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Water Stress Effects on Leaf Growth and Chlorophyll Content but Not the Grain Yield in Traditional Rice (*Oryza sativa* Linn.) Genotypes of Assam, India II. Protein and Proline Status in Seedlings under PEG Induced Water Stress

Jnandabhiram Chutia[1*], Sailen Prasad Borah[2]

[1]Department of Botany, Darrang College, Tezpur, India; [2]Department of Botany, Gauhati University, Guwahati, India.
Email: [*]cjnan@rediffmail.com

ABSTRACT

Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development. The leaf growth and Chlorophyll content has significantly shown to vary from the control ones while the grain yield was not affected. While many plant species naturally accumulate proline and protein as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive roles in mediating osmotic adjustment and protecting sub cellular structures in stressed plants. Different approaches have been contemplated to increase the concentrations of proline like compounds in plants grown under stress conditions to increase their stress tolerance. Seven different traditional rice varieties of Assam were evaluated for their response to osmolyte production under physiological drought condition through simulation at three levels of osmotic stress of 0.15 bar, 0.25 bar and 0.56 bar of physiological drought initiated by polyethylene glycol (PEG 6000). Along with the evaluation for osmolyte response the different components of genotypic variation for six different drought-sustaining characters in the seven rice varieties were also substantiated. The results indicated that plant height and seed number have significant genotypic coefficient of variability (GCV) and heritability. Verities like *Laodubi*, *Leserihali*, *Beriabhanga* and *Borah* were screened out as the best drought sustaining variety.

Keywords: Abiotic Stresses; Proline; Protein; Osmolyte; Genotypic Coefficient of Variability; Heritability; Traditional Rice Cultivar

1. Introduction

Rice genotypes are known to vary widely in their responses to abiotic stresses. About forty-two biotic and abiotic stresses affect rice production [1]. This is in part due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [2]. Alterations in internal water relations are generally evaluated by investigating the relationships between water potential or its solute and turgor components and relative water content [3].

Simulation of drought stress by polyethylene glycol (PEG) induces drought stress on the plants [4] and significant deviation from the control continues to increase with the increasing solute potential (Ψs) [5]. PEG-6000

has long been utilized as a reliable marker under laboratory conditions for testing the drought tolerant genotypes. This is because polyethylene glycol acts as a non-penetrating osmotic agent resulting into increasing solute potential (Ψs) and blockage of absorption of water by the root system [4,6,7]. Drought screening using some seed technological parameters has been found to be quite useful in a number of crops [8] under laboratory conditions. This technique can be further extended to test drought tolerance in other genotypes, [9].

Length of leaf is negatively related with osmotic stress. Thus the length of the leaf decreases with the rise in solute potential. Reduction in leaf length in higher osmotic stress is due its negative correlation with leaf angle [8]. The longer leaves have wide angle while the shorter ones have smaller angles. The leaf angle is a character usually associated with plasticity in leaf rolling when internal

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water deficit occurs.

Grain yield under stress environment is the primary trait for selection in breeding for drought tolerance. Drought effect on seed yield is due to the relation with duration of watering from flowering until physiological maturity [10].

Osmotic stress generated by polyethylene glycol (PEG-6000) generally reduce photosynthetic rate [11]. Exposure to drought stress leads to a significant effect in Chlorophyll-*a* and Chlorophyll-*b* contents [5].

Response to water stress in plants at the molecular level undoubtedly constitutes an area of major interest for a complete understanding of the process. The major strategy for gaining such understanding is through the approach of proteomics. Differential expression of genes under water stress conditions can reveal a picture as to what are the biochemical pathways that are instrumental in enabling the cells to elicit the right response [12-18]. While there are several reports of expression of a number of genes only under water stress, a much more comprehensive approach is to profile the total protein contents and kinds of protein under normal and stressed conditions.

Many plant species naturally accumulate protein and proline as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play adaptive role in mediating osmotic adjustment and protecting sub cellular structures in stressed plants [19,20]. Thus, different approaches have been contemplated to increase the concentrations of these compounds in plants grown under stress conditions to increase their stress tolerance. The present investigation, thus is aimed at elucidating the drought sustaining character of some traditional rice cultivars of Assam to drought stress based on some established protocols related to screening of rice cultivars in response to drought stress. Since breeders are still looking for traits that are suitable for screening rice germplasm for characters affecting plant water relations under drought conditions [21].

2. Materials and Methods

The present study, initially 12 verities were considered viz., *Bengunguti*, *Beriabhanga*, *Borah*, *Jahinga*, *Kesamani*, *Kolajoha*, *Laodubi*, *Leserihali*, *Pattesari*, *Rangadaria*, *Sakuakumal* and *Solpuna*. After initial studies related to germination index (GI) in PEG initiated drought and the whole plant behaviour under three water regimes then subsequently only seven traditional varieties *viz. Laodubi*, *Borah*, *Jahinga*, *Beriabhanga*, *Pattesari*, *Leserihali* and *Kolajoha* of Assam, India, were screened for their response to osmolyte production under physiological drought condition simulated by PEG 6000.

Three levels of osmotic potential ($\Psi\pi$) of 0.15 bar, 0.25 bar and 0.56 bar induced by PEG-6000 were used for simulation of physiological drought. Seeds of the experimental rice varieties were treated with different solutions of PEG-6000. After the PEG-6000 treatment, the germination index was determined and the seedlings were subsequently grown under three different water regimes-1) normal irrigated condition considered as non-stress (control), 2) unirrigated water stress upland condition and 3) unirrigated water stress potted condition.

The experiment was conducted in a randomized block design (RBD) with three replications. Hundred healthy seeds each of the 7 different cultivars was pre soaked in distilled water for 12 hrs. Forty eight pairs of clean and sterilized petri plates were used for the experiment. In each replication there were 16 petri plates. The presoaked seeds were first air-dried to eliminate the surface water. They were then placed over blotting paper in the petri-plates and were allowed to germinate aseptically under three different osmotic potentials *i.e*., 0.15 bar, 0.25 bar and 0.56 bar using appropriate concentration of PEG-6000 [5]. Deionised water was used for the control and applied similarly. At regular intervals of 12 hrs, 5 - 6 drops of different solutions of PEG-6000 were administered to the seeds in the petri plates. The treated and controlled seeds were allowed to germinate in a BOD incubator at 25°C ± 2°C for seven days. The lid of the petri-plates were opened and replaced for exchange of fresh air to the growing seedlings at regular intervals. The seeds soaked in PEG-6000 solutions were kept under observation for 7 days and the germination index was calculated out. The number of germinating seeds were counted and continued up to seven days at a regular interval of 24 hrs.

Collected data were analyzed for determining the 1) seed germination index, 2) leaf protein content and 3) leaf proline content.

The germination index (**GI**) was calculated by using the formula as suggested by the Association of Official Seed Analysis [22].

$$\textbf{GI} = \frac{\text{No. of germinated Seeds}}{\text{Days of first count}}$$
$$+ \frac{\text{No. of germinated Seeds}}{\text{Days of Second count}}$$
$$+ \cdots + \frac{\text{No. of germinated Seeds}}{\text{Days of final count}}$$

The petroleum ether extract of the leaves were prepared and analysed using UV based spectrophotometer (Hitachi grade) at a wavelength of 663 nm, 645 nm and

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663 nm. The amount of chlorophyll present in the extract mg chlorophyll per tissue was calculated out using the following equations:

1) For mg chlorophyll a/g tissue

$$= 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

2) For mg chlorophyll b/g tissue

$$= 22.9(A_{665}) - 4.68(A_{645}) \times \frac{V}{1000 \times W}$$

3) For total chlorophyll/g tissue

$$= 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

where, A is the absorbance at specific wavelengths; V is the Final volume of chlorophyll extract in 80% Acetone and W is the fresh weight of tissue extract.

For estimation of protein and proline content, young leaves from 20 days old seedlings grown under osmotic potentials of—0.15 bar, 0.25 bar and 0.56 bar were taken. The proline content was assayed by the method described by Bates *et al.* [23] and Chinard *et al.* [24]. For the experiment, 0.5 gm of freshly collected leaves were homogenized in 10 ml of 3% aqueous sulphosalicylic acid. Control sample was consisted of leaves from seedlings grown in deionized water alone. The homogenate was filtered through Whatman No. 2 filter paper. 2 ml of the filtrate was taken in a test tube and 2 ml of glacial acetic acid was added to it. To the mixture freshly prepared 2 ml of acid ninhydrin was added. The final solution was subjected to heat for 1 hr in a boiling water bath. After one hour of boiling the reaction was terminated by placing the test tube in an ice bath. Now to the test tube 4 ml of toluene was added and stirred for 20 - 30 seconds. Subsequently, the toluene layer was separated and the final mixture was again warmed to room temperature and the red colour (slightly red colour) was measured at 520 nm.

A standard curve was prepared using 0.1, 0.2, 0.3, 0.4 and 0.5 µmol of pure proline and used for conversion of absorbance values into proline content.

Protein content in the leaf samples was determined by following Lowry's method [25].

Proline and protein content were estimated from the seedlings grown under simulated drought condition induced by—0.15 bar, 0.25 bar and 0.56 bar of PEG 6000. Seeds grown under osmotic stress induced by 0.56 bar of PEG 6000 failed to yield sufficient number of seedlings enough for the biochemical assays. Thus proline and protein could be estimated only in those seedlings grown under 0.15 bar and 0.25 bar of artificial drought.

The genotypic and phenotypic coefficients of variabilities for the characters were calculated according to the formulae of Burton [26]. The heritability in broad sense was estimated according to Johansson *et al.* [27, 28].

3. Results and Discussion

The leaves of modern semi dwarf varieties have relatively small leaves with acute angles often folded into a tube form [29]. In the traditional rice varieties like *Laodubi, Leserihali, Pattesari and Beriabhanga*, the exceptionally long and droopy leaves with larger leaf angle were more susceptible to rolling due to their ability to intercept relatively more radiant energy, greater extensibility and might help conserved water in plant tissues (**Table 1**). Chang *et al.*, [30] also worked on rice and advocated the role of larger leaf angle in conservation of water in plant cells. Thus it can be substantiated that long and droopy leaves (*i.e.*, with higher leaf angle) results into leaf rolling decreasing the area intercepting radiation resulting into decrease in rate of transpiration (Gates 1968) as leaf rolling is an adaptive response to water deficit in rice (Singh, 2000).

The present study revealed that the reduction in grain yield was maximum in the variety *Laodubi* (23.78 ± 0.40 number of grain) under unirrigated potted condition. On the contrary the same variety exhibited a yield of 117.58 ± 0.30 and 145.81 ± 0.18 number of grain under unirrigated upland and irrigated rainfed condition respectively (**Table 2**). Thus the results indicated that grain yield under stress was limited due to decreased production and translocation of assimilates as the sink size, is not affected (Chaturvadi and Ingram, 1989; Ahmed, 1992). The results clearly indicate that water deficit during booting to anthesis initiation is more detrimental than anthesis stage stress. Ingram, (1989) also reported reduction in grain production due to moisture stress during booting stage to flowering or early grain filling stage in rice. These observations support the hypothesis that selection for yield under reproductive-stage drought stress is effective in rice, and that choice of donor is very important in breeding drought-tolerant rice (**Table 3**).

In the present investigation there was a significant decrease in the Chlorophyll-*a* and Chlorophyll-*b* and total chlorophyll content in the plants of unirrigated upland and potted condition while under normal rained condition the ratios of chlorophyll was higher as evident in *Laodubi, Kolajoha* and *Pattesar* (**Table 4**). An increasing trend of osmotic adjustment ($\Delta\psi_\pi$) with decreasing solute (ψ_s) is a mechanism developed for the plant to survive in dry conditions (Heralde, *et al.*, 1998). The higher amount of

Table 1. Mean values of various plant characters studied under three different water regimes in 12 different rice genotypes.

Variety	Culture condition induced by PEG-6000 bar	Rate of Germination GI ± SE	Culture condition	Plant height cm ± SE	Length of the flag leaf cm ± SE	Flag leaf angle degrees ± SE	Green leaf duration days ± SE	Panicle length cm ± SE	Total seeds (Fertile & Sterile) numbers ± SE
LAODUBI	Ψs – 0.15	19.57 ± 0.36	Control	120.41 ± 0.42	33.55 ± 0.29	30.38 ± 0.31	137.93 ± 0.52	23.51 ± 0.29	145.85 ± 0.15
	Ψs – 0.25	15.75 ± 1.4	Upland	114.51 ± 0.29	21.62 ± 0.52	70.25 ± 0.25	117.75 ± 0.38	23.51 ± 0.29	117.73 ± 0.26
	Ψs – 0.56	4.13 ± 0.27	Potted	85.51 ± 0.51	14.73 ± 0.37	59.89 ± 0.42	97.92 ± 0.08	13.49 ± 0.25	24.63 ± 0.47
BORAH	Ψs – 0.15	17.58 ± 0.43	Control	118.52 ± 0.52	50.66 ± 0.33	80.26 ± 0.27	136.85 ± 0.46	17.85 ± 0.15	121.92 ± 0.08
	Ψs – 0.25	16.28 ± 0.9	Upland	145.55 ± 0.29	33.51 ± 0.51	74.88 ± 0.48	114.85 ± 0.45	23.85 ± 0.45	82.40 ± 0.41
	Ψs – 0.56	2.04 ± 0.22	Potted	74.85 ± 0.46	18.65 ± 0.33	50.84 ± 0.45	96.79 ± 0.21	11.75 ± 0.38	14.55 ± 0.55
JAHINGA	Ψs – 0.15	9.37 ± 0.53	Control	112.45 ± 0.29	29.92 ± 0.08	60.32 ± 0.33	133.85 ± 0.45	22.38 ± 0.38	136.51 ± 0.29
	Ψs – 0.25	5.77 ± 0.6	Upland	133.51 ± 0.52	26.73 ± 0.45	60.25 ± 0.25	113.85 ± 0.46	20.38 ± 0.38	108.73 ± 0.26
	Ψs – 0.56	0.97 ± 0.12	Potted	62.43 ± 0.30	23.45 ± 0.29	53.56 ± 0.30	95.91 ± 0.08	11.69 ± 0.17	11.42 ± 0.43
BERIABHANGA	Ψs – 0.15	12.94 ± 0.40	Control	128.78 ± 0.40	21.76 ± 0.24	60.55 ± 0.56	135.51 ± 0.29	20.85 ± 0.15	116.78 ± 0.22
	Ψs – 0.25	8.80 ± 0.9	Upland	119.6 ± 0.31	27.55 ± 0.29	79.98 ± 0.56	112.783 ± 0.40	19.59 ± 0.30	47.96 ± 0.04
	Ψs – 0.56	1.50 ± 0.25	Potted	55.48 ± 0.29	11.73 ± 0.27	65.51 ± 0.52	96.88 ± 0.12	18.98 ± 0.57	6.81 ± 0.56
PATTESARI	Ψs – 0.15	6.38 ± 0.52	Control	121.66 ± 0.33	33.51 ± 0.52	90.51 ± 0.51	134.85 ± 0.45	22.75 ± 0.38	98.88 ± 0.11
	Ψs – 0.25	2.51 ± 0.6	Upland	132.45 ± 0.45	28.95 ± 0.54	70.59 ± 0.30	108.75 ± 0.38	18.98 ± 0.02	87.99 ± 0.01
	Ψs – 0.56	0.33 ± 0.05	Potted	86.55 ± 0.29	21.74 ± 0.26	52.92 ± 0.51	73.87 ± 0.27	21.52 ± 0.24	6.62 ± 0.32
LESERIHALI	Ψs – 0.15	10.34 ± 0.31	Control	110.71 ± 0.36	26.85 ± 0.45	79.94 ± 0.53	133.75 ± 0.38	22.38 ± 0.31	100.40 ± 0.4
	Ψs – 0.25	0.76 ± 0.2	Upland	97.7 ± 0.54	24.45 ± 0.29	85.55 ± 0.29	105.85 ± 0.46	16.70 ± 0.30	95.86 ± 0.46
	Ψs – 0.56	0.3 ± 0.03	Potted	71.41 ± 0.42	23.88 ± 0.48	70.23 ± 0.40	71.74 ± 0.25	17.77 ± 0.23	4.743 ± 0.38
KOLAJOHA	Ψs – 0.15	6.4425 ± 0.49	Control	120.88 ± 0.12	26.55 ± 0.29	80.55 ± 0.29	131.75 ± 0.38	22.75 ± 0.25	132.77 ± 0.22
	Ψs – 0.25	4.3 ± 0.9	Upland	112.41 ± 0.30	25.51 ± 0.29	70.29 ± 0.30	110.65 ± 0.33	23.84 ± 0.15	74.51 ± 0.52
	Ψs – 0.56	0.67 ± 0.06	Potted	84.81 ± 0.43	20.41 ± 0.42	55.45 ± 0.45	75.85 ± 0.15	19.55 ± 0.29	4.51 ± 0.52

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Continued

Genotype		Germination index	Culture condition	Plant height	Flag leaf length	Flag leaf angle	Green leaf duration	Panicle length	Seed number
SOLPUNA	Ψ_s - 0.15	6.45 ± 0.51	Control	114.49 ± 0.49	40.81 ± 0.43	80.52 ± 0.52	130.75 ± 0.38	22.86 ± 0.14	98.99 ± 0.01
	Ψ_s - 0.25	4.08 ± 0.9	Upland	139.52 ± 0.29	23.48 ± 0.29	70.28 ± 0.28	108.95 ± 0.53	22.42 ± 0.30	55.67 ± 0.51
	Ψ_s - 0.56	0.91 ± 0.06	Potted	75.85 ± 0.15	24.85 ± 0.15	59.72 ± 0.27	75.25 ± 0.21	12.56 ± 0.22	3.48 ± 0.48
BEGUNGUTI	Ψ_s - 0.15	0.86 ± 0.12	Control	121.74 ± 0.38	25.81 ± 0.52	75.55 ± 0.29	119.73 ± 0.37	23.58 ± 0.30	126.40 ± 0.30
	Ψ_s - 0.25	1.48 ± 0.4	Upland	73.45 ± 0.45	23.51 ± 0.52	59.37 ± 0.32	101.88 ± 0.48	20.70 ± 0.30	56.44 ± 0.29
	Ψ_s - 0.56	0.74 ± 0.01	Potted	55.92 ± 0.51	20.43 ± 0.43	45.88 ± 0.48	66.246 ± 0.25	9.723 ± 0.36	2.99 ± 0.57
KESAMANI	Ψ_s - 0.15	1.72 ± 0.26	Control	103.51 ± 0.52	17.92 ± 0.08	69.78 ± 0.40	120.85 ± 0.45	21.38 ± 0.31	125.77 ± 0.52
	Ψ_s - 0.25	1.51 ± 0.4	Upland	80.88 ± 0.12	20.05 ± 0.05	81.85 ± 0.46	104.85 ± 0.45	19.39 ± 0.40	75.55 ± 0.55
	Ψ_s - 0.56	0.69 ± 0.02	Potted	57.77 ± 0.23	15.85 ± 0.15	59.65 ± 0.33	70.90 ± 0.10	18.92 ± 0.51	2.79 ± 0.41
SAKUAKUMAL	Ψ_s - 0.15	2.27 ± 0.54	Control	111.92 ± 0.08	29.73 ± 0.37	69.85 ± 0.15	119.92 ± 0.51	20.72 ± 0.36	146.37 ± 0.32
	Ψ_s - 0.25	3.42 ± 0.6	Upland	87.59 ± 0.30	25.85 ± 0.35	80.41 ± 0.30	101.77 ± 0.40	18.66 ± 0.33	72.73 ± 0.27
	Ψ_s - 0.56	0.16 ± 0.02	Potted	60.55 ± 0.29	22.78 ± 0.22	65.7 ± 0.53	68.92 ± 0.08	16.80 ± 0.18	1.76 ± 0.39
RANGADARIA	Ψ_s - 0.15	1.60 ± 0.53	Control	139.51 ± 0.29	31.41 ± 0.30	40.52 ± 0.52	117.92 ± 0.51	27.40 ± 0.30	150.51 ± 0.52
	Ψ_s - 0.25	2.05 ± 0.8	Upland	87.76 ± 0.52	23.55 ± 0.29	70.25 ± 0.26	105.73 ± 0.37	24.59 ± 0.30	63.65 ± 0.33
	Ψ_s - 0.56	0.13 ± 0.01	Potted	64.55 ± 0.55	23.84 ± 0.15	50.51 ± 0.52	72.27 ± 0.29	9.85 ± 0.46	1.43 ± 0.43

Table 2. Mean sum of squares for various plant characters in 12 traditional rice cultivars grown under three different water regimes.

Source of variation	Degrees of freedom	Character studied	Degrees of freedom	Plant characters					
		Germination index		Plant height	Flag leaf length	Flag leaf angle	Green leaf duration	Panicle length	Seed number
Variety	11	196.40**	11	1154.932**	163.01**	555.36**	571.383**	15.16**	1240.633**
Replication	3	0.00	2	0.194	1.0275	0.675	0.037	0.23	0.285
Culture condition	2	562.90**	2	24770.58**	982.11**	2238.785**	22164.48**	534.565**	77155.4**
Error	127	6.59	92	438.9389	57.25663	221.2238	156.0795	12.2341	454.1803

**Significance level P = 0.1.

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Table 3. Estimates of different genetical parameters in 12 different rice varieties.

Plant characters	Mean ± SE	Range	Genotypic variance	Phenotypic variance	Genotypic co-efficient of variability %	Phenotypic co-efficient of variability %	Heritability %
Germination index	6.276667	19.85 - 0.14	63.26699	69.86	168.9656	177.5532	90.56062
Plant height	99.36111	139 - 56	238.6643	677.6032	15.5481	26.19819	35.22184
Flag leaf length	25.22222	51 - 15	35.25112	92.50775	29.08998	47.12442	38.10613
Flag leaf angle	65.96296	80 - 45	111.3787	332.6025	15.9993	27.64793	33.48704
Green leaf duration	106.01	137 - 66	138.4345	294.514	11.09886	16.18859	47.0044
Panicle length	19.37963	24 - 9	0.975301	13.2094	5.095934	18.75409	7.383385
Seed number	69.96296	150 - 1	262.1508	716.3311	23.14235	38.25505	36.59632

Chlorophyll-*a* and Chlorophyll-*b* attributes to the accumulation of solutes in the cell sap through passive accumulation resulting from reduced cell size (Morgan, 1984) which significantly does osmotic adjustment ($\Delta\psi_\pi$). Non-stomatal restrictions on CO_2 assimilation under drought stress can be effectively assessed through measuring Chlorophyll based parameters. Energetic status of the chloroplast increases as a consequence of the drought stress which has a direct relationship to that of increased amount of total chlorophyll and Chl *a* and Chl *b* [5] among the stressed induced verities.

Germination and seedling development under laboratory conditions have been accepted as suitable growth stages for testing the response to abiotic stresses [31] and thus it was employed to evaluate the drought sustaining character of the local varieties of Assa. A positive correlation between germination index (GI) in PEG initiated drought and the whole plant behaviour under three water regimes were observed in the present investigation (**Table 2**). This was evident from the results exhibited by *Laodubi*, *Leserihali* and *Pattesari* with higher germination index while these same varieties showed good response to other drought sustaining characters under three water regimes. Thus the determination of germination index (GI) can be used just as an easy and reliable parameter for measuring drought sustenance among the traditional rice cultivars of Assam.

The low germination rate in *Jahinga*, *Pattesari* and *Kolajoha* as observed in the present study was due to the osmotic stress induced by PEG 6000 which had mark effect in both shoots and roots parameters. The reduction in seed germination may be due to the less availability of free water to the seeds during early hours of inbibition, thus leaving the hydrolytic enzymes inactive [32,33]. Inhibition of germination at higher osmotic potential may possibly be attributed to moisture deficit in the seed below the threshold requirement for germination [34]. The reduction in shoot and root growth is important as PEG induced stress affects root volume and root length [10]. The reduction of root volume under induced osmotic stress originates not only from growth inhibitions but also from a loss of turgidity [35].

Total protein content decreases due to abiotic stress Baruah *et al.* [36]. As synthesis of proteins occur during dehydration stress a class of proteins called late embryogenesis abundant globular protein known as osmotin or dehydrin [19] are known to accumulate in dry seeds, which play an important role in the regulation of dehydration in seeds. The protein content among all tolerant genotypes was found higher than susceptible ones [37]. Water stress condition caused a marked change in protein synthesizing apparatus of plant tissue [38] and the capacity for protein synthesis also decreases considerably as observed in response to water stress [39]. In the present study the results obtained with higher protein content in *Borah*, *Beriabhanga*, *Laodubi* and *Solpuna* (**Table 5**) are in agreement with the findings of Chinoy *et al.* [40] who also reported a high protein content in drought stressed rice plant. Ashraf and Foolad [20] had reported that higher protein content in tolerant genotypes under water stress condition is due to higher DNA and RNA content, which stimulate synthesis and inhibit protein decomposition.

Decrease in osmotic potential under stress reflects the increased hydrolysis of macromolecules into simpler ones like mono- and disaccharides, amino acids specially proteins etc. and consequently higher osmolite concentration [41]. Thus under higher solute potential, *Laodubi*, *Leserihali*, *Beriabhanga* and *Pattesari* accumulated higher proline (**Table 6**), which acted as a osmoticum and accounted for higher drought tolerance due to greater relative water content and leaf water potential [42]. This is because proline is a major organic osmolyte that accumulates in a variety of plant species in response to environmental stresses such as drought which is thought to have positive effects on enzyme and membrane integrity

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Table 4. Chlorophyll content in 12 different rice varieties grown under three different water regimes.

Sl. No.	Variety	Culture condition	Amount of chlorophyll		
			CHL. a/g tissue	Chl b/g tissue	Total chlorophyll
1	Laodubi	Control	12.39	10.39	9.17
		Upland	6.870	5.541	4.88
		Potted	5.53	4.831	4.26
2	Borah	Control	8.06	6.34	5.59
		Upland	8.45	7.02	6.20
		Potted	7.20	6.82	6.02
3	Jahinga	Control	30.89	22.78	20.13
		Upland	16.76	3.15	2.78
		Potted	12.28	2.79	2.46
4	Beriabhanga	Control	21.48	17.58	15.51
		Upland	18.26	15.98	14.10
		Potted	17.04	13.46	11.87
5	Pattesari	Control	18.19	13.92	12.28
		Upland	5.44	4.67	4.12
		Potted	4.81	4.44	3.919
6	Leserihali	Control	24.23	19.348	17.07
		Upland	7.911	11.128	9.81
		Potted	7.175	8.815	7.77
7	Kolajoha	Control	6.65	5.33	4.70
		Upland	2.42	2.15	1.89
		Potted	1.24	1.46	1.29
8	Solpuna	Control	6.65	5.33	4.70
		Upland	2.42	2.15	1.89
		Potted	1.24	1.46	1.29
9	Bengunguti	Control	6.48	4.58	6.70
		Upland	8.26	5.98	4.10
		Potted	7.04	3.46	1.87
10	Kesamani	Control	1.02	1.76	3.04
		Upland	4.46	6.08	7.13
		Potted	4.36	6.36	5.61
11	Sakuakumal	Control	8.19	3.92	2.28
		Upland	5.44	4.67	4.12
		Potted	4.81	4.44	3.91
12	Rangadaria	Control	8.19	3.92	2.28
		Upland	5.44	4.67	4.12
		Potted	4.81	4.44	3.91

along with adaptive roles in mediating osmotic adjustment in plants grown under stressed conditions. Exogenous application of proline to plants, before, during, or after stress exposure, has been shown to increase the internal levels of these compounds and generally enhances plant growth and final crop yield under stress conditions [20]. This can be also summed up that over all water loss causes increase in concentration of solutes leading to high concentration of cell sap and intercellular fluid causes a greater decrease in the water potential of the fluids. This causes stress on the protoplasm [43].

Tolerance to abiotic stresses is very complex at the whole plant and cellular levels [44-47]. Putting these observations under consideration the subsequent phases of analysis was done so as to establish the complexity of

Table 5. Total leaf protein content (mg/g of leaf tissue) in 7 different rice cultivars grown under simulated physiological drought stress condition.

Sl. No	Variety	Culture condition		
		Control (deionized water)	Simulated osmotic drought of 0.15 bar	Simulated osmotic drought of 0.25 bar
1	Laodubi	0.12	0.16	0.15
2	Borah	0.15	0.18	0.14
3	Jahinga	0.12	0.19	0.15
4	Beriabhanga	0.11	0.21	0.15
5	Pattesari	0.119	0.18	0.13
6	Leserihali	0.15	0.19	0.12
7	Kolajoha	0.12	0.16	0.11

Table 6. Proline content (μmol/g of leaf tissue) in 7 different rice cultivars grown under simulated physiological drought stress.

Sl. No	Variety	Culture condition		
		Control (deionized water)	Simulated osmotic drought of 0.15 bar	Simulated osmotic drought of 0.25 bar
1	Laodubi[**]	0.003	0.132	0.253
2	Borah[**]	0.001	0.0131	0.161
3	Jahinga	0.0003	0.173	0.145
4	Beriabhanga[**]	0.061	0.068	0.171
5	Pattesari	0.069	0.079	0.135
6	Leserihali[**]	0.057	0.053	0.204
7	Kolajoha	0.052	0.075	0.083

[**]Varieties selected as the best performing ones.

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Table 7. Estimates of different genetical parameters in 7 different rice varieties.

Plant characters	Mean ± SE	Range	Genotypic variance	Phenotypic variance	Genotypic co-efficient of variability GCV %	Phenotypic co-efficient of variability PCV %	Heritability %
Plant height	107.4286	129 - 55	40427.44	40521.63	187.16	187.38	99.76
Flag leaf length	26.44444	50 - 11	55.09	77.91	28.06	10.31	70.71
Flag leaf angle	65.38095	90 -51	104.53	219.88	15.63	22.68	47.54
Green leaf duration	111.3016	138 - 73	87.65	109.26	8.41	9.39	80.23
Panicle length	19.5873	24 - 11	4.49	11.41	10.83	17.25	39.39
Seed number	70.80952	146 - 5	649.40	769.26	35.99	39.17	84.42

interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [2]. In correlation to this it has been observed that the different components of genotypic variation for six drought-sustaining characters in seven experimental rice varieties indicates that plant height and seed number have less environmental influences with high GCV as 187.16 and 35.99 respectively with high heritability (**Table 7**).

From this screening procedure it has been *Laodubi*, *Leserihali*, *Beriabhanga* and *Borah* cultivars were screened out as the best drought sustaining variety among the ones considered in this investigation. This paves the way for further investigation into the inherent characters of drought sustenance characters of the traditional cultivars of Assam.

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