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Evaluating Resilience of Upland Rice to Water Stress through Study of Physiological Responses in Subtropical hills of North East India

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ABSTRACT

The present study sought to investigate the effects of water stress in few physiological factors, leaf area index (LAI) and grain yield of upland rice under rainfed subtropical hill condition of North East India. Four high yielding rice cultivars were subjected to artificially imposed water stress, of 15 days duration each, during vegetative (active tillering) and reproductive stages (grain filling) in a rainout shelter. Cell membrane stability (CMS), chlorophyll content, soil moisture, LAI and grain yield were measured. Water stress markedly increased cell electrolyte leakages in both vegetative (9.4 to 25.2%) and reproductive (12.7 to 29.6%) stages. CMS decreased with the age of the plants and the tolerant cultivars exhibited better CMS. Total chlorophyll content of water stressed plants reduced to the tune of 6.1 to 20.9% (vegetative stage) and 7.2 to 17.3% (reproductive stage). Water stress also caused drastic reduction in LAI, which ranged from 11.1 to 22.5% (vegetative stage) and 12.2 to 18.8% (reproductive stage) over the controls. The impact of water stress on physiological and growth factors of the four cultivars were reflected through differential reduction in grain yield. These results confirm that CMS and chlorophyll retention capacity are suitable indicators of water stress tolerance capability of plants and hence, screening of resilient cultivars.

1. Introduction

Under changing climatic condition, agriculture under assured irrigation is likely to have better adaptive capacity, compared to the rainfed areas. The North Eastern region of India has total annual water resources of 42.5 million hectare meter, but due to lack of proper water harvesting measures, only 20.7% of the net sown area is irrigated leaving remaining to the mercy of seasonal rainfall. Around 44% of the total geographical area in North East India is hilly and summer rice, maize, ginger and vegetables are the predominant crops cultivated here (ICAR, 2011). Agricultural productivity in the North Eastern region is constrained by several factors such as difficult terrain, acidic and poor soils, high soil degradation, intra-seasonal water stress *etc.* due to which, despite having ~8% of the total geographical area and ~13% of the total rainfall of the country, the region contributes only 1.5% to the total food grain production in the country. Due to rapid global climate change, alarming deficits in annual as well as monsoon rainfall has led to droughts of varying intensities across the region and subsequent crop failures (Kumar, 2011). Due to increased warming of the atmosphere, the annual agricultural water requirement for the North Eastern region is further anticipated to increase from approximately 20 km^3 in 2001 to 25.2 km^3 in 2021 (Sharma, 2003). However, it is projected that climate change is likely to impact the rice production scenario differentially. Under such projections, rice yield is likely to be changed by -10 (decrease) to +5% (increase)

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under irrigated condition and by -35 (decrease) to +5% (increase) under rainfed condition by 2030 (MoEF, 2010).

To reduce the impact of climate change on crop production, it is widely advised to select climate adaptive crop cultivars through screening of their physiological, growth and yield traits. Water stress causes definite modification in plant metabolisms and some of the plants have a set of physiological adaptations that allow them to tolerate water stress. The degree of adaptations to the reduction in plant cell water potential caused by drought may vary considerably among species (Save *et al.*, 1995). Effects of water stress in plant include morphological and biochemical changes at initial level and functional damage and loss of plant parts, when stress becomes acute (Sangtarash, 2010). Researchers have linked various physiological responses of plant to drought with their tolerance mechanisms, such as: chlorophyll content and stability; and high relative water content (Clarke and McCaig, 1982). Almeselmani *et al.* (2011) reported that chlorophyll content, membrane stability index (MSI) and relative water content (RWC) are few good physiological indices of drought tolerance and can be used for improvement in drought tolerance in wheat. In open field condition, it is not always possible to sufficiently quantify the effect of water stress on plant growth and development at critical growth stages. In open condition, results of such field experimentation are likely to be undone by any large or untimely precipitation event, which may lead to incomplete and erroneous conclusions. To overcome such problems 'rain shelters' or 'rainout shelters' have been designed for the first time in 1962 at Iowa State University (Horton, 1962). Rainout shelters have provided a mechanism to bridge the gap between greenhouse or growth chamber and field experiments by modification of the field environmental conditions, primarily precipitation (Ries and Zachmeier, 1985). Considering importance of the issues discussed above, and vulnerability of rainfed upland rice to intra seasonal prolonged dry spells, this experiment was conducted to evaluate climate resilience of upland rice cultivars to water stress through study of the effects on few important physiological parameters in a subtropical hill condition of North East India.

2. Materials and Methods

The experiment was conducted during summer season of 2014 at ICAR Research Complex for NEH Region, Umiam, Meghalaya located at $91^{o}55^{/}$ N latitude and $25^{o}41^{/}$ E longitude and at an altitude of 1010m above MSL. Four nos. of popularly grown and high yielding upland rice cultivars, *viz.* Bhalum 1, Bhalum 3, RCPL 1-412 and IURON 514 (all are of medium duration, 110-120 days) were selected for the study. The average yield of these cultivars is in the range of 2.0 to 2.5 t ha^{-1}. The experiment was conducted in an adjustable rainout shelter. The rainout shelter was 9.14 m long and 7.62 m wide. Height of the shelter was 3.66 m at the centre and 2.44 m at the sides. The top of the shelter was covered with 8 mm thick triple walled ultraviolet ray stabilized polycarbonate sheet fixed with polycarbonate H&U profile, aluminium strip with gasket/silicon sealant. The sheet was fixed in a manner to escape rains from the sides. Individual plot sizes in the rainout shelter were 18 m^2 with a provision to make two halves of 9 m^2 each to distinguish between control and rainout plots. Dry seeds were sown on 27th June 2014, as soil moisture was enough to support germination and seedling establishment, with a row to row spacing of 30 cm. Normal recommended dose of $N:P_2O_5:K_2O$ were applied in the form of urea, SSP and MoP @ 80:60:40 kg ha^{-1}, respectively. Urea was applied in two splits. 1st split was applied basally and the 2nd split was applied at 35 days after sowing (DAS). Two rainouts of 15 days each were imposed, to bring in water stress artificially, at vegetative (active tillering, 28 DAS) and reproductive (grain filling, 85 DAS) stages (Table 1). The plots were kept completely under open sky condition except during the rainouts. The control plots were never brought under rainouts during the entire growth period. The physiological traits studied were cell membrane stability (CMS) and chlorophyll pigmentation status (total chlorophyll content and chlorophyll a/b ration) of the cells. CMS was estimated following the method of Leopold *et al.* (1991) and chlorophyll a, b and total chlorophyll (a+b) were estimated spectrophotometrically (Misyura*et al.*, 2013). Growth and yield parameters measured were leaf area index (LAI) and grain yield, respectively.

Table 1. Crop management and rainout activities during the rice growing period

Activities	Dates	Days after sowing (DAS)
Date of sowing	27/06/2014	0
Date of germination	02/07/2014	5
1st rainout (Active tillering)	25/07/2014	28
1st rainout withdrawn (15 days)	08/08/2014	42
2nd rainout (grain filling)	20/09/2014	85
2nd rainout withdrawn (15 days)	04/10/2014	99
Harvesting	20/10/2014	115

Table 2. Rainfall, temperature and rainy days during the rice growing period

Parameters	Values
Total rainfall (mm) during growth period (27th June to 20th October 2014)	1499.6
Total rainy days (rainfall > 2.5 mm) during growth period	63
Rainfall (mm) during 1st rainout (25th July to 8th August 2014)	190.6
Total rainy days (rainfall > 2.5 mm) during 1st rainout	9
Rainfall (mm) during 2nd rainout (20th September to 4th October 2014)	265.9
Total rainy days (rainfall > 2.5 mm) during 2nd rainout	10
Average maximum air temperature (°C) during growth period	28.1
Average minimum air temperature (°C) during growth period	18.6
Average temperature (°C) during growth period	23.3

LAI was measured with a digital plant canopy imager (model: CI-110; CID Inc., Camas, WA 98607, USA). Volumetric soil moisture content of the top 15 cm soil was measured with a time domain Reflectometer (model: Minitrase-6050X3K5B; Soil Moisture Equipment Corp. Santa Barbara, CA 93130, USA). Triple replicated measurements/samplings were done on the days of imposition of rainouts and their withdrawals. Grain yield was measured at the time of harvesting.

3. Results and Discussion

3.1 Weather and soil moisture status in control and rainout plots

Total rainfall and rainy days during the entire crop growth period (115 days) was 1499.6 mm and 63 days, respectively. During the 1st and 2nd rainouts, the sheltered crops received 190.6 and 265.9 mm (total: 456.5 mm) less rainfall, respectively, compared to their controls, which was 30.4% less than the total rainfall received by the control. Average maximum and minimum temperature were 28.1 and 18.6°C, respectively, with a mean of 23.3°C, which were very much favourable for the growth and development of rice (Table 2). Rainout condition caused reduction in available soil moisture by 24.0 to 39.4% during the vegetative and 18.8 to 36.9% during reproductive stages compared to their controls. Reduction in soil moisture was found highest under Bhalum 3 cultivar in both the periods of rainout (Table 3). Availability of soil moisture was found less during the reproductive stage compared to the vegetative stage, irrespective of the treatments.

3.2 Effect of water stress on cell membrane stability (CMS)

Water stress inflicts damage to the cell membrane in the initial stage itself (Levitt, 1972) and the ability of the plants to maintain membrane integrity determines its level of tolerance (Vieira da Silva *et al.,* 1974). Hence, CMS is being widely used to determine the tolerance of crop cultivars to prolonged water stress/drought. When exposed to various kinds of stresses, plant cell membrane got damaged and that results in leakage of cell electrolytes. The membrane damage could be due to physical and/or biochemical changes. CMS was measured in terms of percent (%) leakage of electrolytes from the cell membrane. Among different cultivars, Bhalum 3 exhibited lowest magnitude of electrolyte leakage *i.e.* highest level of stability against stress. Bhalum 3 was followed by Bhalum 1, RCPL 1-412 and IURON 514, in decreasing order of stability. Rainouts resulted in cell electrolyte leakage ranging from 9.4 (Bhalum 3) to 25.2% (IURON 514) in vegetative stage and 12.7 (Bhalum 3) to 29.6% (IURON 514) in reproductive stage (Table 4). Assaha *et al.* (2016) reported reduction in CMS and increase in rate of electrolyte leakage in a leafy vegetable, huckleberry, under increasing levels of induced water stress and our findings are also in line with it. It was also observed that CMS decreased with the age of the plants. The tolerant cultivars showed better CMS and it is in agreement with other researchers (Vasquez-Tello*et al.*, 1990; Almeselmani *et al.*, 2011).

Table 3. Soil moisture content in control and rainout conditions in two growth stages

Cultivars	Soil moisture (%/volume)			
	At withdrawal of 1st rainout(Active tillering)		At withdrawal of 2nd rainout(Grain filling)	
	Control	Rainout	Control	Rainout
Bhalum 1	33.8	25.7 (-24.0)	26.1	21.2 (-18.8)
Bhalum 3	34.0	20.6 (-39.4)	27.1	17.1 (-36.9)
RCPL 1-412	34.1	21.8 (-36.1)	26.7	18.1 (-32.2)
IURON 514	35.2	24.1 (-31.5)	27.6	21.6 (-21.7)

* Values in parentheses are % change in soil moisture content in rainout treatments over their controls at withdrawal of rainouts

3.3 Effect of water stress on chlorophyll content

Water stress causes reduction in chlorophyll content (Iturbe *et al.,* 1998, Saraswathi and Paliwal, 2011) and high content of chlorophyll during stress indicates better mechanism of plant tolerance to any stress (Farquhar *et al.*, 1989). In this study, similar results were observed on total chlorophyll content and chlorophyll a/b ration of different rice cultivars. Total chlorophyll content got reduced by 6.1 (Bhalum 1) to 20.9% (IURON 514) during the vegetative stage and 7.2 (Bhalum 1 and Bhalum 3) to 17.3% (IURON 514) during reproductive stage rainouts (Table 5). Ration of chlorophyll a/b was found higher under rainouts in at both vegetative and reproductive stages compared to their controls. The cultivars, which recorded higher magnitude of total chlorophyll loss, were in match with higher values of chlorophyll a/b ratio. Randall and Thornber (1977) observed that, in water stressed maize crop, reduction in the chlorophyll content was governed by reduction in the lamellar content of the light-harvesting chlorophyll a/b-protein and that led to the elevated chlorophyll a/b ratio in stressed plant. Almeselmani *et al.* (2011) reported that under stress condition chlorophyll content differ significantly among various cultivars and hence, it was also a good parameter for screening of water stress tolerance in plants.

3.4 Effect of water stress on LAI

LAI is one of the most important plant growth parameters that regulate realization of ultimate economic yield. RecepCakir (2004) reported that maize plants maintained higher LAI under favourable soil moisture conditions but water stress during vegetative growth and tasselling stages reduced LAI due to reduced size of the leaves; and during the ear formation and milk stages, caused early loss of lower leaves and decreased dry matter weight and grain yield as a result of reduced intercepted radiation. Drought also suppresses leaf expansion and tillering (Kramer and Boyer 1995), and reduces leaf area due to early senescence (Nooden 1988).In this study, among all the cultivars, Bhalum 3 had maximum values of LAI under both vegetative (0.75) and reproductive (1.72) stages, in control plots (Table 6). Rainout condition found to cause reduction in LAI in all the cultivars. Water stress caused drastic reduction in LAI in cases of RCPL 1-412 (-13.9%) and IURON 514 (-18.8%) during the reproductive stage. Higher reduction in soil moisture in case of Bhalum 3 at grain filling stage might be due to high transpirational losses caused by high LAI of that cultivar.

Table 4. Effect of water stress on leaf cell membrane stability of rice cultivars under control and rainout conditions in two growth stages

Cultivars	**Cell membrane stability** (expressed in terms of %leakage of electrolytes)			
	At withdrawal of 1st rainout(Active tillering)		**At withdrawal of 2nd rainout**(Grain filling)	
	Control	**Rainout**	**Control**	**Rainout**
Bhalum 1	11.6e	14.2d (22.4)*	12.8e	16.3d (27.3)
Bhalum 3	9.6e	10.5e (9.4)	11.8e	13.3e (12.7)
RCPL 1-412	16.7c	19.7b (17.9)	18.2cd	22.3b (22.5)
IURON 514	19.4b	24.3a (25.2)	19.6c	25.4a (29.6)
SEM	**0.78**		**0.81**	
LSD (0.05)	**2.36**		**2.46**	

* Values in parentheses are % change in cell electrolyte leakage in rainout treatments over their controls at withdrawal of rainouts

3.5 Effect of water stress on grain yield

Rice, as a field crop, is susceptible to water stress (Yang *et al.*, 2008) and around 50% of the world's rice production is affected by drought (Bouman*et al.*, 2005). Drought stress during vegetative growth, flowering and terminal period of rice cultivation, can interrupt floret initiation (which cause spikelet sterility) and grain filling, respectively (Kamoshita*et al.*, 2004). Usually, water stress at grain filling process induces early senescence and shortens the grain filling period but increases remobilization of assimilates from the straw to the grains (Asseng and van Herwaarden, 2003). Low soil moisture content might be responsible for inhibiting photosynthesis and decrease in translocation of assimilates to the grain which may lower grain weight (Van Heerden and Laurie, 2008; Mostajeran and Rahimi-Eichi, 2009). In this experiment, spectacular reduction in grain yield was observed under the influence of imposed water stress during vegetative and reproductive (Table 7). Under control, highest grain yield was recorded in case of RCPL 1-412 (2.74 t ha^{-1}) followed by Bhalum 3 (2.62 t ha^{-1}), IURON 514 (2.56 t ha^{-1}) and Bhalum 1 (1.99 t ha^{-1}). Once the rainouts were imposed, the performance of various plant metabolisms were affected due to stress caused by below optimal water availability. The impacts were noticed by means of changes in CMS and chlorophyll contents of the plant cells. Considering CMS and chlorophyll retention capacity, Bhalum 3 was found better

Table 5. Effect of water stress on total leaf chlorophyll content and chlorophyll a/b ration of rice cultivars under control and rainout conditions in two growth stages

Cultivars	**Total chlorophyll content** (mg per g of fresh weight)				**Chlorophyll a/b**			
	At withdrawal of 1st rainout(Active tillering)		**At withdrawal of 2nd rainout** (Grain filling)		**At withdrawal of 1st rainout**(Active tillering)		**At withdrawal of 2nd rainout**(Grain filling)	
	Control	**Rainout**	**Control**	**Rainout**	**Control**	**Rainout**	**Control**	**Rainout**
Bhalum 1	2.63d	2.47e (-6.1)*	2.76b	2.56c(-7.2)	1.48c	2.05a (38.5)	2.01a	2.06a (2.5)
Bhalum 3	2.92b	2.74c(-6.2)	2.90a	2.69b(-7.2)	1.37cd	1.77b (29.2)	1.70bc	1.76bc (3.5)
RCPL 1-412	3.03a	2.52e (-16.8)	2.96a	2.56c(-13.5)	1.27d	1.67b (31.5)	1.76bc	1.80b (2.3)
IURON 514	2.73c	2.16f(-20.9)	2.71b	2.24d(-17.3)	1.11e	1.69b (52.2)	1.66c	1.69bc (1.8)
SEM	**0.03**		**0.03**		**0.04**		**0.04**	
LSD (0.05)	**0.09**		**0.08**		**0.13**		**0.11**	

* Values in parentheses are % change in chlorophyll content in rainout treatments over their controls at withdrawal of rainouts

Table 6. Effect of water stress on leaf area index (LAI) of rice cultivars under control and rainout conditions in two growth stages

Cultivars	**Leaf area index (LAI)**			
	At withdrawal of 1st rainout(Active tillering)		**At withdrawal of 2nd rainout**(Grain filling)	
	Control	**Rainout**	**Control**	**Rainout**
Bhalum 1	0.63abcd	$0.56 (-11.1)^{cd}$	1.53^{b}	$1.34 (-12.4)^{c}$
Bhalum 3	0.75a	$0.65 (-13.3)^{abcd}$	1.72^{a}	$1.51 (-12.2)^{b}$
RCPL 1-412	0.72ab	$0.60 (-16.7)^{bcd}$	1.51^{b}	$1.30 (-13.9)^{c}$
IURON 514	0.71abc	$0.55 (-22.5)^{d}$	1.54^{b}	$1.25 (-18.8)^{c}$
SEM	**0.04**		**0.04**	
LSD (0.05)	**0.13**		**0.12**	

* Values in parentheses are % change in LAI in rainout treatments over their controls at withdrawal of rainouts

among all and it was also found better equipped to extract enough soil moisture for transpirational purposes during the periods of stress. In contrast, cultivars, *viz*. RCPL 1-412 and IURON 514, though performed well under normal (controlled) condition, but due to absence of better adaptation mechanism might have led to loss of grain yield to the tune of 22.3 to 26.9%, respectively, under stressed condition. Under water stress, the other two cultivars could retain the extent of losses to the least *i.e.* up to maximum 6.1%.

Conclusion

In conclusion, considering all the findings described above, it may be stated that rainout shelter facility is a useful tool to screen cultivars for their resilience against abiotic stresses, like, prolonged water stress/drought conditions. Also confirms that CMS and chlorophyll retention capacity are very good physiological indicators of water stress tolerance capability of plants, which regulates the growth performance of the crop and thereby, the grain yield. It also helps in screening of water stress tolerant and location specific crop cultivars. As rice constitute the main staple food for majority of the population, the anticipated decline in its yield under climate change puts the future prospects of food security in jeopardy.Thus, the farmers can adopt best varietal options to sustain their production and profitability. Hence, taking into account of increased possibility of prolonged dry spells, upland rice cultivars, *viz*. Bhalum 3 and Bhalum 1 may be recommended for pure rainfed condition, while the other two cultivars may be adopted with provision of supplemental irrigation from harvested rain water in the subtropical hills of North East India.

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Table 7. Effect of water stress on grain yield of rice cultivars under control and rainout conditions

Cultivars	Grain yield (t ha^{-1})		
	Control	Rainout	% Change in rainout over control
Bhalum 1	1.99e	1.90f	-4.5
Bhalum 3	2.62b	2.46c	-6.1
RCPL 1-412	2.74a	2.13d	-22.3
IURON 514	2.56b	1.87f	-26.9
SEM	**0.02**		
LSD (0.05)	**0.08**		

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