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Research Article

Analysis of ABA Regulation of Antioxidant Metabolism Under ABA and Water Stress in Wheat Cultivars

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Abstract

ABA-regulation under ABA and WS was studied in two wheat cultivars PBW343 (drought susceptible) and C306 (drought tolerant) by using tiron (T), specific scavenger of superoxides, under ABA and using sodium tungstate (ST), ABA biosynthetic inhibitor, under WS where ABA+T treatment was compared to ABA and WS+ST treatment was compared to WS. ABA+T treatment decreased biomass, ascorbate to dehydroascorbate ratio, proline and increased malondialdehyde in both cultivars; decreased antioxidant enzymes in PBW343 only while in C306, it increased antioxidant enzymes accompanied with increased H2O2. WS+ST treatment decreased biomass, ascorbate to dehydroascorbate ratio, antioxidant enzymes and increased H2O2, malondialdehyde in both cultivars but more pronounced in C306. It did not decrease proline in both cultivars. These results showed that ABA-pathway under WS was higher in C306 but lesser in PBW343, hence may be the reason of drought susceptibility of PBW343. However, under ABA, this pathway showed some inhibitions in C306 which may be due to ABA-higher sensitive nature of cultivar.

Keywords: Abscisic Acid; Antioxidant; Reactive Oxygen Species; Triticum aestivum; Water Stress

Abbreviations

ABA: Abscisic Acid; APX: Ascorbate Peroxidase; AA: Ascorbate; CAT: Catalase; DHA: Dehydroascorbate; GPOX: Guaiacol Peroxidase; GR: Glutathione Reductase; MDA: Malondialdehyde; ST: Sodium Tungstate; T: Tiron; WS: Water Stress

Introduction

Abscisic acid (ABA) regulates many plant physiological processes like dormancy, germination, vegetative growth and environmental stresses. Antioxidant mechanism of the plant plays an important role in providing stress tolerance as otherwise overproduced reactive oxygen species (ROS) lead to cellular toxicity or cell death [1]. ABA is found to activate antioxidant potential under exogenous ABA in maize [2-5], *Stylosanthes guianensis* [6], triploid bermudagrass [7], rice [8], wheat [9], *Atractylodes macrocephala* [10], transgenic tobacco overexpressing ABA-biosynthetic gene [11]; under water stress in maize [12,13], triploid bermudagrass [7]; under lead stress in *Atractylodes macrocephala* [10]. In almost all of these studies, by using chemicals (inhibitors of ABA biosynthesis, inhibitors of NADPH oxidases, ROS scavengers, ROS producers), it has been found that ABA activates NADPH oxidase to produce superoxide anion radicals which are converted into H_2O_2 and H_2O_2 activates antioxidant potential.

Previously, wheat cultivars PBW343 and C306 contrasting in drought tolerance were compared for various stress parameters (biomass, antioxidant enzymes, ascorbate to dehydroascorbate ratio, MDA, H2O2 levels) under ABA, WS and ABA plus WS [14] where it was found that both cultivars showed increased antioxidant potential under ABA but under WS, C306 showed increased but PBW343 showed decreased antioxidant potential which was improved on supplying ABA. So it was concluded that PBW343 might be lacking or having lesser level of ABA-pathway for upregulation of antioxidant potential. In this study, it was aimed to analyse ABA-pathway under ABA as well as under WS. Under ABA, it was done by using tiron (specific scavenger of $\bullet -O_2$) as ABA act through superoxide production. Under WS, sodium tungstate (inhibitor of ABA biosynthetic enzyme aldehyde oxidase) was used as stresses increase ABA level mainly due to increased ABA biosynthesis [15]. Both chemicals (sodium tungstate and tiron) have been used in literature for these purposes [3,12,13]. Same stress parameters were planned to compare between ABA and ABA+T to see their ABA regulation if through superoxide production, between WS and WS+ST to see their regulation under WS if through ABA-pathway. Same experimental set up was used and analysis was performed in shoots and roots at 24 and 48h of treatment given to 4-day old seedlings. ABA sensitivity of the cultivars was also checked by testing their seed germination under 20 µM ABA [16].

Materials and Methods

Plant material

Seeds of wheat (*Triticum aestivum*) cultivars PBW343 and C306 were collected from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (India). These were sterilized and grown over moistened filter paper in sterilized petri dishes in dark at 25ºC for 4 days. Treatment was applied on 4th day as 20 µM ABA (ABA), 10 mM tiron in 20 µM ABA (ABA+T), 6% mannitol (WS), 5 mM sodium tungstate in 6% mannitol (WS+ST). Samples were collected from 24 and 48 h after treatment.

Seed germination

ABA sensitivity of the cultivars was checked by germinating seeds (100/petridish) in water (CT) and 20 µM ABA (ABA). Experiment was performed in triplicates. Data was collected as % germination on 7th day after imbibition.

Biomass measurement

Fresh biomass of shoots and roots were measured in triplicates of 25 seedlings each at 48h of treatment given to 4-day old seedlings.

Antioxidant enzymes

Antioxidant enzymes were extracted (in triplicates) and measured as in Kaur., *et al* (2014). In brief, common extraction was made in 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 2% PVP and 0.05% triton-X-100. Ascorbate peroxidase (APX) was assayed in 0.3 mM ascorbate, 0.1 mM EDTA, 1 mM H_2O_2, 50 mM potassium phosphate buffer pH 7 at 290 nm for disappearance of ascorbate. Catalase (CAT) was assayed at 240 nm for the disappearance of H_2O_2 in 50 mM potassium phosphate buffer pH 7, 25 mM H_2O_2. Guaiacol peroxidase (GPOX) was assayed in 50 mM guaiacol, 32 mM H2O2, 100 mM potassium phosphate buffer pH 6.5 at 470 nm for appearance of tetraguaiacol. Glutathione reductase (GR) was assayed in 0.7 mM oxidized glutathione (GSSG), 0.07 mM NADPH, 50 mM potassium phosphate buffer pH 7 at 320 nm for disappearance of NADPH. Enzyme activities were calculated using molar extinction coefficient of ascorbate as 2.8 mM^{-1} cm^{-1} (for APX), of H_2O_2 as 0.0394 mM^{-1} cm^{-1} (for CAT), of tetraguaiacol as 26.6 mM^{-1} cm^{-1} (for GPOX) and of NADPH as 6.22 mM^{-1} cm^{-1} (for GR).

Antioxidants and other related metabolites

These were extracted (in triplicates) and measured [14]. In brief, H_2O_2 was extracted in 0.1% TCA, incubated in 1.3 M potassium iodide, 33 mM potassium phosphate buffer pH 7 at room temperature for 1hr in the dark and read at 390 nm. Ascorbate was extracted in 5% TCA, incubated in 0.8% H_3PO_4, 0.04% $FeCl_3$, 0.2% bipyridyl, 80% ethanol at 37°C for 40 minutes and read at 525 nm. Dehydroascorbate was extracted in 5% metaphosphoric acid, 1% thiourea, incubated at 37°C in 1% dinitrophenyl hydrazine, 0.2% thiourea, 0.025% $CuSO_4 \bullet 5H_2O$, 4.5N H_2SO_4 for 3h, further incubated at room temperature in 61% H_2SO_4 (cold) and read at 530 nm. Proline was extracted in 3% sulphosalicylic acid, incubated in 48 mM ninhydrin, 9.3M acetic acid, 0.8 M phosphoric acid at 100°C for 1h, phase-separated at room temperature by adding equal volume of toluene, read upper layer of pink-red color at 520 nm. MDA was extracted in 0.1% TCA, incubated at 100°C in 0.4% thiobarbituric acid, 16% TCA for 30 minutes, read at 532 nm and 600 nm where absorbance at 600 nm was subtracted from absorbance at 532 nm and calculated using molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical analysis

Mean ± SD was calculated. Data was analyzed by Student's t-test at $P \leq 0.05$ to test for statistical differences between ABA and ABA+T; WS and WS+ST pairs at each stage.

Results and Discussion

Seed germination experiment showed that 20 µM ABA inhibited germination in both cultivars but inhibition level was almost five times higher in C306 than PBW343 (Table 1). This showed ABA higher sensitive nature of C306 and ABA-lesser sensitive nature of PBW343. C306 is a drought tolerant cultivar [14,17-20]. ABA higher sensitivity has been related to drought and cold stress tolerance in different plants including wheat [16,21]. Biomasses were found decreased under ABA+T as well as under WS+ST in both cultivars (Figure 1), indicating the involvement of ABA pathway in biomass maintenance under stress. Previously [14], C306 showed higher maintenance of biomass under WS as compared to PBW343. Biomass maintenance is a stress tolerant trait [22].

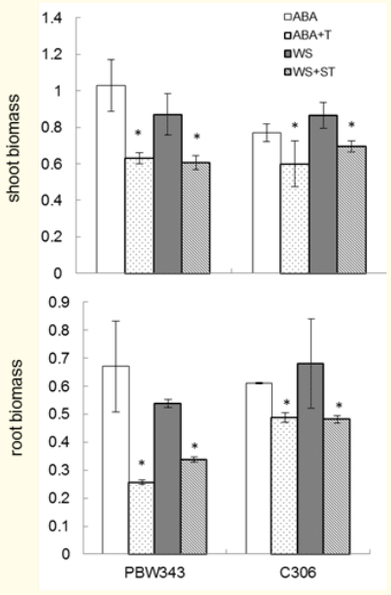

Figure 1: Effect of tiron (T) under ABA and effect of sodium tungstate (ST) under water stress (WS) on shoot and root biomass (g per 25 seedlings) of wheat cultivars PBW343 and C306 at 48h of treatment. ABA, ABA+T, WS, WS+ST treatments given to 4-day old seedlings. *: Represents significant difference between ABA and ABA+T, WS and WS+ST pairs as analysed by Student's t-test ($p < 0.05$).

% germination on 7th day after imbibition		
	PBW343	C306
CT	100 ± 0	82.7 ± 2.08
ABA	83.7 ± 1.52*	18.3 ± 4.16*

Table 1: Effect of ABA (20 µM) on seed germination of wheat cultivars PBW343 and C306. Data is analysed by Student's t-test ($p \leq 0.05$).

Antioxidant enzymes analysis under ABA+T showed that these enzymes decreased in PBW343 while increased in C306. For example, APX and CAT (Figure 2) decreased in shoots (APX at 48h, CAT at 24 and 48h) and roots (APX at 24 and 48h, CAT at 24h) of PBW343 while increased in shoots (APX at 24 and 48h, CAT at 48h) and roots (APX at 48h, CAT at 24 and 48h) of C306. Similarly, GPOX and GR (Figure 3) decreased in shoots (at 48h) of PBW343 while increased in shoots and roots (GPOX at both stages, GR at 48h) of C306. Ascorbate analysis under ABA+T showed that ascorbate (Figure 4) decreased in shoots of both cultivars (at 48h) and in roots of PBW343 (at 24 and 48h), similarly, ascorbate to dehydroascorbate ratio decreased in shoots of both cultivars (at 24h) and in roots of PBW343 (at 24 and 48h). H_2O_2 (Figure 5) under ABA+T increased in shoots (at both 24 and 48h) and in roots (at 48h) of C306 while in PBW343, it increased in shoots at 24h only. Proline (Figure 5) decreased under ABA+T in roots of both cultivars (24 and 48h in PBW343, 24h in C306) and in shoots of C306 (at 48h). MDA (Figure 5) under ABA+T increased in roots of both cultivars (24h in PBW343 and 48h in C306) and increase was higher in PBW343. Decrease of ascorbate, ascorbate to dehydroascorbate ratio, proline and increase of MDA and H_2O_2 in both cultivars showed that these parameters are regulated by ABA through superoxide production. Decrease of antioxidant enzymes and increase of H_2O_2 in PBW343 indicated the working of ABA pathway through superoxide production to upregulate antioxidant enzymes to decrease H_2O_2. However, increase of antioxidant enzymes accompanied by higher increases of H_2O_2 in C306 is hard to explain but this can be related to a report in literature for a wheat cultivar kitakei (ABA-hypersensitive and dormant) where some LEA genes were downregulated under ABA-supply as compared to other cultivar (ABA-sensitive and non-dormant wheat cultivar) where these genes were upregulated, however under low temperature, these genes were equally upregulated in both cultivars [16]. We also found in our previous study [14] that exogenous ABA increased LEA genes' expression in both of these cultivars but when ABA added under WS, it downregulated such expression in C306 only not in PBW343. There may be a chance that ABA when over-accumulates, it may suppress its own pathway [14,16] or opt for its another pathway which may downregulate some of antioxidant enzymes or LEA genes.

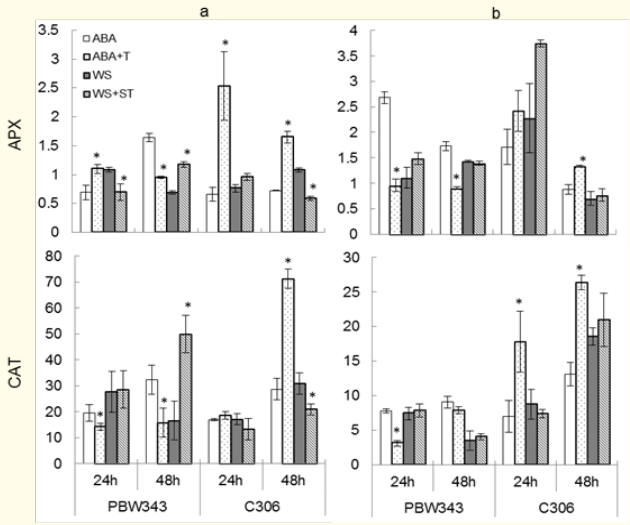

Figure 2: Effect of tiron (T) under ABA and effect of sodium tungstate (ST) under water stress (WS) on ascorbate peroxidase, APX (µmole of ascorbate changed min-1 mg-1 protein), catalase, CAT (µmole of H2O2 changed min-1 mg-1 protein) activities in shoots (a) and roots (b) of wheat cultivars PBW343 and C306 at 24 and 48h of same treatments (as in figure 1) given to 4-day old seedlings. *: Represents significant difference between ABA and ABA+T, WS and WS+ST pairs at each stage as analysed by Student's t-test ($p < 0.05$).

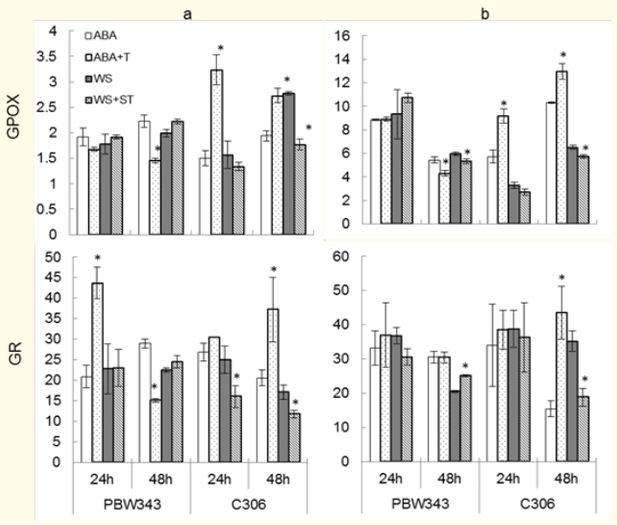

Figure 3: Effect of tiron (T) under ABA and effect of sodium tungstate (ST) under water stress (WS) on guaiacol peroxidase, GPOX (µmole of tetraguaiacol changed min-1 mg-1 protein) and glutathione reductase, GR (ηmole of NADPH changed min-1 mg-1 protein) activities in shoots (a) and roots (b) of wheat cultivars PBW343 and C306 at 24 and 48 h of treatment given to 4-day old seedlings where treatments and statistical analysis are same as in figure 2.

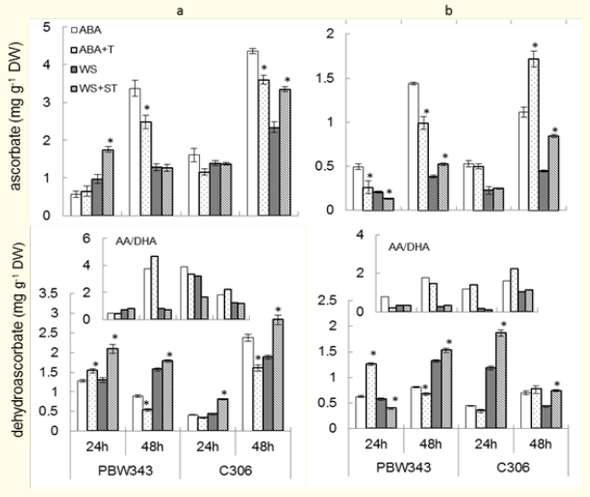

Figure 4: Effect of tiron (T) under ABA and effect of sodium tungstate (ST) under water stress (WS) on reduced ascorbate and oxidised ascorbate (dehydroascorbate) in shoots (a) and roots (b) of wheat cultivars PBW343 and C306 at 24 and 48h of treatment given to 4-day old seedlings where treatments and statistical analysis are same as in figure 2. Inset figures represent ascorbate to dehydroascorbate ratios (AA/DHA).

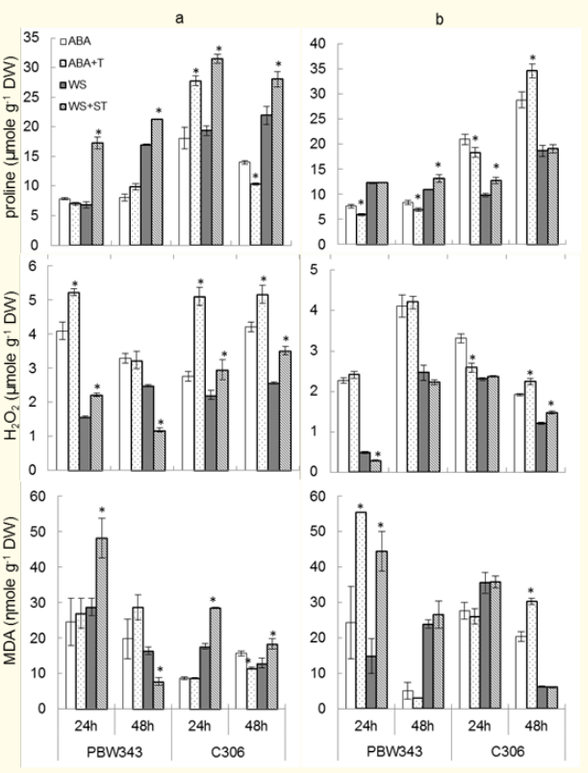

Figure 5: Effect of tiron (T) under ABA and effect of sodium tungstate (ST) under water stress (WS) on proline, H2O2, malondialdehyde (MDA) in shoots (a) and roots (b) of wheat cultivars PBW343 and C306 at 24 and 48h of treatment given to 4-day old seedlings where treatments and statistical analysis are same as in figure 2.

Analysis under WS+ST indicated that C306 showed higher decreases of antioxidant enzymes, ascorbate to dehydroascorbate ratios accompanied with higher increases of H_2O_2 under WS+ST as compared to PBW343. For example, only APX in shoots (at 24h) and GPOX in roots (at 48h) were found decreased in PBW343, while in C306, almost all antioxidant enzymes decreased under WS+ST (APX and CAT in shoots at 48h, GPOX in shoots and roots at 48h, GR in shoots at 24 and 48h, GR in roots at 48h) (Figure 2, 3). Though no decrease of ascorbate was observed under WS+ST in both cultivars but increases of dehydroascorbate and decreases of ascorbate to dehydroascorbate ratio were observed in both cultivars and again higher in C306 specially in shoots (Figure 4). Similarly, H_2O_2 (Figure 5) under WS+ST increased in shoots (at both 24 and 48h) and roots (at 48h) of C306 while in PBW343, it increased in shoots at 24h only. This indicated the presence of higher level of ABA-pathway of upregulation of antioxidant potential to decrease ROS (H_2O_2) level in C306. C306 in many studies including field as well as laboratories studies [14,17-20], showed higher antioxidant enzymes and lesser oxidative stress (H_2O_2 and

MDA) under heat stress and drought when compared to PBW343 or other susceptible wheat cultivars. ABA is reported to activate antioxidant enzymes and decrease oxidative toxicity under water stress in maize [12,13] and triploid bermudagrass [7]. Ascorbate being an important non-enzymatic antioxidant in plants, detoxifies ROS directly as well as through glutathione-ascorbate cycle. Ratio of reduced ascorbate to oxidized ascorbate is essential for the ability of plant to fight oxidative stress [23]. Previously [14], C306 showed improved such ratio under WS than PBW343. MDA (Figure 5) under WS+ST increased in shoots and roots (24h) of PBW343 and in shoots (24 and 48h) of C306 where comparative increases of MDA appeared to be higher in PBW343. Compared to H_2O_2, MDA is marker of cellular toxicity while H_2O_2 is the marker of ROS level. So, higher increase of MDA in PBW343 on inhibiting ABA pathway under WS may indicate the operation of ABA-pathway to remove cellular toxicity and C306 may not need it due to less level of cellular toxicity due to its higher antioxidant potential.

Proline playing role of osmolyte as well as ROS scavenger, its accumulation is contributed by both ABA-dependent and –independent pathways [24]. Previously, both cultivars showed increases in proline under WS. In this study, proline was not decreased under WS+ST in both cultivars (Figure 5). Proline accumulation may be contributed more by ABA-independent pathways under WS in these cultivars.

Conclusion

This study showed that ABA-pathway for upregulation of biomass, antioxidant enzymes, redox state of ascorbate and reduction of oxidative toxicity, is mediated through superoxide (ROS) production. Such pathway occurs at higher level in C306 than PBW343 under water stress. PBW343 does not lack it but has its lesser level under water stress. This may be the reason of its drought susceptibility. This can also have relation with ABA-sensitivity. Drought tolerance of ABA-higher sensitive cultivar may be due to higher induction of ABA-pathway of upregulation of antioxidant potential.

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