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Effect of drought stress on protein and proline metabolism in seven traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India.

Authors:
Chutia J, Borah SP, Tanti B.

Institution:
1. Department of Botany, Darrang College, Tezpur -784001 Assam.

2. Department of Botany, Gauhati University, Guwahati - 781014 Assam.

Corresponding author:
Chutia J.

Email:
cjnan@rediffmail.com.

Phone No:
+91-94350-82261.

Web Address:
http://jresearchbiology.com/Documents/RA0208.pdf.

ABSTRACT:

Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development. 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.

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INTRODUCTION

Rice genotypes are known to vary widely in their responses to abiotic stresses. About forty-two biotic and abiotic stresses affect rice production (Sarkar et al. 2006). Recent studies indicate this in part due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu 2002). 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 (Cutler et al. 1980).

Simulation of drought stress by polyethylene glycol (PEG) indeed induced drought stress on the plants (Jiang et al.1995) significant deviation from the control continues to increase with the increasing solute potential (Ψs) (Ranjbarfordoei et al. 2000). 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 (Chezen et al. 1995 and Jiang et al. 1995). Therefore, PEG solutions are often used to induce water stress in higher plants (Ashraf and O'Leary 1996). Drought screening using some seed technological parameters has been found to be quite useful in a number of crops (Singh and Afria 1988) under laboratory conditions. This technique can be further extended to test drought tolerance in other genotypes, (Karan Singh et al. 2001).

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 (Holmberg and Bulow 1998, Kasuga et al. 1999, Serrano et al. 1999, Hasegawa et al. 2000, Zhu 2001a, Prabhavathi et al. 2002, Rontein et al. 2002). 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.

It has also been seen that 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. However, not all plants accumulate protein or proline in sufficient amount to help averting adverse effects of abiotic stresses. It has also been observed that some stress proteins are synthesized during the dehydration stress (Singh 2003, Ashraf et al. 2007). 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 of the investigations conducted previously by others. Since breeders are still looking for traits that are suitable for screening rice germplasm for characters affecting plant water relations under drought conditions (Jha et al. 1997). The lack of a reliable method for identifying stress tolerant genotypes and the magnitude of factors involved in tolerance to water stress makes it difficult to choose traits conferring an advantage under such stressed conditions.

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 0.15bar, 0.25bar and 0.56bar 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- (i) normal irrigated condition considered as non -stress (control), (ii) unirrigated water stress upland condition and (iii) 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 (Ranjbarfordoei et al. 2000). 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 + 20C 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 (i) seed germination index, (ii) leaf protein content and (iii) leaf proline content.

The germination index (GI) was calculated by using the formula as suggested by the Association of Official Seed Analysis (AOSA 1983).

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

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. (1973) and Chinard et al. (1952). For the experiment, 0.5 gm of freshly collected leaves were homogenized in 10ml 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. 2ml of the filtrate was taken in a test tube and 2ml of glacial acetic acid was added to it. To the mixture freshly prepared 2ml 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 520nm.

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 (Lowry et al. 1951).

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 (1952). The heritability in broad sense was estimated according to Johnson et al. (1955 a, b).

RESULTS AND DISCUSSION

Germination and seedling development under laboratory conditions have been accepted as suitable growth stages for testing the response to abiotic stresses (Sharif-zadeh and Mohsen 2008). 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 1).** 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 (Shah and

Table 1 Mean sum of squares for various plant characters in twelve 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

Loomis 1975, Hadas 1976). Inhibition of germination at higher osmotic potential may possibly be attributed to moisture deficit in the seed below the threshold requirement for germination (Everiff 1983). The reduction in shoot and root growth is important as PEG induced stress affects root volume and root length (Midaoui 2003). The reduction of root volume under induced osmotic stress originates not only from growth inhibitions but also from a loss of turgidity (Huck et al. 1970).

Total protein content decreases due to abiotic stress Baruah et al. (1998). As synthesis of proteins occur during dehydration stress a class of proteins called late embryogenesis abundant globular protein known as osmotin or dehydrin (Singh, 2003) 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 (Serraj and Sinclair 2002). Water stress condition caused a marked change in protein synthesizing apparatus of plant tissue (Genkel et al. 1967) and the capacity for protein synthesis also decreases considerably as observed in response to water stress (Hsiao 1970). In the present study the results obtained with higher protein content in Borah, Beriabhanga, Laodubi and Solpuna **(Table 3)** are in agreement with the findings of Chinoy et al. (1974) who also reported a high protein content in drought stressed rice plant. Ashraf and Foolad (2007) 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 (Tyagi et al. 1999). Thus under higher solute potential, Laodubi, Leserihali, Beriabhanga and Pattesari accumulated higher proline **(Table 2),** which acted as a osmoticum and accounted for higher drought tolerance due to greater relative water content and leaf water potential (Baruah et al. 1998). 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. Although their actual roles in plant osmotolerance remain controversial, it is thought to have positive effects on enzyme and membrane integrity 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 (Ashraf and Foolad 2007).

Table 2 Proline content (µmol/g of leaf tissue) in seven 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**

Table 3 Total leaf protein content (mg/g of leaf tissue) in seven 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

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. Thus most of the biochemical processes are adversely affected because of water imbalances (Bunting et al. 1998).

Tolerance to abiotic stresses is very complex at the whole plant and cellular levels (Foolad 1999a,b, Foolad et al. 2003a,b, Ashraf and Harris 2004). Putting these observations under consideration the subsequent phases of analysis was done so as to establish the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu, 2002).

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 4).**

From this screening procedure Laodubi, Leserihali, Beriabhanga and Borah cultivars were screened out as the best drought sustaining variety among the ones considered in this investigation.

Mean values of the drought sustaining characters in seven experimental rice verities are presented (Table 1). Genotypic components of variation for the traits are shown (Table 4).

Table 4 Estimates of different genetical parameters in seven 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

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