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Parveen Fatima
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Pooran Chand
Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

SK Singh
College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Purushottam
College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Anurag Mishra
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Pushpendra Kumar
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Pushpendra Kumar
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Effects of water stress on biochemical character and transcription factors expression in rice (*Oryza sativa* L.)

Parveen Fatima, Pooran Chand, SK Singh, Purushottam, Anurag Mishra and Pushpendra Kumar

Abstract
In this study, the effect of water stress at vegetative stages on biochemical traits, and expression of genes coding for transcription factors OsMYB2, and OsLEA were investigated in two Indian rice cultivars; Nagina22 and Pusa Basmati 1. Activity of proline and chlorophyll content were studied and also found to be different among the rice cultivars in both the conditions. Rice cv. Nagina22 was more efficient in maintaining high Proline content on exposure to water stress as compared Pusa Basmati 1. The Chlorophyll content in both the rice variety decreased significantly under water stress condition as compared to control condition. The expression results demonstrated that there was upregulation of OsMYB2 gene in rice varieties under water stress condition as comparison to control condition. The expression analysis of this gene shows 64 fold upregulation in Nagina 22, however in Pusa Basmati 1 it shows only 8 fold upregulation under water stress condition as comparison to control condition. The expression of OsLEA gene also upregulated in all varieties of rice under water stress condition as comparison to control condition. Biochemical and expression analysis revealed that studied biochemical analysis along with transcription factors OsMYB2 may play an essential role in tolerance to water stress, and therefore may be a useful candidate in developing transgenic plants with improved water stress tolerance.

Keywords: Abiotic stress, water stress, transcription factor

Introduction
Water stress affects morphological, physiological, enzyme activity and biochemical contents of plants. Plant responses to abiotic stresses (salinity, heat and water) are complex involving signal reception and transduction followed by genetic and physiological responses. All plants are capable of perceiving and responding to stress [1, 2]. Water deficit that occurs during reproductive growth often causes more severe yield loss than that in the vegetative phase [3]. Water stress imposes serious influences on growth and development of plants by causing numerous changes at the physiological, metabolic and molecular levels [4]. Plants respond variously to water stress in terms of morphology and physiology under water conditions. Different mechanisms allow plants to survive and even reproduce with a limited water supply, such as the maximization of water uptake by deep, dense root systems, the minimization of water loss by stomatal closure and a reduction in leaf area, osmotic adjustment (OA) or changes in cell wall elasticity as well as other essential processes for maintaining physiological activities throughout extended periods of water [5].
Water stress tolerance is an outcome of a series of molecular, cellular, and physiological processes including induction/ repression of various genes that cause accumulation of various osmolytes, improved antioxidant system, reduced transpiration, inhibited shoot growth, and decreased tillering [6]. Phytohormone, abscisic acid (ABA) is reported to be abundant under water-deficit conditions and this in turn causes stomata closure and induces expression of various stress- related genes [7]. It has been shown that water inducible gene expression is also governed by ABA-independent regulatory system [8]. Plant's ability to cope with water deficit depends largely on its water status, which changes with environmental conditions [9]. In the present study, impact of high temperature on aromatic rice and at biochemical and molecular level were studied for the investigation of their relation with the adaptation with high temperature.

Materials and Methods
The two rice varieties Nagina22 (water stress tolerant) and Pusa Basmati 1 (water stress susceptible) were taken under study.

Seeds were collected from BEDF, Meerut and Zonal Research Station, Nagina, Bijnor and grown at the Crop Research Centre and other laboratory works were conducted at, department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.) India. Water stress treatment was imposed on the plant at vegetative stage. In control condition plants were raised under well watered condition, under water stress condition that was given to plants by withholding water supply for 7 days until symptoms of visible leaf rolling appeared in the plants at vegetative stage. To impose equal level of stress, the amount of soil, pot size and the water were kept constant for all the pots and the same number of seedlings per pot was raised. Plants maintained under optimum irrigation (untreated plants) were used as experimental controls. Leaves were sampled after observation of drought-induced symptoms; such as leaf rolling and wilting with a concomitant loss of chlorophylls, and stored at -80 ˚C.

Biochemical Parameters

Chlorophyll and proline content determination

SPAD (Soil Plant Analytical Development) chlorophyll meter (Minolta) was used to measure the chlorophyll content of the leaves. Data on chlorophyll content of different varieties of rice under control and water stress condition were recorded. Three readings were taken from single leaf and their average were considered for determination of chlorophyll content of each genotype of rice under both control and treatment condition. For the estimation of proline content in the leaves of control and stress plants, leaves were harvested at vegetative stage of all varieties taken under the study. The proline concentration was determined according to the method of Bates *et al*. (1973) [10]. 500mg of fresh leaves were homogenized in 10 ml of aqueous sulfosalicylic acid (3%) and then centrifuge at 4000 rpm for 20 minutes. The 2ml of this aliquot was transferred into test tube and 2 ml of acid ninhydrin reagent were added in each test tube. The mixture was heated on boiling water bath for one hr. After which reaction was terminated by placing the test tubes in an ice box for cooling. Thereafter the reaction mixture was shaken vigorously with 4 ml toluene and kept for 1 hr to make two layered.

Molecular Expression Analysis

Total RNA isolation and cDNA synthesis

The leaf samples were collected for RNA isolation at vegetative stage of both control and stressed plant at same time. All the solutions and glassware were treated with 0.1 per cent DEPC water and sterilized for the RNA work. Total RNA was isolated from leaf tissue at vegetative stage of control and treated water stress rice selected variety using Genei Pure Kit. First-strand cDNA was synthesized according to manufacturer's instructions (with some modifications) using 2 µg of total DNase-treated RNA per reaction in a M-MuLV reverse transcription system. PCR gradient was used to determine the annealing temperature of each set of primers. The quality of all cDNA samples was examined with all sets of primers, and PCR products were tested using 2% agarose gel electrophoresis

Semi-quantitative RT-PCR

For semi-quantitative RT-PCR, the first strand cDNA (1µg) was used a templates and the concerned gene was amplified by the different primers. Actin gene was used as internal control for all the treatments. Different gene-specific primers were used for the amplification of target cDNA (Table 1). The following chemicals indicating the order were added in the respective PCR tubes for the amplification purpose. For all genes tested at different cycles as 26cycle, 29cycle, 32cycle, and 35cycle, were optimal for the expression. The cDNA synthesized from the total RNA of different samples were normalized using primers of actin as housekeeping gene. The cDNA was diluted 10 times prior to PCR amplification. About 20 µl of the amplified reaction mixture from each PCR tube with 3 µl of loading dye were loaded onto 1.2% agarose gel (Appendix I) alongside 100 base pair DNA ladder as molecular weight marker (Merck, Pvt. Limited, India). Electrophoresis was done at 50 volt for initial 30 min. and then 70 volt for 1 hr in a horizontal electrophoresis unit system (Atto, Japan). The buffer used was 1x TAE at pH=8.0. The DNA bands in the gel were visualized on a UV transilluminator and documented using a gel documentation system (Alpha DigiDoc, Alpha Innotech Corporation, USA).

Results and Discussion

The Chlorophyll content ranged from 77.8 (Pusa Basmati 1) and 87.4 (Nagina 22) with an average mean of 87.9 in control condition, while under water stress condition chlorophyll content ranged from 31.7 (PB1) to 71.8 (Nagina 22) with an average mean of 48.9 mg/g (Table 2). Per cent change in chlorophyll content was recorded less in Nagina 22 (17.81) in comparison to Pusa Basmati 1(59.23). In our present study, it was found that Nagina 22 was more efficient in maintaining chlorophyll content on exposure to water stress as compared to PB1 variety taken under the study. Reduction in chlorophyll content is much lower in water stress tolerant varieties as compared to water stress susceptible varieties. Pirdashti *et al.* (2009) [11] also observed decrease in chlorophyll content under water scarcity situation than irrigated environment. Fotovat *et al.* (2007) [12] reported decreased chlorophyll contents in all genotypes under stress conditions and indicated total chlorophyll content measurements as an easy and reliable method to determine a genotype's resistance to water stress at early stages. Dalal and Tripathy (2012) [13] also showed that chlorophyll content was reduced under PEG-induced drought stress in rice seedlings.

The proline content ranged from 0.33 (Pusa Basmati 1) to 0.50 (Nagina 22) with an average mean of 0.37 in control condition, while under water stress condition, proline content ranged from 0.50 (Pusa Basmati 1) to 0.77 (Nagina 22) with an average mean of 0.50 (Table 1). Proline, a highly water-soluble amino acid normally accumulates in higher plants in response to environmental [14]. The present investigation shows that Nagina22 was more efficient in maintaining high Proline content on exposure to water stress as compared Pusa Basmati1. Serraj and Sinclair (2002) [15] reported the increase in the level of Proline content due to water stress. The proline accumulated in plants under water stress can protects the cell by balancing the osmotic potential of cytosol with that of vacuole and external environment [16].

The effect of water stress on expression of the gene coding for OsMYB2 is shown in Fig. 1a. The OsMYB2 gene expression analysis was carried out by semi quantitative RT –PCR at different cycles 26, 29, 32 and 35 on different varieties of rice under both control and water stress condition. The expression results demonstrated that there was upregulation of OsMYB2gene in all varieties of rice under water stress condition as comparison to control condition. The expression analysis of this gene shows 64 fold upregulation in Nagina 22 But in PB1 it shows 8 fold upregulation under water stress

condition as comparison to control condition. Under water stress condition, among all the varieties, Pusa Basmati 1 Rice variety showed minimum transcript abundance and Nagina 22 recorded maximum transcript abundance. Yang *et al*. (2012) [7] suggested that OsMYB2 encodes a stress-responsive MYB transcription factor that plays a regulatory role in tolerance of rice to salt, cold, and dehydration stress.

According to Wang *et al*. (2007) [17], late embryogenesis abundant (LEA) genes code for a diverse group of proteins that accumulate to high levels in seed development LEA genes are a gene family which plays important role in protection of water stress. The effect of water stress on expression of the gene coding for OsLEA is shown in Fig 1b. The OsLEA gene expression analysis was carried out by semi quantitative RT –PCR at different cycles 26, 29, 32 and 35 on different varieties of rice under both control and water stress condition. The expression results demonstrated that there was upregulation of OsLEA gene in all varieties of rice under water stress condition as comparison to control condition. The expression analysis of this gene shows 8 fold upregulation in Nagina 22 under water stress condition as comparison to control condition. In Pusa Basmati 1, variety the expression of this gene was not shown in control condition. The expression of LEA genes can be induced by the application of abscisic acid and by various abiotic stresses such as dehydration, osmotic stress, or cold in both reproductive and vegetative tissues. Among the two varieties, Pusa Basmati 1 showed minimum transcript abundance whereas; Nagina 22 recorded maximum transcript abundance. Rice variety Nagina 22 showed biochemical and expression of transcription factors under water stress and hence had better adaptation in comparison to susceptible one. The present study may lead towards better understanding of abiotic stresses. Findings of the research may help in the development of water stress resistant plants.

Table 1: Transcription factors and gene primers used in the semi quantification

Sr. No.	Gene Name	Forward Sequence	Reverse Sequence
1	OsMYB2	5'-GGGCTGAAACGCACAGGCAAGA-3'	5'-CTGCTTGGCGTGCTTCTGC-3'
2	OsLEA	5'-TAGCAGCAGAAGAACTGAAGAAG-3'	5'- CCGCCATTGCAAATAACTCAAAC-3'
3	Actin	5'-ATTTGGCACCACACATTCTAC-3'	5'-TAACCTTCGTAGATTGGGACT-3'

Table 2: Chlorophyll and proline content of rice genotypes under control and water stress condition.

S.N.	Chlorophyll content (mg/g)				Proline (µg/g)		
	Genotype	Control	Treatment	% Change	Control	Treatment	% Change
1	PB1	77.8	31.7	59.23	0.33	0.40	17.91
2	Nagina22	87.4	71.8	17.81	0.50	0.77	34.64
	Mean	87.9	48.9		0.37	0.50	
		C	T	C X T	C	T	C X T
	S. Em.±	0.52	0.91	2.11	0.01	0.02	0.03
	C.D. @ 5%	1.08	1.88	4.35	0.02	0.03	0.07
	C.V. (%)	10.08			27.81		

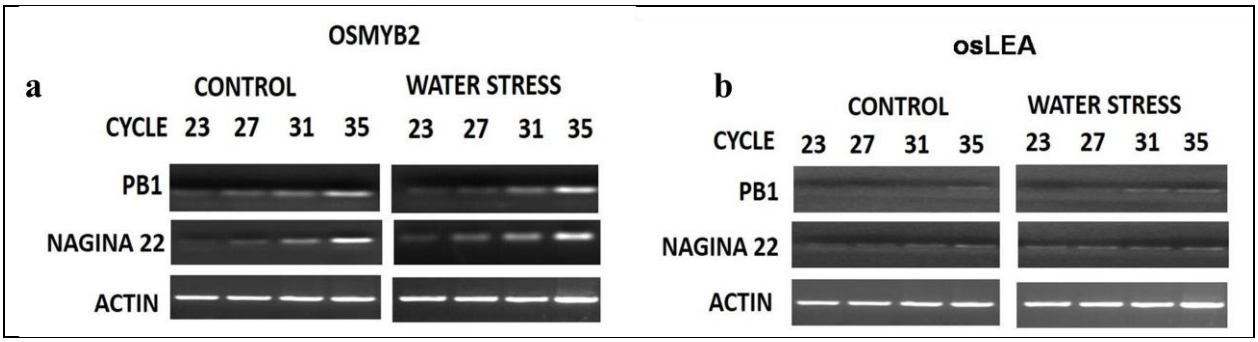

Fig 1a: Semi-quantitative expression analysis of osMYB2 and **b:** osLEA gene in the leaves of Pusa Basmati1 and Nagina 22 rice varieties grown under control and water stress condition.

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