

Screening of Rice Germplasm for Tolerance to Drought Stress using Morphological and Physiological Parameters

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ABSTRACT

Identification of rice genotypes for drought stress condition is one of priority research area therefore, in order to quantify the drought tolerance genotypes, the rate of water response and contribution of yield components due to water availability of rice with different drought tolerance obtained from the yield data under drought stress and irrigated sets of experiment were examined with 500 rice genotypes consisting 7 checks varieties Dagad deshi, RRF 127, RRF 140, DRR 42, Annada and susceptible MTU010, and Swarna subjected to moisture stress before reproductive stage. Yield data at different growth stages were used for the analysis of drought tolerance. Different drought indices probably measure similar aspect of drought

tolerance. The drought susceptibility index (DSI) were superior in genotype IC386140, IC217358, IC516693, IC203364, IC216830, IC459860 and IC464270 indicating that they can be used as alternative for each other to select drought tolerant genotypes with high yield performance in both conditions IC 218251, IC206864, IC135899, IC379170, 61762X, IC 206758 and IC 449598.

Keywords Rice, Drought stress, Seed yield, Tolerant, Drought susceptibility index.

INTRODUCTION

Rice (*Oryza sativa* L.) is cultivated under diverse ecologies, ranging from irrigated to rain-fed and upland to lowland and deep water system. The frequent occurrence of abiotic stresses has been identified as the key to the low rice productivity of rain-fed ecosystem. Drought is the most severe abiotic constraint reducing rice yield on more than 23 million ha of rain-fed area in south and south-east Asia (Kumar *et al.* 2014). Out of the 20.7 million ha of rain-fed rice area reported in India, approximately 16.2 million ha lies in eastern India (Singh and Singh 2000) of which about 6.3 million ha of upland and 7.3 million ha of lowland areas are highly drought prone (Manjappa and Shailata 2014). Future crop improvement depends on the genetic variation from traditional varieties and related wild species to cope with the

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many biotic and abiotic stresses that challenge rice production around the world. In this plain, losses due to reproductive-stage drought stress are most severe in the key rice producing states of Eastern India, viz., Chhattisgarh, Orissa, Jharkhand, Bihar, and eastern Uttar Pradesh. In severe drought years, total losses to rice production in Chhattisgarh, Orissa, and Jharkhand have been reported to be as much as 40 %. Most traditional as well as high yielding varieties of the Eastern region are highly susceptible to drought, particularly at reproductive stage. Understanding of physiological and morphological that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and using these traits in breeding program (Monkham *et al.* 2015). In this context, a field experiment was conducted for kharif seasons of 2020 to study the effect of water stress on morpho-physiological traits associated with drought tolerance in rice genotypes under rainout shelter moreover irrigated normal condition.

MATERIALS AND METHODS

Experimental site and plant materials

The experiments were carried out at the Research cum Instructional farm, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur (CG) during *kharif* season 2020. The experiment was done in rainout shelter and irrigated normal both conditions. 500 rice germplasm accessions were received from IARI, New Delhi under the “Mainstreaming rice landraces diversity in varietal development through genome wide association studies: A model for large scale utilization of gene bank collection of rice” project. Check varieties with tolerance checks viz., Dagad deshi, RRF 127, RRF 140, DRR 42, Jaya, Annada and susceptible MTU010, and Swarna for testing under stress and irrigated conditions.

Field and lab experiments

The field experiments were conducted under reproductive stage water stress (rainout shelter) and normal condition (irrigated). The experiment was laid out in augmented design. Twenty one days old seedlings were transplanted. Each genotype was raised in a 3 m²

plot by transplanting. The single rice seedlings were transplanted manually in puddled field spaced 15 cm apart. Row to row space was maintained at 20 cm. In each plot a uniform plant stand were maintained and standard agronomic practices were followed for raising and maintenance of plants. Both water stress and non-stress control field were fertilized at the N, P₂O₅ and K₂O, respectively. Irrigated condition experimental field was kept continuously flooded with 5 cm water after transplanting until 25 days before harvest. Under drought stress experimental field the crop was grown under normal irrigation for four weeks after transplanting and then irrigation was withdrawn for next one month and beyond, till the susceptible checks showed permanent wilting. During the reproductive stage stress period soil moisture content status was monitored through periodical soil sampling at 15 and 30 cm soil depth after suspension water. Water table depth was also monitored during the stress period. Observations of yield and yield contributing traits i.e., days to 50% flowering (DFF), plant height (PH), tiller numbers /plant (TN/P), percentage spikelet sterility relative water content, leaf rolling, leaf drying, drought recovery score, drought susceptibility index and grain yield (GY/pl.) in g. recorded. The observations were taken as per SES (IRRI 2013) method.

Studies of drought trait

Soil moisture

Soil moisture was measured by collecting duplicate soil samples from the soil depth of 0-10, 11-20, 21-30 cm. The soil sample was taken by the gravimetric. The fresh weight of soil was noted and the soil was dried in an oven at 100°C up to the constant weight. The gravimetric soil moisture was determined as follows:

$$\text{Soil moisture} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight}} \times 100$$

Relative water content (RWC)

Leaf Relative water content (RWC) was estimated by recording the turgid weight of 0.5 g fresh leaf sample by keeping in water for 4h, followed by drying in hot air oven till constant weight is achieved (Weatherly, 1950). It is given as below Fully expanded leaves

were excised at 10.00 AM and relative water content was determined. The unit of RWC is $\mu\text{ mol CO}_2/\text{m mol H}_2\text{O}$.

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{turgid weight} - \text{Oven dry weight}} \times 100$$

Where,

FW = Fresh weight of the leaf samples taken immediately after excision.

TW = Fully turgid weight determined upon re-hydration of leaves by immersing them in a petri dish containing distilled water for two hours.

DW = Dry weight obtained after drying at 80° C for 2 days until no further weight change occurred.

Chlorophyll reading by soil plant analysis development (SPAD)

The SPAD plus determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength. SPAD reading is equivalent to chlorophyll content in g/cm^2 .

Leaf rolling

Leaf rolling decreases leaf surface area, reducing transpirational losses, and is a common response to low water availability in drought tolerant species. Leaf rolling is scored visually in rice either in morning or at mid-day with the help of scales (0-9) as per SES is given below :

Score 0	Leaves healthy
Score 1	Leaves start to fold (shallow V-shaped)
Score 3	Leaves folding (deep V-shaped)
Score 5	Leaves fully cupped (U-shaped)
Score 7	Leaf margins touching (O-shaped)
Score 9	Leaves tightly rolled

Leaf drying

A scale of 0-9 is used to visually score leaf desiccation for each entry at levels of imposed drought using standard evaluation system (SES) 0-9 scale developed at IRRI is given below :

Drought score: Leaves appearance

0	No symptoms of stress effect
1	Slight leaf tip drying
3	Tip drying extended up to ¼ lengths in most leaves
5	One-fourth to 1/2 of all the leaves fully dried
7	More than 2/3 of all leaves fully dried
9	All plants apparently dead

Drought recovery score

1	90%–100% of plants recovered
3	70%–89% of plants recovered
5	40%–69% of plants recovered
7	20%–39% of plants recovered
9	20% of plant recovered

Drought susceptibility index

$$\text{DSI} = (1 - Y_s/Y_i) / (1 - (Y_s)^{\bar{}} / (Y_i)^{\bar{}})$$

Where, Y_s = Yield in stress condition, Y_i = Yield in irrigated condition,

$(Y_s)^{\bar{}}$ = Mean yield in stress condition, $(Y_i)^{\bar{}}$ = Mean yield in irrigated condition.

Therefore, selection using morpho-physiological traits can improve the drought tolerance at reproductive stage in rice. Leaf rolling is one of the visible physiological responses to plant water deficit. It is an adaptive response to water deficit which helps in maintaining favorable water balance within plant tissues with resultant benefit to plants under conditions of water scarcity and depleting soil moisture (Upadhyaya *et al.* 2019). Plant recovery from desiccation in agricultural crops is primarily a function of the capacity for maintaining higher RWC during desiccation.

RESULTS AND DISCUSSION

Out of 500 core rice germplasm accessions were evaluated as per SES 2013 in *kharif*-2020 under rainout shelter drought condition with control environmental conditions. The frequency distribution of various

Table 1. Frequency distribution for various characters in rice germplasm 500 accessions under rainout shelter.

Sl. No.	Characters	Category	No. of Acc. Recorded	Frequency
1	Days to 50% flowering	Early (71-90 days)	500	02
		Medium (91-110 days)		252
		Late (111-130 days)		70
		Not flowered		176
2	Plant height	Semi dwarf group (>90 cm)	500	22
		Intermediate group (90-125 cm)		154
		Tall group (<125 cm)		148
		Not flowered		176
3	Panicle length	Short group (16-20cm.)	500	23
		Medium group (21-25 cm)		214
		Long group (26-30 cm),		83
		Very long group (>30 cm).		4
		Not flowered		176
4	Productive tiller/plant	Very low group (<5tiller/plant)	500	188
		Low group (5-10 tiller/plant)		114
		Medium group (11-19 tiller/plant)		22
		Not flowered		176
5	Spikelet fertility %	Highly fertile (>90%)	500	05
		Fertile (75-89%)		76
		Partly sterile (50-74%)		143
		High sterile (<50%)		276
		Not flowered		176
6	Leaf rolling (in days)	Leaves healthy - scale -0	500	76
		Leaves start to fold (shallow V-shaped)-scale-1		124
		Leaves folding (V-shaped)-scale-3		38
		Leaves fully coped (u-shape)-scale -5		52
		Leaves margins touching (O-shape) scale-7		94
		Leaves tightly rolled - scale -9		
7	Leaf drying (in days)	No symptoms scale-0	500	68
		Sight tip drying scale -1		89
		Tip drying extended up to 1/4 length in most leaves Scale-3		88
		One fourth to 1/2 of all leaves fully Dried- scale-5		69
		More than 2/3 of all leaves folly Dead- Scale -7		186
		All plant Apparently Dead -Scale-9		
8	Drought recovery score`	(90-100% plant recovered) score-1	500	88
		(70-89% plant recovered) score-3		108
		(40-69% plant recovered) score-5		28
		(20-39% plant recovered) score-7		19
		(0-19 % plant recovered) score-9		257
9.	Drought susceptibility index	Score (<50)	500	85
		Score (0.51-1.00)		168
		Score (>1.00)		247

yield and yield contributing traits are summarized below; following observations were recorded in all 500 accessions during *kharif*- 2020.

Days to 50% flowering is a major effect in stress. The plant reproductive phase involves many processes, including floral initiation, anther and pollen devel-

opment, pistil development, blossom, fertilization and seed development. All the reproductive processes the stage of anther and pollen developments is the most sensitive to drought (Pantuwan *et al.*2019). Rainout shelter condition flowering ranged from 89 days resistance check Dagad deshi to 128 days (IC 114897) with a grand mean of 108 days.

Plant height was categorized into three (3) groups. Out of 500 accession 22 accessions cum under semi dwarf group (>90 cm), 154 accessions cum under intermediate group (90-125 cm.), 148 accessions under tall group (<125 cm). Maximum and minimum value for plant height was found 170 cm in IC 377261 and IC377240 in 76.0 cm. respectively with a grand mean of 121.32 cm (Table 1). The IC 458657X was found tallest accession with 142.62 cm in rainfed condition, whereas was found shortest was found in susceptible check Swarna 64.0 cm in rainfed. The accession categorized in five groups. 23 accession cum under short group (16-20 cm), 214 accessions under medium group (21-25 cm), 83 accessions cum under long group (26-30 cm), and 4 accessions in very long group (>30 cm).

The range of panicle length was recorded to a range of 17.63 cm. (minimum) to 33.07cm (maximum) with a mean of 24.88 cm. The minimum and maximum measurements were recorded in accession IC216759 and IC554860 respectively.

Productive tiller per plant categorized in four groups. Out of 500 accessions 188 accessions cum under very low group (<5tiller/plant), 114 accessions under low group (5-10 tiller/plant), 22 accessions was found under medium group (11-19 tiller/plant) and none of the accession was found under high (20-25 tiller/plant) group. The maximum and minimum tiller was found 14 and 03tiller/plant respectively with a mean of 5.0. Accessions was found highest tiller ie. IC 386140 (14 tiller /pl.) followed by IC126121 (13 tiller /pl.) and IC 458657X (12 tiller/plant) and minimum tiller (03 tiller/plant) IC206999 (Table1). Spikelet fertility, an important component of grain production, is influenced by environmental conditions and genetic background. Out of 500 accessions, 93 accessions were found under high sterile (<50%), 145 accessions cum under partly sterile (50-74%), 80 accessions cum under fertile group (75-89%), only six accessions cum under highly fertile group (>90%). The maximum and minimum percentage for spikelet fertility was found 93.77% and minimum 11.02% with a grand mean of 59.36% (Table 1).The rice crop is very sensitive to drought during the reproductive stage, when it can lead to various degree of sterility. Numerous Morpho-Physiological traits putatively

contribute to drought tolerance. Thus, the spikelet fertility percentage was recorded under rainfed as well as irrigated condition.

The increase in the pollen sterility leads to a decline in grain set. In rice large proportion of the water stress affected anthers small, shriveled, unable to dehiscence and contain sterile pollen (Raman *et al.* 2012, Monkham *et al.* 2015, Wassmann *et al.* 2019).

The maximum percentage for spikelet fertility in irrigated exhibited by IC 459738 (93.77%), followed by IC 464157 (93.45%), IC 516681(90.52%), IC 86020 (89.57%) and IC 206758 (88%).

The soil moisture recorded during onset of stress period. The soil moisture content was recorded at 15–30 cm and it was varied under rainout shelter drought stress conditions and depth of soil. The moisture content were recorded 40.3 %, 28.5 %,14.0 and 12.6 % at 0-10 cm, 10-20 cm and 20-30 cm soil depth respectively with the grand mean of 23.6%.

Soil moisture%= FW-ODW/FWX100
FW=Fresh weight, ODW= Oven dry weight
Soil moisture % from onset of stress at
terminal stage from
08 Oct 2020 to 16.Nov 2021

Soil moisture % during stress		
1	08 Oct 2020	40.3
2	16 Oct 2020	28.5
3	23 Oct 2020	14.0
4	03 Nov 2020	12.6

Leaf rolling is used as an important selection criterion for drought tolerance in rice. Lines with good drought tolerance have leaf rolling character in stress condition to prevent the water loss but it have also faster recovery ability after the stress were removed in rice because flag leaf in cereal crops plays the important role in grain filling and development (Upadhyaya *et al.* 2019). Therefore, the selection proceeds to identify genotypes which had almost erect flag leaf enabling photosynthesis for longer duration. The leaf rolling were recorded with 4 days interval in 500 accessions and categorized in 6

groups, 76 accessions cum under score-0 (Leaves healthy), 124 accessions cum under score -1 (Leaves start to fold (shallow V-shaped), 116 accessions cum under score-3 (Leaves folding (deep V-shaped), 38 accessions cum under score-5 (Leaves fully coped (u-shape), 52 accessions cum under score-7 (Leaves margins touching (O-shape) and 94 accession was found under the score-9.

It indicates that the selection was very tight. Leaf rolling are significantly associated with rapid ability to recover after drought stress. This trait is still widely used to screen drought tolerant of rice. Same result was reported by Serraj *et al.* 2011, Sio-Se *et al.* 2006.

Leaf drying is one of the visible morphological traits which responses to plant during water deficit, which helps in maintaining favorable water balance within plant tissues under conditions of water scarcity and depleting soil moisture. The leaf drying were recorded with 4 days interval in 500 accessions and categorized in 6 groups, 68 accessions cum under score-0 (No symptoms) 89 accessions cum under score-1 (Sight tip drying), 90 accessions cum under score-3 (Tip drying extended up to 1/4 length in most leaves), 70 accessions cum under score-5 (1/4 to 1/2 of all leaves dried) 190 accessions cum under score-7 (More than 2/3 of all leaves fully dried) and non of the accessions was not found under the score-9 (All plant Apparently Dead) (Table1).

Leaf greenishness level was measured using a chlorophyll meter SPAD 502 Plus. The principle of this tool is to record the greenishness level of leaves and relative total of chlorophyll molecules present in leaf in a single value based on the amount of light leaf transmitted (Verulkar and Verma 2014). Chlorophyll value was recorded to be the highest in IC 218251(45.97 SPAD unit) and the lowest value of the chlorophyll was observed in IC 461742X (24.10 SPAD unit). The grand mean for chlorophyll value in all the accessions were found (36.60 SPAD units). Following accessions were identified based on SPAD value accessions having chlorophyll value which have above 37.0 SPAD value IC114628, IC217501, IC387810, IC461762X, IC463878, IC465008, IC390528 IC85885 IC116037, IC537485, IC206864, IC135899 IC379170 IC125800 IC215125 IC379003

and IC378190. The maximum and minimum values for RWC percentage were recorded 94.02% in IC 206954 (maximum) and 55.27% in IC 126468 (minimum) with a grand mean of 87.24%. The maximum RWC % was found in IC 386140 (89.14%) and IC 114664 (88.76%). A significant difference in RWC was observed among genotypes between drought stress and irrigated condition. Under water stress condition, higher value of RWC was recorded in water deficit stress tolerant rice genotypes as compared to susceptible one at reproductive stage.

The recovery score was recorded after re-watering after stress in 500 accessions and categorized in 5 groups, 92 accessions cum under score-1 (90-100% plant recovered) 109 accessions cum under score-3 (70-89% plant recovered), 28 accessions cum under score-5 (40-69% plant recovered), 19 accessions cum under score-7 (20-39% plant recovered) 259 accessions cum under score-9 (0-19 % plant recovered).

Ys and Yp are the mean yield of genotypes under drought stress and irrigated conditions and the genotypes with lowest value of DSI are more resistant to drought conditions. Result indicated that the genotype IC386140, IC217358, IC516693, IC203364, IC216830, IC459860 and IC464270 had the lowest DSI followed by while, genotypes IC458657X, IC449598, IC386358, IC205951, IC460497. IC463894 and IC378408, IC463878, IC121872, IC579766, IC114270, IC114897 resistance check DRR42 (IR64 Drought) and Jaya exhibited resistance to drought (Verulkar and Verma 2014, Monkham *et al.* 2015).

Drought susceptibility index was calculated by the grain yield of the genotype under terminal stage drought stress and grain yield of the genotype under irrigated condition. High values for DSI represent drought susceptibility. 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 85 accession including 2 checks RRF127 and RRF 140. Drought susceptibility index was recorded (<0.50), 163 moderately drought tolerant (DSI: 0.51-1.00) and 247 accessions was come under drought susceptible.

Drought susceptibility index was recorded (>1.00). Yield under stress and DSI are negatively correlated (Pantuwan *et al.* 2002, Redona *et al.* 2009). The maximum and minimum value of yield/ plant were recorded in IC217358 (31.70 g) and IC461762X (5.0 g.) with the grand mean of 13.45g. The accessions were found highest grain yield /plant under drought stress viz., IC 206758 (29.6g.), IC 449598 (28.7g), IC 114413 (27.5g), IC126121 (26.4), IC579766 (24.4) IC514466 (21.3) and IC463878 (20.8g), Whereas in irrigated maximum and minimum grain yield / plant was recorded IC114413 (55.3g) and IC386334 (17.3g) with the grand mean of 23.10g. The accessions were found highest grain yield /plant IC388692 (46.7g), IC514384(45.7g), IC379102(44.3g), IC463096 (42.0g), IC378813 (40.7g), IC377474 (39.7g), IC458677 (38.0g) and IC217772 (36.5g).

CONCLUSION

The present study showed the existence of variation among the genotypes for grain yield and physiological traits under drought stress environment at reproductive stage. The yield difference between water stress (rainout shelter) and irrigated rice between 29 and 78 %. Further selection of promising drought tolerant rice genotypes with desired physiological attributes gives better performance under target drought stress environments. Rice genotypes IC217358, IC206758, IC516693, IC458657X, IC449598, IC114413, which showed significant yield advantage, higher values of desired physiological traits such as plant RWC, leaf rolling, leaf drying, drought recovery score and drought susceptibility index as compared to check varieties under drought stress condition can be adopted in stress ecosystem, where drought is frequent, particularly at reproductive stage. Further, these morpho-physiological traits can be used as direct or indirect selection criteria to improve grain yield stability of rice genotypes under drought stress condition.

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