CHARACTERIZATION OF SOME UPLAND RICE CULTIVARS UNDER MOISTURE STRESS CONDITION

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SUMMARY

A trial was conducted to asses the performance of some locally grown upland rice cultivars of Assam, *viz.* Kolong, Luit, Nilajee, IR-50 and Gunni under different moisture regimes; 0 bar, -2 bar and -6 bar. Moisture deficit resulted in lowering of the seed germination percentage and seedling vigour in all the cultivars but the cultivars varied significantly in their response to moisture stress in regards to these traits. The local cultivars (Kolong and Luit) had comparatively higher seedling vigour and profuse root growth. Higher chlorophyll content was recorded in Kolong and Luit as compared to IR-50 and other genotypes. The leaf anatomical characteristics such as thickness of cuticle, palisade, and spongy parenchyma cells were found to be higher in Luit; however, the stomatal index and stomatal size were lower in this cultivar followed by Kolong. Higher values of RWC and chlorophyll stability index were also recorded in Luit followed by Kolong. These local cultivars also registered higher xylem number and xylem area in root. From the observations, it can be concluded that the local cultivars are having better adaptive characters for moisture deficit situation compared to the high yielding cultivar IR-50.

***Key words*: Broadcasted summer rice, chlorophyll stability index, germination percentage, leaf anatomy, moisture deficit, relative water content**

INTRODUCTION

Ahu rice (broadcasted summer rice) is the second major important rice in the state of Assam, which provides the buffer stock of food grain and fodder during the flood and post flood period. It covers an area of 0.54 million hectares and has the productivity of only 755 kg ha^{-1}. Low soil moisture during germination, intermittent moisture stress during seedling and panicle initiation (Goswami 1991, Kalita 1996) are the major factors that contribute to lower productivity. It has been suggested that the possession of unique qualities and plant architecture in traditional rice cultivars of Northeast India confer resistance to biotic and abiotic stresses (Patnaik and Panda 1990). On the other hand improved cultivars are unable to perform well under unfavourable condition. The *Ahu* soils of Assam are generally sandy loam with uneven topography, poor native fertility and low moisture retention coupled with erratic rains during March to June cause severe moisture deficit (Goswami 1991, Kalita 1996). The upland rainfed *Ahu* rice suffers from varying levels of early intermittent moisture deficit during seed germination to vegetative stage, though rainfall is plenty during grain filling and ripening stages of the crop (Goswami 1991, Seetharaman *et al.* 1991, Singh and Singh 1983).

Drought is considered as one of the most important factors limiting crop yields in the world. The deficiency of available soil water often affects the yield of cereals. The intensity of the response depends on the stress severity and its duration, as well as the plant

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developmental stage. One view is that water stress is less detrimental to grain yield when occurring early in the crop cycle (Blum 1996). It is well known that stress during seed germination and seedling stage has an influence on seedling vigour, which ultimately reflects the number and size of plant. The number of grains per panicle, panicle length and final number of panicle per square meter can also be reduced when dry conditions prevail (Morgan and Riggs 1981, Blum and Penuel 1990). Hence, the present study aimed at physiological characterization of some upland rice cultivars during germination and seedling stage under moisture stress condition in order to identify genotypes having better tolerance to moisture stress so that these genotypes can be used in future breeding programmes for moisture stress tolerance.

MATERIALS AND METHODS

The experiment was conducted with five rice cultivars, viz. Kolong, Luit, IR-50, Nilajee and Gunni in the Department of Crop Physiology, Assam Agricultural University, Jorhat. Poly ethylene glycol (PEG-6000) was used to induce water stress. For preparation of –2 MPa and –6 MPa solutions PEG-6000 was dissolved in 100 ml of double distilled water respectively following the method of Yaniv and Werker (1983). For 0 MPa treatment, distilled water was used. Seeds were placed on moistened filter paper in glass petriplates. Filter papers were moistened at the beginning, and at regular intervals with above-mentioned solutions. After germination, Hoagland solutions were provided to the seedlings till the end of the experiment. Plants were grown in laboratory in the month of March under natural day-length. There were four replication in each treatment. The germination percentage and relative parameters such as relative vigour index, seedling mortality, relative stress injury and membrane stability index and dry weights of roots and shoots and biochemical characters of different genotypes of rice were measured. For the measurement of leaf thickness and root anatomy, leaf and root segments of each treatment were initially placed in fixative, comprising of formalin: acetic acid: ethyl alcohol: water (10: 5: 50: 35 V/V). It was then dehydrated in ethyl-butyl alcohol series and embedded in paraffin wax. Cross section was made by sharp razor and stained with safranine (0.5%. w/v) in 50%, (v/v) alcohol. Leaf thickness and xylem and phloem tissues were characterized after staining with safranine. For stomatal studies clear nail polish was applied to both leaf surfaces and allowed to dry for approximately five minutes. Double sided cellophane tape was used to peel dried impression from the leaves. Five cm long section of the impression at the tip, center and base positions of each leaf surface were mounted on microscope slides and cover slip was placed. Stomata of each surface were counted in 1.85-mm^2 microscopic field (100 x magnifications) (Cohen *et al.* 1982). Leaf chlorophyll was extracted by non-maceration method using dimethyl sulphoxide (DMSO) (Hiscox and Israelstam 1979) and light absorption at 663 nm and 645 nm was read in a spectrophotometer. The amount of chlorophyll was calculated using absorption coefficients. Proline was estimated according to method of Bates *et al*. (1973). Membrane stability index was calculated and was expressed in percentage using the formula of Premachandra *et al.* (1989). Relative stress injury (RSI) was calculated using the formula suggested by Goyal *et al.* (2001) and it was expressed as percentage. Relative water content (RWC) was calculated following Weatherly and Barrs (1962) method. Statistical analysis of data was done by analysis of variance (ANOVA) (Panse and Sukhatme 1967). The critical difference (CD) values were calculated at 5 per cent probability level.

The germination was recorded 4 days after treatment, which was found to be significantly inhibited due to moisture stress in both –2 MPa and –6 MPa osmoticum (Table 1). Highest reduction was observed in –6 MPa indicating the germination inhibition intensity was increasing as the stress increased. There was no significant difference among the cultivars in terms of germination in 0 bar. Kolong and Luit showed germination even in –6 MPa whereas there was complete absence in germination in other cultivars.

RESULTS AND DISCUSSION

Moisture stress reduced seedling vigour in rice (Fig. 1A). The highest (58.7%) seedling vigour was observed in cultivar Luit followed by 53.66% in cultivar Kolong.

Fig. 1. **Effect of water stress on seedling vigour (A) and seedling mortality (B) in rice cultivars 20 days after treatment.**

Rice cultivars with greater seedling vigour may contribute to reducing crop losses due to competition from drought (Namuco *et al*. 2009). Moisture stress significantly enhanced seedling mortality (Fig. 1B). The mortality percentage was lowest (11.82%) in, Kolong followed by Luit (13.56%). Increase in tiller mortality in rice cultivars with increased moisture deficit has been reported by Konwar (2009).

The relative water content (RWC) of leaves was significantly lower for water-stressed plants compared to 0 MPa. RWC declined progressively with increased concentration of PEG. (Fig.1). In the present investigation rice seedlings subjected to water stress at -2, -6 MPa showed decrease in RWC by 28.17 and 54.42%, respectively. The highest reduction in RWC was observed in the cultivars IR-50, Nilajee and Guni (Fig. 2A). Choudhury *et al.* (2005) reported gradual decrease in RWC tolerant genotypes of rice but the decrease was drastic in susceptible one. Similar results were reported by Goyal *et al.* (2001) in pearl millet.

Moisture stress significantly reduced leaf thickness (Fig. 2 B). The Lowest leaf thickness was observed in cultivar Kolong followed by Luit. This observation was however in contrary to the report of Hofstra and

Table 1. Effect of water stress on seed germination (%) in rice cultivars.

Cultivars	0 MPa				-2 MPa				-6 MPa			
	4 DAT	7 DAT	10 DAT	Mean	4 DAT	7 DAT	10 DAT	Mean	4 DAT	7 DAT	10 DAT	Mean
Kolong	10.20	60.00	95.22	55.14	2.35	12.11	47.33	20.60	0.00	7.21	14.21	7.14
Luit	12.21	62.12	90.33	54.89	4.46	16.21	49.36	23.34	0.00	8.88	16.23	8.37
IR-50	14.32	65.14	92.76	90.33	0.00	7.21	28.78	17.99	0.00	0.00	4.57	1.52
Nilajee	8.45	60.67	90.89	92.76	0.00	6.33	20.67	9.00	0.00	0.00	2.22	0.74
Gunni	7.23	64.89	92.56	90.89	0.00	4.11	16.23	6.78	0.00	0.00	7.34	0.75
Mean	10.48	62.36	92.35		1.36	9.19	32.47		0.00	3.22	2.91	
CD (0.05)												
T			NS				1.27				1.23	
V			NS				3.22				5.22	
TXV			NS				6.45				8.51	

Fig. 2. Effect of water stress on relative water content (%) of leaf (A) and leaf thickness (B) in rice cultivars

Hesketh (1975) where they recorded higher leaf thickness under moisture stress.

Under water stress conditions the chlorophyll content of leaves decreased significantly (Table 2). The lowest (0.68 mg g^{-1} fw) chlorophyll content was observed in Nilajee, whereas, it was highest (0.83 mg g^{-1} fw) in Kolong. Reduction in chlorophyll content under stress was comparatively less in the cultivar Kolong. Similar results of decreased chlorophyll content under moisture stress was also reported by Reddy *et al.* (2007) in rice genotype; they opined that this may be due to low rate synthesis of chlorophyll which may be casually related to lesser absorption of nitrogen under moisture stress condition.

The stress enhanced proline content in leaves. The highest proline content was observed in the cultivar Luit followed by Kolong (Table 2). Proline may possibly play an important role in the osmoregulation under moisture stress condition (Choudhury 2005, Kale 2006). Demand in soluble protein is reported to be associated with an increase in free amino acids like proline. Stress induced proline accumulation in rice with different varietal response has also been reported by Dingkuhn *et al.* (1991).

The membrane stability index (MSI) in leaf discs under 0 MPa conditions did not differ significantly. In contrast, the water-stressed (-2 MPa and -6 MPa) plants showed significant difference. MSI in non-stressed plant was 78.08% whereas it was 23.08% in stressed plant

Table 2. Effect of water stress on chlorophyll and proline content in the leaves of rice cultivars.

Cultivars	Total chlorophyll (mg/g fw)				Proline (mg/g dw)			
	0 MPa	-2 MPa	-6 MPa	Mean	0 MPa	-2 MPa	-6 MPa	Mean
Kolong	0.91	0.82	0.74	0.82	2.68	4.68	5.02	4.12
Luit	0.93	0.78	0.78	0.83	2.46	4.76	5.58	4.27
IR-50	1.01	0.62	0.52	0.72	2.07	2.22	3.80	2.70
Nilajee	0.96	0.54	0.54	0.68	2.68	2.68	3.02	2.79
Gunni	0.97	0.59	0.56	0.71	2.46	2.76	2.58	2.60
Mean	0.96	0.67	0.63		2.47	3.02	4.00	
CD (0.05)								
T			0.02				0.72	
V			0.08				1.02	
TXV			NS				1.98	

Table 3. Effect of water stress on membrane stability index (MSI), relative stress injury (RSI), chlorophyll stability index (CSI), and stomatal index (SI) in the leaves of rice cultivars.

Cultivars	MSI (%)				RSI (%)				CSI (%)				SI (%)			
	0 MPa	-2 MPa	-6 MPa	Mean	0 MPa	-2 MPa	-6 MPa	Mean	0 MPa	-2 MPa	-6 MPa	Mean	0 MPa	-2 MPa	-6 MPa	Mean
Kolong	79.23	51.56	40.78	57.19	1.64	3.68	5.44	3.59	82.10	57.29	25.17	54.85	78.35	59.65	50.25	62.75
Luit	78.33	50.11	35.44	54.62	1.96	4.02	5.12	3.70	83.72	49.95	27.04	53.37	77.75	56.45	58.25	64.15
IR-50	76.09	44.21	24.34	48.21	1.97	6.22	8.10	5.43	84.07	38.04	18.21	46.77	78.15	39.25	15.75	44.38
Nilajee	80.22	45.56	21.54	49.44	2.68	6.68	8.62	5.99	82.40	37.24	17.07	45.57	77.45	36.25	18.15	43.95
Gunni	76.56	43.21	19.56	46.44	2.46	6.76	8.48	5.90	81.71	28.94	16.84	42.50	76.65	53.25	49.45	59.78
Mean	78.08	46.93	28.33		2.14	5.47	5.96		82.80	42.29	20.87		77.67	48.97	38.37	
CD (0.05)																
T			4.62				0.48				1.02				1.13	
V			6.42				1.02				3.19				4.17	
TXV			12.16				2.01				5.67				8.23	

(Table 3). There was significant decrease (57.19%) in MSI in Guni whereas it was 46.44% in Kolong under water stress condition. Complete opposite trend was observed in case of relative stress injury (RSI). The highest RSI (5.99%) was observed in Nilajee whereas it was lowest (3.59%) in Kolong. Chlorophyll stability index also decreased with increasing water stress in most of the cultivars (Table 3). Reduction in MSI, CSI and SI was also reported by Konwar (2009) in upland rice under moisture stress condition. The loss in MSI is related to production of reactive oxygen species which causes damage to membrane lipid and protein. Sairam *et al.* (2008) reported existence of variation among wheat cultivars MSI and CSI. They observed that the genotype that showed higher MSI and CSI under water stress also possessed higher glutathione reductase and peroxidase activity.

Moisture stress reduced the stomatal index (SI) significantly. Among the cultivars, lowest SI was observed in cultivar Kolong and Luit. The stress-induced reduction in SI was recorded highest in Luit whereas it was lowest in Nilajee (Table 3). Decreased number of stomata under higher moisture deficit condition has been reported by Xu and Zhou (2008) in grasses. Reports also suggest the reduction in leaf net photosynthetic assimilation (A_n) by both stomatal and metabolic limitations under moisture stress situation (Ghannoum *et al.* 2003, Ripley *et al.* 2007)

Moisture stress was found to significantly change the root: shoot ratio in rice (Fig. 3). On an average root: shoot ratio was increased by 32.35% due to the moisture stress. Among the cultivars, the root: shoot ratio was more in cultivar Kolong and less in cultivar IR-50. Acceleration of root growth in cultivar Kolong and Luit under moisture stress condition could also result in the establishment of seedlings more rapidly and avoidance of moisture stress. The greater root dry weight in the cultivar Kolong and Luit may be due to more root growth, root number and root volume, which help in the absorption of available water. Turner and Begg (2005) were also of the opinion that moisture stress caused increase in root: shoot ratio. Morission (1983) suggested greater allocation of carbon to root system which resulted in higher root volume, root proliferation and root activity.

Fig. 3. Effect of water stress on root: shoot ratio of rice cultivars

Table 4. Effect of water stress on number of root xylem and area of root xylem in rice cultivars.

Cultivars	Xylem number/100 mm^{-2}				Xylem area (μm^2)			
	0 MPa	-2 MPa	-6 MPa	Mean	0 MPa	-2 MPa	-6 MPa	Mean
Kolong	23.67	24.12	26.15	24.65	179.45	223.68	215.94	206.36
Luit	23.67	25.88	26.27	25.27	181.06	224.22	225.22	210.35
IR-50	24.11	24.24	22.44	23.53	175.97	166.22	158.10	166.76
Nilajee	24.19	25.12	21.34	23.55	172.68	156.28	148.22	159.66
Gunni	24.45	24.21	23.23	23.96	182.06	152.76	138.41	157.74
Mean	24.02	24.72	23.88		143.05	184.63	177.18	
CD (0.05)								
T			NS				1.02	
V			NS				3.12	
TXV			NS				5.42	

The highest xylem size was observed in the cultivar Luit followed by Kolong under moisture stress condition (Table 4). This might have been of help in the entry of water and nutrient from the soil more efficiently. Acceleration of root growth in cultivar Kolong and Luit under moisture stress condition might have resulted in establishment of seedlings more rapidly under water deficit condition. Similar results were also reported by Konwar (2009).

From the results of the study, it can be concluded that Kolong and Luit possess higher tolerance capacity among the cultivars studied and this could be attributed to higher accumulation of proline and adjustment of anatomical features like xylem size, stomatal modification, leaf thickness and morphological modification such as increment of root volume during seedling stage. The cultivars like IR-50, Nilajee and Gunni were susceptible to moisture stress. The susceptible cultivar invariably reflected less favourable plant water status, more metabolic de-arrangements in terms of chlorophyll loss and depressed level of membrane stability. The capacity to sustain plant function at low plant water status is a rare occurrence in crops. Therefore, genotypic differences in plant function under moisture stress can be utilised for enhancing plant productivity.

REFERENCES

Bates, L.S., Waldren, R.P. and Tear, I.D. (1973). Rapid determination of free proline in water stress studies. *Plant Soil* **39:** 205-207.

Blum, A. (1996). Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul*. **20:** 135-148.

Blum, A. and Penuel, Y. (1990). Physiological of attributes associated with drought resistance of wheat cultivars in a Mediterranean environment. *Aust. J. Agric. Res*. **41:** 799-810.

Choudhury, N.L., Sairam R.K. and Tyagi, A. (2005). Expression of Ä[1]-pyrroline -5 – carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). *Indian J. Biochem & Biophysics* **42:** 366-370.

Cohen, C.J., Chicote, D.O. and Frakes, R.V. (1982). Leaf anatomy and stomatal characteristics of four tall Fescue selections differing in forage yield. *Crop Sci*. **22:** 704-708

Dingkuhn, M., Cruz, R.T., O· Toole, J.C., Turner, N.C. and Doerffling, K. (1991). Responses of seven diverse rice cultivars to water deficit III. Accumulation of proline in relation to leaf water potential and osmotic adjustment. *Field Crop Res*. **27:** 103-117.

Ghannoum, O. Conroy, J.P., Driscoll S.P., Paul, M.J., Foyer, C.H. and Lawlor, D.W. (2003). Nonstomatal limitations are responsible for drought-induced photosynthetic inhibition in four C_4 grasses. *New Phytologist.* **159:** 599–608.

Goswami, R.K. (1991). Physiological potential of some rice (*Oryza sativa* L) cultivars for tolerance to moisture stress. M.Sc. Thesis, Assam Agricultural University, Jorhat.

Goyal, V., Jai, S., Bishonoi, N.R. and Munjal, R. (2001). Leaf water relations, diffusive resistance and proline accumulation in hybrid pearl millet under depleting soil moisture content. *Indian J. Plant Physiol.* **6:** 41-45.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57:** 1332-1334.

Hofstra, G. and Hesketh J.D. (1975). In: Environmental and biological control of photosynthesis. Marcelle, R. (ed.). Amsterdam : W Junk : 71-80.

Kale, M.V. (2006). Assessment of proline contents in relation to stress conditions of certain pteridophytes of Western Ghats South India. *Indian Fern J*. **23:** 11-14.

Kalita, U.C. (1996). Genetic studies on improvement adaptive traits of Rice under rainfed upland situation. Ph.D. Thesis Assam Agricultural University, Jorhat.

Konwar, P.B. (2009). Characterization of some selected Ahu rice cultivars under moisture stress condition. M.Sc. Thesis Assam Agricultural University, Jorhat.

Morgan, A.G. and Riggs, T.J. (1981). Effects of drought on yield and on grain and malt characters in spring barley. *J. Sci. Food. Agric.* **32:** 339-346.

Morission, J.I.L. (1983). Sensitivity of stomata and water use efficiency to high CO_2. *Plant Cell Environ.* **8:** 89-95.

Namuco, O.S., Cairns, J.E. and Johnson, D.E. (2009). Investigating early vigour in upland rice (*Oryza sativa* L.): Part I. Seedling growth and grain yield in competition with weeds. *Field Crops Research.* **113:** 197-206.

Panse, V.G. and Sukhatme, P.T. (1967). Statistical methods for agricultural workers, 2nd edn. Indian Council of Agricultural Research, New Delhi.

Patnaik, S. and Panda, D. (1990). Technology for increasing the production of rainfed rice in eastern India. *Indian Farming*, December, pp. 33-38.

Premachandra, G.S., Saneoka, H. and Ogata, S. (1989). Nutro-physiological evaluation of the polyethylene glycol test of cell membrane stability in maize. *Crop Sci*. **29:** 1287-1202.

Reddy, A.M. Shankhdhar D. and Shankhdhar, S.C. (2007). Physiological characterization of rice genotypes under periodic water stress. *Indian J. Plant Physiol.* **12:** 189-193.

Ripley, B.S., Gilbert, M.E., Ibrahim, D.G. and Osborne, C.P. (2007). Drought constraints on C_4 photosynthesis: stomatal and metabolic limitations in C_3 and C_4 subspecies of *Alloteropsis semialata*. *J. Exp. Bot.* **58:** 1351-1363.

Sairam, R.K., Deshmukh, P.S. and Shukla, D.S. (2008). Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron. Crop Sci.* **178:** 171-178.

Seetharaman, R., Sharma, S.D. and Sastry, S.V.S. (1991). Germplasm conservation and use in India. Rice Breeding. Int. Rice Res. Inst. Los Banos, Philippines, pp 187-199.

Singh, K.P. and Singh, K. (1983). Seed germination and seedling growth responses of some rice cultivars to water potential treatments. *Indian J. Plant Physiol*. **25:** 182-188.

Turner, N.C. and Begg, J.E. (2005). Plant-water relations and adaptation to stress. *Plant Soil.* **58:** 97-131

Weatherly, P.F. and Barrs, H.D. (1962). A re-examination of relative turgidity techniques for estimating water deficit in leaves. *Aust. J. Biol. Sci*. **15:** 413-428.

Xu, Z. and Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **59:** 3317-3325.

Yaniv, Z. and Werker, E. (1983). Absorption and secretion of polyethylene glycol by solanaceous plants. *J. Exp. Bot.* **34:** 1577-1584.

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