potato have high GI as well as GL (Ludwig, 2002). Some foods despite having low GI may show intermediate to high GL, possibly due to higher portion size selected for estimation of GL. Thus, the long-term consumption of a carbohydrate rich diet with high GL value is correlated with increased risk of type-2 diabetes and coronary heart diseases. Thus, GL is an additional biochemical parameter along with GI to classify the foods for their ability to raise the blood sugar level. Formula to calculate GL: $[(GI \times \text{amount of available carbohydrate})/100]$.

8. BIOCHEMICAL INDICATORS FOR STARCH DIGESTIBILITY

8.1. *Resistant starch*

Apart from amylose and amylopectin as major starch component, some starch fraction present in rice bypass the enzymatic digestion called as Resistant Starch (RS). The RS is the residual fraction of starch, resistant to enzyme hydrolysis, entering the large intestine along with dietary fibre. Though the RS accounts only a small proportion of the total calorie intake, its effect is similar to those of other fibre components (Bjorck, 1996). Recently, RS has received more attention for both its functional properties and health benefits. It positively influences the digestive tract functioning and blood cholesterol level, thus help in the control of diabetes (Fuentes-Zaragoza et al., 2010). RS has similar physiological functions of dietary fiber which is completely resistant to digestive enzymes in the small intestine of human. Further, RS reaches to the large intestine where it acts as substrate for fermentation by microflora. As rice is consumed, the starch is usually hydrolyzed by α -amylases at the mouth and in the small intestine. However, RS which generally comprises less than 3% of cooked rice (Yang et al., 2006) escape digestion almost entirely and therefore, its calories are unavailable for cells to use (Sajilata et al., 2006). The RS content in rice grain have been established as an important indicators of starch digestibility (Kumar et al., 2018b). Englyst et al. (1992) proposed a classification system based on starch digestive rate. This system divides starches into rapidly digestible starches, slowly digestible starches and resistant starches



based on the results of their *in vitro* digestion. On the basis of enzymatic resistance and structural characteristics, RSs are categorized into five types: Type 1 RS is physically inaccessible starch fraction such as in whole or coarsely milled grains, Type 2 RS is resistant to enzymatic digestion such as native and raw starch granules of potato, green banana, high-amylose maize, Type 3 RS is retrograded form of starch made by recrystallization of starch chains during storage after cooking and Type 4 RS is chemically modified form of starch such as cross-linked with phosphate bonded starch. Latest categorization of RS is Type 5 RS which is formed in rice by association between amylose and lipids to form immiscible structure resistant to digestive enzymes (Table 2).

Table 2 Types of resistant starch in starchy foods

Form of RS	Description	Food source	References
Type 1 RS	Physically not accessible for digestive enzymes	Milled cereal grains and legume seeds	Sullivan et al., 2017
Type 2 RS	Ungelatinized form of starch	Raw potatoes, green raw bananas, high amylose maize	Zaman and Sarbini, 2016
Type 3 RS	Recrystallization of amylose (Cooked foods allow to cooled)	Potato and pasta salads	Zhang et al., 2013
Type 4 RS	Chemically modified starch	Modified starches not occurring naturally	Zaman and Sarbini, 2016
Type 5 RS	Amylose-lipid complex	Rice and other foods with high amylose content	Kumar et al., 2020

8.2. Amylose content

Physicochemical and metabolic properties of rice are influenced by several factors. Among them, AC is one of the important factors, which is often used to predict the rate of starch digestion, blood glucose and insulin responses to rice. The AC in rice grain starch is of great importance when determining the quality of the rice grain. It affects the stability, dietary fiber content, texture and viscosity of processed rice (Morita et al., 2002). Generally, starches with higher amount of AC are more resistant to digestion (Hu et al., 2004). The cooking and eating characteristics of rice which are the bases of choice for the consumers is determined by the AC and gelatinization temperature. For instance, there is good linear correlation between hardness and AC. Also, a positive correlation exists between AC and length/breadth ratio in relation to elongation of cooked rice. The AC of starches usually ranges from 15 to 35% (Okonet al., 2004). Rice low in AC are generally known to be sticky and moist, whereas those high in AC are non-sticky, flaky and dry. However, deviations from this correlation exist, which include low amylose rice that are non sticky and *vice versa*. Also, rice containing the same AC may differ substantially in hardness (firmness) and stickiness (Perez and Juliano, 1979). Amylose/amylopectin ratio of rice

cultivars is one of the three main factors that appear to explain most of the variation in glycemic and insulinaemic responses to rice; the other two are post-harvest processing (particularly parboiling) and consumer processing (cooking, storage and reheating) (Frei et al., 2003). Starchy foods rich in AC are associated with lower blood glucose levels and slower emptying of human gastrointestinal tract compared to those with low levels of amylose (Behall et al., 1988). Based on AC, milled rice is classified in "amylose group" as follows: waxy (1 - 2% amylose), very low AC (10 - 20% amylose), intermediate AC (20 - 25% amylose) and high AC (25 - 33% amylose). Waxy and low-amylose starches are desired for their influence on cooking quality and seed softness, especially in rice (Liu et al., 2008). Many investigators have reported that high AC rice exhibited lower GI values than low AC varieties (Hu et al., 2004; Denardin et al., 2012).

8.3. *Phytic acid*

Phytic acid (PA), a phosphorous storage compound comprising ~1 to 5 % in cereals, oilseeds and legumes. It is generally considered as an anti-nutritional factor because it reduces minerals bioavailability in both ruminants and non-ruminants (Kumar et al., 2017). It is also reported to have inhibitory effect on the activity of digestive enzymes where it binds to some amino acid needed for catalytic activity. Thus, changes in PA content in rice may affect starch digestibility and influence blood glucose level. Few other beneficial roles of PA have been reported as an antioxidant and anti-cancer substance. It also plays a role in stress tolerance against both abiotic and biotic stresses (Saha and Reddy, 2016). Though not much information is reported on the interaction between starch and PA, both can bind through phosphate linkages. Therefore, foods with high PA are of major concern, since a negative relationship between PA intake and blood glucose response has been reported (Yoon et al., 1983). As rice is a starchy crop, its digestibility could also be affected by binding of PA with digestive enzymes or starch associated proteins. In addition, PA can bind with Ca^{++} (a co-factor required for α -amylase activity) in the gut and reduce starch digestibility. Thus, higher the PA, lesser is the rate of starch hydrolysis and consequently lower is the GI of food (Fig. 2). A negative correlation between GI and PA was reported in the rice grain due to the presence of high PA which resulted into reduced starch digestibility (Kumar et al., 2020). The effect of PA on reduction of starch hydrolysis is beneficial for the diabetics as it reduces the level of blood glucose and improvises colon health by reducing the gastric emptying (Kumar et al., 2021).

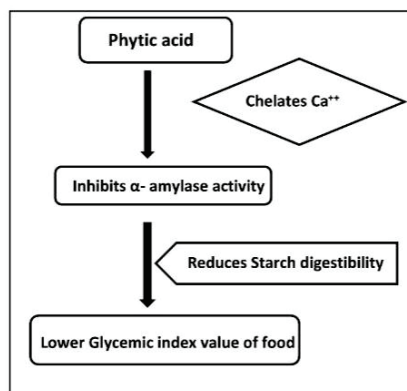


Fig. 2 Starch digestibility reducing mechanism of Phytic acid.

9. METHOD OF GLYCEMIC INDEX ESTIMATION

The GI of a food can be estimated through both *in vivo* and *in vitro* methods (Fig. 3). The *in vivo* method has long been used for the determination of GI in food products. Here, volunteers of the same age group fed with a certain amount of food after an eternal night fast then the increasing blood glucose level is checked and measured for 2 to 3 hrs at regular intervals. The *in vivo* method for GI estimation of food depends upon the metabolic rate and physiology of an individual which include the digestion of food and absorption of released glucose in the gastrointestinal tract (Hur et al., 2011). In contrast, the *in vitro* GI estimation is a rapid screening method developed in laboratory for determining starch digestibility of foods. It is a good alternative of *in vivo* method. The major disadvantage of *in vivo* method is that it is laborious, time taking and costly. Apart from handling the human volunteers, physiological differences of different volunteers make this method complicated with lack of precision and poor reproducibility. Further, *in vivo* method for GI estimation needs ethical committee approval and a complicated infrastructure of relatively large tested foods. Therefore, some laboratories developed digestive enzymes-based *in vitro* methods for GI estimation (Englyst et al., 2003). Thus, *in vitro* GI estimation method is better alternative as it is quick, cost-effective and large number of samples can be screened within a short period of time. The *in vitro* GI estimation method provides results with high reproducibility and is less time consuming. Hence, it is useful as a quick and rapid screening method of a large number of starchy food products and helps breeders to develop low GI rice varieties.

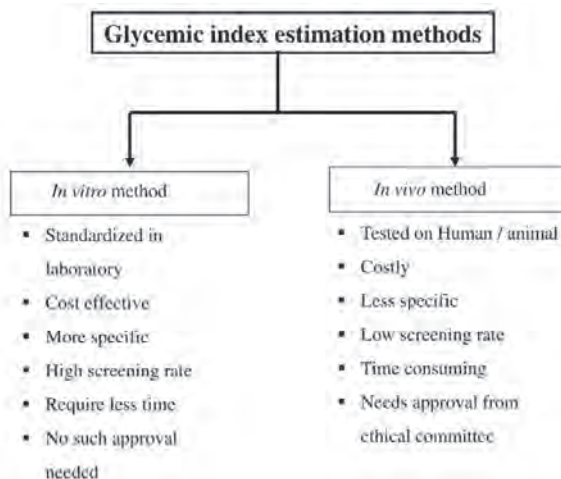


Fig. 3 Comparison between *in vivo* and *in vitro* GI estimation methods.

10. CONCLUSIONS

Rice is an integral part of the diet of about half of the global population and is a major source of calories consumed by more than three billion Asians. The demand for good quality rice has been increasing as it provides higher returns to farmers. Proper water, nutrient and pest management practices add not only to grain yield but also to grain quality. Most of the rice quality traits are genetically controlled except few physical and sensory qualities. Understanding the genetics and gene manipulation of these traits is very important to develop a promising variety. The concept of rice grain quality varies with the consumer's choice, purpose and the preference. Normally, the physical (grain size, shape and appearance), milling (capacity to withstand the milling pressure) and chemical qualities which determine cooking, nutritional properties and starch digestibility are the major determinants of rice grain quality. As more than 95% of rice produced is consumed directly as cooked whole grain, the grain qualities assume greater importance for rice compared to other food grains regarding the market price.

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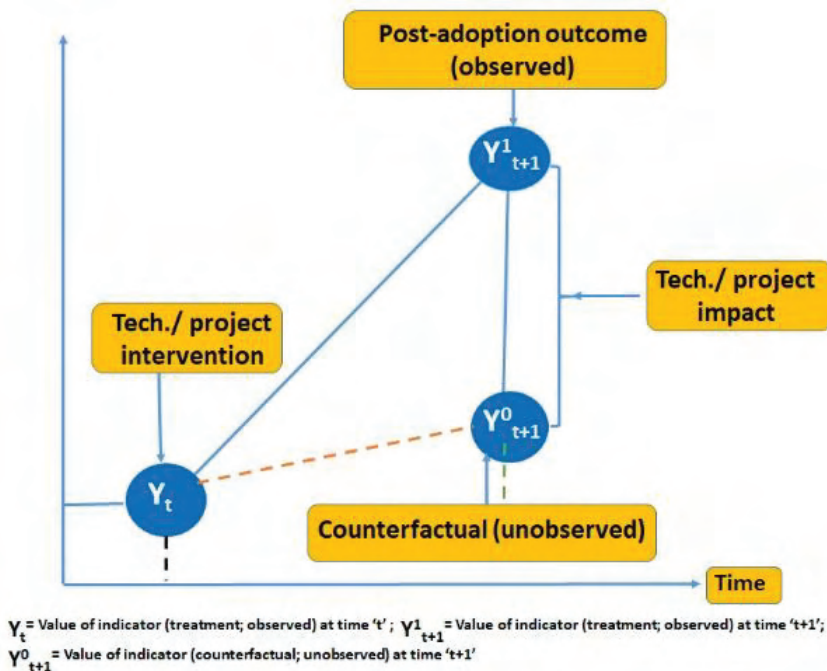
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RECENT APPROACHES OF IMPACT EVALUATION FOR RICE PRODUCTION TECHNOLOGIES: A METHODOLOGICAL PERSPECTIVE

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GRAPHICAL ABSTRACT



SUMMARY

Impact evaluation techniques using suitable economic and econometric criteria are important and support to justify the investment on research as well as technology adoption by the intended user. The evaluation may be of *ex-ante* or *ex-post* type and many mid-term evaluations within the period of *ex-ante* and *ex-post* assessment (concurrent). Evaluation of impact of any agricultural/farm technology is a continuous process, starts with the generation of technology itself and comprises of setting priority or *ex-ante* assessment, on-farm appraisal



of technology, implementation or adoption, and *ex-post* impact. A coherent method is desirable to identify the suitable indicators, quantify and value the returns (benefits), select true counterfactual for comparison and incorporate the spill-over effects. Various experimental and non-experimental approaches are available to evaluate the impact of technology or program and the choice of right technique to be adopted depends on the data obtainable.

1. INTRODUCTION

Impacts are the long-term outcomes generated from a programme, policy or project, typically in combination with other causes and actions by other interventions. These may be anticipated or unintentional consequences, affirmative or harmful, straight or secondary effects, which are larger than the aims specified. With the objective of responsibility, knowledge, worth for inputs/investment and moral behaviour, it is imperative that the word ‘impact’ comprises also the unintentional outcome, which may be undesirable in many cases. Impacts not only denote to what has occurred-but in few situations, the impact can be indicated as ‘to prevent negative effects’. Further, impact also embraces the concepts of decrease, evasion or inhibition of damage, peril, expenses or other adverse consequences (Warwick and Nolan, 2014). Negative effects, which have been stopped, are hard to identify and estimate, as by explanation, they have not been happened.

There are four motives for undertaking an impact evaluation (Jones et al., 2013; Jones et al., 2017): (i) advocacy (establishing the worth of a technology or a program), (ii) allocation of asset (fund, workforce and other inputs), (iii) analysis to update the information on continuous upgrading (including forthcoming programme strategy), and (iv) accountability (as needed for legal purposes and improved management of performance). Any specified impact assessment is expected to have a blend of these motives, though each may necessitate diverse data and dissimilar approaches for its collection.

Impact evaluation of projects/ technologies/ activities is the process of organizing and analyzing information about them, accomplishments, results and of determining and weighing the costs and benefits. It provides information about the actual achievements in the form needed by the planners/ managers/ program administrators/ policy makers and so on. An evaluation of an agricultural project/ technology attempts to:

- Determine the adequacy of the technology to overcome the identified constraints on agriculture
- Compare the actual attainments with the target set and identify the reasons for short-falls or over-achievements

- Assess the efficiency of technology and quality of managerial performance
- Determine the efficiency of the technology in economic terms
- Present the lessons learned and the recommendations that follow from them

For evaluation of a technology, all basic data at all stages *i.e.* from innovation to adoption by the end user and its uses are required. Hence, proper sampling technique is to be adopted for ensuring representation of the users and the progress at various levels should be monitored. For collection of data, a suitable questionnaire should be adopted and various components such as productive, protective, environmental etc. should be computed. Evaluation process often seeks to analyze a situation to determine why some thing happened, and suggest what might be done to correct undesirable situation.

2. TYPES OF EVALUATION

Evaluation denotes a uninterrupted practice, which starts at assessment, enduring with mid-term appraisals and at the end, followed by an *ex-post* evaluation, and preferably a supplementary assessment after five, ten or fifteen years later, or at the termination of the lifespan of the technology.

The evaluation may be of both *ex-ante* and *ex-post* type. *Ex-ante* assessment is done prior to real acceptance of the technology, so as to evaluate its financial viability in advance and to identify if the said technology is able to solve the problem or augment the outcome. *Ex-ante* evaluation is done for estimation of the anticipated output from the current or existing research activities. These assessments deliver valued evidence on the possible effect of the knowledge generated or technology developed and support to convince for sustained efforts and expenditure in promotion of the technology. But, substantial doubts remain in the creation of prospective situations as well as in the forecasts of understanding of the probable pay-outs from the investigation and the likelihoods of attainment (Alston et al., 1995). *Ex-post* evaluation is done after the technology has been adopted, so that it is known whether the technology really attained its intended aims and to aid in alteration/modification for corrections of deficiencies if any, in the existing technology. *Ex-post* impact evaluations provide the assurance to the researchers and stakeholders and present the report with the hope of higher research funding (Bantilan and Dar, 2001). There are one or many mid-term evaluation within the period of *ex-ante* and *ex-post* assessment (concurrent), so that it is known whether the manner in which actions are initiated and results are obtained during the technology acceptance period is working on the specified track or not. This process of appraisal would support in placing the implementation



procedure on precise way and aid in sinking the difference between the anticipated and observed output of the technology concerned.

3. IMPACT EVALUATION AS A PROCESS

Evaluation of impact of any agricultural/farm technology stands as a non-stop procedure (Manyong et al., 2001), which is well-postulated as a sequence linking various kinds of investigation at different phases. Impact evaluation started since the generation of technology and depending upon the process of development of technology. So, four phases of impact appraisal would comprise the impact cycle, which consists of (i) setting of priority (*i.e.*, *ex-ante* impact), (ii) on-farm appraisal of technology, (iii) implementation or adoption, and (iv) *ex-post* impact. There are various kinds of impact evaluation studies available, which are not mutually exclusive; instead these assist different as well as complementary roles simultaneously during the generation and diffusion of the technology. An impact assessment is a set programme of activities and required to be scheduled by adopting the typical stages as following:

- Identification of key users of the assessment and the particulars of uses and involving them in the planning process itself
- Identification of related outcomes and in what way they might be impacted. Reports about earlier research and assessment, key informants, and documentation of process, developing the steps indicating how different activities are expected to make the projected effects, and a negative concept viewing what would happen in the absence of the technology
- Developing a list of key assessment enquiries in brief, and developing probable answers against those key questions, using a suitable mixture of procedures and strategies
- Reporting of findings to the key anticipated users and provides them to practice the outcomes

3.1. Identification of indicator for evaluation/ assessment

Indicators are variables, statistics or measures, which assist to assess deviations in a particular condition, changes in state of something valued or changes in quality. Alternatively, indicators are intended to offer a threshold against which we judge and quantify the improvement of an action in contrast to specified goals. They give evidence and define the situation, which are beneficial to observe variations and indicates the way for comparing the movements and advancement over certain period. These are used as pointers of the development to achieve short-term, medium-term or long-term purposes. The selection of indicators is of crucial importance and is a highly skilled or professional job.

The foremost obstacles to identify the indicators are selecting those items, which are adequately demonstrative, simple to comprehend and quantify on regular basis. It depends on the type of the purposes and anticipated results and effects of the technology. Thus, the choice or selection of an indicator will depend on users who will be served, reporting period and data collection. Generally the choice of indicators is also guided by common sense, experience, knowledge, statistical data sources and what the project analyst wants to know. Ideal indicators should be of:

- *Specific* (clearly and unambiguously defined)
- *Measurable* (either qualitatively or quantitatively)
- *Achievable* (must be cost effective to monitor; result should be commensurate with the value of time and investment made to apply them)
- *Relevant* (consistent with the objectives and clearly reflect the goals)
- *Time-bound* (should be quite sensitive to change in the situation to be documented and sensitive to important changes such as in policy, programmes, and institutions)

3.2. Identification of benefits/ criteria and important steps for evaluation

A coherent method is desirable to identify, quantify and value the societal returns (benefits) and shortcomings (costs) through common monetarist components. The stream of common financial unit in respect of time is carried to a net present value (NPV). Unvalued possessions/ effects (intangibles) are defined in qualitative terms and weighed contrary to measurable substances. It is important that only additional or incremental benefits and costs impacts that are attributed from or to the technology should be considered. Comparison of benefits and costs is a normative approach which provides policy relevant conclusions and not dictating results. It also helps in priorities setting among investment alternatives. Therefore, benefit-cost analysis is a marginal analysis and required one base/ comparable data, upon which marginal costs or benefits should be estimated. The following seven steps are to be followed in conducting benefit-cost analysis.

Establishment of evaluation criteria: Maximization of social welfare is the general goal of agricultural technology and in maximization of profit/ income is taken as proxy for this.

Determining distributional consideration: Since project outcome is weighted, summation of costs and benefits of individual items after assigning weights, and the weights of respective additional unit of income may differ to each individual. In most of cases all monetary units are treated equally, regardless to



whom they occur. However, there is a scope for consideration of distributional aspect in technology evaluation but required more skill, time and costs.

Period of analysis: It is the economic life of the project (financial analysis from private point of view) or technical life (till benefits and costs occurred). Two important factors which decide the economic life of the technology are: (i) the anticipated useful physical/practical lifespan of the technology, and (ii) the rate of discount used (inverse relation i.e. if discount rate lower, higher will be the life span).

Identification of relevant input and output: The quantification of benefits that arises or likely to arise from adoption of agricultural technologies are generally treated as difference in benefits from, before and after the adoption. But the first point is that without adoption, situation is not generally synonymous with the before adoption situation and needs special consideration. Only additional benefits that are attributed to the technology be counted in evaluation. Similar is the case for input used with the technology.

Valuation of inputs (costs) and outputs (benefits): The input used in and output produced differs in physical terms and very difficult to judge the resource efficiency without price tag attached to use of resources and the production achieved. Therefore, label of price needs to be integrated to individual item. It may be market price under perfect competition, shadow price under imperfect competition and surrogated market price if the input used and/or output produced had no value in the market, but there are strong alternatives for them against which there is some market value attached. Cost price (assign some value on the basis of personal judgment) method is employed when there is no substitute. Cost accounting table for each enterprise is to be prepared separately for each year after adoption of the technology. Year-wise costs and benefits are then summed up to prepare cash-flow table for the technology as a whole.

Discounting: The rate of discount is an indicator to denote the rate of interest charged by the society as opportunity cost of time. Actually, time is the greatest irreversible loss to everybody. As time proceeds, prospects are inevitably lost. Thus, every individual desires to consume time in utmost competently. The appropriate discount rate is that rate, which signifies the rate of return on subsequent best alternate investment option having identical risks situation.

Considering uncertainty and risks (sensitivity analysis): Benefits and costs estimates are projected for future events which cannot be forecasted with certainty because (i) the bio-physical output from the technology adopted are determined by climatic sequences and random process, (ii) availability of limited data from which estimates of probability can be made, (iii) uncertainty in cost estimates due to unforeseen construction and implementation problems, and (iv) population growth, demand and supply may change the prices of

products and inputs in question. To evaluate the financial feasibility of a technology in the face of uncertainty, sensitivity analysis is undertaken. Sensitivity analysis is defined as analysis of magnitude of change in outcome to change in assumptions concerning input-output relationship and values associated with them. Sensitivity analysis can be done for technology life, discount rate, input output relations, prices and political uncertainty. By sensitivity analysis, the evaluator provides a set of switching values of the technology worth for different values of decision variables to the decision maker. These values provide the strength of the technology with respect to the level of changes in decision variables it can absorb and ultimate decision remains unaltered.

3.3. Selection of counterfactual

In an earlier section it is mentioned that impact evaluation process requires a true counterfactual that signifies the condition of beneficiary farmers without the project. In the comparison of a pre and post-project situation, the counterfactual is the participating farmers themselves, but prior to the initiation of the project. This may look like a sensible approach, as the participants at one time period are alike to themselves during a later period. However, the difficulties arise exactly due to the time dimension. Making comparison of the status of participating farmers before and after a project, assuming that there were no changes for other aspects except the commencement of the interventions under the project and, hence, the variations in impact indicators can be credited to the project alone.

The assumption is strong predominantly in projects related to farming. Weather parameters shifts each year which influence the important indicator like yields, which will change undoubtedly over time. Moreover, prices of output and input also change every year that impact choices of enterprise which in turn changes the returns from agriculture. A pre and post-project assessment is able to capture not only the effect of the project, but all of the additional deviations. Any *ex-post* assessment using this approach is expected to be biased and it is not possible to isolate actual project impact from the general time trends. So, using of such a method for impact evaluation of agricultural projects is not desirable.

3.4. Incorporating spill-over effects

As said earlier, it is more likely that agriculture related projects will experience spill-over effects at least inside local groups and perhaps outside the local groups also. This may be the potential reason for facing problems to identify the direct project effects, as it makes the contamination of the control group, and lead to underestimation of the impact. Therefore, if spill-over effects are



anticipated, assessment strategy should make actions for their incorporation into the evaluation strategy.

The foremost step to incorporate the spill-over effect into an assessment strategy is to consider the theoretical framework, why the spill-over effects are predictable. The two main explanations for spill-over effects are the presence of externalities and the effect of general equilibrium. It is precarious to comprehend why spill-over effects happen as it indicated that the non-beneficiaries, who are likely to be affected by the project indirectly. These indirect recipients of benefits should not be inducted into the control group as they are “contaminated” from the project effect, but they will get the attention of the analysis. For instance, in a project related to technology transfer, the main group which is expected to benefit indirectly from a project is non-participants in the same societies, where the project is running. Even though they are not recognized as beneficiaries in the project, they may absorb the skill about the technology or even receive the technology in proper from participants and choose to adopt the technology themselves.

Like estimation impact of the project for the direct beneficiaries of a project, capturing the impact on non-beneficiaries also challenging as there also we need to take a counterfactual—that is, a cluster of farmers with comparable features but who did not obtain even indirect benefits. Following Angelucci and Di Maro (2010), two approaches are taken care of: (i) double randomization, (ii) using eligibility criteria. These are measured distinctly though it is conceivable to have a mixture of these approaches to recognize indirect effect. As the aim is to quantity both direct and indirect benefits from the project, four sets of farmers are needed: (i) direct beneficiaries, (ii) a control group for direct beneficiaries (iii) indirect beneficiaries, and (iv) a control group for indirect beneficiaries. The direct recipients are those, who will receive the project benefits. The indirect beneficiaries are the farmers, who live in the vicinity to the beneficiaries and are expected to benefit through spill-over effect, for instance, farmers in the same community, who are more likely get the benefit for the fellow farmers who are the beneficiaries. The residual farmers should not be influenced directly or indirectly by the project and are considered as the control group. Since all the farmers fulfill the suitability standards, the random assignment should guarantee that the farmer groups are similar in all respect except that the direct beneficiaries get the benefits of the project and the indirect beneficiaries obtain indirect benefits from the beneficiaries. Comparison of the direct beneficiaries with the control group and the indirect beneficiaries to the control group offers the estimates of direct project impact (average treatment effect) and indirect project impacts (indirect treatment effects).

In certain cases, indirect beneficiaries are not part of the project as they are not entitled. In spite of ineligibility, they might be predisposed by the

program due to positive externalities or general equilibrium effect. For instance, if a project targets the poor farmers and decide to provide support to the farmers only with less than one hectare of land, farmers who are having more than one hectare of land living in the same community may incidentally benefit from the project. They may acquire the knowledge about the new valuable technology (positive externality) or because the project may impact the land value, labour, inputs or outputs (general equilibrium effect).

4. ECONOMIC EVALUATION OF TECHNOLOGY

The basic objectives of economic evaluation(s) is to measure the worth of a technology by comparing the values of goods and services produced or conserved with the cost incurred after taking all the effects of action into account. There are two broad categories of measures of worth of technologies: (i) undiscounted measures, and (ii) discounted measures.

4.1. Undiscounted measures of worth

In this measure, time value of money is not taken into account and it comprises:

- Ranking by visual inspection
- Cut-off period, which specifies the period within which cost/capital has to be recovered
- Undiscounted sum of benefits per unit of initial capital investment
- Average annual benefits per book value of capital investment
- Net average rate of return, which is defined as benefits minus cost divided by useful life of the technology

4.2. Discounted measures of worth

Appraisal and evaluation criterion falls under this category, when one essential element in common is that they account for the time value of money. The measure comes under this are described below:

Net Present Worth (NPW) or Value (NPV)

This is merely value of gross benefits (discounted) minus the costs (discounted).

$$NPV = \sum_{t=1}^n \frac{B_t - C_t}{(1 + i)^t}$$



where, B_t = value of incremental production out of the technology during time t ;
 C_t = incremental costs during time t ; i = rate of discount and t = expected life
of technology.

Decision rule: Accept the technology as worthy, if NPW or NPV >0 , otherwise
reject. Higher the NPV, better is the technology.

Demerits: This does not reflect the return per rupee invested on the technology.

Benefit-Cost Ratio (BCR)

It can be defined as the proportion of value of total production at current
period to the value of total costs at the same period.

$$BCR = \sum_{t=1}^n \frac{B_t/(1+i)^t}{C_t/(1+i)^t}$$

Decision rule: If $BCR >1.0$, accept the technology, otherwise reject. The
technology with higher B-C ratio is selected on the basis of their ranking till the
budget is exhausted. Among a set of alternatives we can choose the greatest
expensive alternative, if the incremental benefit-cost ratio surpasses one;
otherwise we can select less costly alternatives.

Demerits: It favours low-cost technology.

Internal Rate of Return (IRR)

This is specific rate of discount at which the value of the benefits during present
period equal to value of costs at the same period. In other words, IRR is that rate
of discount 'r', when

$$\sum_{t=1}^n \frac{B_t - C_t}{(1+i)^t} = 0$$

Decision Rule: Select the technology, if $r > i$, otherwise reject. The 'r' is internal
rate of return and 'i' is rate of interest to be paid on the money to be invested.

Demerits: Method is cumbersome and least suited to *ex-ante* assessment.

Pay Back Period (PBP)

It can be defined as the maximum time that will be taken by a technology to
recover the investment made on it. It is estimated by working out cumulative
NPV of the technology. It is that year of the project life of the technology when
cumulative NPV out of it become first time positive.

Decision rule: Select the technology with least payback period.

Demerit: The criterion does not favour the technology with long gestation period.

The benefit-cost decision tree under different situations is described in Table 1.

Table 1 Benefit-cost decision tree under different project situations

Decision	Constraint	Criterion
Accept or reject one technology	-	Accept if $NPW > 0$ or $BCR \geq 1.0$, $IRR \geq i$
Select one of several technology	-	Select technology with greatest NPW
Select several of many technologies	Budget constraint	Select in order of BCR till budget is exhausted
	No budget constraint	Select all technologies with $NPW > 0$

BCR: Benefit-Cost Ratio; NPW: Net Present Worth; IRR: Internal Rate of Return

4.3. Impact evaluation of new varieties

Assessment of returns to agricultural research investment is needed to convince donors and policy makers. The important parameters which are taken into consideration in research evaluation process (in this case development of new variety) are probability of success, time lags (innovative research, adaptive research and adoption by farmers), spill-over effects (technology and price) and external factors (economic, social, agro-climatic, government policies). The impact of new cultivars is assessed through adoption studies. Adoption studies can be broadly classified into two categories, viz., a) adoption of an innovation over time, and b) adoption of an innovation across farmers. The adoption path of new varieties is generally S shaped. However, in some cases, this may take linear or step wise paths. The adoption reaches maximum level, after which it becomes parallel to horizontal axis for some time. After some years, dis-adoption takes place due to availability of better varieties/technologies for that ecosystem.

The factors affecting adoption can be found out by regressing explanatory variables on the explained variable. Once factors/constraints are identified, policies/actions are initiated to relieve them, so that adoptions become faster.



4.3.1. Adoption over time

The logit model is commonly used to find out the rate of adoption over time. The model can be specified as follows:

$$A_t = \frac{K}{1 + e^{-(a+bt)}}$$

where, A_t = Area under modern varieties as a percentage of total area in year t ;

K = Ceiling or saturation level of percentage area;

a = Constant or intercept parameter;

b = Growth rate of adoption; and

t = Year code which varies from 1, 2, 3,....., n .

In case of time series data, the factors affecting adoption can be identified by regressing appropriate independent variables on the percentage area under modern varieties as dependent variable.

4.3.2 Adoption across farmers / land types from cross section data

The percent area under modern varieties across farmers / land types can be computed for a few years according to availability of data. Very often, extension workers and policy makers are interested to know the factors affecting adoption for which, one has to carry out regression analysis. Once factors are identified through this technique, steps are taken to relieve constraints, which will facilitate adoption. Here two things need attention, first, specification of variables and second, choice of functional form. In case of cross section data, in extension studies, often the dependent variables are found to be dichotomous *i.e.* adoption (1) or non-adoption (0). To handle such cases, the following three models are used: (i) the linear probability model; (ii) logit model, and (iii) probit model. In cases, where the dependent variable is continuous along with 0, tobit model is used.

5. EVALUATION DESIGN AND RECENT APPROACHES

There are various methodologies that need to be considered for evaluating technologies and agricultural programs. After selecting the option for the evaluation, additional information needs to be acquired from other sources. The key factor which has to be kept in consideration during designing the assessment plan is the close relation between the data and the methodology for ensuring unbiased impact estimate. Data need to be collected from representative samples of experimental and control units or respondents,



suitable statistical techniques can be employed to assess the impact. In fact the finest technique for impact assessment of a technology or project relies upon the quality of available data which needs less sophisticated econometric procedures. As such models essentially depends upon few assumptions, greater emphasis should be given on better data collection for avoiding use of more difficult econometric techniques.

Largely, impact assessment approaches are categorized as experimental and non-experimental methods. Experiments denote the process where the treatment and control groups are allocated randomly for judging that both are similar from every respect excluding the treatment effect. Actuality, seamless experiments are tough to plan and if designed also, it frequently faces problem in execution. In certain situations, modifications are generally required during analysis for ensuring that effect can be identified correctly. Both the methods are elaborated under the following sub-section:

5.1. Experimental approaches

The crucial concern in impact assessment is the situation, which is not imaginable in the sense that have occurred to the technology adopters or participants of the project without adoption of technology or its implementation. This is considered as the true counterfactual. The nearby to this perfect situation would be a cluster that matches with all the attributes to join in the project or adopt the technology and has a comparable degree of similarity with the treatment groups. For instance, a department is apprehensive that lack of cash during cropping season are inhibiting the farmers to procure the inputs and contributes to food security through adequate production of grains. The department can think to transfer the cash directly assuming that the support would enable them to procure the inputs as per their necessity. Now if the impact of such project is to assess, eligible farmers need to be identified first using some kind of standards, say having five acres area and making about 50% of their earning from farming activities. Once the eligible farmers identified, they can be randomly allocated to one category who will be receiving the cash benefit and a control group who would not receive the particular benefit. Considering the experiment is big enough, the experimental group and control group are expected to be similar in every respect excluding one group obtained the cash benefit, while others didn't. Both the categories were eligible to receive the benefits and the group who received selected randomly and eliminating systematic variations, if any among the two groups. If the sample is large enough, analysis of collected data from treatment and control group will provide unbiased estimates of impact in terms of various indicators like use of input, food security, and farmers' well-being in the above case.



5.2. *Non-experimental approaches*

Various non-experimental approaches can also be adopted to evaluate the impact of technology or program in case conducting an experiment is not feasible. The suitable technique to be adopted depends on the data available. If we use secondary data, the finest non-experimental method again depends upon the type of data available. If primary data is being used, the best technique depends on details of the project, including the stakeholders of the project and the way the project will be initiated and carried out. Finest method needs seeing the existing choices and choosing the best conceivable one or set of choices.

5.2.1 *Difference-in-difference*

The difference-in-difference (*DID*) method is one of the most widespread non-experimental methods in impact evaluation as it permits adjusting for certain kinds of selection in a direct and instinctive means, as long as benchmark information is obtainable. In DID method, the pertinent contrast is alterations of indicator along with the time. Therefore, the contrast in a DID method is among the movements in the control group during pre and post -project versus the movements in the experimental cluster (Fredriksson, 2019). The difference-in-difference then denotes the changes with time (the first difference) and shift from the control to the experimental (the second difference). If the movements are considerably larger towards the experimental group (in a statistical sense), this advocates for the technology/program exerts an influence. Therefore, the estimators of DID pools temporal and cross-sectional deviation to exact for changes among the categories, when treatment and controls initiate from dissimilar levels.

5.2.2. *Instrumental variables approach*

The instrumental variable method is a procedure, which is frequently applied when a program comprises some kind of self-selection and there is an apprehension that un-noticed variations among the group who obtained the benefits and other group who didn't direct to a biased estimations of impact (Pokropek, 2016). This kind of situation arises in agricultural technologies/programmes in which farmers select themselves as beneficiaries or adopters. For example, if we consider a program aiming to enhance income from agriculture for farmers who self-select themselves as a beneficiary of technology or a participant of a training course. Now, it is anticipated that some inherent attributes, which influences farmers' adoption of technology or involvement in training programme might also affect their income, such as managerial skills, entrepreneurial capabilities, inspirations, ambition to prosper, and so on. Comparison among the beneficiaries and non- beneficiaries will comprise the bearing for the program and an inherent characters of the

participants. More specifically, in this situation, applying regression analysis is not advisable for identification of contributory factors for the participation (P_i) on the variable of interest (Y_i). This is due to the unnoticed features, captured by random term (ϵ), which would be correlated with both participation (P_i) and income (Y_i).

5.2.3. Propensity-score matching

In the case where an experimental design is not available, assigning the treatment is often non-random, and therefore, adopter/participant (of technology or a program) receiving the benefits from technology/projects and those missed the benefits may vary in their treatment level as well as in other attributes which affect both participation and the consequences. Propensity-score Matching Method (PSM) desires to get non-treated farmers who are comparable to treated farmers in all other respect except the situation that they did not receive the benefits out of the program. The method performs this by identifying matches with the experimental and control farmers by generating propensity scores (Imbens, 2004). Identification of control using propensity scores which is alike the experimental group, PSM makes what is referred to as a “quasi-experiment” as the control set is comparable to the experimental set statistically. Precisely, it assumed that the outcome variable cannot be detected for the treated cluster with and without the program, so it appears indispensable to find a control group to identify what is the likely effect to the receivers without the program. Alternatively, a set of farmers, unit of production or farm households are required who are very alike statistically in every noticeable feature against who received the benefits of the program.

5.2.4. Regression discontinuity

This is also a non-experimental method which is employed for assessing the programs where the target based on an explicit variable that describes a perfect threshold for participation. For instance, a program that is targeted for small farmers only with the holding size less than 5 acres of, BPL (below poverty line) farmers or those farmers who earn lower than the regional average. Following this technique, any farmer beyond this threshold will not be eligible, alternatively all farmers less than the threshold will be provided with the benefits. Certainly, the variations among the farmers on both sideways of the threshold might be very low or might be noticeable. The process of targeting advocates a strong case for fixing the thresholds that defines the treatment and permits to apply regression discontinuity design (David and Lemieux, 2010). The main idea behind this technique is that near to the threshold, the assignment to either of the group-treatment and control is likely to be random.



Alternatively, farmers who are remain near the cut-off (above or below) are almost parallel, thus, signify a correct counterfactual for the treated category.

5.2.5. Decomposition analysis

The introduction of New Technology (NT) in agriculture has many beneficial effects like increased output, employment, input saving, etc. The NT may be perceived in terms introduction of High Yielding Varieties (HYV's) of crops, high yielding breeds of livestock, improved cultural and management practices, etc., which may result in shifts of production function upwards and production parameters. To measure the contributions from various sources like technology and inputs to the output growth, Bisalialah (1977) developed Decomposition Model.

The information on the 'technology contribution' to increased output is very useful in determining the returns to research investment while switching over to NT from traditional technology. Many studies in Indian agriculture used this model in finding the sources of output growth in the areas like HYV v/s local varieties, Bt Crops v/s Non-Bt Crops, IPM v/s Non-IPM farms, Contract farms v/s Non-contract farms, Organic farms v/s In-organic farms, Exotic breeds v/s Local breeds of Cows, Yield Gap Analysis, etc. For using this model, one need production parameters for the same number of variables under two situations and geometric mean levels of both output and inputs. A Cobb-Douglas production function can be used for the analysis as slope coefficients directly represent the elasticity of production in this functional form. The production function (per hectare) fitted and estimated using the OLS method used for decomposition analysis (Fig. 1).

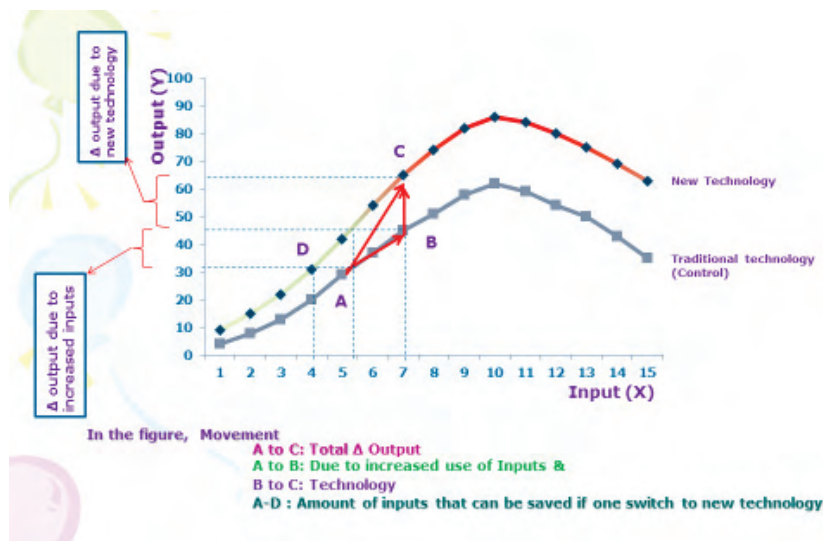


Fig. 1 Decomposition of output growth into technology and input components

If we desire to decompose the effect of an improved variety (new technology) over traditional variety (old technology), initially a production function used to be fitted using pooled data from both experimental as well as control plots (equation 1):

The general form of the function can be used as below.

$$\ln Y_i = \alpha + \sum_{j=1}^5 \beta_j \ln X_{ij} + u_i \dots\dots\dots(1)$$

Where, Y_i denotes the gross returns from the improved variety for i^{th} farmer, X_{ij} 's are the expenditure on j^{th} input with respect to i^{th} farmer, R and β 's are designated as scale and slope coefficients and u_i is independent random errors, which is identically distributed with normal distribution $[N(0, \sigma_u^2)]$.

The variables to denotes various inputs (X_j 's) comprised in the model were expenditure on seed (X_1), agrochemicals (X_2), labour (X_3), machinery use (X_4) and other miscellaneous expenses (X_5). The production function can be fitted and estimated separately for each category of treated and control.

It is to be assessed whether production function of two varieties shows any 'structural break' which can be examined using either the 'dummy variable technique' or Chow's 'F' test considering the value 1 for improved variety and zero for traditional variety (equation 2) as:

$$\ln Y_i = \alpha + \sum_{j=1}^5 \beta_j \ln X_{ij} + dD + u_i \dots\dots\dots(2)$$

If the coefficient of dummy variable [D] found to be significant, then that indicates that there is a structural break in production relations among the two categories of plots and suffices justification to go for decomposition. Then, two separate production functions can be estimated for improved variety (equation 3) and traditional variety (equation 4) and parameters obtained are plugged into the decomposition model to find out the various sources contributing to the output difference.

$$\text{Log}Y^* = \text{log}R^* + \beta_1^* \text{log}X_1^* + \beta_2^* \text{log}X_2^* + \beta_3^* \text{log}X_3^* + \beta_4^* \text{log}X_4^* + \beta_5^* \text{log}X_5^* + u^* \dots\dots\dots(3)$$

$$\text{Log}Y = \text{log}R + \beta_1 \text{log}X_1 + \beta_2 \text{log}X_2 + \beta_3 \text{log}X_3 + \beta_4 \text{log}X_4 + \beta_5 \text{log}X_5 + u \dots\dots\dots(4)$$

By re-arranging the above equations, the decomposition equation was specified as below.



$$\text{Log } [Y^*/Y] = \text{log } [R^*/R] + [(\beta_1^* - \beta_1) \text{log } X_1 + (\beta_2^* - \beta_2) \text{log } X_2 + (\beta_3^* - \beta_3) \text{log } X_3 + (\beta_4^* - \beta_4) \text{log } X_4 + (\beta_5^* - \beta_5) \text{log } X_5] + [\beta_1^* \text{log}(X_1^*/X_1) + \beta_2^* \text{log}(X_2^*/X_2) + \beta_3^* \text{log}(X_3^*/X_3) + \beta_4^* \text{log}(X_4^*/X_4) + \beta_5^* \text{log}(X_5^*/X_5)] + (u^* - u) \dots \dots \dots (5)$$

Where, Y^* and Y are the gross returns from the improved variety and traditional variety; X_j^* 's and X_j 's are the expenditure on j^{th} input for treatment and control.

The decomposition equation (5) includes decomposing the natural logarithm of the ratio of gross income of the treatment group and control group. The expression inside the first bracket on the right hand side is a size of changes in returns (in percent) due to shift of the scale parameter (R) of the production function and this is considered as the neutral component of the technology or program. The expression inside the second bracket, which is the addition of the changes in output elasticities, which further weighted by the logarithm of inputs used in production functions for control group and considered as the degree of alteration in production/income results from shifts in slope parameters (non-neutral component of technology). The term under (third bracket) denotes the gap which is attributable to variations in input use weighed by the slope coefficients of the production function, fitted for treatment groups and explains the effect of inputs.

5.2.6. Estimation of technical efficiency

Technical efficiency (TE) referred as the capability and preparedness of an improved technology to get the highest probable production by an indicated arrangement of resources (which is signified by a frontier production function), with a given technology and environmental set-up (Aigner et al., 1977). Considering a farm with a plan of production (Y° , X°), where the former term is one or a group of outputs and the later term characterizes the group of inputs. With a given production function $f(\cdot)$, the farm is said to be technically efficient, if $Y^\circ = f(X^\circ)$ and technically inefficient, if $Y^\circ < f(X^\circ)$. Thus, the TE can be estimated by the relation $0 \leq Y^\circ / f(X^\circ) \leq 1$.

This estimation accepts that the production function of the unit, which is fully efficient is known in some manner. Since this baseline of frontier production function was not ever recognised in exercise, it is recommended that it can be assessed from the sample information using either a non-parametric approach or by a parametric function like a Cobb-Douglas function. The disturbance term in stochastic frontier is decomposed into two parts; a symmetric part that measures randomness beyond the farmer's control and the statistical noise which can be found in all experimental association and another single-sided component which is under the farmer's control (*i.e.*, inefficiency).

Officially, $Q_i = f(X_i) \exp(e_i)$

Where, $e_i = v_i - u_i$ and $i = 1, 2, 3, \dots, n$; $-\infty \leq v_i \leq +\infty$ is the symmetric component; and $u_i \geq 0$ is the single-sided component. Q_i signifies the production of the i^{th} farm; X_i denotes the amount of i^{th} input used in the production process. The v_i 's are expected to be independent and identically distributed random error, follows normal distribution; $N(0, \sigma v^2)$ and not related to the u_i 's. The u_i 's are called technical inefficiency which are presumed to be non-negative truncation and follows half-normal distribution. A larger value of u_i specifies greater technical inefficiency. If u_i becomes zero then it is considered that the farmer is perfectly technically efficient. FRONTIER 4.1 is the available and open-access software used to fit and estimate (through maximum likelihood method) the stochastic model (Coelli, 1996).

5.2.7. Economic surplus approach

Economic surplus, also called total welfare or Marshallian surplus (named after Alfred Marshall), talk about to two associated measures, viz., Consumer surplus and Producer surplus. Consumer surplus is the gain in monetary terms attained by customers or consumers as they are capable to buy an item at a price which is lower than the maximum amount which they are ready (willing) to pay. Producer surplus is the quantity that producers received by selling at a price which is greater than the minimum amount at which they would be ready (willing) to sale which is approximately equivalent to profit (as sellers are not usually ready to sale incurring a loss, and are generally uninterested to marketing at a break-even price also). The goal of the economic surplus technique is to quantify the combined benefits to the society due to embracing a new technology (Briones et al., 2005). Using this

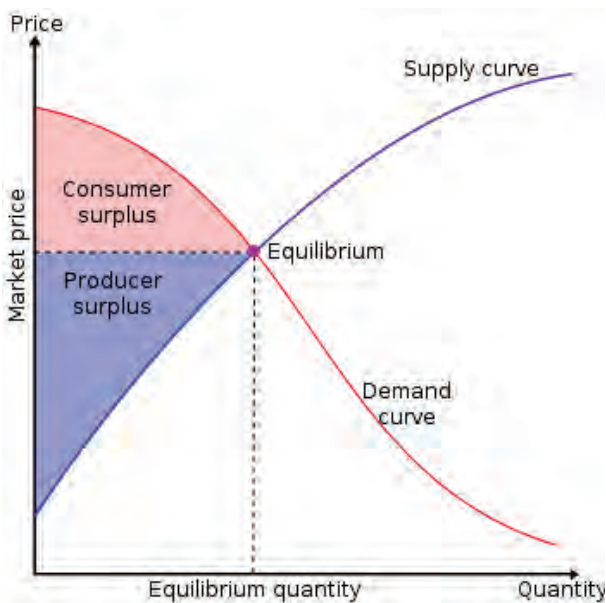


Fig. 2 Economic surplus as sum of consumer and producer surplus



technique, it is imaginable for estimation of the return over investments by calculating the increment in both the surplus in terms of technological shift created by research. Subsequently, the economic surplus is employed in association with the investment on research for estimating the net present worth (NPW), the benefit-cost-ratio (BCR) or the internal rate of return (IRR). The key benefit of employing the economic surplus technique is that it requires fewer data in comparison to other techniques. The economic surplus method allows the assessment of the financial paybacks created by acceptance of scientific inventions, related to the condition earlier, i.e. without the adoption, when only traditional technology was available and used.

Consumer surplus: It is the variations among the highest price a customer/consumer is ready/willing to offer and the genuine price they do pay. If a consumer is ready to pay higher price than the asked price, then they are receiving extra advantage out of the procured items than they paid initially. For instance, a product with normally great consumer surplus is drinking water. People are ready to pay large amount for drinking water, since it is needed for survival. The variation in the price that they are ready to pay, if they had to, and the amount that they are paying actually is their consumer surplus. It is to be noted that the usefulness of the first few bottles of drinking water is unusually larger (as people die without it), thus, the first few bottles will be having higher consumer surplus than the successive bottles.

The highest price a customer would be ready to pay for a certain amount of the commodity is the summation of the highest price they would pay for the first unit, then they would be ready to pay little lower for the second unit, then even lower for the subsequent units, etc. Usually, these prices are diminishing, which can be explained by the individual demand curve. The total of consumers' surplus is the addition of the consumer's surplus for every separate consumer. The concept can be explained graphically as shown in the Fig. 2, where the consumer surplus (individual or aggregated) is the area under the individual or aggregated demand curve and above a horizontal line at the actual price (in the aggregated case it is the equilibrium price).

Producer surplus: This is an economic measure of the changes between the amount that a producer of a commodity obtains and the minimum amount that he or she would be willing to receive for the commodity. The variation, or surplus amount, is the benefits obtained by the producer for selling the good in the market. This can also be indicated in Fig. 2 as the area above the supply curve of the producer that it receives at the price point. The extent of this area rises as the price for the commodity moves upwards.

6. WRITING AN EVALUATION PLAN

To decide choices associated with collection of data and procedure for the impact assessment of a particular technology or project, an evaluation plan need to be prepared. The plan helps in scheduling the detail of the activities, it is necessary to conduct the appraisal as well as giving direction for the project authorities, officials of government department or assessors who conduct the assessment for knowing what was envisioned. Levy and Cossens (2011) have composed a guideline for preparation of an appraisal strategy in which three key elements were emphasized, *viz.*, (i) selection of indicators, (ii) choice of appraisal technique, and (iii) formulation of a plan for sampling and collection of data. Alongside the theoretical discussion about these concerns, the plan should also comprise the feasibilities of the appraisal techniques. The three main facets need to be considered during designing phase to guarantee a fruitful execution of the appraisal process are: (i) building the plan of work and choosing the evaluation group/team, (ii) preparation of budget for the appraisal, and (iii) getting information from counterparts. The appraisal plan offers a detail direction to the evaluation process of the agricultural technology or project. Further, it is also necessary to indicate clearly so that if leader or members of project team changes, rest of the members or new leader reading the plan can comprehend the envisioned method to implement and do the appraisal without any hindrance.

7. CHALLENGES IN EVALUATING AGRICULTURAL TECHNOLOGIES AND PROJECTS

The usual challenge of any impact assessment process is to determine what will be the effect to the beneficiaries/participants without the project. To comprehend the project impact on a set indicator, data would preferably be obtainable from recipients of project benefits with the project and the same recipient without the project. Whether the project had an impact could then be equated between these two states with respect to a set of indicators. In fact, participating farmers cannot be present simultaneously as the project beneficiaries and out of the project at the same time period, making it essential to catch a supernumerary cluster of farmers to act as the counterfactual—that is, likely effect without the project. To become eligible as a genuine counterfactual, this group would require to be precisely the same as beneficiaries of the project, or treatment group, excepting they are not receiving the project benefits. Therefore, any variations in the indicator can be accredited to the project only. Making a true counterfactual by recognizing a rational control group and guaranteeing that a recognized effect can be credited to the said project is really a challenging task. The most prevalent question with appraising the agricultural



projects is that they frequently comprise self-selection of the beneficiaries. For instance, agricultural extension schemes usually benefitting the self-formed group of farmers as they enjoy better contacts with the extension agencies. Projects that give loan or insurance facilities to farmers might inspire the farmers to receive the proposal but a few of them only will accept. Self-selection indicates that some confident farmers may choose to participate in certain project. If an appraisal tries to estimate the effect of a project by comparing those who chose to be in the project with other groups who did not, alteration in the indicator of attention may reveal not only the project impact, but also any inherent variations among the participants and non-participants. The situation may also arise, when a better doing farmers decide to participate in a project, meaning the farmer is advanced and like to experiment in his field, such farmers are more expected to reap greater harvests even without participating in the project. Now a judgment of production between these advanced, treated farmers and non-participant, control farmers is expected to display higher yield difference due to the project. The problematic issue is that it is difficult to assess how much of the productivity variation is owing to the project and how much to the changes in farmer type. The situation makes any estimation of impact of the project biased as the estimate cannot exclusively be credited to the project. Evidently, choice of beneficiary or participants is also an important issue since a farmer with some specific characteristics is selected under the project. If a project emphasizes on farmers with limited land resources, persons with greater holding size are not likely to be a better comparison. Nevertheless, these characteristics are likely to be noticeable as the project must detect them to recognize who will really participate. With the careful appraisal strategy, predominantly if completed in grouping with the project design before the implementation of the project, it is probable to generate a rational counterfactual and evade biased estimates of impact.

Alongside the issues in selection, another serious concern for agricultural projects is related to incidental or spill-over effect. Spill-over effect denotes the impact of a project on individual or households who are not directly intended as the project beneficiary. Spill-over effects comprise interaction effects and externalities as well as overall equilibrium effect. Interaction effect and externalities are prevalent in agriculture as the practices in agriculture, both productions related as well as instruments for networking with the market, are frequently shifted from farmer to farmer. In fact, projects on agricultural extension and transfer of technology projects are generally planned to have spill-over effect. For example, the programme is designed to train a small group of persons about a certain technology to offer knowledge and train them so that the participants can further spread it and train other farmers.



Spill-over effects make two challenges; first, if a project expected to have spill-over effects on the recognized control group, this might result in inappropriate impact estimates. Suppose a technology, say a new seed variety which increases productivity. A project has been initiated to distribute the seeds randomly to the farmers in a pre-defined area. Those chosen to collect the seeds without any fee comprised the treatment group and those not selected are the control. This looks like a fine appraisal strategy because with a sample of large size, the random assignment should guarantee treatment and control is alike in every aspect, barring the benefits from the project. The problematic situation arises if those who received the new variety part it with their neighbors, who were considered as control. This may happen instantly or over time as the worth of the new variety becomes known. If seed has been shared with the control group, the control group will be reaping the paybacks of the new variety and any evaluation will undervalue the project impact—the assessment would be a comparison of a treatment group who got full benefits of the new variety to a control group who at least got some benefits. So, we can say that the control group is “contaminated”. Another issue may also crop up when the spill-over effects can be fairly larger, spread to the better-off area, and then it can exceed the direct impact of the project, especially in projects related to agriculture, where technology is easily transferred.

In the given situation, not recognizing these effects can intensely undervalue the inclusive effect of the project. In certain cases, an assessment process which flops to identify the spill-over effect of a project may result in the discontinuation of the project, while considering the spill-over effects properly leads to the situation that the project could have been watched as very fruitful. So the real challenge is to design an assessment strategy that captures both direct and spill-over effects and estimates the total project effect.

Some other precise concerns are there in evaluating agricultural projects, which make them unlike appraisals carried out in the social sector. First, agricultural projects are normally planned to increase production or the income to agriculture and thus impact assessments of such projects emphasized the production-based indicators such as: agricultural investment, spending on agricultural inputs, technology adoption, changes in land use patterns, crop and varietal diversification, yields, productivity, gross margins, crop prices, and food for home production, etc. Gathering this sort of data is challenging. The challenge is even larger when trying to assess the effect of a project on diverse categories of families, such as small-farmer and large-farmers, who relatively have very different production arrangements. Secondly, impact assessments frequently emphasized on investigating a set of indicators in order to get an image of the complete typical effects of the activities as well as the instrument by which these impacts were achieved. In analyzing agricultural



production, the association among inputs and outputs or viability is frequently inspected through production or profit functions. Seemingly, agricultural projects have an effect not just on inputs and outputs of productions, but on the way they are used and pooled, that too needs to be measured in an assessment process.

8. CONCLUSIONS

The operational method depends on the features of the technology or project as well as the data which is available or need to be collected. The common practice amongst the evaluators is to work with many procedures or diverse stipulations to estimate the influence of the project. Though the fund and the data available limit the options, but in case it is unconstrained, then it is considered to be a worthy exercise to choose a particular method or bunch of methods as it aids to guarantee that the assessed impact is properly known. The anticipation is that any effective estimation procedure should get similar outcomes and replicate the accurate effect of the technology or project. Using various approaches or altered stipulations also aid to check the robustness of the impact estimates. As part of the project design, if evaluation strategy also designed, it is helpful to consider not only the best individual strategy, but also the options that might be required or necessary to confirm the correctness of the estimates and in turn, the impact of a technology or a program.

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