2. Drought tolerance

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2.1 Introduction

Rice is grown under a diverse range of ecosystems and gets exposed to different environmental stresses like drought, salinity, submergence, cold *etc.*, wherein drought constitutes an important yield limiting determinant. Food security and prosperity of India are challenged by increasing demand and threatened by declining water availability. Out of 42.63 million ha area under rice in India, drought is one of the major abiotic constraints in around 8.0 million ha of rainfed upland and rainfed lowland situations. Eighteen per cent of total rice area of India and 20% of Asia are drought prone. The irregularities in South-west monsoon do result in moderate to severe drought in rainfed rice growing areas, especially in eastern India, a number of morphological, physiological and phenological traits have been reported to improve the performance of rice challenged by drought. Drought is a multifaceted stress condition with respect to timing and severity, ranging from long drought seasons where rainfall is much lower than demand, to short periods without rain where plants depend completely on available soil water (Lafitte *et al.*, 2006). Incorporation of drought tolerance has always been a challenge to plant breeders, because of the complexity of the trait that involves several physiological and molecular mechanisms and different mechanisms often combine to confer drought tolerance (Wang *et al.*, 2001; Parida and Das, 2005). Drought at the vegetative stage can cause a moderate reduction in yield but the reproductive stage (from panicle initiation to flowering) is recognized as the most critical stage at which drought stress can cause serious damage to the crop and can even entirely eliminate the crop yield (O'Toole, 1982; Zhang, 2007). Evaluation of genotypes under field conditions in the dry season is ideal for identification of drought tolerant genotypes that are able to retain a large proportion of green living tissues under soil water deficit both at vegetative and reproductive stages (Chang *et al.*, 1974; De Datta *et al.*, 1988).

2.2 Screening for drought tolerance

To identify rice germplasm lines with built-in tolerance to vegetative and reproductive stage drought, large number of rice germplasm suitable for upland, lowland, deep water and wild rice, aromatic rice and fixed lines are being screened at NRRI, Cuttack under field conditions during the dry season (Plate 2.1 and 2.2). Details of protocol standardized and followed are described below for convenient and easy way of screening varieties/germplasm lines.

2.2.1 Selection of experimental sites

Generally, for large-scale screening experiments are to be conducted under field condition during dry season where interference of rain is negligible during the cropping period. Using controlled facility like rainout shelter, screening can be done in wet (*Kharif*) season. Depending on the soil type the irrigation/stress schedule is to be managed.

2.2.2 Soil properties

Before the initiation of experiment information regarding soil type, pH, EC, and available NPK content is to be measured. Soil moisture content at 0.03, 0.05, 0.10, 0.50 and 1.50 MPa matric potential to be measured to

make a soil moisture release curve. The field should be properly tilled and leveled to avoid variation in soil moisture content within the experiment.

2.2.3 Experimental design

Large number of varieties (>1000 entries) are to be sown in the field following augmented design. Using higher number of tolerant and susceptible checks is generally encouraged.

2.2.4 Seed sowing

Seeds of all the entries are to be seeded directly in dry soil with 4-5 seeds per hill in a spacing of 20 × 10 cm. The line length should be 3 m per genotype or 1.5 m × 2 lines per genotype. After 15 to 20 days of sowing, thinning/gap filling should be done to maintain uniform plant population.

2.2.5 Stress imposition

After germination, plants are allowed to grow with sprinkler irrigation at 3-4 days interval for 25-30 days (4 weeks). Irrigation should be withdrawn for 30 days or beyond, till the susceptible check shows permanent wilting and maximum number of lines show leaf rolling and tip drying symptoms. Phenotypic observations will be recorded during the stress period and then the plants should be re-watered for recovery after stress.

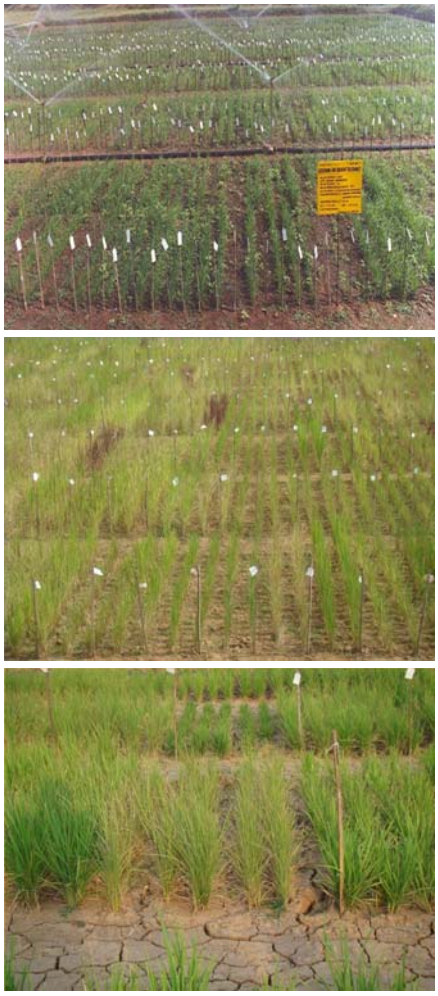

Plate 2.1: Large scale field screening for drought tolerance during dry season

For the vegetative stage, the stress will be imposed at active tillering stage (4 week crop growth stage) and for reproductive stage; the stress will be imposed at booting stage. During the period of stress, piezometer needs to be fixed to monitor the ground water table depth on daily basis.

Plate 2.2: Screening facility under rainout shelter during wet (Kharif) season

2.2.6 Soil sampling for SMC and SMT

Soil moisture should be recorded at periodical interval of 5-7 days at 15 and 30 cm soil depth from the day of suspension of sprinkler irrigation till the susceptible check shows the symptoms of wilting. Soil sample should be collected in a zigzag fashion with the help of auger from the whole field at least from 2 different places in each block. Collected soil samples should be kept in aluminum boxes, fresh weight of the soil + box is to be recorded and then the soil to be dried in an oven at 100°C at least for 48 h. Then dry weight of soil is to be determined with the box and by deducting the blank box weight the soil moisture content (%) to be calculated as

$$Soil\ moisture\ (\%) = \frac{FW - ODW}{FW} \times 100$$

where, FW: fresh weight of soil, ODW: over dry weight of soil.

Soil moisture tension (soil matric potential) to be recorded with tensiometer tubes installed at 30 or 45 cm soil depth in both control and stress blocks. At least 10 tensiometers should be installed to cover the field properly. When soil moisture tension reaches -40 to -50 kPa and -50 to -60 kPa at 30 cm soil depth under vegetative stage and reproductive stage stress, respectively, the plots need to be irrigated. A stress level that reduces yield by >50% will help in amplifying the genetic differences between genotypes (Fischer *et al.*, 2003). Phenotypic observations will be recorded in control and stress plots, and then the plants allowed to be recovered by watering. The stress and recovery cycles need to be continued till maturity (Plate 2.3).

Plate 2.3: Soil sampling and drying, tensiometer reading

2.2.7 Flow chart for vegetative stage screening

No stress for 30 DAS, then withdraw irrigation

⇩

Leaves start rolling with some leaf drying at moderate to severe stress

⇩

Continue the stress untill susceptible check shows complete wilting (severe stress)

⇩

Record leaf rolling and tip drying before irrigation, recovery after re-irrigation

⇩

Record water table depth and soil moisture content and other morphological and biochemical observations during the stress period

2.2.8 Flow chart for reproductive stage drought screening

Dry direct seedling in the field (genotypes of same duration to be grouped)

⇩

The seedlings to be allowed to grow up to 70-75 days after sowing (15 days before flowering) of 120 days duration group with normal irrigation in 3 days interval with no standing water

⇩

Withdraw irrigation until most of the varieties attain dough stage (15 days after flowering)

⇩

Soil moisture content (SMC) and soil moisture tension (SMT) during the stress period to be monitored. SMC% to be 10-12% at 30 cm depth and SMT to be -50 to -60 kPa at 30 cm depth

⇩

Irrigation to be given after releasing stress and to continue in 7 days interval till maturity with surface irrigation only

⇩

Observations on days to 50% flowering, days to physiological maturity, flowering delay and reduction in grain filling duration to be recorded

2.3 Phenotypic and biochemical observations

Some of the important morpho-physiological and phonological observations to be taken during stress period for vegetative stage drought tolerance screening.

2.3.1 Leaf rolling and death score

Leaf tissues may die (showing desiccation) because of extreme loss of water or because of heat stress when the leaf temperature rises as a result of inadequate transpirational cooling. All leaves in the canopy should be observed when leaf death is scored. Desiccation may not occur throughout a given leaf in a uniform fashion unless the water deficit is acute. More typically, it begins at the tip of the leaf, which is usually under greater water deficit than the basal part closer to the stem. Leaf rolling, death and drought score can be recorded as per the following procedure:

i) Leaf rolling to be recorded in the stressed plots between 12:00 to 14:00 h. Visual scales of 0 (no rolling) to 5 (complete rolling) to be used to record leaf rolling (Courtois *et al.*, 2000).

ii) Leaf death score ranges from 0 (no senescence) to 5 (complete leaf drying) to be recorded visually during the morning time, preferably before 10:00 AM (Fischer *et al.*, 2003).

iii) Leaf rolling and tip drying (drought score) and recovery data also can be recorded following International Rice Research Institute Standard Evaluation System (IRRI SES) method, 1 to 9 scales (IRRI, 1996).

The varieties/lines with early leaf rolling after suspension of sprinkler irrigation showed a higher score for drought tolerance (7-9) will be considered as susceptible ones. Varieties/lines with delayed leaf rolling (SES '1-3') and recovered faster after re-watering will be considered as tolerant ones (Table 2.1).

Table 2.1: Standard Evaluation System (SES - IRRI, 1996) for vegetative stage drought tolerance

Leaf rolling score at vegetative stage		Tip drying score at vegetative stage		Recovery score after releasingstress (24 h, 72 h and 10 days after stress)	
Scale	Symptoms	Scale	Symptoms	Scale	Recovery
0	Leaves healthy	0	No symptoms	1	90-100%
1	Leaves start to fold (shallow)	1	Slight tip drying	3	70-89%
3	Leaves folding (deep V-shape)	3	Tip drying extended up to 1/4	5	40-69%
5	Leaves fully cupped (U-shape)	5	One-fourth to 1/2 of the leaves dried	7	20-39%
7	Leaf margins touching (O-shape)	7	More than 2/3 of all the leaves fully dried	9	0-19%
9	Leaves tightly rolled	9	All plants apparently dead. Length in most leaves fully dried		

Leaf sample collection: Top-most fully expanded leaf (in the case of vegetative stage stress) or flag leaf (in reproductive stage stress) will be sampled between 11:00 to 14:00 h. At least three or more plants will be sampled per genotype. Half of the leaf sample will be used for relative water content, while rest half of the leaves will be used for leaf water potential, cell membrane stability, chlorophyll, and proline measurements. Chlorophyll and proline will be expressed on the fresh/dry weight basis of leaf.

2.3.2 Relative water content (RWC)

Relative water content (RWC) is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most useful index for dehydration tolerance. RWC is related to water uptake by the roots as well as water loss by transpiration. A decrease in the RWC in response to drought stress has been noted in a wide variety of plants when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. Normal values of RWC range between 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves. RWC to be measured by following the procedure described by Barrs and Weatherley (1962).

- Cut fresh leaf into slices of 1-1.5 mm size.
- Weigh the samples immediately to record the fresh weight of the leaf sample.
- Hydrate the samples to full turgidity by floating on de-ionized water in a closed petridish for 8 h under normal room light and temperature.
- After 8 hours take leaves out of water and remove any surface moisture quickly and lightly with filter paper and immediately weigh to obtain fully turgid weight.

- Oven dry the samples at 80°C for 24 h and weigh to determine dry weight of the sample and then calculate RWC by following the formula

$$\text{RWC}\,(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

The mean lethal RWC (RWC_{lethal}) is 38% (Lilley and Ludlow, 1996).

2.3.3 Leaf water potential (LWP)

Leaf water potential (LWP) is a measure of the whole plant water status and maintenance of high LWP is associated with drought avoidance mechanisms. Genotypes with high LWP had less spikelet sterility and produced higher yield than genotypes with lower LWP under drought stress conditions in both upland and lowland conditions.

Fully expanded youngest leaf should be collected in polyethylene bag during the 12:00 to 14.00 h and kept in ice bucket. Cut small leaf disc with the help of a punching machine and keep inside the leaf chambers attached to the Water Potential System, Psypro, WESCOR (Germany). The chamber with the leaf disc will be left for 5 minutes to stabilize the temperature, then switch on the machine to record the water potential of leaf discs. Eight observations can be taken at a time within 15 minutes.

The leaf water potential threshold for stomatal closure in rice is between -0.8 to -1.2 MPa, depending on the genotype (Henson, 1982; Dingkuhn *et al.*, 1989b) and presumably on the level of osmotic adjustment. O'Toole and Moya (1981) identified -1.7 MPa as the leaf water potential threshold below which water deficit affects rice growth. Reduction in photosynthesis at a leaf water potential of -1.7 MPa varied from 20 % (Fukai *et al.*, 1985), to 70-80 % (Dingkuhn *et al.*, 1989a), to a complete stop (Furuya *et al.*, 1994).

2.3.4 Membrane stability index

The cell membrane is one of the main cellular targets common to different abiotic stresses. The extent of its damage is commonly used as a measure of tolerance to various stresses in plants. Critical features of desiccation tolerance depend on the abilities to limit membrane damage during water stress and to regain membrane integrity and membrane-bound activities quickly upon rehydration. It is a physiological index widely used for the evaluation of drought and temperature tolerance. Cell membrane stability (CMS) is to be measured from electrolyte leakage of control and drought stressed leaf tissue following Tripathy *et al.* (2000).

2.3.5 Water use and transpiration efficiency

Water use efficiency of selected genotypes is to be studied following the method described by Kholova *et al.* (2010). Water use efficiency (WUE) refers to the ratio of water used in plant metabolism to water lost by the plant through transpiration. Water use efficiency of productivity (also called integrated water use efficiency), which is typically defined as the ratio of biomass produced to the rate of transpiration. Increases in WUE is commonly cited as a response mechanism of plants to moderate to severe soil water deficits, and has been focused to increase crop tolerance of drought. It may be calculated as:

$$WUE\ (kg\ ha^{-1}mm^{-1}) = \frac{GY}{WS - E} \times 100$$

where GY: grain yield (kg ha^{-1}), WS: crop water supply (mm), E: soil evaporation (mm).

TE is measured in well-watered (WW) and water stressed (WS) plants and calculated as the production of biomass per amount of water transpired during the dry down as:

$TE = (HB - DB)/T$

where, HB: final harvest biomas, DB: predry down biomass, T: water transpired.

Water transpired is the sum of daily transpiration measured in the dry down, assessed by regular weighing of pots and recording of water added. The final harvested biomass is that of WW and WS at the end of the dry down.

However, other than this method advanced methods are also followed like carbon/oxygen isotope discrimination methods:

- The flag leaf/grain samples to be analyzed for ^{13}C isotope discrimination to measure the WUE (Impa *et al.*, 2005) and
- $\delta^{18}O$ to be measured from leaves as a surrogate for mean transpiration rate (Sheshshayee *et al.*, 2005).

2.3.6 Chlorophyll content and chlorophyll stability index

Total chlorophyll content is to be estimated by the method described Arnon (1949).

- Dip 25 mg of leaf samples (fully matured leaf) in 10 mL of 80% acetone in a graduated glass tube.
- Incubate the glass tubes in dark at 4°C for 48 h.
- Take absorbance at 645 nm and 663 nm after 48 h.
- Quantify the total chlorophyll, chlorophyll 'a' and chlorophyll 'b' content from the 80% acetone extract by following the calculation:

  Total chlorophyll (µg mL^{-1}) = 20.2 (A_{645}) + 8.02 (A_{663})

  Chlorophyll a (µg mL^{-1}) = 12.7 (A_{663}) - 2.69 (A_{645})

  Chlorophyll b (µg mL^{-1}) = 22.9 (A_{645}) - 4.68 (A_{663})

  Where, A_{663} is the solution absorbance at 663 nm and A_{645} is the absorption at 645.

  The Chlorophyll content is expressed as mg g^{-1} fresh weight.

Chlorophyll stability index (CSI) to be calculated as the per cent of chlorophyll content under stress as compared with that under normal conditions.

$$CSI = \frac{C_S}{C_N} \times 100$$

where, C_S: Chlorophyll content under water stress, CN: Chlorophyll content under normal condition.

2.3.7 Proline content

Proline acts as an osmoregulator and its concentration in many plants or tissues exposed to a variety of abiotic stresses has been frequently studied (Hare and Cress 1997; Mohammadkhani and Heidari 2008). According to Soraya *et al.*(2004) genotypes with high proline content in leaf tissues were more dehydration tolerant, a relatively high water content was maintained, and leaf rolling and senescence was delayed under severe water deficit. However, the ability of rice roots to penetrate deep into the soil was negatively correlated with proline accumulation in leaf tissue. Proline content to be measured following the method of Bates *et al.* (1973).

- Homogenize the 0.5 g fresh leaf sample in 10 mL of 3% aqueous sulphosalicylic acid.
- Filter the homogenate through Whatman No. 2 filter paper.
- Prepare the reaction mixture by adding 2 mL of filtrate, 2 mL of glacial acetic acid and 2 mL acid ninhydrin (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6*M* phosphoric acid).
- Heat the mixture to boiling for 1 h.
- Terminate the reaction by placing the tubes in ice bath, then add 4 mL of toluene to the reaction mixture and stir for 20-30 sec.

- Separate the red colour toluene layer and then measure its intensity at 520nm using spectrophotometer (Genesys 200).
- Prepare the standard curve by running a series of standard with pure proline.
- Calculate the proline amount in the test sample from the standard curve using the following formula:

$$\mu\text{moles per g tissue} = \frac{\mu\text{g proline mL}^{-1} \times \text{mL toluene} \times 5}{11.55 \times \text{g sample}}$$

Where 11.55 is the molecular weight of proline.

2.3.8 Gas exchange parameters

Measure the gas exchange parameters in the fully expanded leaf second from the top of the plant by using a portable infrared gas analyzer (LI-6400; LI-COR, Lincoln, NE) (Natural and Jacob, 1999). The measurements are made at an ambient CO_2 concentration of 390 µmol mol^{-1} and photosynthetic photon flux density of 1,500 µmol m^{-2} s^{-1} by using LICOR light source and a chamber temperature of 28°C. The parameters net photosynthetic rate (*A*) and transpiration rate (*T*) are used to calculate the instantaneous WUE (ratio *A*/*T*), which is the amount of CO_2 fixed per unit amount water lost by transpiration.

2.3.9 Chlorophyll fluorescence

For chlorophyll fluorescence measurement, the same intact leaf is used. Chlorophyll fluorescence is measured using a plant efficiency analyzer (Handy *PEA*, Hansatech Instruments Ltd., Norfolk, UK). To measure the Chl a fluorescence transients, leaves are maintained in darkness for 30 min and data is recorded from 10 µs up to 1 s with data acquisition every 10 µs for the first 300 µs, then every 100 µs up to 3 ms and later every 1 ms. The maximal intensity of the light source, providing an irradiance saturating pulse of 3500 µmol photons m^{-2} s^{-1}, is used.

2.3.10 Photosystem II yield

The quantum yield of photosystem II to be measured from the chlorophyll fluorescence yield (Maxwell and Johnson, 2000). PS II yield of dark adapted leaf or PSII yield at constant light to be measured on the topmost fully expanded leaf (second leaf from the top) at vegetative and flag leaf at flowering stage during 10:00 to 14:00 h by using a Plant Efficiency Analyzer, Handy *PEA* (Hansatech Instruments, Norfolk, UK) and recorded from 10 µs up to 1 s, with a data acquisition every 10 µs for the first 300 µs, then every 100 µs up to 3 ms, and after that 1 ms. The signal resolution was 12 bits (0–4 000). All measurements are to be taken on fully dark-adapted attached leaves. The maximal irradiance of 3000 µmol (photon) m^{-2} s^{-1} is to be used. The measured light and dark adapted parameters are minimum fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v), and maximum quantum efficiency of photosystem II (F_v/F_m).

2.3.11 Phenotyping for root morphological traits

A dynamic root system is fine-tuned to soil moisture status and is known to regulate the amount of water available to the plant depending on its distribution in the soil. Since root traits are associated with drought tolerance under field condition, germplasm lines differing in their response towards drought can also be evaluated for root traits in polyvinyl chloride (PVC) pipes under moisture stress at vegetative stage (Plate 2.4 and 2.5).

- Among the root morphological traits, maximum root length, root diameter, and root: shoot dry weight ratio were found to be associated with drought resistance in upland conditions (O'Toole and Soemartono, 1981; Yoshida and Hasegawa, 1982).

- Root thickness was found to confer drought resistance, as roots are capable of increasing root length density and water uptake by producing more and larger root branches (Ingram *et al.*, 1994).
- Shoot growth is reported to be more inhibited than root growth when soil water is limited (Westgate and Boyer, 1985; Sharp *et al.*, 1988).

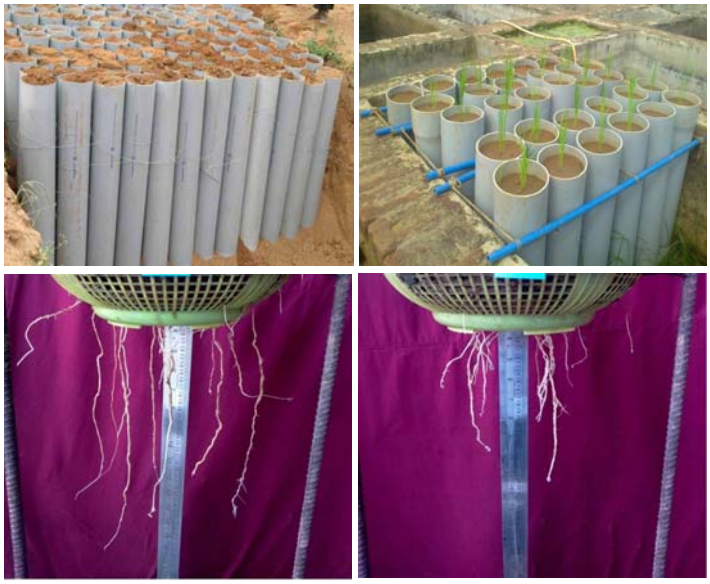

Plate 2.4: Evaluation of deep rooting by PVC cylinders and basket method

- This differential response/sensitivity of root and shoot growth to low-water potential is considered to be a means of avoiding excessive dehydration (Sharp and Davies, 1989; Hemamalini, 2000).
- Increased root: shoot ratio (Sharp & Davies, 1989), high total root length (Ingram *et al.*, 1994) and high root elongation rates (Sharp *et al.*, 1988) enable plants to maintain relatively high rate of water uptake under water stress conditions.

Plate 2.5: Washed roots for different traits

2.3.12 Specific traits for reproductive stage drought tolerance screening

The following observations are to be taken during the reproductive stage drought tolerance screening.

- *Phenology:* Days to 50% flowering, days to physiological maturity, flowering delay and reduction in grain filling duration to be recorded.
- *Spikelet fertility/grain filling %:* Number of filled and unfilled spikelets to be recorded at physiological maturity to calculate spikelet fertility (%) both in control and stress treatments (Fischer *et al.* 2003).
- *Yield and its components:* One meter row or 10 hills to be harvested at maturity. Plant number, tillers per plant, panicles per plant, Biomass, grain yield and 1000 grain weight data to be recorded. Relative yield reduction (RYR) and Drought susceptibility index (DSI)

(Fischer and Maurer, 1978) analysis to be used to rank the genotypes for drought tolerance in yield.

$$RYR\ (\%) = \frac{Y_S - Y_C}{Y_C} \times 100$$

$$DSI\ (\%) = \frac{1 - Y_S/Y_C}{1 - \bar{Y}_S/\bar{Y}_C} \times 100$$

where, Y_S: yield under stress, Y_C: yield under control, $\bar{Y}_S$: mean yield under stress, $\bar{Y}_C$: mean yield under control.

Or DSI= $(1-Y_{WS}/Y_{WW})/D$

D=1-(Experimental mean under WS / Experimental mean under WW)

Y_{WS}= Grain yield of the genotype under vegetative stage drought stress

Y_{WW}= Grain yield of the genotype under well watered condition

High values for DSI represent drought susceptibility (Winter *et al.* 1988). The DSI for grain yield or any other trait close to or below 1, indicates the relative tolerance of that trait to drought. Based on the value and direction of desirability, ranking was done for different genotypes as highly drought tolerant (DSI<0.50), moderately drought tolerant (DSI: 0.51-1.00) and drought susceptible (DSI>1.00). Yield under stress and DSI are negatively correlated.

2.4 Breeding for drought tolerance

Drought is one of the major abiotic stresses that affects rice production in rainfed areas of India. From 1965 till 2009, on 14 occasions, rice production failed to achieve the expected production level. Drought was the factor for lower production on 11 of these 14 occasions. The country's rice production declined to 89.13 Mt in 2009-10 from record 99.18 Mt in the previous year due to severe drought. The estimated average loss in rice production during drought years for the 3 states (Jharkhand, Odisha and Chhattisgarh) of eastern India is 5.4 million tons (Pandey *et al.*, 2007). Drought tolerance is a quantitative trait and considered to be complex in nature from genomics point view (Blum, 2011). Low heritability for grain yield under drought stress and high genotype × environment interaction variation and unpredictability of environmental conditions have hindered breeder's efforts to select for drought tolerance. However, recent studies at IRRI and elsewhere have reported that with precise screening protocols and large breeding populations, it is possible to achieve moderate to high heritability for grain yield under reproductive stage drought stress. Breeders have taken up their approach to coax this situation by their own methods of breeding between the best-suited varieties for generating high-yielding drought tolerant varieties.

2.4.1 Breeding strategies

The conventional approach of breeding rice varieties for drought tolerance is based on selection for grain yield and its components in a given drought environment. Even in drought-prone areas, farmers do not usually want varieties that are drought tolerant but low in yield potential under favorable conditions. They want varieties that produce grain yield at least at par with popular varieties under favorable conditions and out-yield it under drought conditions. Therefore, it is necessary that segregating breeding lines should be screened under both stress and non-stress conditions to combine high yield potential with drought tolerance. Choice parents for hybridization are very important in conventional breeding and at least one parent used for crossing should be highly tolerant to reproductive stage drought. F_1 plants are developed by crossing a high-yielding variety and a drought-tolerant cultivar. Double or three-way crosses may be used to combine tolerance for other biotic/ abiotic stresses prevalent in the target environment or other important traits. For a single cross an F_1population of 25-30 plants per cross is considered desirable and size will increase proportionately if more

numbers are involved in the crossing. F_1 plants are grown under irrigated condition to harvest seeds for large F_2 population. The optimum population size for F_2 generation is 2500 to 3000 per cross. In this generation individual plants are grown in non-stress condition with recommended package of practices and single plant selection carried out based on duration, plant type, grain type, yield components, visual yield rating and reaction to biotic stresses. Selection criteria may differ a little bit based on the target ecosystem. The pedigree method of breeding is mostly used. The F_3 lines (single plant selections made in F_2) are divided into two sets. One set is used for drought screening under stress condition and another set is grown in non-stress condition to evaluate for yield potential. The F_3 lines are planted in 2-row plots of 2 m length. The progenies performing well under both conditions are selected for next cycle of selections. In uplands, individual panicles are selected because of continuous seeding, whereas in lowlands individual plants are selected in a progeny row. The selected plants/panicles are advanced to the F_4 generation, grown under non-stress condition. The generations advanced lines in F_4 are grown as pedigree nursery in F_5 generation for selections. In uplands, selections are made under rainfed stress condition only whereas, in lowlands lines are grown both under stress and non-stress condition for further selection. In F_6 generation, the single panicle/plant selections are generation advanced under non-stress condition. In F_7 generation the generations advanced lines are again grown as in F5 and uniform progenies performing well under rainfed stress condition in uplands and both under stress and non-stress conditions in lowlands are selected as bulks and advanced to the preliminary yield trial (PYT) (Fig 2.1).

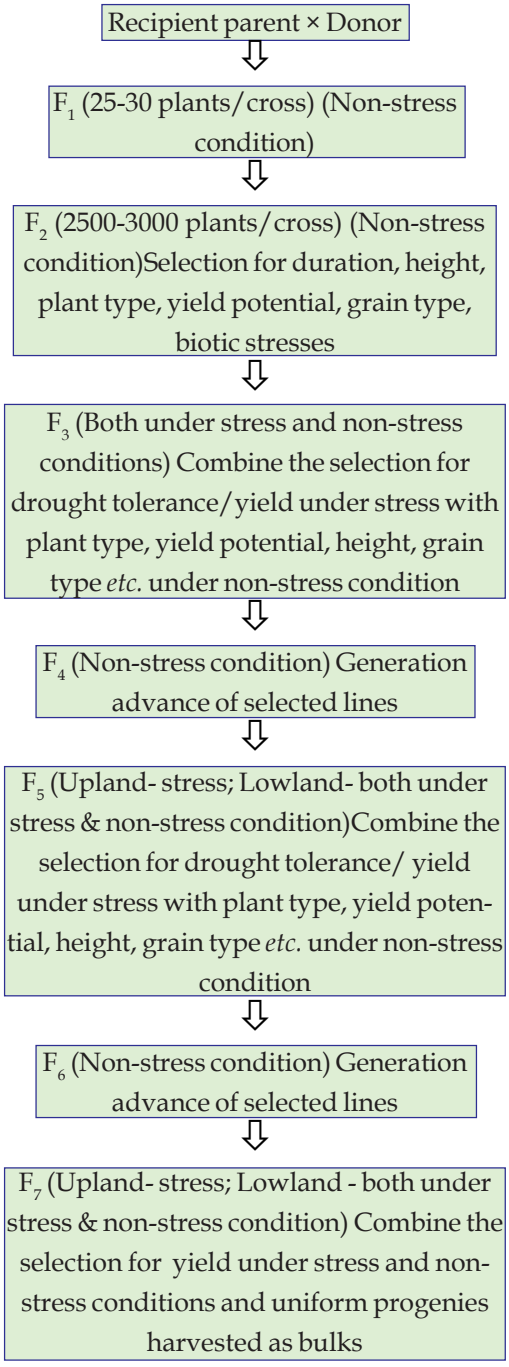

Fig 2.1. Schematic diagram of conventional breeding programme to develop high yielding drought tolerant varieties

2.4.2 Marker-assisted breeding

Till recent past marker-assisted breeding (MAB) is not very successful in improving drought tolerance in any crops because none of the identified QTLs had large enough effect on grain yield (Bernier *et al.*, 2008), and selection for secondary traits don't realize the desired progress. Steele *et al.* (2013) use marker-assisted backcross breeding to introgress four QTLs for root traits, previously identified from japonica variety Azucena into an upland rice cultivar Kalinga III. Though the introgression lines showed improved root traits throughout tillering than the recurrent parent but QTL effect is not detected for yield improvement under drought stress conditions. The recent identification and fine mapping of large and consistent effect QTLs for grain yield under drought stress at IRRI present an opportunity to improve high-yielding but drought-susceptible varieties through MAB of large-effect QTLs. Two such QTLs (*qDTY2.2* and *qDTY4.1*) identified from backcross inbred lines of IR64 x Aday Sel cross are introgressed into the IR64 that shows enhanced grain yield under drought in multi-location evaluations in the target environment, thereby confirming the value of these QTL for sustainable yield under drought stress (Swamy and Kumar, 2013). The major issue of concern with these QTLs is large QTL × genotype background effect. One of the major drought QTLs, *qDTY1.1* have shown an effect in a number of backgrounds and identified as one of the candidate QTLs for introgression. In a DBT supported QTLs introgression project a number *DTY* QTLs have introgressed into popular varieties like IR64 and Swarna along with submergence tolerance QTLs *Sub1*. Two QTLs (*qDTY1.1* and *qDTY2.2*) are successfully introgressed into IR64 - *Sub1* and introgressed lines shows improved performance under reproductive stage drought over the recurrent parent in controlled testing using rainout shelter. The MAB strategy used for the introgression of the QTLs is given below (Fig. 2.2).

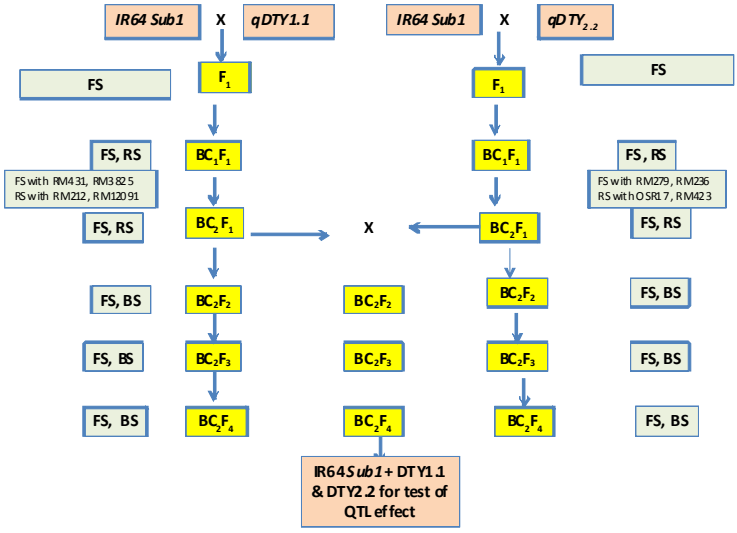

Fig. 2.2: Schematic diagram for marker-assisted breeding strategy with large effect QTLs for grain yield under drought stress in rice. FS = foreground selection; RS = recombinant selection; BS = background selection

2.5 Germplasms and varieties for drought tolerance

- *Numbers of genotypes screened:* 689
- *Vegetative stage drought tolerance:* 424 (IC568024, IC568009, IC568114, IC568060, IC568016, IC568030, IC568083, IC568112, IC568065, AC42297, AC42994, AC43020, Ranga Bora, CR143-2-2, AC43012, AC43025, Joha)
- *Reproductive stage drought tolerance:* 265 (CR143-2-2, IR55419-04, Mahulata, IR77298-14-1-2-10, IR83614-1001-B-B, CT9993-5-10-1-M, IR72667-16-1-B-B-3, IR80461-B-7-1, AC43030, AC43013, Kokua, IC337574, IC267416, IC337576, EC545088, IC311011, IC426044, IC382660, IC311130, IC337605)
- *Promising lines:* CR 143-2-2, Sarjoo-52, Mahulata,Brahman Nakhi and Salkain
- *Promising varieties:* Sahbhagidhan, Satyabhama, Ankit, Vandana, Anjali, CR Dhan 40

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