

Differential Responses of Non-enzymatic Antioxidative System under Water Deficit Condition in Rice (*Oryza sativa* L.)

Anirudh Kumar, Minu Marry John, Mir Zahoor Gul, Waikhom Bimolata and Irfan A. Ghazi*

Dept. of plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad- 5000 46

*Corresponding author: irfan@uohyd.ernet.in, Tel.: +91-40-23134511, Fax: +91-40-23010120

Abstract. Drought stress is one of the foremost causes for crop yield reduction in many agricultural regions of the world. Present study gives insight into the relationship between oxidative damages caused by drought stress and variations in the level of antioxidants as part of the plants' protective mechanism in three rice genotypes IRBB21, Pusa Basmati 1 (PB1) and *O. longistaminata*. Augmentation in antioxidants, predominantly flavanoids, phenolics and total antioxidants were many folds higher in IRBB21 over PB1 and *O. longistaminata* during drought stress. The drought tolerant genotype *O. longistaminata* might have accumulated smaller amounts of free radicals and hence it produced lesser amount of antioxidant. The novelty of present work is that IRBB21 (resistant to biotic stress i.e., Bacterial blight (BB)) has produced more antioxidant, phenolics and flavanoids compared to Pusa Basmati1 (susceptible to BB) and *O. longistaminata* (tolerant to drought) to dehydration stress. Such observation suggests that increased water deficit induces a severe oxidative stress in rice plants, which apparently lead to enhance the antioxidant defense.

Keywords: Drought, antioxidant, free radicals, *Oryza sativa*, susceptible, flavanoids

1. Introduction

Rice is a major staple food on which more than half of the world's population depends on. In Asia, rice supplies 30 – 80% of the daily calories consumed (Narciso and Hossain 2002). It is a semi-aquatic tropical C₃ crop and hence it requires high level of soil moisture (Mckersie and Lesham 1994). Drought stress is one of the major constraints reducing crop yield in various water limited regions of the world. There are some stress specific generic responses of plants particularly production of excess reactive oxygen species (ROS). Reactive oxygen species play a major role in energy production, phagocytosis, regulation of cell growth, intercellular signalling and synthesis of biologically important compounds. Its production and removal is balanced under tight control which is disturbed by a number of adverse abiotic stress factors such as high light, drought, low temperature, high temperature and mechanical stress. However, its overproduction causes lipid peroxidation, protein denaturation and membrane deterioration in plants (Yu and Rengel 1999; Prochazkova et al. 2001). It also inhibits the photochemical performance and decreases the activities of enzymes in the Calvin cycle (Monakhova and Chernyadev 2002). A common effect of drought stress is an imbalance between the generations and quenching of ROS resulting in oxidative stress (Smirnov 1998). Therefore, survival of plants under stress depend on the ability of recognition, generation and transmission of the stimulus, and initiation of various physiological as well as biological changes accordingly (Nguyen et al. 2004). Plants have developed its own machinery to overcome drought stress through production of enzymatic (catalase, peroxidase, superoxide dismutase) and non enzymatic (flavanoids, anthocyanins, carotenoids) free radical scavengers.

This study was conducted to assess the extent of the changes in the activities of non-enzymatic antioxidants and its concentration in rice genotypes under drought stress. A detailed study of the behaviour of antioxidant activities induced under drought tolerance was essential in order to identify genes encoding

them, which may give new leads to produce transgenic drought tolerant rice crops employing biotechnological approaches.

2. Material and methods

Seeds of *Oryza longistaminata*, Pusa Basmati 1 and IRBB21 were germinated for 21 days and the healthy seedlings were transplanted in 20 litres earthen pots (three plants in each pot) filled with a mixture of clay and peat. The experiment was carried out at the greenhouse complex of University of Hyderabad, Hyderabad, India. The plants were subjected to drought stress after 20 days of transplantation by withholding water. In growth room, the plants were maintained at 25±2°C (day/ night) temperature in natural photosynthetically active radiation. The control plants were kept at 100% pot water holding capacity (PWC) by supplying water regularly (Zero stress), while the test plants were subjected to mild (4 days), moderate (5 days) and severe (6 days) drought stress.

Ethanol extracts (80%) were prepared from 500mg leaf sample for each rice genotypes. Different antioxidant assays were carried out to determine the antioxidant nature of the extracts. The flavanoids content was determined by aluminium chloride method using quercetin standard solution (Zhishen et al. 1999). Flavanoids form internal complex (chelate) with the aluminium ion (Al³⁺) and give yellow colour whose intensity determine the content of flavanoids spectrophotometrically. The total phenolic content of the extract was estimated by well known Folin–Ciocalteu method (Osawa and Namiki 1981; Singleton and Rossi 1965) and it was expressed as gallic acid equivalents. We estimated total non-enzymatic antioxidants as described by Preito et al. (1999) taking ascorbic acid as standard. Statistical analysis was performed using one way analysis of variance (ANOVA) and data presented are mean ± standard errors (SE).

3. Results and discussion

In the present study, there was significant increase in polyphenol content in all the three genotypes. IRBB21 treated (IRBB21T) showed the maximum polyphenol content (4.84 mg GAE/g) followed by Pusa Basmati 1 (4.29 mg GAE /g) and *O. longistaminata* (3.85mg GAE / g) at 6 d of drought stress. Almost same pattern was seen in the case of flavanoids content as well, where it was induced maximally in IRBB21T (16.35 mg QE / g) at 6 d followed by Pusa basmati 1 and *O. longistaminata* (15.30 mg QE / g and 11.20 mg QE / g; 5 d, respectively). Plant extracts and plant-derived antioxidants can elicit a number of *in vivo* effects which amplifies the synthesis of endogenous antioxidants or act directly as antioxidants. It is also reported that composition of antioxidant varies with duration and nature of stress. Antioxidant enzymes and metabolites increases under various environmental stresses, with their comparatively higher activity in stress tolerant genotypes, suggesting that higher antioxidant activity impart tolerance (Sairam and Saxena 2000). The concentrated action of low molecular weight antioxidants like polyphenol (Sgherri et al. 2004) and flavanoids (Hernandez et al. 2004) can effectively scavenge harmful radicals and stabilize lipid oxidation.

IRBB21 is a near isogenic line (NILs) developed by Khush et al. (1990) which contain *Xa21* gene, confer resistance to bacterial blight (BB), is also responding to drought stress by increasing significant amount of antioxidants during water deficit. Higher production of non-enzymatic antioxidants such as phenolics and flavanoids in susceptible genotype (Pusa Basmati 1) compared to tolerant genotype (*O. longistaminata*) is in contrast to the previous reports (Jagtap and Bhargava 1995; Sairam et al. 1998). IRBB21T has appreciably more amount of antioxidant content in its leaves facing severe drought stress at 6 d (8.28 mg ASE /g). With increase in stress degree there was no significant changes in the antioxidant content of *O. longistaminata* (6.04, 6.36 and 6.99 mg ASE / g at 4, 5 and 6 d respectively). There was also less variation of antioxidant content between control and treated Pusa Basmati 1 at 5 d and 6 d of drought stress whereas, there was enhancement in antioxidant content on 4 d and 5 d. (Table 1). *O. longistaminata* was showing much lesser production of phenolics, flavanoids as well as total antioxidants, may be due to appreciably higher repair mechanism to free radical damage, which is a prominent feature of drought tolerant genotype. Alternatively, other antioxidants might be compensating the need of low molecular weight antioxidants in drought tolerant genotype (*O. longistaminata*).

4. Conclusion

The information about the comparative role of plant antioxidant systems in relation to drought tolerance in rice is limited. Therefore, to gain more insight on drought response of PB1, IRBB21 and *O. longistaminata* in terms of oxidative damages, its relation to antioxidant capacity with drought stress was compared. PB1 behaved as drought sensitive which support earlier study whereas the corresponding parameters were few in *O. longistaminata*, which therefore showed appreciable repair to free radical damages and hence prominent drought tolerance. The above mentioned antioxidants might be limited up to the defence mechanism of the susceptible genotypes under drought. Although laboratory conditions may not always reflect the behaviour of the plants exposed to water stress under field conditions, but such finding may help to understand the mechanism of drought stress management and selection or development of rice genotypes resistant to drought stress.

5. References

- [1] B. Srivalli, C. Viswanathan and K. C. Renu. Antioxidant defense in response to abiotic stresses in plants. *J. Plant Biol.* 30, 121-139, 2003.
- [2] B.D. Mckersie, and Y.Y. Lesham. *Stress and Stress Coping in Cultivated Plants*. Kluwer Academic Publishers, Dordrecht, 1994.
- [3] C. Sgherri, B. Stevanovic, and F. Navari-Izzo. Role of phenolic acid during dehydration and rehydration of *Ramonda serbica*. *Physiologia Plantarum*. 122, 478–485, 2004.
- [4] D. Prochazkova, R.K. Sairam, G.C. Srivastava, and D.V. Singh. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.* 161: 765–771, 2001.
- [5] J. Narciso, and M. Hossain. *World Rice Statistics*. In. IRRI, 2002.
- [6] J. Zhishen, T. Mengcheng, and W. Jianming. The determination of flavanoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64:555-559, 1999.
- [7] J.A. Hernández, C. Escobar, G. Creissen, and P.M. Mullineaux. Role of hydrogen peroxide and the redox state of ascorbate in the induction of antioxidant enzymes in pea leaves under excess light stress. - *Funct. Plant Biol.* 31: 359-368, 2004.
- [8] G.S. Khush, E. Bacalangco, and T. Ogawa. A new gene for resistance to bacterial blight from *O. longistaminata*. *RGN* 7: 121-122, 1990.
- [9] L. T. Fridovich, and R. Sreenivasa: Oxygen radicals, hydrogen peroxide and oxygen toxicity. *Free Radical in Biology*, 1, 239-277, 2000.
- [10] N. Smirnoff. The role of active oxygen in the response to water deficit and desiccation. *New Phytol.* 125:27–58, 1993.
- [11] O. F. Monakhova, and I. I. Chernyadev. Protective role of kartolin-4 in wheat plants exposed to soil drought. *Appl. Biochem. Microbiol.* 38:373-380, 2002.
- [12] P. Preto, M. Pineda, M. Aguilar. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 269:337-341, 1999.
- [13] Q. Yu, and Z. Rengel. Drought and salinity differentially influence activities of superoxide dismutases in narrow-leaved lupins. *Plant Sci*, 142: 1–11, 1999
- [14] R.K. Sairam, and D.C. Saxena. Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *J. Agron. Crop Sci.* 184: 55-61, 2000.
- [15] R.K. Sairam, P.S. Deshmukh and D.C. Saxena. Role of antioxidant system in wheat genotypes tolerance to water stress. *Biologiae Plantarum* 41, 387-394, 1998.
- [16] T. Osawa, and M. Namiki. A novel type of antioxidant isolated from leaf wax of Eucalyptus Leaves. *J. Agric Food. Chem.* 45: 735-739, 1981.

- [17] T.T.T. Nguyen, N. Klueva, V. Chamareck, A. Aarti, G. Magpantay, A.C.M. Millena, M.S. Pathan, and H.T. Nguyen. Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Mol. Genet. Genomics*, 272:35–46, 2004
- [18] V. Jagtap, and S. Bhargava. Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L) Moench exposed to high light, low water and high temperature stress. *J. Plant Physio.* 034, 084-086, 1995.
- [19] V.L. Singleton, and J.A. Rossi. Colorimetry of total phenolic substances. US: American Chemical Society Symposium series. 26: 47-70, 1965.

Table 1: Effect of drought treatment on phenolics (mg GAE /g), flavanoids (mg QE /g) and total antioxidant (mg AAE /g) fresh weight.

Variety	Phenolics						Flavonoids						Total antioxidants					
	4 th Day		5 th Day		6 th Day		4 th Day		5 th Day		6 th Day		4 th Day		5 th Day		6 th Day	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Pusa basmati 1	0.8	0.9	2.1	2.6	3.2	4.2	5.8	6.6	7.9	12.2	8.2	15.3	3.6	4.9	7.7	6.1	6.5	7.5
	4 ±	6	4 ±	9 ±	4 ±	9 ±	7 ±	4 ±	5 ±	8 ±	1 ±	0 ±	5 ±	8 ±	3 ±	6 ±	4 ±	5 ±
	0.1	±	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.13	0.1	0.13	0.1	0.1	0.1	0.1	0.1	0.1
	2		7	4	4	2	3	9	4		5		1	2	7	5	3	4
		0.1																
		4																
IRBB 21	3.0	3.2	3.1	3.5	4.5	4.8	5.2	7.2	6.3	12.8	8.1	16.3	3.4	4.4	6.8	6.8	7.5	8.2
	4 ±	3 ±	9 ±	5 ±	4 ±	4 ±	1 ±	0 ±	7 ±	0 ±	2 ±	5 ±	8 ±	5 ±	1 ±	5 ±	5 ±	8 ±
	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.17	0.1	0.13	0.1	0.1	0.1	0.1	0.1	0.1
	3	1	0	7	2	7	3	8	3		8		3	4	6	5	5	4
Oryza longistaminata	0.4	1.1	2.3	3.1	3.0	3.8	4.1	7.4	5.2	11.2	6.2	13.3	3.7	6.0	4.7	6.3	6.4	6.9
	6 ±	3 ±	0 ±	3 ±	6 ±	5 ±	1 ±	7 ±	5 ±	0 ±	9 ±	4 ±	5 ±	4 ±	8 ±	6 ±	5 ±	9 ±
	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.18	0.1	0.11	0.1	0.1	0.1	0.1	0.1	0.1
	5	4	6	3	1	3	4	3	7		4		3	5	3	4	4	3