Oryza Vol. 53 No.4, 2016 (422-427)

Response of physiological and biochemical parameters in deeper rooting rice genotypes under irrigated and water stress conditions

RK Panda[2], E Pandit[1], A Swain[1], DP Mohanty[1], MJ Baig[1], M Kar[2] and SK Pradhan[*1]

[1]National Rice Research Institute, Cuttack-753006, Odisha, India

[2]College of Agriculture, Bhubaneswar-751003, Odisha, India

****Corresponding author e-mail: pradhancrri@gmail.com***

Received :06 March 2016 Accepted :13 November 2016 Published :03 February 2017

ABSTRACT

An experiment was conducted under vegetative stage drought stress condition with the objective to determine the effect of water deficit stress on various physiological and biochemical traits associated with drought tolerance attributes of twelve promising rice genotypes selected on the basis of deeper rooting abilities. Drought stress at vegetative stage caused reduction in relative water content (RWC) (31.57 %), grain yield (55.31 %), number of effective tillers (37.70 %), plant biomass (23.65 %), and increase in grain sterility (51.5 %) and proline content (55.9 %) in rice genotypes. However, the responses varied among genotypes. Out of the twelve rice genotypes, Bamawypan, showed superiority in terms of grain yield, 1000-grain weight, total plant biomass, RWC, leaf area index (LAI), proline content, catalase activity, peroxidase activity and total chlorophyll content. Significant and positive correlations were observed between yield and physiological attributes like proline content, LAI, relative water content, catalase activity, peroxidase activity, total chlorophyll content and plant biomass under drought stress condition. The current study indicated that the physiological and biochemical traits have direct or indirect effect on yield performance of rice genotypes under water stressed environment at vegetative stage.

Key words: *Drought stress, relative water content, proline content, catalase activity, peroxidase activity, total chlorophyll content, leaf area index*

Rice (*Oryza sativa* L.) is one of the important primary cereal crops in the world. It is grown under environmental adjustment diverse conditions than any other crop. Out of the entire coverage under rice, nearly 13% is devoted to upland rice and 28% to rainfed low lands (Sadasivam *et al*. 2000; Babu *et al*. 2003). About half of the world's rice is grown in rainfed tracts, where production is dependent on a good and evenly distributed rainfall. Drought, a period of no rainfall or irrigation that affects plant growth, is a major constraint of rice production in the rainfed areas. Drought effects in lowland rice can occur when soil water contents drop below saturation (Bouman and Tung 2001). Rice plants respond to drought through alternation in morphological, physiological and metabolic traits. A deep root system with high root length density at depth is useful in extracting water thoroughly in upland conditions, but does not appear to offer much scope for improving drought resistance in rainfed lowland rice where the development of a hard pan may prevent deep root penetration. Osmotic adjustment is promising because it can potentially counteract the effects of a rapid decline in tissue water potential and there is large genetic variation for this trait. There is genotypic variation in expression of green leaf retention which appears to be ideal character for prolonged droughts, but it is affected by plant size which complicates its use as a selection criterion for drought resistance. It has been contemplated that general lack of drought related research for rice in rainfed lowland conditions needs to be rectified, particularly considering their importance relative to upland conditions in Asian countries. In this context, an experiment was conducted in dry season of 2014 to study the effect of water stress well watred condition on physiological and biochemical traits associated with drought tolerance in deeper rooting drought tolerant rice genotypes.

MATERIALS AND METHODS

Plant materials

Ninety six rice genotypes comprising of national, international and landraces collections were screened under water stress conditions. Seeds of these rice genotypes were collected from International Rice Research Institute (IRRI), Philippines, National Bureau of Plant Genetic Resources (NBPGR), New Delhi and National Rice Research Institute (NRRI), Cuttack. On the basis of genotyping results, 9 genotypes showing positive to Dro1 markers and three check genotypes were selected for deeper rooting phenotyping study.

Experimental site

The experiment was carried out in a raised brick structured tank of 18ft inside length, 6.5ft inside breadth, 3ft height above ground and 1.5ft below ground with low proportion of sand to cement of 20:1 so that it was easy to dismantle the structure. Each tank was partitioned into two sub-tanks by a middle wall with size 18'x3"x3" (above ground) at the experimental farm of the National Rice Research Institute, Cuttack, Odisha, ($20^0$27'10.99"N, $85^0$56'26.46"E) during dry season, 2014. The tanks were filled with soil (around 660cft) collected from other lands having loamy sand of pH 4.21, organic carbon 0.573 % and with available nitrogen, phosphorous, exchangeable potassium of 150, 14.08 and 25.54 kg ha^{-1}, respectively. The soil height was maintained up to 3ft in each tank. The tanks were watered for two days to allow proper compaction of soil and to maintain the uniform level. Steel baskets of 7.5cm upper, 5cm low radius and 5cm height having 2mm mesh were placed inside the soil with a gap of 45cm between baskets in each row containing 12 baskets. Six moisture meter probes were inserted in each tank to assess the moisture content of the tank. One/two seeds of individual genotype were sown 2cm below the soil exactly in the middle of the basket. The experiment was replicated twice with split plot design in two main plots (stress and no stress) and twelve genotypes in subplots. After germination, single seedling was maintained in each basket. Both water stress and no stress control tanks were fertilized at the rate of 80, 40 and 40 kg ha^{-1} N, P_2O_5 and K_2O, respectively. Nitrogen was applied on three occasions, *viz.*, 1/3rd as basal, maximum tillering and panicle initiation stages, while full P_2O_5 and K_2O were applied as a basal application. Stress was imposed on the tanks after 45 days of sowing till the susceptible checks showed permanent wilting. But the no-stress (control) tank was kept continuously watered. Water table depth was also monitored during the stress period. The drought scores, leaf rolling, leaf drying and stress recovery observations were taken as per SES method on a 0-9 scale (IRRI 1996). Leaf rolling score description '0' indicates leaves healthy. '1' leaves starts to fold, '3' Leaves folding (deep V-shaped), '5' leaves fully cupped (U- shaped), '7' leaves margins touching (O-shaped), '9' leaves tightly rolled. Studies of physiological and biochemical parameters on the basis of growth performance of 12 rice genotypes under vegetative stage drought stress condition, *viz*., Bowdel, Lalsankri, Karni, Dinoroda, N-22, Bamawypan, Tepiboro, Dular, Surjamukhi, and three check varieties Kasalath, IR64, and Kalinga-III were made on total dry matter content, LAI, RWC, proline content, catalase, peroxidase activity, isozyme activity and total chlorophyll content. Proline content was determined on acid nin-hydrin method as per Bates LS *et al*. (1973). Catalase activity was observed as per hydrogen peroxide oxidoreductase EC 1.11.1.6 and peroxidase was also determined as per donor H_2O_2 oxidoreductase E.C.1.11.1.7. Isozymes were separated on native gel as per the method of Laemmli (1970) by non-denaturating polyacrylamide gel electrophoresis. The total chlorophyll content with Chlorophyll-a and Chlorophyll-b content of leaf was measured by fresh weight basis by acetone extraction method as per Sadasivam *et al.* (1992).

Statistical analysis

Physiological and biochemical data were analyzed following the split plot design as outlined by Gomez and Gomez (1984) and Panse and Sukhatme (1985). Simple correlation co-efficient between physiological and biochemical parameters were calculated. Fisher and Yates table were consulted for comparision of 'F' values and't' values for determination of critical difference at 5% level of significance.

RESULTS AND DISCUSSION

The results related to physiological and biochemical parameters performance of 12 rice genotypes under well watered condition and water stress at vegetative stage have been presented in Table 3. Rice genotypes grown under water stress condition produced significantly lower grain yield than non-stress situations. Yield decline was observed in almost all the rice genotypes grown under stress condition. The yield reduction under drought stress over non-stress (irrigated) control ranged from 29 to 78%. The expected yield reduction under drought stress condition has been reported by many workers. In another study in Cambodia, Basnayake *et al*. (2004) estimated 9-51 % yield reduction due to drought in rice genotypes in multi-locational trials conducted over 3 years in the target environment. Similar finding has also been reported by Kumar *et al.* (2009).

Significant variations were observed among genotypes for drought tolerance parameters, *viz*., leaf rolling, leaf drying and stress recovery. Genotypes like Dinoroda and Tepiboro showed less leaf rolling, leaf drying and better stress recovery. However Dular, Surjamukhi and Kasalath showed little morphological symptoms of stress. Leaf rolling is induced by the loss of turgor and poor osmotic adjustment in rice and delayed leaf rolling is an indication of turgor maintenance and dehydration avoidance (Blum 1989). Physiological and biochemical traits like leaf area index (LAI), relative water content (RWC), proline content , catalase activity, peroxidase activity and isozyme activities were significantly increased by drought stress at vegetative stage. Gupta and Guhey (2011) also reported similar type of finding. Gloria *et al.* (2002) reported that the water deficit in rice caused a larger reduction in leaf area than shoot dry matter, demonstrating the greater sensitivity of leaf

Table1. Leaf rolling score of 12 studied genotypes at different days after sowing under drought stress condition

Leaf rolling score	45 DAS	50 DAS	55 DAS	60 DAS
0:healthy				
1:V shape	Kasalath, Bowdel	Kalinga III, Bowdel		
3:Deep V shape	Kalinga III, Lalsankri, Tepiboro	KalingaIII, Lalsankri, Karni, Tepiboro	KalingaIII, Lalsankri, Karni, Tepiboro, N22, Dinoroda	
5:U shape				Kasalath, Tepiboro, Dinoroda
7:O shape				
9:tightly rolled				Lalsankri, Karni, N22, Bamawypan

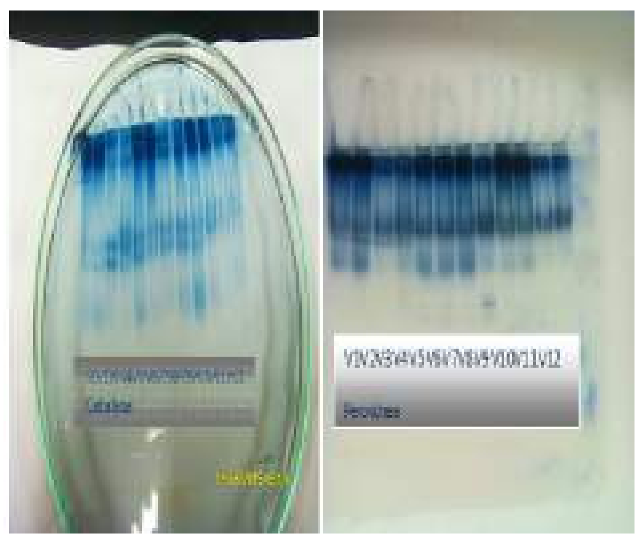

Fig. 1. Banding pattern of Isozyme catalase and peroxidase using the 12 studied genotypes. V_1-Bowdel; V_2-Lalsankri; V_3-Karni; V_4-Dinoroda; V_5-N-22; V_6-Bamawypan; V_7-Tepiboro; V_8-Dular ; V_9-Surjamukhi; and three check varieties V_{10}-Kasalath; V_{11}-IR64 and V_{12}-KalingaIII

Table 2. Drought score of 12 studied genotypes at 45 days after sowing during vegetative stage

Scale	Description	Rate	Genotypes
0	No symptoms	Highly resistant	-
1	Slight tip drying	Resistant	-
3	Tip drying extended to ¼ length in most leaves	Moderately resistant	Kasalath, Dinorado
5	¼ to ½ of the leaves fully dried	Moderately susceptible	KalingaIII, Lalsankri, Karni, Tepiboro, N22, Dinoroda
7	More than 2/3 of all leaves fully dried	Susceptible	Karni, N22, Tepiboro, Lalsankri, Bamawypan
9	All plants apparently dead	Highly susceptible	-

Table 3. Estimates of various physiological and biochemical parameters of 12 rice genotypes under water stress and well watered conditions as well as on phosphorus application and without phosphorus status of soil

Genotype	Total Biomass(g)		RWC		Leaf area index		Total chlorophyll (mg/g FW)		Proline accumulation (µg g-1 FW)		Catalase activity (µmole min-1 g-1 protein)		Peroxidase activity (µmole min-1 g-1 protein)		Unfilled grains/plant		Filled grains/plant		Test Weight (g.)	
	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress	No Stress	Stress
Bowdel	53.8	34.9	74.0	70.5	1.6	2.0	1.4	1.5	6.4	16.0	0.5	0.4	3.4	6.0	211.0	227.3	603.0	50.3	21.2	11.2
Lalsankri	76.6	34.0	75.5	79.1	1.9	2.5	1.2	1.7	9.3	26.8	0.8	0.6	4.5	6.2	132.0	209.3	109.5	36.3	15.8	9.9
Karni	77.5	19.4	65.1	69.9	1.7	2.0	1.3	1.9	7.2	40.9	0.6	0.6	4.0	6.0	523.8	699.8	599.3	133.0	20.0	9.5
Dinoroda	75.9	52.6	72.4	65.5	1.4	2.1	1.0	0.9	5.2	10.2	0.7	0.5	4.5	6.3	320.5	316.8	612.0	140.5	12.1	7.1
N22	30.5	32.9	69.6	63.7	1.7	2.6	1.3	1.3	5.6	31.8	0.7	0.6	4.1	6.5	230.8	407.8	405.5	73.3	12.8	7.6
Bamawypan	39.8	51.6	68.8	60.4	1.3	2.1	1.4	1.5	5.9	25.7	0.8	0.5	4.7	6.5	72.8	110.8	88.8	37.0	14.6	8.0
Tepiboro	38.6	30.7	83.5	67.8	1.5	2.6	1.1	1.7	6.9	37.0	0.7	0.6	4.4	6.1	373.5	420.0	373.0	113.0	17.9	9.5
Dular	45.0	38.8	82.8	74.1	1.3	2.2	1.3	1.9	6.5	23.1	1.1	0.6	4.7	7.9	301.0	321.5	833.5	154.3	22.5	13.2
Surjamukhi	36.8	17.5	63.5	54.5	1.6	1.7	1.5	1.5	6.4	25.6	0.8	0.4	4.3	7.7	114.0	157.0	1133.8	241.0	13.3	7.3
Kasalath	104.4	51.7	70.8	64.6	1.8	2.7	1.1	1.5	7.7	24.4	0.5	0.6	2.7	7.5	589.3	600.0	482.5	111.5	16.6	7.8
IR64	104.8	31.1	78.4	79.2	2.0	1.7	1.1	1.6	10.1	28.5	0.7	0.5	2.6	6.5	227.3	286.3	582.3	138.5	16.5	8.6
KalingaIII	122.9	25.2	61.6	44.9	2.2	1.5	1.4	1.1	8.2	20.9	0.8	0.7	3.6	6.7	244.0	225.5	607.8	66.5	17.8	9.1
	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%	CD(5%)	CV%
Variety	1.65	0.54	6.30	2.04	0.19	0.06	0.17	0.06	1.78	0.58	0.06	0.02	0.30	0.10	60.39	19.56	147.27	47.69	1.14	0.37
Variety within Stress	2.34	26.37	8.92	2.94	0.27	0.34	0.24	0.06	2.52	5.10	0.09	0.06	0.43	0.29	85.40	566.07	208.28	553.98	1.61	1.13

enlargement to water stress than dry matter accumulation. A significant difference in RWC was observed among genotypes between drought stress and irrigated condition. Highest value of RWC was observed in Surjamukhi (65.2 %) followed by Bamawypan (64.1 %) and Bowdel (63.4 %) (Table 3). The capacity to maintain higher RWC under drought stress condition has been suggested as a possible water scarcity tolerance mechanism in rice (O'Toole and Moya 1978). Gupta and Guhey (2011) and Jongdee *et al.* (1998) also reported similar findings. Drought stress condition caused an average increase of 61.45 % in proline content across the genotypes as compared to irrigated condition. Highest value of proline content was observed in Bamawypan followed by Surjamukhi and Bowdel under drought stress condition (Table 3). Maibangasa (1998) also reported similar increase in proline content in rice under water stress condition. Isozymes of the selected genotypes study also revealed the same accumulation pattern in case the Dinoroda and Tepiboro.

Results of this experiment suggest that genotypes had the capability in expressing their genetic yield potential under these conditions. It appears that the yield advantage observed under favorable conditions of semi-dwarf (Bowdel and Lalsankri) which required less assimilate for vegetative organs was not maintained under water limiting conditions.

The results also suggest that Dinoroda and Tepiboro genotypes have drought-tolerance mechanism and are able to retain green leaves longer than others under drought conditions (Table 3). Retention of green leaves in seedlings under drought conditions has been used as a selection criterion for drought resistance (De Datta *et al*. 1988). Alternatively, cultivars with green leaf retention may process dehydration-tolerance mechanism which allows the plants to maintain metabolic activity, despite low leaf water potential, for example, as a result of high osmotic adjustment (Fukai and Cooper 1995). From the above results, it is concluded that cultivars required for Odisha conditions, where frequent drought occurs, should have those with appropriate phonological development to escape mid drought with choice of genotypes like Dinoroda and Tepiboro which has the ability to maintain growth late in the season. Consideration of these characters in plant-breeding programs should increase the efficacy of plant improvement in the region.

Table 4. Estimates of correlation coefficients of various physiological and biochemical parameters of 12 rice genotypes under water stress and no stress situation

	biomass	RWC	LAI	T CHL	Proline	Catalase	Peroxidase	Unfilled	Filled	Test wt
Biomass										
RWC	-0.239									
	(-1)									
LAI	**0.757	-0.345								
	(-0.440)	(-0.440)								
TCHL	-0.324	-0.541	-0.014							
	(-0.318)	(-0.318)	(-0.214)							
Proline	*0.668	0.143	**0.758	-0.213						
	(-0.515)	(-0.515)	-0.255	(*0.650)						
Catalase	-0.254	0.236	-0.266	0.227	-0.036					
	(-0.039)	(-0.039)	(-0.267)	(-0.050)	(-0.345)					
Peroxidase	*-0.65836	0.083	*-0.6389	0.214	-0.549	*0.6066				
	(-0.060)	(-0.061)	(-0.050)	(-0.111)	(-0.177)	(-0.007)				
Unfilled	0.360	0.069	0.071	-0.492	0.026	-0.432	-0.377			
	(-0.059)	(-0.059)	(-0.417)	(-0.292)	(-0.509)	(-0.419)	(-0.080)			
Filled	-0.030	-0.187	-0.043	0.290	-0.166	0.178	-0.106	0.094		
	(-0.342)	(-0.343)	(-0.286)	(-0.114)	(-0.063)	(-0.311)	(-0.568)	(-0.150)		
Test wt	-0.030	-0.187	-0.043	0.290	-0.166	0.178	-0.106	0.094	1.000	
	(-0.136)	(-0.137)	(-0.011)	*0.601)	(-0.017)	(-0.158)	(-0.083)	(-0.008)	(-0.146)	

* significant at 5%,** significant at 1%

(Parentheses contain correlation coefficient on water stress condition)

REFERENCES

Babu RC, Naguyen BD and Chamarerk V *et al.* 2003. Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Science 43: 1457-1469

Basnayake B.R.S.B., Rathnasiri J and Vithanagn JC 2004. Rainfall and Temperture Scenarios for Sri Lanka under the anticipated climate change. Paper presented at 2nd AIACC regional workshop for Asia and the Pacific, Manila, Phillipines

Bates LS, Waldren RP and Teari D 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207

Beena R, Thandapani V and Chandrababu R 2012. Physiomorphological and biochemical characterization of selected recombinant inbred lines of rice for drought resistance. Indian Journal of Plant Physiology 17(2): 189-193

Blum A 1989. Osmotic adjustment and growth of barley cultivars under drought stress. Crop Science 29: 230-233

Bouman BAM and Toung TP 2001. Field water management to save water and increase its productivity in irrigated lowland rice. Agriculture Water Management 49: 11-30

De Datta SK, Malabuyoc JA and Aragon EL 1988. A field screening techniques for evaluating rice germplasm for drought tolerance during the vegetative stage. Field Crops Research 19: 123-134

Fukai S and Cooper M 1995. Development of drought - resistant cultivars using physio-morphological traits in rice. Field Crops Research 40: 67-86

Gloria CS, Ito O and Alejar AA 2002. Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. Plant Science 163: 815-827

Gomez KA and Gomez AA 1984. Statistical procedures for agricultural research (2nd ed.). John wiley and sons, New York, pp. 680

Gupta R, and Guhey A 2011. Responses of rice genotypes to water stress imposed at early seedling stage. Oryza 48(4): 366-369

Jongdee B, Fukai S and Cooper M 1998. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crop Research 76: 153-156

Kamoshita A, Babu RC, Boopathi NM and Fukai S 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rain-fed environments. Field Crop Research 109: 1-23

Kumar A, Verulkar S, Dixit S, Chauhan B, Bernier J, Venuprasad R *et al.* 2009. Yield and yield-attributing traits of rice (*Oryza sativa* L.) under lowland drought and suitability of early vigour as a selection criterion. Field Crop Research 114: 99-107

Laemmli UK 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (15 August 1970): doi:10.1038/227680a0

Mackill DJ 1991. Varietal improvement for rain-fed lowland rice in south and south east Asia: results of a survey. Progress at rain-fed lowland rice, pp. 115-144, Manila: International Rice Research Institute

Maibangasa S 1998. Morpho-physiological, biochemical and molecular mechanisms of water stress tolerance in rice (*Oryza sativa* L.). Ph.D. Thesis, Tamil Nadu Agricultural University,Coimbatore

O'Toole JC and Moya TB1978. Genotypic variation in maintenance of leaf water potential in rice. Crop Science 18: 873-876

Ozdemir F, Bor M, Demiral T and Turkan I 2004. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regulation 42: 203-211

Panse VG and Sukhatme PV 1985. Statistical methods of agricultural workers. Indian Counsil of Agricultural Research Publication, 87-89

Sadasivam S and Manickam A 1992. Biochemical methods for agricultural sciences, pp. 11-14, New Delhi: Wiley Eastern Limited

Sairam RK, Deshmukh PS and Shukla DS 1997. Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. Journal of Agronomy and Crop Science 178: 171-177

Singh S and Singh TN 2000. Morphological, chemical andenvironmental factor affecting leaf rolling in rice during water stress. Indian Journal of Plant Physiology 5: 136-141

Weatherley PE 1950. Studies in water relation of cotton plants. The field measurement of water deficit in leaves. New Phytology 49: 81-87

Zaharieva M, Gaulin E, Havaux M, Acevedo E and Monneveux P 2001. Drought and heat responses in the wild wheat relative. Aegilops geniculata Roth. Crop Science 41: 1321-1329