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Development of advanced breeding lines of rice for drought tolerance based on physiological and yield traits

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ABSTRACT

The traditional drought tolerant cultivar 'Banglami' was crossed with high yield drought susceptible variety 'Ranjit'. A total of 49 true F_{1s} were raised and selfed. The advanced F_4 lines were screened for physiological and yield traits under drought stress. Among these, the maximum root length ranged from 19cm to 59cm with the average value of 38.96. It was observed highest in 'Banglami' (59cm) followed by 'ARC10372' (51cm) and three F_4 lines B-18, B-22 and B-23 with the root length of 50cm under drought stress. The maximum root length showed positive correlation with relative leaf water content (RLWC) and grain yield under drought stress. High heritability was observed for the various physiological and yield traits under drought stress. The two F_4 lines (B-15 and B-23) were selected based on various physiological and yield traits under drought stress. The selected lines may be used in the development of drought tolerant rice variety for upland drought affected areas of Assam.

Key words: *Rice, physiological traits, grain yield, drought tolerance*

INTRODUCTION

In India, the North Eastern (NE) region is considered to be one of the hot spots of rice genetic resources in the world and a potential region with extremely diverse rice growing conditions as compared to other parts of the country. Being the secondary centre of origin of rice, the NE region is rich in diverse accessions which are believed to be evolved from diverse sources under different agro-climatic situation and have adapted to particular growth conditions. In Assam, rice is the most important crop and covers 2.54 million (M) ha of the total 3.3 M ha cropped area. Traditionally, rice is grown in three overlapping seasons in the state as a consequence of agro-climatic conditions and is dependent on rainfall. Among the three rice seasons, *ahu* or autumn rice is grown predominantly as direct seeded under rainfed upland ecosystem (0.3M ha) during February/March to June/July. Despite of heavy rainfall in this region, the main rice crop suffers from prolonged drought in *ahu* season and intermittent drought in *sali* rice growing season.

The mega rice variety Ranjit, predominantly grown in Assam during *kharif* season is susceptible to drought stress. In eastern India, *kharif* rice is mainly grown in rainfed condition. Due to continuous change of rainfall distribution pattern as a result of global warming and climate change, the variety 'Ranjit' is being exposed to drought and suffers high yield loss, leading to significant decline in the rice production in Assam. The drought tolerance was observed in some of the traditional *ahu* rice cultivars such as 'ARC10372' and 'Banglami' but due to low grain yield and poor plant types these are not very popular among the farmers. Therefore, in the present investigation 'Banglami' was crossed with 'Ranjit' and advanced breeding lines (F_4) were evaluated for physiological and yield traits under drought stress in order to identify superior line(s) that may be used for the development of drought tolerant rice variety in elite genetic background.

MATERIALS AND METHODS

Plant materials and crossing scheme

A tall, short bold grains and medium duration (120-130 days) drought tolerant cultivar (Banglami) was used as a female parent. A semi dwarf, long duration (150-155 days), weekly photosensitive, medium slender grain-types and drought susceptible variety (Ranjit) was used as a male parent for development of Advanced breeding lines (F_4). A total of 50 seeds were obtained from the cross between 'Banglami and Ranjit'. One hundred eighty F_2 plants were raised from selfing of true F_{1s}. The seeds from only 120 F_2 plants were harvested and bulked to raise F_3 plants. The 120 $F_{2:3}$ lines consisting of 1268 F_3 plants were raised and harvested individually. The randomly selected 24 $F_{2:4}$ lines consisting of 96 F_4 plants were phenotyped for various physiological and yield traits under drought stress using 'ARC10372' as a tolerant check by PVC pipe method as described by Shashidhar et al. (2012).

Genotyping using SSR markers

In order to confirm the hybridity of F_{1s} using SSR marker the total genomic DNA of samples was extracted following the protocol of Plaschke et al. (1995). For PCR analysis, 10 µ L of reaction mixture consisting 50ng DNA, 1X PCR buffer, 2.5 mM dNTPs, 20pM each primer and 1 unit Taq DNA Polymerase enzyme was made for PCR analysis. The amplified products were resolved in 3.5 percent agarose gel.

Phenotyping

The F_4 plants raised from 'Banglami x Ranjit' were evaluated under drought stress following Randomized Complete Block Design (RCBD) with four replications using PVC pipe method as described by Shashidhar et al. (2012). Three to five germinated seeds were directly sown in each pipe and only one healthy plant was kept at 30 days after sowing. The drought stress was imposed from panicle initiation to panicle emergence period (reproductive stage) by withholding irrigation. The pipes were irrigated only when the soil water tension fell below -50 kPa and moisture content to 10% at 30 cm of soil depth. The observation on the traits associated with photosynthesis was recorded using portable gas-exchange systems (LI-6400; LI-COR, Lincoln, NE, USA) at the full heading stage. ANOVA and genetic diversity parameters were estimated by using Microsoft Excel statistical tools. The Pearson correlation among the traits were analysed by using SPSS version 15.0.

RESULTS AND DISCUSSION

Hybridity confirmation

The SSR marker RM16030 exhibiting clear polymorphism between the parents was used for hybridity confirmation. The allele size of 100bp and 140bp were detected in 'Banglami and Ranjit' respectively. Out of 50 putative F_1 plants raised, the hybridity of 49 F_{1s} were confirmed proving their heterozygosity at the respective loci representing two specific alleles of both parents. Only one plant was confirmed as selfed type as it exhibited only one of the alleles of the parents and was discarded. The true F_{1s} were used for selfing to develop F_2 plants (Fig.1).

Variation among the physiological and yield traits

Observations were recorded on various physiological and yield traits and it indicated the presence of genetic variability among the F_4 plants (Fig. 2; Table 1). The traditional drought tolerant cultivars 'ARC10372' and 'Banglami' showed deep root system of 51cm and 59cm followed by the three F_4 plants (B-18, B-22 and B-23) with the root length of 50cm under drought stress (Fig. 3; Table 2) however, shallow root system of 19cm (B-17) and 25cm was observed in 'Ranjit'. Similarly, the greater root length has also been reported in drought tolerant plants such as 'Dular', 'Azucena' and 'Rayada' compared with high-yielding drought susceptible varieties such as 'IR64' (Henry et al., 2011).

The maximum root length showed positive correlation with Relative Leaf Water Content (RLWC)

Table 1. ANOVA for physiological and yield traits of the F4plants derived from 'Banglami and Ranjit'

Source	d.f	PH	NOT	MRL	RLWC	RDW	SDW	RV	RN	Phot	gsw	Ci	Trmol	GY
Treatment	26	1135.71*	204.69*	346.60*	240.44*	2055.17*	3352.10*	1141.33*	25275.79*	217.23*	1.05*	7366.87*	35.74*	38.04*
Replication	3	57.44*	53.87*	42.49*	0.429	7.92	78.39*	4.46	38.49	26.60	0.025	131.38*	0.059*	0.15
Error	78	17.59	9.79	7.62	1.37	3.17	12.72	11.26	44.57	11.28	0.035	36.12	0.014	0.13

*Significant at 5% level

Table 2. Mean performance of F_4 plants for physiological and yield traits under drought stress

Plants	PH	NOT	MRL	RLWC	RDW	SDW	RV	RN	Phot	gsw	Ci	Trmol	GY
Ranjit	103.00	19.00	25.00	62.61	65.31	90.00	50.00	310.00	7.47	0.18	289.00	1.73	4.50
Banglami	132.00	39.00	59.00	70.00	73.95	75.00	90.00	212.00	8.67	0.13	298.00	1.35	10.00
ARC10372(C)	136.00	20.00	51.00	75.68	22.89	99.00	20.00	261.00	13.23	1.01	319.00	7.45	9.15
B-15	115.00	34.00	40.00	62.64	50.00	143.00	30.00	258.00	16.10	0.16	261.00	2.43	15.50
B-23	107.00	36.00	50.00	71.08	93.33	105.00	60.00	329.00	8.76	0.31	260.00	3.31	17.50
Population Mean	120.72	34.33	38.21	70.82	52.41	117.50	34.08	274.75	16.78	0.56	266.25	4.42	8.38
C. D.@ 5%	5.93	4.42	3.90	1.65	2.51	5.04	4.74	9.44	4.75	0.26	8.50	0.17	0.53

PH= Plant height (cm), NOT= Number of tillers, MRL= Maximum root length (cm), RLWC= Relative leaf water content (%), RDW= Root dry weight (gm), SDW= Shoot dry weight (gm), RV= Root volume (ml), RN= Root number, Phot= Rate of photosynthesis (µmol $CO_2 m^{-2} s^{-1}$), gsw= Stomatal conductance (µmol $H_2O\ m^{-2}s^{-1}$), Ci= Intercellular CO_2 concentration (µmol $CO_2\ mol^{-1}$), Trmol= Rate of transpiration (µmol $H_2O\ m^{-2}\ s^{-1}$), GY= Grain yield (gm/plant), CD= Critical difference

under drought stress indicated that the cell turgidity was maintained through mining water from deeper layers of soil. Therefore, higher RLWC was maintained in 'ARC10372' (75.68%) and 'Banglami' (70%) and some deep rooted F_4 plants 'B-18' (73.74%) and 'B-23' (71.08%) apart from this, some moderate rooted plants (B-3, B-10 and B-11) also showed high RLWC under drought stress. The maintenance of a relatively high RLWC in the plants under moisture deficient condition is an indicator of drought tolerance (Altinkut et al., 2001; Colom and Vazzana, 2003). However, 'Ranjit' and some F_4 plants maintained relatively low RLWC due to its shallow root system that causes loss in cell turgidity and appearance of leaf rolling after 15 days of drought stress. It showed symptoms of severe leaf rolling after 25 days of drought stress. Whereas, leaf rolling was not observed in 'ARC10372', 'Banglami' and some of the F_4 plants therefore, these are tolerant to drought stress. Leaf rolling, exhibiting medium to high heritability was reported to be directly correlated with yield under stress (Deokar et al., 2007).

The observed rate of photosynthesis had significant positive correlation with stomatal conductance, intracellular CO_2 and rate of transpiration. Kramer and Boyer (1995) reported the rate of leaf photosynthesis in rice plants is affected by the carboxylation rate and the rate of diffusion of CO_2 from the atmosphere into the leaf at the ambient atmospheric

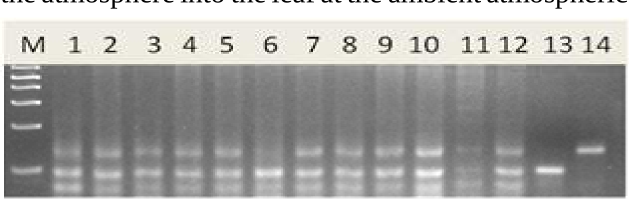

Fig. 1. Confirmation of F_1 hybrids using SSR marker RM16030. M=100bp ladder, 1-12= F_1 samples, 13= Banglami, 14= Ranjit

concentration of CO_2. The deep rooted plants showed higher rate of photosynthesis under drought stress resulting in improved grain yield as compared to the shallow rooted plants. The development of cultivars with thicker and deeper roots is expected to increase yield under drought as reported by Steel et al. (2013). The deep root system enables plant to absorb more water and nutrients, and thereby increase photosynthesis and carbohydrate synthesis and mobilize assimilates to grain yield even under water scarcity situation. Therefore, the maximum root length showed positive correlation with grain yield under drought stress. Similar findings were also reported by Mambani and Lal 1983; Lilley and Fukai, 1994. In the present study high grain yield was recorded in 'ARC10372', 'Banglami' and the selected F_4 plants (B-15, B-23). Whereas, the lowest grain yield of 4.5 gm/plant was recorded in 'Ranjit' under drought stress (Table 2). The adaptability of farmer preferred variety 'Ranjit' can be increased by incorporating drought tolerance from the local tolerant cultivars. High heritability was observed for the various physiological and yield traits under drought stress indicating high component of heritable portion of

Ranjit Banglami ARC10372 F_4 plants

Fig. 2. Plants (F_4) under reproductive stage drought stress

Fig. 3. Root morphology of F_4 plants raised from 'Banglami x Ranjit' under drought stress. 1= Banglami, 2= ARC10372, 3= Ranjit, 4-26= F_4 plants

variation. The estimate of genetic advance is more useful as a selection tool with heritability estimates. High heritability accompanied with high genetic advance as percent of the mean in the case of root dry weight, root volume, rate of photosynthesis, stomatal conductance, rate of transpiration and grain yield under drought stress. The two advanced lines (B-15, B-23) were selected based on phenotypic performance under drought stress (Table 3). These lines may be used in the development of drought tolerant rice variety in the elite genetic background.

Table 3. Variability, heritability and genetic advance for physiological and yield traits in 'Banglami and Ranjit'

Characters	GCV	PCV	Heritability	Genetic advance	GA% of mean
PH	13.81	14.23	0.969	34.44	28.45
NOT	20.89	22.89	0.912	14.37	43.04
MRL	23.62	24.66	0.957	18.96	48.67
RLWC	10.94	11.06	0.988	15.92	22.53
RDW	43.06	43.20	0.996	46.65	88.72
SDW	25.29	25.48	0.992	59.52	52.10
RV	46.40	47.31	0.980	34.62	95.59
RN	29.06	29.17	0.996	163.60	59.88
Phot	44.82	49.49	0.905	14.78	92.34
gsw	92.53	98.68	0.937	1.04	190.61
Ci	15.84	15.99	0.990	88.18	32.63
Trmol	69.20	69.26	0.999	6.15	142.57
GY	36.99	37.26	0.99	6.34	76.21

PH= Plant height (cm), NOT= Number of tillers, MRL= Maximum root length (cm), RLWC= Relative leaf water content(%), RDW= Root dry weight (gm), SDW= Shoot dry weight (gm), RV= Root volume (ml), RN=Root number, Phot= Rate of photosynthesis (µmol $CO_2m^{-2}s^{-1}$), gsw=Stomatal conductance (µmol H_2O $m^{-2}s^{-1}$), Ci=Intercellular CO_2 concentration (µmol CO_2mol^{-1}), Trmol=Rate of transpiration (µmol $H_2Om^{-2}s^{-1}$), GY= Grain yield (gm/plant), GCV=Genotypic coefficient of variation, PCV=Phenotypic coefficient of variation, GA=Genetic advance.

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REFERENCES

Altinkut A, Kazan K, Ipekei Z and Gozukirmizi N (2001). Tolerance to paraquate is correlated with the traits associated with water stress tolerance in segregating F_2 populations of barley and wheat. Euphytica 121: 81-86

Colom MR and Vazzana C (2003). Photosynthesis and PSII functionality of drought-resistant and drought sensitive weeping lovegrass plants. Environ. Exp. Bot. 49: 135-144

Deokar AA, Kesawat MS, Thorat AS, Sahapurwad AB, Kadam SB, Deshmukh RK, Sharma V and Verulkar SB (2007). Identification of QTLs for leaf rolling and relative water content under water stress conditionin rice (*Oryza sativa*). Indian J. Crop Sci. 2: 345-348

Henry A, Gowda VRP, Torres RO, McNally KL and Serraj R (2011). Variation in root system architecture and drought response in rice (*Oryza sativa*): phenotyping of the Oryza SNP panel in rainfed lowland fields. Field Crops Res. 120: 205-214

Kramer PJ and Boyer JS (1995). Photosynthesis and respiration. In: Water relations of plants and soils. San Diego, CA: Academic Press 313-343

Lilley J and Fukai S (1994). Effect of timing and severity of water deficit on four diverse rice cultivars I. Rooting pattern and soil water extraction. Field Crop Res. 37: 205-213

Mambani B and Lal R (1983). Response of upland rice varieties to drought stress. 1. Relation between the root system development and leaf water potential. Plant Soil 73: 59-72

Plaschke J, Ganal MW and Röder MS (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91: 1001-1007

Shashidhar HE, Henry A and Hardy B (2012). Methodologies for root drought studies in Rice. International Rice Research Institute. Los Banos

Steele KA, Price AH, Witcombe JR, Shrestha R, Singh BN, Gibbons JM and Virk DS (2013). QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. Theor. Appl. Genet. 126: 101-108