

Foliar Application of Abscisic Acid Improves Drought Tolerance of Sugarcane Plant under Severe Water Stress

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Abstract – An experiment was set up to investigate the interrelationship between drought induced abscisic acid (ABA) biosynthesis and antioxidative defense system, and to confer the role of foliar application of ABA in imparting drought tolerance to the sugarcane plant. A drought tolerant variety ROC22 was treated with T1 (drought-water withholding), T2 (drought + foliar application of 15 μ M ABA) and C (control, normal irrigation). Drought treatment (T1) enhanced the ABA concentration in leaves but it was significantly higher in combined treatment (T2), suggesting its biosynthesis was triggered in leaves by the ABA application. Both T1 and T2 resulted in an increase in proline, H_2O_2 and MDA content but the plants applied with ABA were found to resist the increase in MDA content. Overproduction of H_2O_2 in plants treated with T1 was followed by higher activities of CAT, GPX, GR and APX enzymes. A decrease in H_2O_2 level with increasing stress in T2 treated plant showed effectiveness of ABA induced highly expressed antioxidative defense system which was found to be vanished progressively, in the plants treated with the drought only. The results clearly suggests that though the tolerant variety showed an enhanced protective system against drought conditions, the foliar application of ABA further improved its tolerance by continuously triggering the over expression of antioxidative defense system.

Keywords – ABA, Sugarcane, Drought Tolerance, H_2O_2 , Antioxidative Enzyme.

I. INTRODUCTION

One of the greatest challenges for future crop research is the ever decreasing water resources and occurrence of drought, time to time due to continuously changing environmental regimes. During the course of development plants have developed a number of strategies that increase tolerance or adaptation to stress conditions. One of the mechanisms in the series of developments was the alteration in internal growth regulation system and the most important phytohormone mediator that responds as alteration of gene expression in plants is abscisic acid (ABA). ABA has been shown to

mediate plant responses not only to drought [1] but also to number of environmental stresses like cold, salinity, water logging *etc.*, in addition to the regulation of other growth and developmental processes [2].

The basic problem in understanding the mode of action of ABA in crop plants is the still strange as most of the products of plant ABA response that generally confer protective or adaptive roles are also induced by abiotic stresses [3]. Although all stress induced expressions are not regulated by application of indigenous as well as exogenous ABA, a large number of them are responsive to it as shown by their impaired induction in ABA deficient mutants [4]. Increased ABA biosynthesis is itself a stress response [5].

The plant hormone ABA, as a stress signal, increases as a result of water stress [6] and plays important role in the regulation of plant responses from the whole plant level to the cellular level. Increasing evidence indicates that one mode of ABA action may be related to its role in the oxidative stress in plant cells. It has been documented that ABA can cause an increased generation of O_2^- and H_2O_2 [7], and induce the expression of antioxidant genes encoding Cu, Zn-SOD, Mn-SOD [8] and catalase [9], *etc.* ABA also increases the activities of other antioxidant enzymes such as the APX and GR in plant tissues [7]. However, it is not yet clear whether there exist the mode of action described by scientists [7, 9] among ABA, ROS and antioxidative defense under water stress. Also, the molecular signals and pathways that govern most of the abiotic stress responses in sugarcane are still poorly understood [10].

Due to important role of ABA in avoiding deleterious leaf dehydration by closing stomata [11], decreasing transpiration rate [12], controlling root water uptake and/or plant water status via root growth and root hydraulic conductivity [13], the consequence of foliar application of ABA or its analogues in sugarcane fields under drought conditions has been felt and discussed in this paper. We have explored the time course of ABA accumulation under drought conditions alone as well as

under combination of drought stress and foliar ABA application in relation to production of ROS and antioxidative defense enzymes, especially to work out the capability of tolerant variety for resisting the drought conditions *via* ABA mediated pathway and also to know whether ABA application can further improve the tolerance in sugarcane or not.

II. MATERIALS AND METHODS

A. Plant material and treatments

The experiment was performed in a completely randomized block design using the soil pot culture system. The single bud sets of sugarcane variety ROC22 were initially raised by standard culture techniques. The 45 days old settlings have been transplanted in the experimental pots. The soil in the pots contained a mixture of clay soil, organic manure and sand (in 70:20:10 ratio, w/w) with a basal dose of NPK fertilizer. The drought treatment was given at the 5 month growth stage of the plants. The treatments included T1 (drought-water withholding), T2 (drought + foliar application of 15 μ M Abscisic acid) and C (control, normal irrigation).

The drought treatment in pots was maintained by withholding the water during the course of experiment (7days). The soil moisture content in drought treated pots was maintained at $50\pm 2\%$ less compared to the control (irrigated) pots. The total moisture content in control and drought treated pots was maintained at $20\pm 2\%$ and $9\pm 2\%$, respectively. The samples of last transverse marked (LTM) leaves from each treatment were taken in three replicates at 3rd, 5th and 7th days after treatment (DAT) and frozen in liquid nitrogen for analyzing physiological and biochemical parameters.

B. Hydrogen peroxide concentration analysis

The hydrogen peroxide concentration was determined as hydroperoxide-titanium complex by the method of Brennan and Frenkel [14]. Frozen leaf samples were homogenized with acetone and centrifuged at 6000 \times g for 15 min, the supernatant was used to analyze H_2O_2 content. The hydroperoxide titanium complex formed by reaction of tissue H_2O_2 with $TiCl_4$ reagent (20% $TiCl_4$ in HCl) was precipitated using concentrated ammonia solution. The precipitate was repeatedly washed with cold acetone to remove the pigments. The precipitate was solubilized in 2 N sulphuric acid and absorbance of the solution was read at 415 nm against distilled water blank. Hydrogen peroxide contents were calculated by comparing with a standard curve drawn with known hydrogen peroxide concentrations.

C. Malondialdehyde concentration analysis

The level of lipid peroxidation production was estimated following the method of Ohkawa *et al.* [15]. Approximately 0.5 g of frozen leaf sample was cut into small pieces, homogenized with the addition of 2.5 ml of 5% trichloroacetic acid, and centrifuged at 10 000 g for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid in 20% trichloroacetic acid were added in a new tube and incubated at 98°C for 25

min. The tubes were transferred into an ice bath and then centrifuged at 8000 g for 5 min. The absorbance of the resulting supernatant was recorded at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of lipid peroxides was quantified and expressed as MDA content in terms of μ mol g^{-1} FW using an extinction coefficient of $155\text{ mM}^{-1}\text{ cm}^{-1}$.

D. Proline content analysis

Proline content was measured by the method as described by Bates *et al.* [16].

E. Abscisic acid content analysis

ABA concentration was estimated by using the ELISA kit procured from Bei Nong Wei Tian Biological Technology Co. Ltd. (Beijing, China). One gram fresh leaf was homogenized in chilled pestle and mortar using the extraction buffer (containing 80% methanol and 1 mM/L 2,6- di-tert-butyl-4-methylphenol). The homogenate was kept at 4°C for 4 h and centrifuged at 4000 rpm for 5 min. The supernatant was collected in another tube and the residue was again mixed with extraction buffer and centrifuged at 4000 rpm for 5 min. The combined supernatant was used to measure the ABA concentration following the procedure given in user's manual of the kit.

F. Enzyme assay

Catalase activity was estimated using the method of Aebi [17]. The reaction mixture contained 50 mM potassium phosphate (pH 7) and 10 mM H_2O_2 . After enzyme addition, the reaction was monitored by following decomposition of H_2O_2 at 240 nm (extinction coefficient of $H_2O_2 = 43.6\text{ mM}^{-1}\text{ cm}^{-1}$).

GPX was determined according to Zheng and Van Huystee [18]. The reaction mixture contained 10 mM sodium phosphate (pH 6), 0.1 mL of 0.3% (v/v) H_2O_2 , and 0.1 mL of 1% (v/v) guaiacol. Reaction was initiated by the addition of H_2O_2 and followed at 470 nm (extinction coefficient of guaiacol = $26.6\text{ mM}^{-1}\text{ cm}^{-1}$) at 30°C.

GR was measured according to Foyer and Halliwell [19]. The reaction was developed in 50 mM Tris-HCl (pH 7.5) containing 2.5 mM $MgCl_2$, 0.5 mM GSSG, and 0.2 mM NADPH. Oxidation of NADPH was followed at 340 nm (extinction coefficient = $6.2\text{ mM}^{-1}\text{ cm}^{-1}$).

The APX activity was measured by the method of Nakano and Asada [20]. The reaction mixture contained 50 mM phosphate buffer (pH 7), 1 mM sodium ascorbate, and 2.5 mM H_2O_2 . After the addition of ASC to the mixture, the reaction was followed at 290 nm (extinction coefficient of ASC = $2.8\text{ mM}^{-1}\text{ cm}^{-1}$).

Data were subjected to an analysis of variance (ANOVA) and mean separation was performed using the least significance difference (LSD; $P\leq 0.05$) procedures of the SPSS statistical package (SPSS Student Version 15.0).

III. RESULTS

ABA content

No significant difference was observed in controls during the course of study. At 3 DAT, ABA content

increased significantly in T2 (19%) but this increase was not significant in case of T1 (9%) compared to the control. The plants treated with T2 showed 10% higher ABA content than T1. At 5 DAT, the increase in ABA content continued and it was 65% and 13% higher than the controls significantly, in T2 and T1 treatments, respectively. At this stage, ABA treatment (T2) recorded

a 60% increase over the T1. At 7 DAT, ABA content fell down significantly in T2 (from 65 at 5 DAT to 56 % increase over control), while in T1, a significant increase (from 13% at 5 DAT to 33% increase over control) was observed. Nevertheless, the plants treated with T2 showed 35% higher ABA content than in T1 (Fig. 1A).

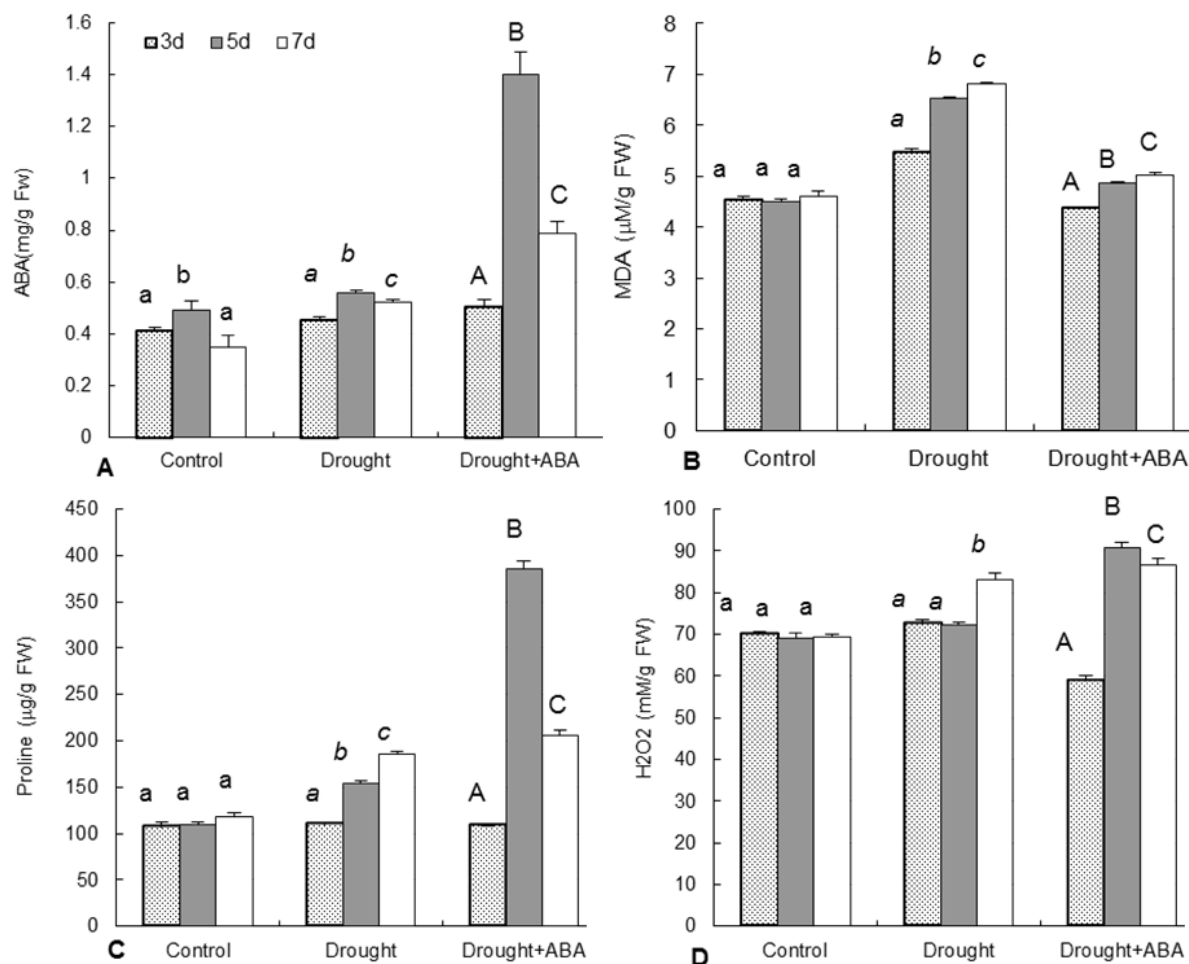


Fig.1. Effect of drought and drought+abscisic acid treatments on endogenous ABA (A), malondialdehyde (B), proline (C), and hydrogen peroxide concentration (D) in sugarcane variety ROC 22 at 3, 5 and 7 days after treatment. Mean values \pm SD (n=3). Different small, capital and italicized alphabets are showing significant difference ($P \leq 0.05$) at 3rd, 5th and 7th day within the control, drought and drought+ABA treatments, respectively.

Malondialdehyde (MDA) content

As a result of drought stress MDA content in T1 increased with increase in the severity of stress, compared to the control. While in the plants with T2 treatment, MDA content first decreased (-4%) at 3 DAT and then increased slightly but not significantly by 7% and 9% at 5 and 7 DAT, respectively, compared to the control. A decrease in MDA content by -25%, -35% and -36% has been recorded in plants with T2 treatment, when compared with T1 treatment (Fig. 1B).

Proline (PRO) content

Generally, no significant difference amongst T1, T2 and control treatments has been observed at 3 DAT. At 5 DAT, PRO content increased significantly in both, T1 and T2 compared to the control, but this increase was

higher (72%) in T2 than the T1 (29%). Also, the plants with T2 treatment showed a 60% increase over those with T1 treatment. At 7 DAT, the PRO content was found to be higher in the both treatments (43% and 36% increase over control in T2 and T1, respectively) but this increase was less than the PRO content at 5 DAT. Also, only 10% increase in T2 (compared to 60% at 5 DAT) as compared to T1 was recorded at the most severe stress level (Fig. 1C).

Hydrogen peroxide (HP) content

Initially at 3 DAT, the plants with T2 treatment showed a 19% decrease in H₂O₂ content, while those with T1 treatment increased by 4%, not significantly, compared to control. Increase in the duration of drought (5 DAT) caused a significant increase in H₂O₂ content which was

24% higher in T2 than in T1 (4%) compared to control. It was found that T2 increased H₂O₂ by 20% compared to T1. Further enhancement in stress duration (7 DAT) caused an additional increase in H₂O₂ content in T1 (16 % compared to 4% increase at 5DAT, compared to control), but it decreased in plants with T2 treatment (20% compared to 24% at 5 DAT, compared to control) (Fig. 1D).

Specific catalase activity (CAT)

During the initial stage of drought, *i.e.*, at 3 DAT, no significant difference was observed amongst T1, T2 and control treatments. However, advancement of stress to 5 DAT abruptly and significantly increased CAT activity in T2 (47%), while the plants with T1 treatment recorded only 6% increase compared to control which was non-significant. The ABA (T2) treatment caused a 44% increase over drought treatment alone (T1). At 7 DAT, there was a significant increase in CAT activity in T1 compared to that at 5 DAT, but in T2, though it increased compared to the control but remained the same as that at 5 DAT (47% and 44% at 5 and 7 DAT, respectively). The increase in T2 over T1 fell down from 44% (at 5 DAT) to 33% at 7 DAT (Fig 2A).

Guaiacol peroxidase activity (GPX)

In contrast to CAT activity which showed no significant increase initially, at onset of the drought stress, a significant increase in the activity of GPX was observed in T1 (9%) and T2 (21%) compared to control. ABA treatment (T2) recorded 14% higher GPX activity than drought (T1) treatment alone. A swift increase in GPX activity, irrespective of treatment was recorded at 5 DAT. Also the trends in GPX activity relatively varied with those exhibited by the CAT enzyme. Here also, the increase in activity was higher in T2 (45%) than T1 (30%) compared to control. However, ABA treatment (T2) recorded 21% increase in activity over drought (T1) treatment alone. At 7 DAT, a decrease in GPX activity was recorded in both treatments compared to that at 5 DAT. This decrease in activity was significant in both treatments, the T1 (from 45 to 38% increase over control) and T2 (from 30 to 23% increase over control). Vanishing effects of ABA treatment were also observed here (Fig. 2 B).

Glutathione reductase (GR) and ascorbate peroxidase (APX) activities

At 3 DAT, on one hand, the GR activity significantly rose in T1 and T2 (42 and 32%, respectively) compared to control, though there was no any significant difference between T1 and T2 (Fig. 2C). On the other hand, no significant differences between T1, T2 and control were recorded in case of APX (Fig. 2D). At 5 DAT, the activity of GR increased significantly in both treatments (45 and 73% in T1 and T2, respectively), the rise in activity in T2 was abrupt, and a 50% increase over T1 has been recorded in this treatment. While in APX, the plants with T2 treatment did not showed any differences from those with T1 treatment, but a significant increase (19%) was observed in T2 compared to control. Furthermore, this

treatment showed 16% increase over T1. Progression of drought resulted in the maintenance of GR activity as shown by T1 and T2 treatments, which represented a non-significant difference as compared to 5 DAT. However, the effect of ABA started to diminish at the end of the experiment (50% increase at 5 DAT followed by just 43% increase at 7 DAT in T2 compared to control). But, APX activity at 7 DAT continued its increasing expression with the increasing severity of stress. A 32 and 40% increase in T1 and T2 was observed compared to the control, respectively. The increase was found 12% higher in T2 than in T1. Here also, the diminishing effect of ABA application was observed (Fig 2 D).

IV. DISCUSSION

The data showed that foliar application of ABA increased leaf internal ABA content significantly. The accumulation of ABA in relation to various stresses is now a well-known phenomenon [5], and it has been known to occur in number of monocot plants including wheat, rice, barley, sorghum and maize [21]. Though, it was also increased in plants treated with drought only (T1), the plants treated with T2 responded with an abrupt increase in ABA concentration, especially at 5 DAT. These effects were continued, even at higher severity level (as shown by significant increase in ABA content in T2 over the T1), but as a matter of fact, ABA is metabolized very swiftly in plant system, the effect vanished slightly at the end of experiment. In earlier study, Zhang *et al.* [22] showed that the rate of ABA catabolism is proportional to the ABA levels in plant tissues. As more ABA is produced during stress conditions, more ABA is degraded into metabolites such as phaseic acid. It might be possible that one additional spell of ABA application at this stage could have been maintained the leaf internal ABA concentration to a constant level (at least up to some extent) to provide defense against the drought stress.

One of the major effect of ABA application in plants is the over expression of antioxidative enzymes [23]. This over expression of enzymes is also caused by the environmental stresses like drought and other stresses [2]. But if the stress conditions prevail for longer duration, the expression of these enzymes cannot keep pace with the production of ROS and may result in plant's death. The data obtained in this study revealed that drought conditions enhanced MDA content which progressively increased with increasing days after treatment, but the plants with T2 treatment (drought + ABA application) showed a constant concentration of MDA throughout the experiment duration (differed insignificantly with the control). This indicated that the overproduction of ROS was subsided by the enhanced expression of antioxidative enzymes. The data further reveals that ABA is also responsible for the overproduction of antioxidative enzymes and it is not the solo effect of water stress (as revealed by higher MDA content in T1).

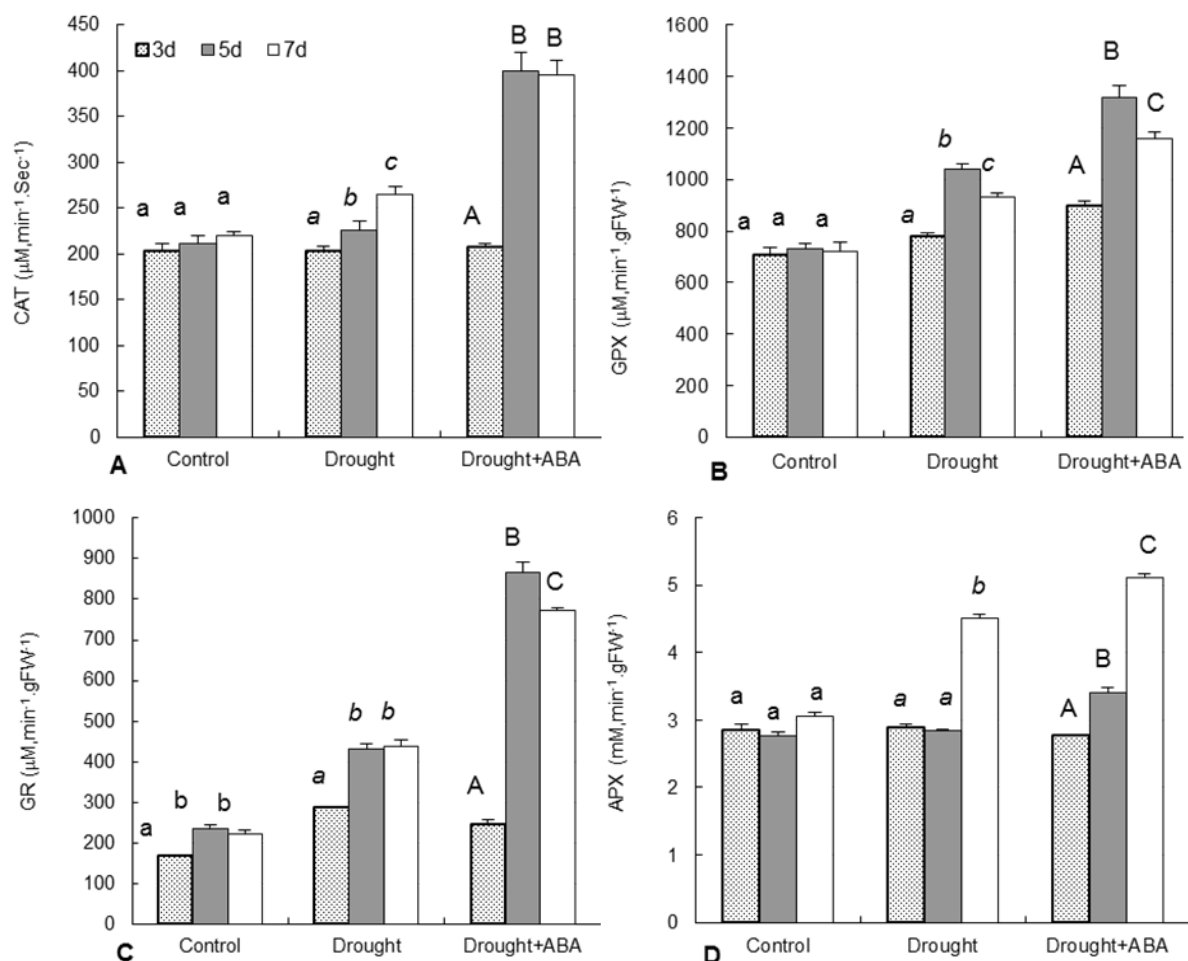


Fig. 2 Effect of drought and drought+abscisic acid treatments on catalase (A), guaiacol peroxidase (B), glutathione reductase (C) and ascorbate peroxidase (D) enzyme activities in sugarcane variety ROC 22 at 3, 5 and 7 days after treatment. Mean values \pm SD (n=3). Different small, capital and italicized alphabets are showing significant difference ($P \leq 0.05$) at 3rd, 5th and 7th day within the control, drought and drought+ABA treatments, respectively.

ABA application has been found to enhance the biosynthesis of free PRO contents in plants. In the present study, initially at 3 DAT, neither drought stress (T1) nor the application of ABA (combination of drought + ABA application, T2) enhanced the PRO content, rather kept it constant and *at par* with the PRO concentration in control plants. This might be due to the tolerant nature of the sugarcane variety ROC 22 which was not affected by the drought conditions up to this stage (supported by morphological observations, data not shown). The plant showed an abrupt increase in PRO content at 5 DAT, due to over production of ROS with increasing severity of drought. The higher increase in T2 (60% over T1) showed the infallible role of ABA in enhancing the antioxidative defense system and strengthened its function under stress conditions. Furthermore, a slight but not significant decrease in PRO content at 7 DAT, compared to 5 DAT in plants with T2 treatment showed that effects of ABA application persists only up to certain duration and severity of the stress and starts diminishing later on due to rapid metabolism of ABA in plants.

In the present study, the ABA application (T2) was found to induce the production of H_2O_2 up to 5 DAT,

while at 7 DAT, a decrease in H_2O_2 content was observed in this treatment. The increased production of ROS might be the result of water stress at least in part, and this played a vital role in ABA accumulation [24] and its signal transduction pathway finally leads to the activation of catalase enzyme [9]. Jiang and Zhang [7] also reported the increased levels O_2^- and H_2O_2 on ABA treatment of leaves of maize seedlings which lead to the activation of antioxidant enzymes such as SOD, CAT, APX and GR. Combined activities of GPX (H_2O_2 metabolizing) and SOD (H_2O_2 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 [25]. The data obtained in the present study revealed that accumulation of ABA as an effect of drought as well as the foliar application of ABA, together triggered the increase in GPX activity that caused an increase in H_2O_2 concentration which was quenched by the highly expressed CAT and glutathione ascorbate system in T2 (lowering of H_2O_2 in T2 at 7 DAT), while in T1, the expression and activities of antioxidative enzymes (alone caused by drought stress) could not keep pace with the production of H_2O_2 and thus resulted in its accumulation.

Catalase is generally used to reduce H₂O₂ levels in peroxisomes but it is absent in chloroplasts. The role of catalase is filled by ascorbate specific APX which break down H₂O₂ using ascorbic acid as hydrogen donor [26]. The continuous use of ascorbic acid in scavenging H₂O₂ by APX sustains the ABA biosynthesis under stress conditions. While as our studies revealed, under severe drought conditions when oxidative stress precedes over the tolerance mechanism (lower activities of CAT and APX in T1 compared to T2), accumulation of ascorbic acid impedes the biosynthesis of ABA, which otherwise might be facilitated by high production of APX due to ABA application.

Furthermore, this APX operates in cycle with GR which uses NADPH to generate reduced form of GSH from the oxidized disulphide form (GSSG) by the action of APX, thus the complete mechanism operates in a well maintained cycle [27]. The higher GSH (increased GR activity in T2) produced by high GR activity is used to operate other cellular redox reactions and thus does not interfere with the ABA biosynthesis. However, there is still insufficient literature showing up the regulation of GR under abiotic stress, suggesting an uncertain influence of abiotic stress on its expression level [26].

V. CONCLUSION

We have made an effort for understanding of a basic question that whether, the stress induced biosynthesis of ABA is able to initiate and continue the cellular signaling cascades, and if so up to what extent and secondly, how the exogenously applied ABA help the plant to maintain the defensive internal environment under sustained stress conditions. The two most important strategies required to resist the drought conditions are reducing the oxidative stress and facilitating water availability to the plants. Now it is well known that ABA not only hastens the antioxidative defense but also promotes the formation of young roots, increases the hydraulic conductivity. Compared to the other phytohormones and economically available compounds, ABA or recently manufactured ABA analogues which are commercially cost effective compared to ABA, seems to be the best alternative for their use in stress conditions. Our studies showed that most of the enzymes and metabolites which provide the tolerance to plant are not as a whole, but in part the response of biosynthesized and foliar applied ABA. The study clearly suggests that leaves play a major role in the production of ABA in response to acute water shortage. Our studies in the light of above findings reinforce the inevitability of foliar applied ABA. Though, the first alternative to deal with this problem is the use of tolerant sugarcane varieties, but the application of ABA or its analogues (which are more physiologically active and longer-lasting than standard ABA) will further support the plant to stand under prolonged drought conditions for sustaining productivity.

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REFERENCES

- [1] Schlögl, P.S., Nogueira, F.T., Drummond, R., Felix, J.M., De Rosa, V.E., Vicentini, R., Leite, A., Ulian, E.C. and Menossi, M. (2008). Identification of new ABA- and MEJA-activated sugarcane bZIP genes by data mining in the SUCEST database. *Plant Cell Rep*, 27: 335-345.
- [2] Qiu, Y.P. and Yu, D.Q. (2009). Over-expression of the stress-induced *OsWRKY45* enhances disease resistance and drought tolerance in *Arabidopsis*. *Environ Exp Bot*, 65: 35-47.
- [3] Reyes, D., Rodriguez, D., Nicolas, G. and Nicolas, C. (2006). Evidence of a role for tyrosine phosphorylation in the control of postgermination arrest of development by abscisic acid in *Arabidopsis thaliana* L. *Planta*, 223: 381-385.
- [4] Kang, J.Y., Choi, H.I., Im, M.Y. and Kim, S.Y. (2002). *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell*, 14(2): 343-357.
- [5] Zhang, J.H., Jia, W.S., Yang, J.C. and Ismail, A.M. (2006). Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Research*, 97: 111-119.
- [6] Suhita, D., Raghavendra, A.S., Kwak, J.M. and Vavasseur, A. (2004). Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. *Plant Physiol*, 134: 1536-1545.
- [7] Jiang, M. and Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *J Exp Bot*, 53: 2401-2410.
- [8] Kaminaka, H., Morita, S., Tokumoto, M., Yokoyama, H., Masumura, T. and Tanaka, K. (1999). Molecular cloning and characterization of a cDNA for an iron-superoxide dismutase in rice (*Oryza sativa* L.). *Biosci Biotechnol Biochem*, 63: 302-308.
- [9] Guan, L.M., Zhao, J. and Scandalios, J.G. (2000). *Cis*-elements and *trans*-factors that regulate expression of maize Cat1 antioxidant gene in response to ABA and osmotic stress: H₂O₂ is the likely intermediary signaling molecule for the response. *Plant J*, 22: 87-95.
- [10] Trujillo, L.E., Sotolongo, M., Menéndez, C., Ochogavía, M.E., Coll, Y., Hernández, I., Borrás-Hidalgo, O., Thomma, B.P.H.J., Vera, P. and Hernández, L. (2008). SodERF3, a novel sugarcane ethylene responsive factor (ERF), enhances salt and drought tolerance when over expressed in tobacco plants. *Plant Cell Physiol*, 49: 512-512.
- [11] Ikegami, K., Okamoto, M., Seo, M. and Koshiba, T. (2009). Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. *J Plant Res*, 122: 235-243.
- [12] Endo, A., Sawada, Y., Takahashi, H., Okamoto, M., Ikegami, K., Koiwai, H., Seo, M., Toyomasu, T., Mitsunashi, W., Shinozaki, K., Nakazono, M., Kamiya, Y., Koshiba, T. and Nambara, E. (2008). Drought induction of *Arabidopsis* 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. *Plant Physiol*, 147: 1984-1993.
- [13] Kaldenhoff, R., Ribas-Carbo, M., Sans, J.F., Lovisolo, C., Heckwolf, M. and Uehlein, U. (2008). Aquaporins and plant water balance. *Plant Cell Environ*, 31: 658-666.



- [14] Brennan, T. and Frenkel, C. (1977). Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol*, 59(3): 411-416.
- [15] Ohkawa, H., Ohishi, N. and Yagi, Y. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem*, 95: 351-358.
- [16] Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.
- [17] Aebi, H.E. (1983). Catalase. In Bergmeyer HU, Editor, *Methods of Enzymatic Analysis*. Vol.3, Enzymes: Oxidoreductases, Transferases. Verlag Chemie, Weinheim, West Germany, Pp. 273-286.
- [18] Zheng, X. and Van Huystee, R.B. (1992). Peroxidase-regulated elongation of segments from peanuts hypocotyls. *Plant Sci*, 81: 47-56.
- [19] Foyer, C.H. and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.
- [20] Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiol*, 22: 860-867.
- [21] Liu, J., Jiang, M.Y., Zhou, Y.F. and Liu, Y.L. (2005). Production of polyamines is enhanced by endogenous abscisic acid in maize seedlings subjected to salt stress. *Journal of Integrative Plant Biology*, 47: 1326-1334.
- [22] Zhang, J.H., Jia, W.S. and Zhang, D.P. (1997). Re-export and metabolism of xylem-delivered ABA in attached maize leaves under different transpirational fluxes and xylem ABA concentrations. *J Exp Bot*, 48: 1557-1564.
- [23] Khadri, M., Tejera, N.A. and Lluch, C. (2007). Sodium chloride-ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. *Environ Exp Bot*, 60: 211-218.
- [24] Zhao, F.Y. and Xu, Z.J. (2009). Response of antioxidant system to drought and heat stress in transgenic rice roots carrying GST and CAT1. *Acta Botanica Boreali-Occidentalia Sinica*, 29: 1980-1987.
- [25] Veljovic-Jovanovic, S., Kukavica, B., Stevanovic, B. and Navari-Izzo, F. (2006). Senescence- and drought-related changes in peroxidase and superoxide dismutase isoforms in leaves of *Ramonda serbica*. *J Exp Bot*, 57:1759-1768.
- [26] Qureshi, M., Qadir, S. and Zolla, L. (2007). Proteomics-based dissection of stress-responsive pathways in plants. *J Plant Physiol*, 164: 1239-1260.
- [27] Meyer, A. J. (2008). The investigation of glutathione homeostasis and redox signaling. *J. Plant Physiol*, 165: 1390-1403.