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Exogenous Abscisic Acid Mediated Morphological Characteristics, Photosynthetic Pigments and Antioxidant Metabolism under Drought Stress in Rice (*Oryza sativa* L.)

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Drought stress is one of the major abiotic stresses in agriculture worldwide. Drought also produces Reactive Oxygen Species (ROS), triggering a cascade of antioxidative defense mechanisms, and affects many other metabolic processes. It triggers a wide variety of plant responses ranging from physiological, biochemical to molecular levels. The primary challenge for increasing rice production is overcoming a global water shortage, which can severely limit rice yields. In the present study, response of drought stressed plants to abscisic acid (ABA) on antioxidant activities CAT, POD, APX, SOD were studied. The growth was gradually increased with increasing concentrations of ABA. The growth of plant, fresh weight, dry weight and relative water content was higher in the 200 mM abscisic acid. The photosynthetic pigments such as chlorophyll a, b and carotenoids content were increased in the 200 mM ABA as compared to the treated plants and control. The result indicates that increased growth in the 200 mM abscisic acid treated plants compared to control plants due to enhanced antioxidant activities.

Key words*:* Abscisic acid, *Oryza sativa*, Drought, Antioxidant metabolism.

Drought stress is the one of the major abiotic stress and it is limiting factor for plant growth and yield, especially in semiarid areas worldwide (Rizwan *et al.*, 2015). Rice (*Oryza sativa* L.) is the main grain crop grown worldwide and is consumed by more than half of the world population as a staple food (Rizwan et al., 2016). Plant response to water stress include morphological and biochemical changes and later as water stress become more severe to functional damage and loss of plant parts (Sangtarash, 2010). Abscisic acid (ABA) is a plant hormone, it is known as a stress hormone triggers various acclimations processes under drought condition (Zhu, 2002). Under water stress condition exogenously applied ABA stimulated the synthesis of proteins in different species (Riccardi *et al.,* 1998). ABA also expresses the gene encoding enzymes that participate in the repair of spontaneous protein damage (Mudgett and Clark, 1996). Moreover, ABA-induced increase in the osmolyte might also help in stabilizing the proteins under water stress (Noiraud *et al.,* 2001). The present this study was an effort made to investigate the role of ABA under drought stress and also interrelationship between morphological, photosynthetic pigments, antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase and superoxidase dismutase in the seedlings of rice variety in ADT-49 exposed to water stress and ABA condition.

Material and Methods

The seed of rice (*Oryza sativa* L.) variety ADT-49 were obtained from Tamil Nadu Agricultural University, Tamil Nadu, and India. The experiments were conducted at the Department of Botany, School of Life Sciences, Periyar University, Tamil Nadu, India.

Sterilization of seeds

The healthy non-dormant, homogenous seeds were subjected to the surface sterilization with 0.1g Mercury Chloride ($HgCl_2$) for 3 minutes with frequent shaking and the thoroughly washed with de-ionised water to remove the mercury chloride.

Pot culture

The seeds were sown in the pots at greenhouse with triplicates (soil mixture containing red soil, sand and farmyard manure at 1:1:1 ratio) and irrigated with tap water. After 35 days of sowing, the plants were observed under drought condition for 3 days. After drought period, the plants were applied with different concentrations of abscisic acid for 2 days (50, 100, 150, 200, 250 mM, negative control and positive control) and recovery period was 5 days. Plants were collected and analysed at 46th day.

Morphological characteristics

The shoot and root length, fresh and dry weight was calculated at 46th day in control and drought stressed seedlings. Relative water content was calculated by the method of Chen *et al*. (2009). Chlorophyll a and b contents were extracted from a respective leaves and estimated according to the method of Arnon (1949) and the carotenoids content was determined according to the method of Krik and

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Alen (1965). Catalase (CAT) activity was measured by following method of Chandlee and Scandalio (1984). Ascorbate peroxidase was extracted and estimated by the method of Asada and Takahashi (1987). Superoxide Dismutase (SOD) was extracted and estimated by Beauchamp and Fridovich (1971).

Results and Discussion

Growth characteristics

The shoot and root length were decreased under water stress condition and exogenous application of ABA recovered the shoot and root length with increasing concentrations of ABA on drought-induced plants when compared to negative control. Among the concentrations, 200 mM showed enhanced better recovery compared to other treated, control plants (Table 1). Zhang (1987) and Yin *et al.* (2004) reported that ABA treatment increases the stem length when compared to control and other treatments. ABA-induced plants growth was resulted from signal transduction at the single cell level and thereby induces closure of stomata in peas (Creelman *et al.*, 1990).

Table 1. Effect of abscisic acid after drought on Morphological characteristics (plant height, shoot and root length, fresh and dry weight) of rice variety ADT 49 at 46th day

Treatment of ABA (mM)	Plant Height (cm)		Fresh Weight (g)		Dry weight (g)	
	Shoot (ADT 49)	Root (ADT 49)	Shoot (ADT 49)	Root (ADT 49)	Shoot (ADT 49)	Root (ADT 49)
Positive Control Untreated plants	18.2±0.264	2.5±0.360	0.192±0.005	0.138±0.003	0.034±0.004	0.109±0.003
Negative Control	17.1±0.2	2.0±0.264	0.091±0.002	0.102±0.002	0.018±0.003	0.023±0.005
50 mM	18.43±0.351	2.86±0.251	0.102±0.001	0.111±0.003	0.031±0.003	0.031±0.004
100 mM	18.45±0.305	3.13±0.251	0.123±0.002	0.121±0.002	0.032±0.004	0.039±0.002
150 mM	18.40±0.3	4.53±0.404	0.128±0.002	0.132±0.004	0.043±0.005	0.042±0.003
200 mM	19.4±0.608	4.86±0.327	0.141±0.004	0.174±0.005	0.045±0.004	0.050±0.003
250 mM	18.93±0.251	4.23±0.404	0.028±0.003	0.147±0.002	0.038±0.003	0.031±0.003

± Standard deviation (SD)

Drought stress resulted in declined seedling fresh weight; however foliar application of ABA was more effective than the plants treated. Drought with ABA 200 mM was best treatment that showed maximum seedling fresh weight compared to control and other treated plants (Table 1). Dry matter production decreased under drought condition and maximum value was drought-induced with 200 mM exogenous ABA application rice plants compared to other treated plants and control

Table 2. Effect of abscisic acid after drought on relative water content and photosynthetic pigments of rice variety ADT 49 at 46th day

Treatment of ABA (mM)	Relative water content (%)		Photosynthetic pigments (μmol CO_2 m^{-2} sec^{-1})		
	Shoot (ADT 49)	Root (ADT 49)	Chl a	Chl b	Carotenoids
Positive Control Untreated plants	71.46±0.003	83.33±0.004	1.534±0.002	0.923±0.003	1.976±0.002
Negative Control	92.47±0.004	82.35±0.005	1.171±0.003	0.736±0.002	1.625±0.002
50 mM	70.03±0.002	81.60±0.006	1.596±0.003	0.976±0.003	1.989±0.003
100 mM	70.05±0.002	72.07±0.003	1.605±0.004	0.995±0.002	2.008±0.002
150 mM	69.50±0.005	71.26±0.002	1.648±0.002	1.005±0.004	2.067±0.004
200 mM	68.07±0.001	68.18±0.002	1.752±0.001	1.090±0.001	2.233±0.003
250 mM	71.15±0.003	78.91±0.006	1.627±0.004	0.932±0.002	2.114±0.002

± Standard deviation (SD)

RWC were decreased as concentrations of ABA of increased and found low in 200 mM concentration of ABA-treated rice plants (Table 2). Teulat *et al.* (2003) reported that ABA priming ameliorated the drought-induced decrease of RWC of both cultivars. Exogenous application of ABA increased RWC in wheat under water stress by Agarwal *et al.* (2005).

Table 3a. Effect of abscisic acid after drought on antioxidant enzyme activities (CAT, POD) of rice variety ADT 49 at 46th day

Treatment of ABA (mM)	CAT		POD	
	Shoot (ADT 49)	Root (ADT 49)	Shoot (ADT 49)	Shoot (ADT 49)
Positive Control Untreated plants	24.11±0.011	20.46±0.031	10.25±0.025	10.25±0.025
Negative Control	29.05±0.021	26.71±0.021	13.41±0.034	13.41±0.034
50 mM	30.15±0.040	28.67±0.037	14.02±0.044	14.02±0.044
100 mM	33.65±0.047	29.05±0.043	19.48±0.036	19.48±0.036
150 mM	36.05±0.023	31.46±0.046	20.02±0.028	20.02±0.028
200 mM	41.05±0.043	36.75±0.043	22.74±0.033	22.74±0.033
250 mM	38.37±0.048	31.55±0.025	18.44±0.040	18.44±0.040

± Standard deviation (SD)

Photosynthetic pigments

The photosynthetic pigments were decreased in drought induced plants and drought with foliar application of ABA plants was significantly increased with increasing concentration of ABA. The higher content of chlorophyll a, b and carotenoids content was found in 200 mM concentration of abscisic acid when compared to other treated plants and negative and positive control plants (Table 2).

Table 3b. Effect of abscisic acid after drought on enzymatic antioxidant activities (APX, SOD) of rice variety ADT 49 at 46th day

Treatment of ABA (mM)	APX		SOD	
	Shoot (ADT 49)	Shoot (ADT 49)	Shoot (ADT 49)	Root (ADT 49)
Positive Control Untreated plants	0.034±0.004	0.034±0.004	1.246±0.001	0.860±0.001
Negative Control	0.018±0.003	0.018±0.003	1.586±0.004	0.918±0.005
50 mM	0.031±0.003	0.031±0.003	1.626±0.005	1.118±0.006
100 mM	0.032±0.004	0.032±0.004	1.735±0.004	1.147±0.003
150 mM	0.043±0.005	0.043±0.005	1.771±0.003	1.295±0.002
200 mM	0.045±0.004	0.045±0.004	1.980±0.002	1.452±0.004
250 mM	0.038±0.003	0.038±0.003	1.489±0.001	1.251±0.002

± Standard deviation (SD)

ABA plays a direct role in mediating the photosynthesis to respiration in leaves and also the inhibition of lateral root development as reported in *Arabidopsis* (Ren *et al.,* 2007). Sharp *et al.* (2002) reported that under drought condition chlorophyll pigments was decreased and exogenous ABA treatment increases the chlorophyll a, b and carotenoids content when compared to control.

Antioxidants activities

The results of our study showed that the amount of catalase enzyme activity was greatest in 200 mM concentrated plants of ABA compared to negative and positive control and their other treated plants (Table 3a). However, reports on the effect of stresses on CAT activities were varied (Salekjalali *et al*., 2012). Higher levels of antioxidant enzymes are related to drought tolerance in different plants (Khanna-Chopra and Selote, 2007; Hameed *et al*., 2011; Bhagi *et al*., 2013). Increase in CAT activity has been shown to be related with an increase in stress tolerance capacity (Kraus *et al*., 1995; Devi *et al*., 2012). Ismail *et al*. (2005) who found higher catalase activity in drought-tolerant bean plants as compared to drought sensitive.

Peroxidase enzyme activity was measured to determine role of scavenging enzymes in stress tolerance mechanism in rice. However, this enzyme activity was significantly increased with increasing concentration of ABA and mostly significantly increased was 200 mM ABA treated plants compared to control plants (Table 3a). The increase in the activities of antioxidant enzymes by ABA application was reported previously in wheat (Agarwal et al., 2005). The rapid decrease in the level of the POD was observed after withdrawal of drought stress. Previous studies showed that ABA gradually degrades upon removal of drought stress plants Zhang et al. (2006).

The ascorbate peroxidase (APX) activities was found higher in rice shoot and root in drought with 200 mM ABA treated plants compared to other treatment of ABA and negative and positive control plants (Table 3b). Jiang and Zhang (2002) reported the increased levels O2- and H2O2 on ABA treatment of leaves of maize seedlings which lead to the activation of antioxidant enzyme such as APX combined activities of GPX (H2O2 metabolizing) and SOD (H2O2 generating, not studied in the present study) constitute the first line of defense against ROS and changes in their activity and amount have been identified as an indicator of the drought conditions (Veljovic, 2006).

Superoxide dismutase (SOD) enzyme activities under drought condition were significantly decreased. The result showed the highest SOD activities in 200 mM concentration of abscisic acid compared to negative and positive control rice plants (Table 3b). According to Duan *et al.* (2007), ABA increased the activities of SOD, APX, GR, and CAT in comparison to unsprayed control plants. ABA application significantly increased endogenous ABA content and SOD activities under water deficit conditions (Duan *et al.,* 2007).

Antioxidant enzymes could be a useful tool for depicting drought tolerance of rice, useful to plant breeding researchers for developing new drought-tolerant varieties. It suggested that ABA played a positive role for the plant to improve its antioxidant defense mechanism in plants. Further studies are needed to confirm the role of antioxidant enzymes for understanding drought tolerance in a large number of genotypes.

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