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Standard Operating Procedure for Breeder and TL Seed Production of Rice Hybrid at ICAR-CRRI

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P R E F A C E

This Standard Operating Procedure (SOP) outlines the detailed guidelines for breeder seed and truthfully labeled (TL) seed production of rice hybrids. These processes are crucial for ensuring the consistent quality, purity, and genetic integrity of hybrid rice varieties, which play a significant role in meeting the global demand for high-yield, disease-resistant, and nutritious rice crops.

Rice hybridization, which involves the crossing of two genetically distinct rice lines to produce progeny with superior traits, requires meticulous management from seed multiplication to the distribution of certified seeds. Breeder seed production focuses on maintaining the genetic purity and high-quality standards of the parent lines, while TL seed production ensures that farmers receive seeds of known quality, with proper identification and traceability.

The SOP is designed to standardize the practices involved in seed production, to enhance efficiency and reliability, and to minimize the risks of seed contamination or loss of hybrid vigor. It is intended for use by all stakeholders involved in the production, processing, and quality assurance of hybrid rice seeds at various stages of the seed chain.

This document will cover all aspects of breeder seed and TL seed production, including the selection of parent materials, cultivation practices, field management, harvesting procedures, seed processing, storage, and certification. Adhering to these practices will ensure the production of high-quality seeds that contribute to the sustainable growth of rice farming.

By following the SOP, the stakeholders involved in hybrid rice seed production will help ensure the continued success and profitability of hybrid rice farming, fostering improved food security and agricultural development globally.

Authors

Introduction

The success of hybrid seed production however demands higher out-crossing rate to obtain high seed yield. Therefore, hybrid rice seed production requires specialized techniques, which need to be thoroughly understood before embarking upon this venture. Various factors that affect hybrid seed production include choice of field, isolation, seeding time, planting pattern, weather conditions during flowering, rouging, and synchronization in flowering of the parental lines, supplementary pollination and post-harvest operations. The present effort aims at providing critical information for successful hybrid rice seed production, which will of immense use for the agencies/ individuals involved in such a venture.

Breeder Seed (BS) of parents of hybrids is a crucial in the commercial seed production chain of hybrid. It plays an essential role in producing TL and certified seeds of hybrids, while maintaining the genetic purity and integrity of a crop variety. Typically derived from nucleus seed, BS can also originate from itself under certain conditions. When produced from nucleus seed, it is classified as Breeder Seed Stage-I, and the progeny from Stage-I becomes Breeder Seed Stage-II.

BS is vital for preserving the genetic traits of a rice variety, such as yield potential, disease resistance, grain quality, and other agronomic characteristics. Without pure breeder seeds, genetic contamination can occur, leading to a loss of these valuable traits. To ensure quality, breeder seeds are produces under close monitoring of breeder and field management. This ensures the production of high-quality seeds with strong germination potential, genetic purity, and a disease-free status.

Objective

The goal of developing this Standard Operating Procedure (SOP) is to create a clear, standardized, and reproducible framework for producing breeder and TL seeds of rice hybrids at ICAR-CRRI and agencies involves, ensuring the maintenance of their genetic purity and quality.

Scope

This SOP covers all activities involved in the production of breeder and TL seeds of rice hybrids, from seed sowing to harvesting, sampling, testing, and packaging. It is specifically designed for use at ICAR-CRRI to systematically monitor the production and handling of breeder and TL seeds on an ongoing basis.

Standard Operating Procedure	
Stages	**Guidelines**
Variety selection	The selected hybrid must be a released and notified variety of ICAR-CRRI through CVRC or SVRC ➢ Early variety with < 115 days' maturity ➢ Medium variety with 116-135 days' maturity ➢ Medium late variety with 136-150 days' maturity
Producing agency	*Breeder seed of parent:* Breeder seeds of parents will be produced by breeder, or a qualified breeder from the same institute, or breeder seed production unit of the parent institute. *TL seeds of hybrid:* TL/certified seeds of hybrids will be produced by parent institute or licensees involved either public or from private sector.
Nucleus seed source	• Paired crosses of parents will be made and evaluated by original or assigned breeder or Nucleus seed production unit of the parent institute under strict inspection. • Pure seeds of qualified parents will constitute nucleus seed which to be utilized in breeder seed production of A, B & R lines.

Field selection	➢ Seed production plots should have proper irrigation and drainage facilities, be free of volunteer, weedy, or wild rice, and have no record of such infestations in the past years. ➢ For hybrids, breeder seed of the parents is produced under the breeder's strict supervision, while the hybrid is TL or certified seed produced from the breeder seed. • *BS of parents:* to be produced at institute farm, A and S blocks • *TL/certified seed of hybrid:* to be produced at institute farm, A and N blocks; and farmers field through seed agencies and FPOs. ➢ Seeding of the parental lines should be planned in such a way that the flowering in both parents coincides with the most favorable climatic conditions, which are as follows: • Overall daily mean temperature of 24^0– 30^o C • Relative humidity ranging from 70 – 80 % ➢ Seed production plots should have proper irrigation and drainage facilities, be free of volunteer, weedy, or wild rice, and have no record of such infestations in the past years. ➢ For hybrids, breeder seed of the parents is produced under the breeder's strict supervision, while the hybrid is TL or certified seed produced from the breeder seed. • *BS of parents:* to be produced at institute farm, A and S blocks • *TL/certified seed of hybrid:* to be produced at institute farm, A and N blocks; and farmers field through seed agencies and FPOs. ➢ Seeding of the parental lines should be planned in such a way that the flowering in both parents coincides with the most favorable climatic conditions, which are as follows: • Overall daily mean temperature of 24^0– 30^o C • Relative humidity ranging from 70 – 80 % • The differences between day and night temperatures should not be more than 8^0–10 ^{o}C (5^0-7 ^{0}C is optimum). • Sufficient sunshine with moderate wind velocity (2-3m/sec). • There should not be rains continuously for three days during the period of flowering. • Yield will be adversely affected if overall daily mean temperature during flowering is below 20 ^{o}C and above 35 ^{o}C.
Registration of growers and plots	**At institute:** Before transplanting, nodal officer BS production must ensure entry/ registration of seed production plots along with GPS map in seed portal, every season. **By seed agencies:** 1. Grower name with AADHAR No. to be uploaded. 2. GPS location of plots to be recorded and uploaded against the name of growers. 3. In case of FPOs/FPCs, the registration details to be uploaded. 4. Variety name against grower and plot number to be uploaded. 5. The MoU/Agreement for seed production should be completed before supply of breeder seeds of parents to the growers.
Sowing timeline	Breeder and TL seed of hybrid is typically produced during the *Rabi* season. *Rabi sowing:* • Sowing of parental lines must be completed during 1st fortnight of November else flowering will synchronize with high temperature and seed production will suffer. *Kharif sowing:* • During Kharif, sowing of parental lines must be completed before 15th June.

Nursery management	• ~ 600-800m^2 nursery area is required for transplanting one-hectare land. • Before seeding, puddle the seedbed field twice (at an interval of 6-7 days) and keep the water continuously for 4-5 days. • After that drain the excess water and puddle (2-3 times) in wet condition to destroy weeds, weed seeds and germinated rice seeds. • Prepare raised seedbeds (5-10 cm height) of 1m width of any convenient length. • Provide drainage channels (30 cm) in between seedbeds to drain excess water. • Apply recommended fertilizer and manures (500: 500: 500g./100 m^2 N, P, K and 50 kg/100m^{2}FYM) to the nursery beds. Double the phosphorus dose where low temperature retards seedling growth and apply zinc shulphate @ 3-4 kg/1000m^2 in zinc deficient area. • Sow pregerminated seed uniformly on the seedbed (@ of 20-22g seed/m^2) • Use 15 kg of `A' line seed and 5 kg of `R' line seed to produce sufficient seedlings to grow in one hectare each. • Manage the seedbed properly for getting healthy and vigorous seedlings for transplanting. • To avoid the fungal diseases in nursery, seed treatment with carbendazim 50% WP @ 4g/kg seed should be ensured. • For proper seedling growth, maintain the optimum moisture in nursery and apply urea @ 600-800g/100m^2 after 15 days of sowing. • Avoid excess nitrogen application in nursery; it affects the flowering synchronization in parental lines.
Transplanting	• Transplanting of hybrid (BS, TL, CS) rice parental lines in seed production plots must be completed before stipulated timelines in both seasons ➢ *Rabi* transplanting-before 20th December ➢ *Kharif* transplanting before15th of July, every year. • To attain the complete synchronization in parental lines and long duration availability of pollens, male parent (B/R line) must be sown in three staggered date (at 3-4 days interval). • Transplant 21-25 days old seedling of both parent during *Kharif* and 30-35days old seedlings during *Rabi*. • Transplant 1-2 seedlings per hill of the `A' line and 3-4 seedlings per hill of B/R (male lines) lines.
	• Maintain 20cm x 20 cm row spacing between B/R lines, 20cm x 15cm spacing between A and B/R lines and 15cm x 15cm spacing between CMS lines. • Maintain row ration of in hybrid rice 2:8-10 row in hybrid seed production plots and 2: 6 in CMS maintenance plots. • The rows of male and female in the seed production plot must be perpendicular to the prevailing wind direction expected at flowering time of the parents.
Gap filling	Within ten days after transplanting gap filling must be completed
Isolation distance	➢ **Spatial isolation:** maintain 100-meter isolation distance between hybrid seed production plots and plots of other varieties, and 500-meter distance for CMS line maintenance. ➢ **Time isolation:** Keep time isolation of over 21 days, means the heading stage of the parental lines in hybrid seed production plot should be 21 days earlier or later than that of other varieties grown in the vicinity.

	➢ **Barrier isolation:** A crop barrier with maize, sugarcane and *Sesbania* covering a distance of 30 m would also serve the purpose of isolation. Artificial barrier with polythene sheets and seed nets of about 3 m height can also be used in case of small scale seed production. Rouging must be carried out at three key stages of crop growth: tillering, flowering and just before harvesting.
Rouging	• At maximum tillering: Identify off-type plants by examining differences in leaf blade size, shape, and the color of the leaf sheath. • At flowering: Assess the duration of flowering, panicle shape, size, and stigma pigmentation. • Before harvesting: Focus on seed shape and size, fertility percentage, and panicle exsertion.
Out-crossing encouragement	• **Gibberellic acid (GA3) application:** Apply 40ppm - 90ppm GA3 (1 g of GA3 in 25-40 ml of alcohol) in two consecutive days at 10% and 20% flowering. • **Flag leaf clipping:** Cut half or two-third portion of flag leaf from top is recommended for Rabi season. • **Supplementary pollination:** rope pulling or shaking the pollen parent with the help of two bamboo sticks three times during 8.30am to 10.30am morning. **Internal Monitoring:** The Head of the Crop Improvement Division will constitute an interdisciplinary team to monitor breeder seed plots. Internal monitoring should be done at least twice: once at maximum tillering and once at flowering. *Kharif season:* • First monitoring: 15th to 30th August • Second monitoring: 15th September to 20th October (mid November in case of long duration varieties) *Rabi season:* • First monitoring: 25th to 30th February • Second monitoring: 20th to 30th March **External Monitoring** Breeder seed quality is closely monitored by two external teams: • **National Seed Project (NSP):** Includes all Rice Research Institutes involved in rice varietal development and breeder seed production. The central NSP team oversees seed production and facilities at breeder seed centers.
	• **Joint Monitoring Team:** Led by the State Seed Certification Agency, this team inspects breeder seed production plots. It includes representatives from the State Seed Corporation, NSC, the State Agriculture Department, Agricultural University, and the breeding institute's officials.
Monitoring of plots	The Nodal Officer Seeds, ICAR-CRRI will schedule external monitoring dates in consultation with both Central and State Monitoring Teams to ensure the breeder seed plots meet field quality standards (Checklist and proforma attached as *Ann-I & II*). They will also prepare the monitoring report (BSP-III), signed by all team members.

Harvesting, threshing and Seed Processing	**Harvesting:** • All R/B line rows are to be harvested first and harvest is to be kept in a safe place separately. • After R line harvesting, a final roguing in seed parent has to be done carefully, removing the plants showing more than 70% seed setting. Then the seed parent plants are to be harvested. **Threshing:** • All the threshing equipment, threshing floor and tarpaulin to be thoroughly cleaned. • A' line parent and `R' line parent harvests must be kept separate from each other. • Treated breeder seed will be packed in 05 kg or 10 kg bags, each tagged with all relevant information regarding the seed lot. • Store the seed on an elevated wooden platform in a well-ventilated cold room, ensuring no water entry from seepage or rain.
Tags for Quality Seed	Seed lots that meet BS quality should be packed and sealed with a golden yellow tag of 12 cm x 6 cm dimension during sale. Details of tag is attached in (*Annexure III*)
Record maintenance	Detailed records of all activities related to seed production, including field history, seed source, planting details, crop management practices, roguing, harvesting, processing, and bagging should be kept in a standard proforma. It must be made available to monitoring teams on demand.

By following the procedures mentioned above, the quality and integrity of breeder seeds of parents and TL seed of hybrid will be maintained, ensuring they are suitable for further multiplication and distribution.

Annexure I

Checklist for BS monitoring in fields

Name of variety:
Year of release: Area of recommendation:

Stage	Parameters	Remarks
Nursery Transplanting	Variety details record as per the proposal	
	Nucleus Seed Purity Certificate from the developer	
	Sowing to be completed as per the SOP for both season	
	Seed Treatment done before sowing	
	The plot selection for Breeder Seed done as per the SOP?	
	Sowing Plan (Date of Sowing, Number of Panicle Shown, Number of lines shown for each variety)	
	Seedling health	
	Specific remark	
	Transplanting Time as mentioned in the SOP for both season followed or not	
	Isolation: Time isolation/ isolation distance maintained or not.	
	SOP for Breeder Seed transplanting to be followed	
	Seedling age at the time of transplanting	
	Date of Transplanting?	

	Planting method and row spacing as per the SOP	
Field management	Irrigation, Weeding and Fertilizer application as per the SOP	
Rouging	How many times the variety has been rouged out.	
Monitoring	What was the flowering status in the field?	
	Off type plants present or not.	
	Whether management details after transplanting recorded?	
	What is the disease and insect-pest status in the field?	
	Specific remarks, if any?	

Annexure II

Proforma for BS/TL Monitoring Report

Variety	:	
Transplanted Area (m^2)	:	
No. of rows with length (m)	:	
Crop Stage at monitoring	:	
Monitoring report	:	Satisfactory/ rejected
Insect Pest Incidence (if any)	:	
Expected Yield (kg)	:	
Remarks (if any)	:	

Annexure III

Information on the Breeder/TL/CS Tag

While supplying the Breeder seed as per DoAC allotment, a golden yellow colour tag is stitched with the bag with certain mandatory information's written on it. The mandatory information's are as follows:

01. Name of the crop
02. Tag No.
03. Name of the variety
04. Class of seed
05. Lot No.
06. Date of test
07. Pure seed (%)
08. Inert matter (%)
09. Germination (%)
10. Genetic purity (%)
11. Source
12. Signature of the Breeder
13. Seal

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