ICAR-INDIAN INSTITUTE OF RICE RESEARCH
RAJENDRANAGAR, HYDERABAD-500 30

Experimental details of Programme for Kharif - 2023

IMPORTANT:

1. **Strictly adhere to the technical program to the possible extent**
2. **Grain yield and TDM must be reported as g/ m^2 FOR ALL THE TRIALS.**
3. **No. of replications minimum three.**
4. **Check the data before uploading on MIS**
5. **No alphabets or zeros in the data sheet**
6. **Silicon experiment has to be done in 500 m2 area.**
7. **Photographs are to be taken during the life cycle for each of the experiments.**

1) Influence of silicon on improving abiotic stress tolerance in rice genotypes

Objective (a) To study the effect of applied silicon on rice yield.
(b) Uptake of silicon in the tissues at different growth stages.
(estimation of ash content in different plant parts as a surrogate parameter for tissue silicon content)
Total Silicon content to be determined in the leaves and stem at harvest

Locations	:	**CBT, IIRR, KJT, KRK, MTU, PNR, PTB, REWA, TTB & RANCHI**
No. of Varieties	**:**	1)27P37, 2) 27P63, 3)28P67, 4) HRI-174, 5) US-312, 6) US-314, 7) US-380, 8) AZ8433DT, 9) Sahabhagidhan

Design : RBD/Split-plot with 3 reps & 10*20 cm spacing of 500/m2 plot size.
Fertilizer dose : 100N- 45P_2O_5- 60K_2O kg/ha. (P & K as basal dose)
N-Splits : 1st 50% of scheduled N at 10-15 days after planting
2nd 25% of scheduled N at active tillering
3rd 25% of scheduled N at panicle initiation (PI)

Silicon Treatments : 4

T-1 Control (do not apply any chemical and follows the same package of practices)

T-2 Apply silisilic acid (0.08% Ortho silicic acid) @ 80ppm silicon at 15 DAP, 30DAP, 45DAP and 60DAP, total 4 sprays (see the detailed protocol below)

T-3 Apply silisilic acid (0.08% Ortho silicic acid) @ 80ppm silicon at 15 DAP, 30DAP, 45DAP and 60DAP, total 4 sprays (see the detailed protocol below) + water stress (Water stress to be imposed by withholding irrigation 12 days before flowering and again 10 days after anthesis (Total duration of stress will be 22 days)

T4 Water Stress only.

Leaf water potential or Relative water content (RWC) of leaves must be measured during stress imposition (every 2 days) and after relief from the stress.

Silicic Acid Solution for foliar Spray

Materials Provided

1) Solution A (Acid Mix): 1 Number (500 ml)
2) Solution B (Base Mix): 1 Number (500 ml)

Preparation of Silicic acid solution

General procedure

1. Add Solution B (Base Mix) slowly with continuous stirring to equal volume of Solution A (Acid Mix).
2. Dilute 30.0 ml (thirty ml) of the resulting transparent (silicic acid) solution obtained from step 1 to one litre with pure water which will produce a spray solution having about 80 ppm silicon.
3. Different volume of foliar spray solution having silicon strength of 80 ppm can be prepared by mixing the specific quantity of solution A and solution B and water as mentioned in the Table below.

Volume of spray solution to be prepared with 80 ppm silicon concentration	Solution A (Acid Mix) (ml)	Solution B (Base Mix) (ml)	Pure water (ml)
1.0 litre	**15.0**	**15.0**	**970**
5.0 litre	**15.0*5 = 75**	**15.0*5 = 75**	**970*5 = 4850**
10.0 litre	**15.0*10 = 150**	**15.0*10 =150**	**970*10 = 9700**

Example: Preparation of 5.0 litre spray solution having 80 ppm silicon concentration

Step I

Measure **75 ml** of Solution A (Acid Mix) and take in a Beaker. Similarly, measure **75 ml** of Solution B (Base Mix) and take in another Beaker.

Step II

Add measured solution B (Base Mix, 75 ml) slowly with stirring to measured solution A (Acid Mix, 75 ml). This will produce a transparent solution (150 ml) of silicic acid (SA).

Step III

Dilute this 150 ml resulting transparent solution of Silicic Acid (from Step II) to 5.0 litres with pure water and mix well. This resulting spray solution has 80 ppm silicon concentration.

Solution B (Base Mix) = 75

Solution A (Acid Mix) =

Spray schedule **First spray**: After 15 days of Transplantation **Second spray**: After 30 days of Transplantation **Third spray**: After 45 days of Transplantation **Fourth Spray**: After 60 days of Transplantation	1. Always **Base Mix** (Solution B) should be added to **Acid Mix** (Solution A). The addition should be slow with continuous stirring to avoid precipitation. 2. The prepared transparent solution of SA in **Step II** should be diluted for spray within 12 hours. 3. Avoid spray if it is raining or is expected to get rain in 2-3 hours 4. For any clarification regarding the product, contact Dr. Azam (**Mobile No. 8008802860**)

Note:

5. Always **Base Mix** (Solution B) should be added to **Acid Mix** (Solution A). The addition should be slow with continuous stirring to avoid precipitation.
6. The prepared transparent solution of SA in **Step II** should be diluted for spray within 12 hours.
7. Avoid spray if it is raining or is expected to get rain in 2-3 hours
8. For any clarification regarding the product, contact Dr. Azam (**Mobile No. 8008802860**)

Amount of raifall received throughout the crop growth period should be recorded.

Date of Sowing :
Date of Planting :

Observations:

Soil analysis: at initial stage and also at harvest stage:

- Soil pH
- EC
- Organic contents
- Available NPK contents

Agronomic Data:

- Tiller population (number of tillers/m2), Plant height (cm) at flowering
- Phenological: date of sowing & planting, days to 50% flowering and maturity.
- Leaf area**,** leaf wt., culm wt., panicle wt. TDM g/m2 at maximum tillering, PI, flowering & maturity-must
- **Yield attributes at maturity**: shoot wt, panicle wt, total dry matter. g/m2 , pan no/m2, grain no/m2, spk no/m2, grain no/panicle, spk no/panicle, 1000 grain wt (g), grain yield g/m2, HI (%)
- Silicilic acid in leaves at three stages

Sample to be collected at tillering, PI and flowering stages for silicon estimation and to be sent to Head Quarters.

Procedure for silicon estimation provided PDF separately.

If facility not available please collect the sap in eppendorf tubes, properly label and hand over to the ATCRIP team visiting your location. The procedure for collection of cell sap is Small pieces of leaf tissue, poured in a plastic syringe (**Please remove the needle)** and extract by pressurizing the piston. The sap coming out from the syringe 2=5 drops is sufficient and stored at cool temperature (Before handing over a drop of sodium hypo chlorite may be added preferable to avoid pathogenicity).

Estimation of silicilic acid (Not silica): (SEE Kimio saito et al soil Sci and Pl. Nutrition 2005 29-36) from dry samples also can be done

USE ONLY PLASTICWARE WHILE PREPARING SOLUTIONS AND FOR STORAGE. STOCK SOLUTIONS CAN BE STORED IN REFRIGERATOR

1. Extraction solution (Called HF solution)

(1.5 M Hydro Fluoric acid-0.6M Hcl)

Combine 1 Vol of conc HCl (36%) + 1 vol of HF (49%) + 18 mi DD water

To prepare 200 ml of HF add 10 ml of HCl +10 mi of HF + 180 ml of water

2. Reagents for assay

a. 0.5 M boric acid soln (stock)

b. 0.1 M boric acid working soln (0.5M dilute)

c. 0.5 M Sodium Molybdate solution

d. 0.8 M Sulfuric acid (stock soln)

e. 0.25 M working Molybdate soln: 1 Vol of Na molybdate + 1 vol of 0.8 M sulfuric . (Always prepare fresh before assaying)

Extraction: 0.5 g dry tissue or 10 micro litre sap (prefer sap- low vol) immerse in 10 ml HF soln and add 40 ml water (plastic only) stir and keep overnight or more than 6 hrs . Supernatant for assay as follows

Reaction mixture (10 ml total volume)

0.1 M boric acid 2.0 ml: Molybdenum (working soln) 2.0 ml: supernatant extracted 0.1 ml: 0.1 M citric acid 4.0 ml: water (Double distilled 1.9 ml

Mix well for 3-5 mins so that the solution turns yellow. Read OD 400 nm.**Use plastic cuvettes only**

Na silicate as standard solution (details separate mail PDF arranged)

Do not use Glass or Quartz cuvettes

2) Phenotyping of elite rice genotypes for Drought Tolerance

Objective: To screen the elites for their drought tolerance under field condition.

Locations	:	**CHN, NRRI, PTB, REWA, TTB, RPUR & RANCHI**
No. of Varieties	**:**	**30. (RFU 101 to RFU 130)**

Design : RBD with 3 replications and a suitable plot-size

Treatments : 2 (Rainfed and Irrigated). (as decided in the workshop, one irrigated control should be maintained besides normal rainfed condition. This will facilitate to compute required drought indices:

Fertilizer dose: 100N- 45P_2O_5- 60K_2O kg/ha. (P & K as basal dose)

N-Splits: 1st 50% at or just before sowing

2nd 25% at active tillering

3rd 25% at panicle initiation (PI)

Date of Sowing :

Date of Planting :

Observations:

1) Daily Weather data on Rainfall, Temperature, Relative Humidity, Wind speed, Solar Radiation and Sunshine Hours from Jan-Dec, 2016
2) Phenological date of sowing, days to 50% flowering and maturity-must (preferably for individual genotypes)
3) Date of actual harvest (genotype –wise)
4) **MUST**: Soil moisture status at field capacity and wilting point. Please ensure to give soil characteristics and fertility levels wherever possible.
5) **MUST**: Soil moisture status at 20 cm depth during drought spell (Please specify actual dates during each dry spell period with no rain continuously for more than 7-days)-must
6) Plant height (Soil level to the uniform canopy top level) at flowering
7) **MUST**: TDM at flowering and at maturity
8) ***MUST****: Collect 3 plants/hills from each treatment count the tiller number, record the dry weight of leaves, stem and panicle separately (individually for all the 3 plants) at flowering stage and at maturity (very important). Stem weight should be collected after removing the leaves and leaf sheath at both the stages.*
9) ***MUST****: Collect 3 panicles at early grain filling stage (milk stage) and at maturity from each* treatment and record the panicle weight and grain weight individually for each of the 3 samples (very important).
10) *Collect 3 fully grown leaves from each treatment genotype wise at flowering stage and measure the length and width with a scale and record the dry weight of individual leaves after drying (Very important)*
11) Grain yield data at harvest (panicle no/m2, grain no/pan, Spikelet no/pan, grain no/m2, spikelet no/m2, 1000 grain weight, grain yield/(g/m2), total dry matter/ (g/m2) and HI (%)
12) Yield components and grain yield at harvest on sq.m land area basis. Also please convert to Kg/tonnes per hectare as suggested. **Check for any discrepancies before dispatching the data.**

Note*: the observations 9, 10, 11 are very important and must be recorded as they will be used as input to drought susceptibility indexes.*

3) Evaluation of rice genotypes for heat tolerance suitable for future climate

Objective: To investigate the differences in the terminal heat stress tolerance in elite rice genotypes.

Locations	:	**IIRR, MTU, PNR, PTB, REWA, TTB, RANCHI & KAUL**
No. of Varieties	**:**	**26 (HT 101 to HT 126)**

Design : Split-Plot
Treatments : Two (Treated and Control)
Fertilizer dose : 100N- 45P_2O_5- 60K_2O kg/ha.(P & K as basal dose)
N-Splits : 1st 50% at 10-15 DAP
2nd 25% at active tillering
3rd 25% at panicle initiation (PI)
Date of Sowing :
Date of Planting :

Methodology: The genotypes needs to be transplanted in two blocks/strips, one for control and another block/strip for imposing terminal heat stress by covering the block/strip with polythene sheet supported by a metal frame or bamboo sticks like a "tunnel" IMMEDIATELY AFTER PI STAGE (BEFORE ONSET OF FLOWERING) stage until maturity. Control block/strip should be kept uncovered. Leave at least 10 cm space between polythene sheet for sufficient ventilation. Each entry should be sown in 3 rows of 1.5 meter length maintaining 20 cm spacing between rows and recommended plant to plant distance. Leave one blank row between the entries. Each row will be treated as a replication and all the observations needs to be recorded for each row separately. A minimum-maximum thermometer needs to be installed inside the tunnel and both minimum and maximum temperatures needs to be recorded everyday inside the tunnel.

Observations:

1. Daily Weather data on Rainfall, Temperature, Relative Humidity, Wind speed, Solar Radiation and Sunshine Hours during the crop growth period
2. Phenological: date of sowing & planting, days to 50% flowering and maturity.
3. Date of actual harvest (genotype –wise)
4. Plant height, leaf wt., stem wt., panicle wt. and total dry matter (g/m2), at flowering
5. Total dry matter produced at harvest
6. Grain yield data at harvest (panicle no/m2, grain no/pan, Spikelet no/pan, grain no/m2, spikelet no/m2, 1000 grain weight, grain yield/(g/m2), total dry matter/ (g/m2) and HI (%)
7. Chlorophyll content at flowering and at early seed filling and late seed filling stages.
8. Stem wt. to be recorded at flowering stage and at harvest (select 3 plants (hills) at flowering and harvest resume leaves and determine column wt.). PLEASE ENSURE THT ONLY STEM WEIGHT IS RECORDED (WITH OUT LEAF SHEATH AND LEAVES) AT BOTH THE STAGES.

Where facilities exists, Chlorophyll fluorescence parameters, leaf photosynthetic characteristics and chlorophyll a & b t content through solvent extraction method may be recorded at early and late seed filling stages in both control and heat stress plots.

4) Physiological characterization of selected genotypes for multiple abiotic stress Tolerance

Locations: CBT, NRRI, FZB, KJT, KRK, MTU, PNR, PTB, TTB and KAUL

1. Anaerobic germination potential at germination stage
2. Salinity tolerance at early seedling stage (3-4 leaf stage)
3. Drought (osmotic stress) at early seedling stage (3-4 leaf stage)

For salinity and osmotic stress tolerance:
Please grow seedlings in Hoagland/Yoshida solution for 21-25 days (3-4 leaf stage) and then impose the required stress by addition of appropriate levels of NaCl and PEG solutions.

Water stress: 1% Mannitol and 2% Mannitol
NaCl stress: 12 dS m^{-1} of NaCl Stress (about 120 mM NaCl and pH 5.5)

Change solutions once in every two days. Drain completely rinse three –four times with fresh solutions if possible so as to avoid increased stress level due to Mannitol and NaCl. Experiment may be continued for one month and seedling vigour, germination and other related parameters may be taken against control. Maintain uniform temperature and replications (August- September ideal for this). Please keep the number of seedlings same for each replication.

Genotypes: **28 varieties**

1	MAS-401	11	MAS-411	21	MAS-421
2	MAS-402	12	MAS-412	22	MAS-422
3	MAS-403	13	MAS-413	23	MAS-423
4	MAS-404	14	MAS-414	24	MAS-424
5	MAS-405	15	MAS-415	25	MAS-425
6	MAS-406	16	MAS-416	26	MAS-426
7	MAS-407	17	MAS-417	27	MAS-427
8	MAS-408	18	MAS-418	28	MAS-428
9	MAS-409	19	MAS-419		
10	MAS-410	20	MAS-420		

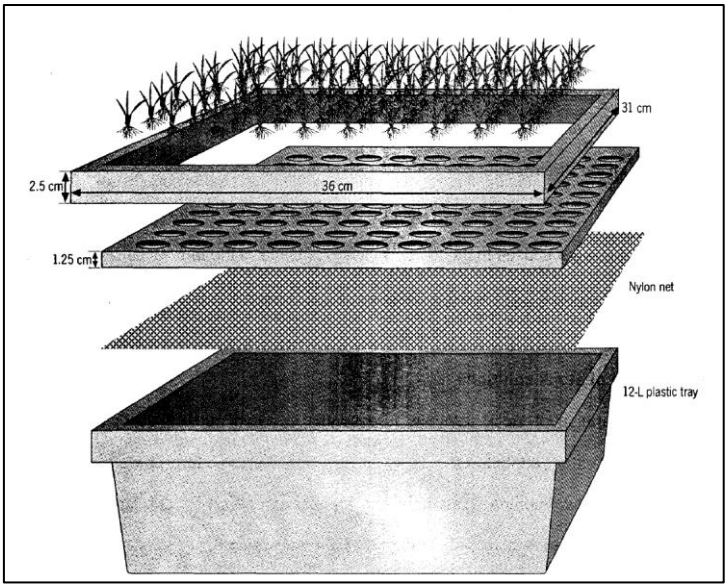
SCREENING FOR ANAEROBIC GERMINATION POTENTIAL:

1. At first, the seeds of different rice genotypes should be preheated at 50 ℃ for 2-3 days.
2. The soil to be used for this experiment should be dried and dusted properly so that there are no clots.
3. Then the plastic tub (15 cm in height) should be filled up with 3 cm of dried soil and the seeds should be directly sown on the soil at a depth of 1 cm. Immediately after sowing, the tubs should be filled with water without disturbing the upper soil, so that there is 10 cm of standing water inside the tub above the soil surface.
4. Inside tubs, there will be a single row for each genotype, where at least 20 seeds should be sown in a row. This way, each genotype should be replicated at least 3 times.
5. The control set should also be sown in similar way, but except standing water, there should be keeping adequate moisture in the soil.
6. The experiment should be continued for 21 days from the date of sowing and germination count to be taken every 7^{th} day in treated and every day in control samples.

SCREENING FOR SALINITY TOLERANCE:

1. Screening for salinity tolerance need to be carried out hydroponically at early vegetative stage of the plants i.e. when the plants reaches 3-4 leaf stage.

2. The centers where salinity screening blocks or fields with saline soil is available the complete experiment may be carried out at such facilities in replications keeping in mind all the observations to be recorded during the experiment. Also, the soil salinity levels (both pH and EC_e) need to be recorded during the course of the experiment.
3. For this floating Styrofoam panel as shown in the figure need to be prepared (as per the size of the tray using thermocol sheet, mosquito nets and adhesive). The Styrofoam panel should have 10-12 × 8-10 holes.



4. Individual accession need to be grown as single line along with standard check lines and will be replicated at least thrice.
5. The tray need to be filled with a nutrient solution comprising of both macro and micro nutrients (as shown in the table) and should have a pH of 5.5

Table:1 Preparation of stock solution

Element (Macronutrient)	**Reagent (AR grade)**	**Preparation (g / L solution)**
N	Ammonium nitrate (NH_4NO_3)	91.4
P	Sodium phosphate, monobasic monohydrate ($NaH_2PO_4.H_2O$)	35.6
K	Potassium sulphate (K_2SO_4)	71.4
Ca	Calcium Chloride, Di-hydrate ($CaCl_2.2H_2O$)	117.35
Mg	Magnesium sulphate, 7- hydrate ($MgSO_4.7H_2O$)	324.00
Micronutrient Dissolve each reagent separately and mix in 500 mL of distilled water, then add 50 mL of conc. H_2SO_4 and make up volume to 1 L		
Element (Micronutrient)	**Reagent (AR grade)**	**Preparation (g / L solution)**
Mn	Manganous Chloride, 4- hydrate ($MnCl_3.4H_2O$)	1.5
Mo	Ammonium Molybdate, 4-hydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$]	0.074
Zn	Zinc Sulphate, 7-hydrate ($ZnSO_4.7H_2O$)	0.035
B	Boric acid (H_3BO_3)	0.934
Cu	Cupric sulphate, 5-hydrate ($CuSO_4.5H_2O$)	0.031
Fe	Ferric chloride, 6-hydrate ($FeCl_3.6H_2O$)	7.7
	Citric acid, monohydrate ($C_6H_8O_7.H_2O$)	11.9

Table 2. Preparation of working nutrient solution

Element	**Reagent**	**mL of stock required solution /1 L of working nutrient solution**	**Concentration of element in ppm**
(Macronutrient)			
N	NH_4NO_3	1.25	40
P	$NaH_2PO_4.H_2O$	1.25	10
K	K_2SO_4	1.25	40
Ca	$CaCl_2.2H_2O$	1.25	40
Mg	$MgSO_4.7H_2O$	1.25	40
(Micronutrient)			
Mn	$MnCl_3.4H_2O$	1.25	0.50
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.25	0.05
Zn	$ZnSO_4.7H_2O$	1.25	0.01
B	H_3BO_3	1.25	0.20
Cu	$CuSO_4.5H_2O$	1.25	0.01
Fe	$FeCl_3.6H_2O$	1.25	2.00

Preparation of working solution:-
Take 1.25 ml/L of each nutrient solution (Micro & Macro) and make up the volume as per need.

6. During the experiment, the solution need to be checked every day for maintaining the pH of the solution. If the pH shifts too far, then it is better to replace the solution.
7. Before starting the experiment, the seeds of each genotype needs to be pre-heated in hot air oven for 3-5 days at 50°C to break seed dormancy (if any).
8. The surface sterilized seeds need to be placed in petridishes with moistened filter papers and incubated at 30°C (or at Room Temperature) for 48 hours to germinate.
9. These pre-germinated seeds should be placed in the individual holes of the Styrofoam panel. Two seedlings per hole.
10. Initially, these seedlings should be kept in normal water for 2-3 days.
11. After that it need to be kept in nutrient solution (as described earlier).
12. After 5 days of growth in nutrient solution, salinity stress need to be imposed in one set of trays, whereas the other set would be kept as such for control.
13. Salt stress need to be imposed as 6 dS m^{-1} NaCl solution (approximately 60 mM NaCl i.e. ~3.0 g NaCl/L solution would give required level of E.C.) for initial two days. After two days it should be increased to 12 dS m^{-1} NaCl solution (approximately 120 mM NaCl i.e. ~6.0 g NaCl/L solution).
14. Visual scoring of the genotypes should be started (as mentioned below) as soon as appearance of the salt specific symptoms. Scoring should be continued until 60% of the plants of most susceptible genotype reaches the score of '9'. Genotypes should be ranked based on the final scoring at this stage.

Table 1: Modified standard evaluation score (SES) of visual salt injury at seedling stage.

Score	**Observation**	**Tolerance**
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant

5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly Susceptible

Observations to be recorded:

1. Visual scoring (SES Scoring in scale of 1-9 as mentioned by Gregorio, 1997 or Chakraborty et al., 2020 https://www.frontiersin.org/articles/10.3389/fpls.2020.00265/full)
2. Root length, shoot length and root & shoot dry weight at the end of the experiment
3. Total chlorophyll content of the leaf at the end of the experiment
4. Na^+ & K^+ content of the root and shoot at the end of the experiment if Flame Photometer is available. Otherwise, dry samples of root and shoot may be sent at ICAR-NRRI, Cuttack for analysis.

Method for estimation of Total Chlorophyll Content:

1. At the end of the experiment, 50 mg of leaf tissue 50 mg should be collected and chopped before suspending it in 10 mL of 80% acetone and mixed well.
2. The test tubes then should be kept inside normal freezer (at 4 ºC) for 48 hours.
3. Concentration of chlorophyll a, b and total chlorophyll should be quantified in samples by reading the optical density of the solution at 663 and 645 nm using a spectrophotometer.
4. The samples need to be analysed in triplicates.
5. The amount of chlorophyll content should be calculated as per the following formula -

$$\text{Chlorophyll a (mg/g)} = 12.21\ (OD_{663}) - 2.81(OD_{645}) \times V/W \times 1000$$
$$\text{Chlorophyll b (mg/g)} = 20.93\ (OD_{645}) - 5.03(OD_{663}) \times V/W \times 1000$$
$$\text{Total chlorophyll content (mg/g)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Method for estimation of tissue Na^+ & K^+ Content:

1. Both root and shoot tissue samples should be washed thoroughly and carefully with distilled water to remove all contaminants before drying.
2. After washing the samples should be oven dried at 65 °C until constant dry weight of the samples is achieved.
3. The dried plant material need to be powdered by grinding and 50 mg dried sample should be placed in a 25-mL test tube.
4. Tissue extraction for Na^+ and K^+ should be done with 1.0 N HCl at 30 °C for 48 h.
5. Thus, obtained tissue extracts need to be diluted and filtered using Whatman #1 filter paper and again the volume should be made up to 25 ml using distilled water.
6. The Na^+ and K^+ contents in the tissue extracts need to be determined using Flame Photometer having Na^+ and K^+ filters.

SCREENING FOR OSMIOTIC STRESS TOLERANCE:

The same protocol as above needs to be followed and during stress imposition 1% and 2% PEG-6000 solution need to be applied instead of NaCl solution.

5) SCREENING FOR SUBMERGENCE TOLERANCE:

Locations: CHN, CBT, NRRI, KRK, PTB and TTB,
Genotypes: 34

1	SUB 501	13	SUB 513	25	SUB 525
2	SUB 502	14	SUB 514	26	SUB 526
3	SUB 503	15	SUB 515	27	SUB 527
4	SUB 504	16	SUB 516	28	SUB 528
5	SUB 505	17	SUB 517	29	SUB 529
6	SUB 506	18	SUB 518	30	SUB 530
7	SUB 507	19	SUB 519	31	SUB 531
8	SUB 508	20	SUB 520	32	SUB 532
9	SUB 509	21	SUB 521	33	SUB 533
10	SUB 510	22	SUB 522	34	SUB 534
11	SUB 511	23	SUB 523		
12	SUB 512	24	SUB 524		

Screening using field tanks (wherever available) or in pots

1. Before sowing the seeds should be pre-heated at 50 °C for 2-3 days for breaking the seed dormancy (if any).
2. The seeds need to be directly sown inside the tanks using wet-bed direct sowing method.
3. Each genotype should be sown in 2 rows (min.) with 3 replications with a row to row spacing of 20 cm and plant to plant spacing of 15 cm.
4. Germinated seedlings should be grown normally till 20–25 days without submergence stress.
5. Plant height (average of 5 plants/genotype per replication) and number of hills per genotype per replication (total numbers) should be recorded before the imposition of submergence stress.
6. Then the plants should be subjected to the submergence stress in the form of standing water, where tanks should be filled with 80-100 cm of water and the level of water must be 20-25 cm above the top of the plant canopy.
7. The level of water should be maintained for 14 days after imposition of submergence stress inside the tanks.
8. After 14 days of submergence stress, water should be drained out from the tanks (de-submergence), and initially plant height, the number of hills will be counted from the plants.
9. Finally, the de-submerged plants should be allowed to grow 5 days in normal condition and the number of survived hills should be calculated for each genotype.
10. Same experiment may be conducted in pots of 10 cm size and small cemented tanks. In case of pot experiment, it is better to put 2-day old germinated seedlings in the pot (3 seedlings per pot) with minimum 5 replications, where 1 pot serves as 1 replication. Rest of the protocol is same.

Observations to be recorded:

1. **Plant height, Number of hills/plants** per genotype should be recorded **before and after imposition of stress.**
2. Survival percentage should be calculated by the following formula:
   Survival Percentage (%) = (No. of hills present after submergence / No. of hills present before submergence) × 100
3. **Total starch content** of the leaf should be estimated **before and after imposition** of stress.
4. Final **yield** and **total biomass** need to be recorded under both **control** and **submergence** treated conditions and the same may be expressed in g plant^{-1} basis.

Method for estimation of Starch Content:

i. Dry leaf samples (~200 mg) should be crushed thoroughly, mixed with 10 ml of 1(N) HCl, and kept in a glycerin bath at 112 ℃ for 30 minutes.
ii. Then filter the sample for 2-3 times.
iii. The extract should be collected and final volume should be made up to 25 mL with the help of distilled water.
iv. An aliquot should be prepared (0.5 mL-1.0 mL) of above extract and volume make up to 2.5 mL with the help of distilled water.
v. Then the samples should be mixed with 10 mL of freshly prepared anthrone reagent. (100 mg of anthrone will be mixed with 100 ml of chilled concentrated sulphuric acid).
vi. Then the mixture should be boiled for 15 minutes in water bath.
vii. Finally, cooled sample should be measured at the wavelength of 620 nm.
viii. A blank (without the aliquot) and appropriate Glucose standard should be run at the same time.
ix. Final value of starch content should be calculated as the Glucose value (obtained from the std. curve of glucose) × 0.9 taking into consideration of appropriate dilution factor used for the estimation.

6) Screening of rice varieties for tolerance to low-light stress

Objective: To screen the elites for their low light tolerance under field condition.

Locations: CHN, NRRI, IIRR, KJT, MTU, PNR, TTB and RAIPUR

Entries: ***18 Varieties***

1	LL-601	9	LL-609	17	LL-617
2	LL-602	10	LL-610	18	LL-618
3	LL-603	11	LL-611		
4	LL-604	12	LL-612		
5	LL-605	13	LL-613		
6	LL-606	14	LL-614		
7	LL-607	15	LL-615		
8	LL-608	16	LL-166		

Design	: Split-Plot
Treatments	: Two (Control and Treated with 50% shade net)
Fertilizer dose	: 100N- 45P_2O_5- 60K_2O kg/ha.(P & K as basal dose)
N-Splits	: 1st 50% at 10-15 DAP 2nd 25% at active tillering 3rd 25% at panicle initiation (PI)
Date of Sowing	:
Date of Planting	:

Methodology: The genotypes needs to be transplanted in two blocks/strips, one for control and another block/strip for imposing low light stress by covering the **shade net preferably white** with supported by a metal frame or bamboo sticks like a "tunnel" after a week of transplanting. Please ensure that two feet gap (open) on all sides so that temperature is not built until maturity. Control block/strip should be kept uncovered. Each entry should be sown in 3 rows of 1.5-meter length maintaining 20 cm spacing between rows and recommended plant to plant distance. Leave one blank row between the entries. Each row will be treated as a replication and all the observations needs to be recorded for each row separately.

Observations:

1. Daily Weather data on Rainfall, Temperature, Relative Humidity, Wind speed, Solar Radiation and Sunshine Hours during the crop growth period**.**
2. Measure light intensity (at least 3 to 4 times) in a cropping season.
3. Phenological: date of sowing & planting, days to 50% flowering and maturity.
4. Date of actual harvest (genotype –wise)
5. Plant height, leaf wt., stem wt., panicle wt. and total dry matter (g/m^2), at flowering
6. Total dry matter produced at harvest
7. Grain yield data at harvest (panicle no/m^2, grain no/pan, Spikelet no/pan, grain no/m^2, spikelet no/m^2, 1000 grain weight, grain yield/(g/m^2), total dry matter/ (g/m^2) and HI (%)
8. Chlorophyll content at PI and flowering stages (chl a, chl b, total chl). Very imp. observation
9. Stem wt. to be recorded at flowering stage and at harvest (select 3 plants (hills) at flowering and harvest resume leaves and determine column wt.). PLEASE ENSURE THAT ONLY STEM WEIGHT IS RECORDED (WITHOUT LEAF SHEATH AND LEAVES) AT BOTH THE STAGES.

Where facilities exist, Chlorophyll fluorescence parameters, leaf photosynthetic characteristics and chlorophyll a & b t content through solvent extraction method must be recorded at early and late seed filling stages in both control and low light stress plots. Please indicate the equations for estimating chlorophyll content.