



Differential Response of Morpho-physiological Traits of Rice (*Oryza sativa*. L) Cultivars under Soil Water Deficit

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Abstract

Moisture stress causes a significant negative impact on rice growth and development. A pot experiment was conducted under a UV sterilized polythene roof rain-out shelter, to study the effect of water stress on five rice cultivars. Water stress was imposed by withholding irrigation for 15 days each at 30, 50 and 70 days after sowing; with another control experiment provided with normal water. There was a significant difference between stressed and non-stressed plants. Root volume, root shoot ratio, and tiller mortality increased due to moisture stress. Improper plant development during moisture stress decreased the leaf area index of plants. Proline accumulation was high during water stress. Chlorophyll 'a' and chlorophyll 'b' content showed a significant reduction in water stressed plants. Accumulation of leaf sugar, starch and total carbohydrates were also affected due to moisture stress. Cultivars showing a higher amount of sugar, starch and carbohydrates were considered as more resistant to water deficit condition. This study envisages that proline content and soluble sugars estimation could be used as the markers for screening of drought tolerance genotype. Among the different rice cultivars screened 'Maibee' and 'ARC- 10372' showed significantly higher resistance to drought as they accumulated the higher amount of osmolytes during water stress condition. In addition, correlation studies showed that accumulation of carbohydrates and sugars increases the drought tolerance traits in rice.

Introduction

Rice is a versatile cereal crop growing across the wide climatic conditions viz, the tropics, subtropics, semi-arid tropics and temperate regions of the world. More than 90%

of the world's rice is produced in Asia, as it is important staple food crop for majority of its population. However, rice cultivation in the region is often threatened by several abiotic stresses, of which the most common is drought. Drought stress seriously affects all stages of crop growth including grain production and its quality. With the unprecedented escalation in human population and burgeoning threat of global climate change, drought is becoming a further more serious and unabated problem. The effect of drought stress is reflected in the chromosomal behaviour of rice cells during meiosis, therefore stress during flowering stage significantly interrupts floret initiation (which cause spikelet sterility) and grain filling. Drought induces pollen sterility and prevents peduncle elongation, panicle exertion and spikelet opening (Hong and Serraj, 2012) all of which leads to the reduction in grain set. Plant growth is greatly affected by water deficit. Both root and shoot are adversely affected by drought. Plants generally develop adaptation to drought by limiting the number and area of leaves during drought stress to curtail the water budget at the cost of yield loss. Since roots are the only path to acquire water from the soil, the root growth, its density, proliferation and size are key factors of plants matter during drought stress (Kavar *et al.*, 2007).

In addition, water stress leads to the disturbances in carbohydrate metabolism of the cell. These metabolic changes are regulated at the level of gene expression in various organs including reproductive structures (Oliver *et al.*, 2005). Non-structural carbohydrates (NSC) can be used as the markers for the indices of drought resistance in plants. The total non-structural carbohydrates are generally sugars, starches, and fructose that can be accumulated and mobilized for metabolism or translocated to other plant structures (Smith, 1981). Pre-heading carbohydrate storage is an important phenomenon in plants that serves as a buffer to support grain filling during unfavourable weather conditions and for rice, estimates of the contribution by pre-heading carbohydrates accumulated in vegetative tissues, range from 24 to 27% (Cock and Yoshida, 1972) and 20 to 30% Accumulation of higher amount of pre-heading carbohydrates can serve as a drought resistance characteristic in rice.

Under drought, both structural growth (sink) and assimilation (source) processes are down-regulated resulting a change in source-sink relations. Drought decreases the source capacity to synthesize adequate leaf starch concentration; in parallel it also increases the accumulation of starch at sink leaves and apex (Luquet *et al.*, 2008). Thus, under drought, carbohydrates are actively accumulated at the apex, which is used as a reserve rather than their utilization for growth and sugars also may act as a signal under water stress, participating in the regulation of organ growth and development. This raises a question, whether the plant phenomic research should consider metabolic markers such as sugars as an indicator of drought tolerance (Rebolledo *et al.*, 2012), however, such markers should be easy to measure having greater genetic diversity and correlation with yield.

Rice cultivars experiencing drought have better osmoregulation, in terms of synthesis and accumulation of increased proline and total sugar content. They tend to accumulate proline over a longer period of time and have higher total sugar accumulation

with better yield stability (Maisura *et al.*, 2014). Lesser reduction in grain yield during drought, accumulation of proline over a longer period of time, and an increase in total sugar accumulation are the major physiological trait that contributes to the drought tolerance in rice. Another important physiological response of plants to drought is its ability to maintain turgor pressure by reducing osmotic potential (Maisura *et al.*, 2014). The decrease in osmotic potential and the ability to accumulate soluble compounds helps to maintain the turgor pressure. The soluble compounds that are usually accumulated during the process are sugars, amino acids and prolines (Szabadoz and Savoure, 2009). Thus, accumulation of soluble sugars like proline can be a good indicator of drought tolerance. The aim of this work was to study the comparative effects of stress on morpho-physiological parameters along with osmolytes and carbohydrate profiling in the leaves of rice cultivars and to analyze the significance of these parameters in drought stress tolerance. Comparison of these parameters in these upland rice cultivars differing in drought tolerance may be helpful in developing a better understanding and provide additional information on the mechanisms of drought tolerance and develop a selection indicator for the breeding program.

Materials and Methods

The present investigation was carried out in the Department of Crop Physiology, Assam Agricultural University, Jorhat, India during the summer season. The experimental site was situated at 6°47' N latitude; 94°12' E longitude having an elevation of 86.6 m above mean sea level. Five rice germplasms collected from Regional Agricultural Research Station, Titabar of Assam Agricultural University were used for the study.

Growth conditions

The experiment was conducted under UV sterilized polythene roof rainout shelter in the earthen pots (28 cm height and 30 cm in diameter) filled with fine and sterilized field soil mixed with shade dried and well decomposed FYM in the ratio of 50:50. The soil in the pots was fertilized with 15:15:15, N: P: K dose. Arresting the irrigation in the half set of the plants set of each cultivar and the other half were kept under regular irrigation throughout the crop season imposed water deficit. In the first round of stress development, water was withheld between 30 and 45 days after sowing for 15 days and then the stressed plants were re-supplied with water for 2 days. Two additional rounds of treatments (15 days-stress/2 days-watering) were imposed on plants throughout the growing period. Soil moisture content was recorded following the gravimetric method. The average moisture content of the soil was maintained at a range from 17-18 per cent under irrigated condition and between 7-8 per cent under moisture stress condition during crop growth period. The physiologically active and fully expanded leaves were used for the study in each treatment.

Phenotypic measurements and data analysis

Roots were dug out when the plants reached panicle initiation stage then soil was separated from the roots by gentle washing. Roots were further washed over a fine sieve.

The length of the fibrous root was measured from root/shoot demarcation point to root tip by metallic measuring tape. The allometry of root growth to shoot growth was expressed by root: shoot ratio. Root volume was determined by water displacement method and was expressed as cc (centimetre cube).

The numbers of tillers were counted at maximum tillering stage and at the flowering stage. Tiller mortality was expressed in percentage by using the following formula:

$$\text{Tiller mortality (\%)} = 1 - \frac{\text{Number of tillers at flowering stage}}{\text{Number of tillers at maximum tillering stage}} \times 100$$

The area of the fresh green leaves for each treatment was measured using leaf area meter (model CI-203) and the leaf area index was calculated at panicle initiation stage using the following formula and was expressed in $\text{cm}^2 \text{ plant}^{-1}$.

$$\text{LAI} = \text{Total leaf area} / \text{Ground area}$$

Net assimilation rate (NAR) was calculated based on the increase in dry weight of plant per unit leaf area per unit time and was expressed as $\text{g cm}^{-2} \text{ day}^{-1}$. NAR was calculated using the following formula,

$$\text{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e LA_2 - \log_e LA_1}{LA_2 - LA_1}$$

Where, W_1 and W_2 were the total dry weight of plant and A_1 and A_2 were leaf area at time t_1 and t_2 respectively.

The rate of transpiration was measured on the leaves using a portable Infrared Gas Analyzer (IRGA). Leaf chlorophyll content was estimated by a non-maceration method using Dimethyl Sulphoxide (DMSO) and light absorption at 663 nm and 645 nm was read in a UV visible spectrophotometer. The amount of chlorophyll content was calculated using absorption coefficients. The chlorophyll content was determined by using the formula given by Arnon (1949) and expressed as mg g^{-1} leaf fresh weight. Proline accumulation was determined by the method as described by Sadasivam and Manickam (1996). Reducing and non-reducing sugar contents were determined using the method mentioned in Allen *et al.*, 1988. The total soluble sugar (TSS) content was computed by adding reducing and non-reducing sugar content and expressed in mg g^{-1} dry weight. Analysis of variance (ANOVA) was used to determine the variation between the treatments and the rice cultivars. The experiment was completely randomized design (CRD) with four replications. Experimental data were analyzed using SAS 9.3. The critical difference (CD) values were calculated at 1 percent and 5 percent probability level for appropriate interpretation of the results.

Results

The Analysis of variance revealed that there was a significant difference between the rice cultivars for all of the assessed morph-physiological traits under the present study (Table 1). It further envisaged a significant difference between the rice plants grown under stress and non-stress conditions, for all of the traits under study. The variety \times treatment effect was significant for all of the traits except chlorophyll 'a' content which was not significant.

Effect of moisture stresses on morpho-physiological traits

Moisture stress caused a significant increase in root volume (9.85%) during the panicle initiation stage in all the cultivars (Table 2). Among rice cultivars 'Bandana' showed the highest root volume during normal and stress grown condition. 'Bandana' also reported the maximum increment in root volume (10.78%) during stress condition followed by 'Kopilee' (10.56%). Averaging over all the cultivars, moisture stress increased the root: shoot ratio by 20.83% during panicle initiation stage (Table 3). Root: shoot ratio was highest in cultivar 'Maibee' (0.27) which was 17.4% higher as compared to cultivar 'ARC-10372', and the later cultivar registered the lowest root: shoot ratio (0.23). Stress induced-increment was the highest in 'Kopilee' (21.79%) and the lowest in 'Bandana' (16.00%).

A perusal of the data in Table 4 reflects that the moisture stress significantly increased the tiller mortality between maximum tillering and flowering stage. Tiller mortality was increased by 101.90% due to the stress. During the normal growth condition, the tiller mortality was observed highest in 'Maibee' (16.69%). 'Maibee' (26.72%) showed the highest tiller mortality in stressed condition followed by 'Lachit' (26.35%). Comparing the stressed and control conditions, the highest percentage increment in tiller mortality was seen in 'Lachit' (176.18%) followed by 'Kopilee' (137.66%). The lowest tiller mortality was observed in 'Maibee' (60.09%).

The leaf area index decreased due to moisture stress. The average decrease in leaf area index was 42.53% during the stress condition (Table 5). During the normal growth condition the maximum leaf area index of $4.64\text{cm}^2\text{ plant}^{-1}$ was observed in cultivar ARC-10732 followed by 'Kopilee' ($4.34\text{cm}^2\text{ plant}^{-1}$). 'Maibee' showed the lowest leaf area index ($3.07\text{cm}^2\text{ plant}^{-1}$). Stress-induced reduction in leaf area index was highest in 'Bandana' (54.83%) and it was recorded to be the lowest in 'ARC-10732' (34.05%) followed by 'Lachit' (40.09%).

Moisture stress increased the plant proline content

Data pertaining to the effect of moisture stress on proline content of rice cultivars are presented in Table 6. Moisture stress significantly increased the proline content (12.73%). On an average cultivar 'Bandana' showed the highest proline content (12.02), which was 24.17% higher as compared to the check cultivar 'ARC-10732' (9.68) and the

lowest content was found in cultivar 'Kopilee' (8.90). The stress induced increase in proline content was the highest in 'Maibee' (17.40%) and the increment was the lowest in 'ARC-10732' (8.05%) followed by 'Lachit' (11.18%).

Effect of moisture stress on transpiration rate and net assimilation rate

Averaging over all the rice cultivars used in the study, moisture stress significantly reduced the transpiration rate. The reduction was 27.70% under moisture stress compared to normal condition (Table 7). There was a significant difference in transpiration rate among the cultivars. The highest transpiration rate was observed in 'Bandana' (10.84) which was 42.06% higher as compared to the check cultivar 'ARC-10732' (6.28) and it was the lowest in 'Maibee' (6.26). A significantly different amount of reduction in transpiration rate was recorded in set of cultivars as affected by moisture stress. Reduction in the rate of transpiration under moisture stress situation was the highest in 'Lachit' (42.56 %) whereas it was the lowest in 'ARC-10732' (10.50%) followed by 'Kopilee' (19.84%). The data in Table 8 reflects that moisture stress significantly reduced the net assimilation rate at the maximum tillering stage by 12.72%. The cultivars differed significantly in terms of net assimilation rate. The highest net assimilation rate was shown by the cultivar 'ARC-10732' (0.0069) followed by 'Maibee' (0.0062). The lowest net assimilation rate was observed in 'Lachit' (0.0044). Stress-induced reduction in net assimilation rate was highest in 'Kopilee' (20.40%) and it was lowest in 'Maibee' (7.81%).

Moisture stress decreased the leaf chlorophyll content

Moisture stress significantly reduced the chlorophyll content in the leaves of rice cultivars (Table 9). Chlorophyll 'b' has higher reduction due to moisture stress than chlorophyll 'a'. 'Lachit' showed higher amount of chlorophyll content than other cultivars in both normal and stress conditions. The highest reduction in chlorophyll content was shown by cultivar 'Bandana'. However, all the cultivar showed less reduction in chlorophyll 'a' content as compared to the check cultivar 'ARC-10372'. Except 'Bandana' all other cultivars showed better performance in terms of percentage decrease in chlorophyll 'b' content as compared to the check. A lesser reduction in chlorophyll content at stress condition was supposed to be a drought resistant. Considering these, the cultivar 'Kopilee' showed the lowest reduction in chlorophyll content followed by 'Lachit'. Cultivar 'Maibee' showed the lowest percentage decrease in chlorophyll 'b' content followed by 'Lachit'.

Effect of moisture stresses on carbohydrate content

Averaging over all the cultivars moisture stress significantly decreased the reducing sugar content (Table 10). On an average the cultivar 'Kopilee' showed the highest reducing sugar content (8.73mg g⁻¹ DW) which was 34.10% higher as compared to the check 'ARC-10372', and the later registered the lowest reducing sugar content (6.51 mg g⁻¹ DW). Moisture stress significantly decreased the reducing sugar content in

cultivars by different degrees. Stress-induced reduction was highest in ‘Kopilee’ (13.52%) whereas it was lowest in ‘ARC-10372’ (9.37%) followed by ‘Maibee’ (10.17%). A significant decrease (10.49%) in non-reducing sugar was observed under moisture stress condition. (Table 8). Moisture stress caused a reduction in non-reducing sugar content in rice cultivar by significantly different degrees. The reduction was highest in ‘Lachit’ (12.13%) and it was lowest in ‘Kopilee’ (8.33%).

The total sugar in rice leaves was measured during the tillering stage and data on total sugar in cultivars pertaining to different treatments was presented in Table 10. Moisture stress significantly reduced the total sugar content and on an average it was 10.76% lower compared to the normal condition. The cultivars and treatments interacted significantly in influencing the total sugar in leaves of rice cultivars. The cultivar ‘Kopilee’ (11.48%) showed the highest reduction in total sugar under moisture stress and the lowest reduction was recorded in ‘ARC-10372’ (9.73%) followed by ‘Maibee’ (10.45%). All the cultivars showed a higher reduction in total sugar content as compared to that of the check. Moisture stress resulted a 11.58% decrease in the starch content in leaf (Table 11). The cultivars differed significantly in their response to moisture stress for this parameter. Starch content was highest in ‘Kopilee’ followed by ‘Lachit’ and the reduction in starch content in stressed condition was also more of these two cultivars. The highest reduction in starch content was recorded in ‘Kopilee’ (12.43%) and it was lowest in ‘ARC-10372’ (10.60%) followed by ‘Maibee’ (11.25%) under moisture stress condition.

There was a high variation in non-structural carbohydrates content among the rice cultivars used in the study. ‘Kopilee’ recorded the highest non-structural carbohydrate content in both stressed and non-stressed condition. ‘ARC-10372’ showed the least reduction in non-structural carbohydrate content (10.40%) whereas, ‘Kopilee’ showed the highest reduction (12.16%). Averaging over the all the cultivars, the total non-structural carbohydrate content was reduced by 11.37% under moisture stress condition than that of normal growth conditions.

Correlation between the traits

Significant correlation was observed between various traits under study (Table 12). Root volume was negatively correlated to chlorophyll ‘b’ whereas not with chlorophyll ‘a’. Root-shoot ratio was negatively correlated with leaf area index, starch, and non-structural carbohydrate content, whereas positively correlated with proline content. Higher tiller mortality was associated with lower leaf area index and lower starch content and higher proline content. Proline content is related to starch and non-structural carbohydrate significantly. The increase in transpiration rate reduced the sugar content in plants. Accumulation of assimilates also reduced the sugar content. Sugar contents were correlated with each other. Reducing sugar, non-reducing sugar, total sugar, starch content and non-structural carbohydrates were positively and significantly correlated with each other.

Table 1. Anova showing the significance of various traits under different treatments and varieties

Traits	Root volume (cm ³)	Root: Shoot Ratio	Tiller mortality (%)	Leaf Area index (cm ² plant ⁻¹)	Proline content (mg g ⁻¹ DW)	Transpiration rate mol (m ² s ⁻¹)	Net assimilation rate (gm ² day ⁻¹)	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Reducing sugar (mg g ⁻¹ DW)	Non-Reducing sugar (mg g ⁻¹ DW)	Total sugar (mg g ⁻¹ DW)	Starch (mg g ⁻¹ DW)	Non-Structural carbohydrate (mg g ⁻¹ DW)
Varieties	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Treatment	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Variety x Treatment	**	**	**	**	**	**	**	Ns	**	**	**	**	**	**

*, ** - Significant at 5% and 1% level of probability respectively, ns - non significant

Table 2. Effect of moisture stress on root volume at panicle initiation stage in rice varieties

Treatments	Root volume (cm ³)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	4.70	6.11	7.51	5.21	6.91	6.09
Stress	5.12	6.65	8.32	5.72	7.64	6.69
% Increment	8.71	8.83	10.78	9.78	10.56	9.85

Table 3. Effect of moisture stress on Root: Shoot ratio at panicle initiation stage in rice varieties

Treatments	Root: shoot ratio					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	0.23	0.23	0.25	0.27	0.23	0.24
Stress	0.27	0.27	0.29	0.32	0.28	0.29
Increment (%)	17.39	17.39	16.00	18.51	21.79	20.83

Table 4 Effect of moisture stress on Tiller mortality (%) at panicle initiation stage in rice varieties

Treatments	Tiller mortality (%)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	10.01	9.53	13.65	16.69	10.54	12.08
Stress	18.78	26.35	25.03	26.72	25.05	24.39
Increment (%)	87.61	176.18	83.36	60.09	137.66	101.90

Table 5. Effect of moisture stress on Leaf area index at panicle initiation stage in rice varieties

Treatments	Leaf area index (cm ² plant ⁻¹)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	4.64	4.29	3.41	3.07	4.34	3.95
Stress	3.06	2.57	1.54	1.74	2.46	2.27
Decrease (%)	34.05	40.09	54.83	43.32	43.31	42.53

Table 6. Effect of moisture stress on Proline content in leaves of different rice varieties

Treatments	Proline (mg g ⁻¹ DW)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	9.68	9.68	12.02	11.78	8.90	10.41
Stress	10.46	10.76	13.58	13.83	10.07	11.74
Decrease (%)	8.05	11.15	12.97	17.40	13.14	12.77

Table 7. Effect of moisture stress on Transpiration rate in leaves of different rice varieties

Treatments	Transpiration rate (m mol m ⁻² s ⁻¹)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	6.28	9.54	10.84	6.26	7.66	8.12
Stress	5.62	5.48	7.48	4.64	6.14	5.87
Decrease (%)	10.50	42.56	30.99	25.87	19.84	27.70

Table 8. Effect of moisture stress on Net assimilation rate in leaves of different rice varieties

Treatments	Net assimilation rate (g cm ⁻² day ⁻¹)					
	ARC-10732	Lachit	Bandana	Maibee	Kopilee	Mean
Control	0.0069	0.0044	0.0052	0.0064	0.0049	0.0055
Stress	0.0063	0.0037	0.0046	0.0059	0.0039	0.0048
Decrease (%)	8.69	15.91	11.54	7.81	20.40	12.72

Discussion

Rice sensitivity to drought depends on the stage and the period of occurrence of drought coinciding with the vegetative and flowering period. This leads to a decrease in production of grain and its productivity. Plant response to drought stress can be analyzed through identification of the characters that play an important role in drought tolerance. Analysis can be done at the morphological, physiological, cellular, biochemical, and molecular levels. Drought response at the cellular stage depends on specific plant stage again when the drought occurs, duration of drought, and plant species (Prasad *et al.*, 2012). Analyzing the root traits, tillers, and biochemical parameters, we can identify 'ARC-10372' and 'Maibee' as the best performing cultivars for drought tolerance.

Present investigation revealed that moisture stress brought about a significant

increase in root: shoot ratio and root volume. Increase in root: shoot ratio was mainly due to the increased root length and shorter shoot as observed in the cultivar 'Maibee'. Morrison (1983) opined that greater allocation of carbon towards the root as a mechanism to improve apparent plant water status of the leaf under moisture stress condition and thereby leads to the increase in root: shoot ratio. The capacity of the root system to uptake water depends not only on root mass but also on root volume (rooting depth), fine root area and fine root activity. Acceleration of root growth in terms of root length and volume as observed in the cultivar 'ARC- 10372' and 'Maibee' under moisture stress condition could also contribute to the establishment of seedlings more rapidly and avoid water deficit. Increase in root volume indicated that more water and nutrient were absorbed from the soil in the cultivars 'ARC-10372' and 'Maibee' which helped them to establish better in moisture stress condition.

The results also suggest that 'ARC-10372' and 'Maibee' were considered to be drought tolerant and were able to retain green leaves longer than other cultivars such as 'Lachit' and 'Kopilee' under drought conditions, which was attributed through maintenance of higher leaf area index. Cultivars with green leaf retention may process dehydration-tolerance mechanism, which allows them to maintain metabolic activity, despite low leaf water potential (Fukai, and Cooper, 1995). Higher tiller mortality in susceptible cultivars ('Lachit' and 'Kopilee') under lower soil moisture levels might be due to the fact that under water stress, plants were not able to produce enough assimilates for photosynthesis. It may be due to the less amount of water uptake to prepare sufficient food and inhibition of cell division in meristematic tissue. Net assimilation rate was adversely affected by decreased soil moisture. Lower soil moisture might inhibit photosynthesis and decrease translocation of assimilates to the grain which lowered grain weight (Liu *et al.*, 2008). In addition, drought stress could curtail the kernel sink potential by reducing the number of endosperm cells and amyloplasts formed (Yang *et al.*, 2006). Ultimately, it reduces the biomass of the plants.

Moisture stress had a significant effect on chlorophyll 'a' content of all cultivars. The decrease in chlorophyll content indicated that drought caused a strong loss of photosynthetic reaction centres. Both chlorophyll 'a' and chlorophyll 'b' are of vital importance for photosynthesis. Chlorophyll 'b' serves as an antenna that collects light and transfers to the reaction centre which is present in chlorophyll 'a'. Light energy is converted into chemical energy in the reaction centre which can then be used in the reduction process of photosynthesis. Both the chlorophyll 'a' and chlorophyll 'b' are susceptible to dehydration (Farooq *et al.*, 2009). He reported that drought in several plant species can cause a change in the chlorophyll a:b ratio and carotenoid content. The increase in chlorophyll 'b' content may be observed in some cases (Jaleel *et al.*, 2009) which are responsible for reducing chlorophyll a:b ratio and thus affects the photosynthetic efficiency. A higher chlorophyll a:b ratio was found in ARC-1032 followed by 'Maibee' which can thus be considered as the resistant cultivars to moisture stress.

Table 9. Effect of moisture stress on Chlorophyll content in leaves of different rice varieties

	Chlorophyll a(mg g ⁻¹ leaf FW)						Chlorophyll b(mg g ⁻¹ leaf FW)					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean
Treatments												
Control	0.32	0.35	0.33	0.23	0.26	0.30	0.38	0.43	0.38	0.40	0.31	0.38
Stress	0.28	0.32	0.29	0.21	0.24	0.27	0.34	0.39	0.27	0.37	0.28	0.33
Decrease (%)	12.5	8.57	12.12	8.70	7.69	10.00	10.53	9.30	28.95	7.50	9.68	13.16

Table 10. Effect of moisture stress on reducing sugar, non-reducing sugar and total sugar content in different rice varieties

	Reducing sugar (mg g ⁻¹ DW)						Non-reducing sugar (mg g ⁻¹ DW)						Total sugar content (mg g ⁻¹ DW)					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean
Treatments	6.51	7.78	6.79	8.26	8.73	7.61	3.15	5.77	3.29	3.60	5.64	4.29	9.66	13.55	10.08	11.86	14.37	11.90
Control																		
Stress	5.90	6.95	6.09	7.42	7.55	6.78	2.82	5.07	2.93	3.20	5.17	3.84	8.72	12.02	9.02	10.62	12.72	10.62
Decrease (%)	9.37	10.67	10.31	10.17	13.52	10.91	10.48	12.13	10.94	11.11	8.33	10.49	9.73	11.29	10.52	10.46	11.48	10.76

Table 11. Effect of moisture stress on starch and non-structural carbohydrate content in leaves of different rice varieties

	Starch (mg g ⁻¹ DW)						Non-structural carbohydrate (mg g ⁻¹ DW)					
	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean	ARC-10372	Lachit	Bandana	Maibee	Kopilee	Mean
Treatments												
Control	31.68	34.83	32.43	32.43	35.31	33.34	41.35	48.39	42.51	44.30	49.69	45.24
Stress	28.32	30.64	28.72	28.78	30.92	29.48	37.05	42.67	37.75	39.41	43.65	40.10
Decrease (%)	10.61	12.03	11.44	11.26	12.43	11.58	10.40	11.82	11.20	11.04	12.16	11.36

Table 12. Correlation coefficient between the various traits in five rice varieties grown under moisture stressed and well-watered condition

	Root volume (cm ³)	Root: Shoot Ratio	Tiller mortality (%)	Leaf Area Index (cm ² plant ⁻¹)	Proline content (mg g ⁻¹ DW)	Transpiration rate (m mol m ⁻² s ⁻¹)	Net assimilation rate (g cm ⁻² day ⁻¹)	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Reducing sugar (mg g ⁻¹ DW)	Non-Reducing sugar (mg g ⁻¹ DW)	Total sugar (mg g ⁻¹ DW)	Starch (mg g ⁻¹ DW)	Non-Structural carbohydrate (mg g ⁻¹ DW)
Root volume (cm ³)	1.00	0.16	0.34	-0.42	0.23	0.42	-0.76**	0.09	-0.60*	-0.02	0.24	0.14	0.01	0.07
Root:Shoot ratio		1.00	0.90**	0.95**	0.80**	-0.58	-0.04	-0.65*	-0.31	-0.23	-0.42	-0.37	-0.79**	-0.64*
Tiller mortality (%)			1.00	0.94**	0.61*	-0.60	-0.35	-0.47	-0.41	-0.29	-0.20	-0.26	-0.77**	-0.58
Leaf area index (cm ² plant ⁻¹)				1.00	-0.80**	0.42	0.26	0.48	0.43	0.31	0.35	0.37	0.77**	0.63*
Proline content (mg g ⁻¹ DW)					1.00	-0.14	0.10	-0.33	-0.10	-0.32	-0.64*	-0.54	-0.63*	-0.63*
Transpiration rate (m mol m ⁻² s ⁻¹)						1.00	-0.23	0.62*	0.18	0.09	0.20	0.17	0.60	0.43
Net assimilation rate (g cm ⁻² day ⁻¹)							1.00	-0.22	0.27	-0.17	-0.69*	-0.50	-0.16	-0.33
Chlorophyll a (mg g ⁻¹ FW)								1.00	0.38	-0.36	0.16	-0.07	0.31	0.15
Chlorophyll b (mg g ⁻¹ FW)									1.00	0.18	0.08	0.14	0.35	0.27
Reducing sugar (mg g ⁻¹ DW)										1.00	0.68*	0.89**	0.74*	0.85**
Non-Reducing Sugar (mg g ⁻¹ DW)											1.00	0.94**	0.70*	0.85**
Total sugar (mg g ⁻¹ DW)												1.00	0.78**	0.93**
Starch (mg g ⁻¹ DW)													1.00	0.96**
Non-Structural Carbohydrate (mg g ⁻¹ DW)														1.00

*, **: Significant at 5% and 1% level of probability



ARC-10372



Lachit



Bandana



Maibee



Kopilee

A: Normal B: Moisture stress

Figure1. Effect of moisture stress on root growth of rice cultivar

An important physiological response of plants to drought is its ability to maintain turgor pressure by reducing osmotic potential as a tolerance mechanism. The decrease in osmotic potential and the ability to accumulate soluble compounds help to maintain the turgor pressure. In the process of osmotic adjustment, the soluble compounds that are usually accumulated are sugar and amino acids, especially proline (Szabadoz and Savoure, 2009). In the present investigation, interaction between drought and cultivar has a significant effect on proline accumulation at the maximum tillering stage. Proline accumulation is an early response when a plant is experiencing a water deficit, in which it decreases cell damage (Anjum *et al.*, 2011). Proline accumulation does not only happen in a tolerant cultivar, but also occurs on susceptible ones. However, drought-tolerant cultivars can accumulate proline for a longer period of time than the susceptible ones. It was shown in ARC -10372, that are categorized as tolerant group as it accumulated a higher proline concentration compared to other cultivars like 'Lachit' and 'Kopilee'. The characters directly related to drought tolerance are the increased accumulation of proline and total sugar during drought stress. Yue *et al.*, (2006) reported the mechanism of drought tolerance through an osmotic adjustment as the increased accumulation of solutes, such as proline and total sugar. According to him, an increase in proline and total sugar accumulation when drought stress occurs is one of the indicators of plant tolerance to drought. Proline also plays an important role as an osmoprotectant, an energy sink to regulate redox potential, and as a radical hydroxyl scavenger (Sharma and Dietz, 2006).

Sugar accumulation in rice cultivars is severely affected by moisture stress. Total sugar accumulation is one of the mechanisms for drought tolerance in plants. Total sugar accumulation also plays an important role in substrate hydrolysis in the biosynthetic process, producing energy and acts as a sensor and signal. It also functions as a typical osmoprotectant to maintain cell stability and to maintain turgor pressure (Kishor *et al.*, 2005). Total sugar accumulation in the organ of a tolerant cultivar is more effective because of the high membrane stability and low water loss than susceptible cultivars (Valentovic *et al.*, 2006). In the present investigation cultivars that are relatively tolerant to moisture stress, especially ARC -1037 and 'Maibee' recorded that total sugar accumulation is significantly different in both the control and drought treatment during the maximum tillering stage. Tolerant cultivars (ARC -1037 and 'Maibee') have recorded higher level of total sugar accumulation during moisture stress than the non-tolerant one. Marisura *et al.*, (2014) reported the susceptibility to drought is due to the blockage in re-translocation of sugar in those cultivars during drought treatment. Therefore, not only the starch and carbohydrate content are responsible for explaining the drought tolerance in plants but its active transportation should also be well studied. Moisture stress-induced reduction in total non- structural carbohydrate content in cultivars differed significantly. The lowest reduction in cultivars 'Maibee' and 'ARC-10372' can be explained due to the active transportation of carbohydrates during moisture stress to various plant parts.

Root-shoot ratio showed a negative correlation to chlorophyll content and accumulated reserves like starch and non-structural carbohydrates. This explains that a decrease in root: shoot ratio increases the carbohydrate content of plants. Lower root: shoot ratio in the plants is due to larger shoot size as compared to that of the root. Since

reserve foods like carbohydrates are accumulated in shoots, larger shoot size is responsible for the accumulation of more amounts of carbohydrates in plants. Correlation studies showed that increase in leaf area index increases the accumulation of more amounts of starch and non-structural carbohydrates. So, leaf area index and carbohydrate content are the positively correlated traits responsible for drought resistance in plants. Although chlorophyll is responsible for photosynthesis, the chlorophyll content is not correlated with the starch or sugar. This shows that photosynthesis is not only dependent on the chlorophyll content of the leaves.

Conclusion

Drought tolerance is a complex mechanism involving various plant adaptive features, which are responsible for producing stable yield. Various morpho-physiological and biochemical traits can be considered as an indicator of drought tolerance. The increase in root volume, root: shoot ratio, and leaf area are some of the mechanisms adopted by plants to cope with drought. Tolerant rice cultivars 'ARC-10372' and 'Maibee' showed resistance to drought due to a high accumulation of osmolytes during stress and easy translocation of these osmolytes to various plant parts. Therefore; it seems that the accumulation rate was correlated with drought tolerance. Other studies showed that the free proline content and soluble sugars can be used as drought tolerance indicators for selecting drought resistant genotypes. Chlorophyll content is not solely responsible for the accumulation of osmolytes in plants; however it is a good indicator of drought tolerance. Plants accumulating the higher amount of sugar and starch are resistant to drought.

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