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ORIGINAL PAPER

Impact of exogenously applied ABA on proline metabolism conferring drought and salinity stress tolerance in wheat genotypes

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Abstract
Drought and salinity are the most catastrophic abiotic stresses that affect plant growth and development. Plant initiates alterations at physiological, biochemical and molecular levels to combat adverse effects of drought in which abscisic acid (ABA) plays a pivotal role. The present investigation aims to study the modulation of enzymatic changes conferring stress tolerance in the 7-day-old shoots of five wheat genotypes influenced by exogenously applied ABA during stress. The results revealed that application of ABA (10 µM) positively influenced the shoot length and biomass of Gladius and Drysdale under water deficit and water withholding condition, while Kharchia was the only genotype showing positive response under salt stress condition and nearly negligible effect being observed in PBW 175 and PBW 660. The content of thiobarbituric acid reactive substances was lowered while proline content along with 2,2 diphenyl-picrylhydrazyl activity increased drastically with exogenous ABA especially in Gladius, Drysdale and Kharchia. The genotypes, i.e., Gladius, Drysdale and Kharchia, also possessed increased activity of proline metabolizing enzymes, i.e., NADH-dependent glutamate dehydrogenase, pyrroline-5-carboxylate synthetase (1.5–2-fold) in response to ABA while proline dehydrogenase remained unresponsiveness to ABA indicating the ABA-dependent and ABA-independent regulation of enzymes, respectively, and depicting the role of ABA and proline in conferring drought and salinity tolerance.

Keywords Abscisic acid · Proline · Wheat · Abiotic stress · Proline metabolism · Salt stress · Water withholding · Water deficit

Introduction

Wheat (*Triticum aestivum*) is the world's most important cereal crop with exceptional agricultural and economic importance, accounting for about 21% of food and 200 million hectares of farmland worldwide (FAO 2011). Drought and salinity are the most important environmental factors that cause osmotic stress and reduction in plant growth and crop productivity (Lobell et al. 2011). Stress responses in plants have revealed significant increases in reactive oxygen species (ROS), including singlet oxygen, superoxide, hydroxyl radical and hydrogen peroxide (H_2O_2) (You and Chan 2015). It has been recognized that products of lipid peroxidation are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, malondialdehyde (MDA) and compounds related to them (Ayala et al. 2014). MDA is a highly reactive three-carbon dialdehydes produced as a by-product of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. Some of these compounds react with thiobarbituric acid to form coloured products called thiobarbituric acid reactive substances (TBARS) (Zeb and Ullah 2016). Acclimation to stress conditions is generally achieved by maintaining lower level of H_2O_2 content and reduced lipid peroxidation. Stress-tolerant plants show reduced H_2O_2 content and reduced lipid peroxidation in contrast to sensitive ones (Hossain et al. 2015).

Plant responses to water and osmotic stress conditions include ABA and proline accumulation. The concurrent accumulation of proline and ABA in response to various stress conditions has resulted in speculation that ABA may trigger proline accumulation (Pál et al. 2018). For proline to accumulate, either synthesis from glutamic acid must be enhanced or the rate of oxidation must decrease, or both. Pyrroline-5-carboxylate synthetase (P5CS) is a

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bifunctional enzyme that catalyzes the first two steps of the glutamate pathway in proline biosynthesis in plants. Activity of P5CS in the chloroplasts can recycle $NADP^+$, the last acceptor of the photosynthetic electron transfer chain, which may reduce ROS production at the photosystem I. NADH-dependent glutamate dehydrogenase (NADH-GDH) though operates in deamination reaction, but it also plays a complementary role in supplying glutamate during proline synthesis under stress conditions (Liang et al. 2013). Oxidative degradation of proline to glutamate is carried out in the mitochondria by sequential action of proline dehydrogenase (PDH).

The objective of the current investigation was to investigate the regulatory role of ABA on the proline metabolism in shoots of five wheat genotypes raised under water deficit, water withholding and salt stress conditions.

Materials and method

Plant material and stress treatments

Seeds of five wheat genotypes comprising Gladius, Drysdale, Kharchia, PBW 175 and PBW 660 were surface sterilized with 0.1% (w/v) mercuric chloride for 5 min, thoroughly rinsed with distilled water and then germinated in Petri dishes on germination paper for seven consecutive days under control, control + ABA, water deficit, water deficit + ABA, water withholding, water withholding + ABA, salt stress and salt stress + ABA conditions. Water deficit conditions were induced by 8% polyethylene glycol (PEG-6000) solution, and water was withheld for 2 days to generate water withholding conditions. Salt stress conditions were maintained using 300 mM NaCl solution. The seedlings were watered with distilled water regularly for 3 days and at the fourth day with PEG solution in water deficit and NaCl solution in salt stress condition, whereas 50% of water was supplied under water withholding condition as compared to control. ABA (10 mM) was supplied at the fourth day under control, water deficit, water withholding and salt stress conditions. The growth measurements, biochemical analysis and proline metabolism were carried out on shoot tissue at the seventh day of post-germination.

Growth parameters

The length, fresh and dry biomass of shoots were measured at seventh day of post-germination (DPG). For the dry weight analysis, shoots were dried at 60 °C in oven till constant weight.

Extraction and estimation of H_2O_2

H_2O_2 was extracted from shoot tissue (0.5 g) with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) in a prechilled pestle and mortar. Homogenate was centrifuged at 12,000 g for 20 min, and H_2O_2 content was estimated in supernatant (Ashraf 2009). H_2O_2 was estimated by adding 0.5–1.0 ml of supernatant to 2 ml of reaction mixture containing 4 mol of potassium iodide and 0.1 mM potassium phosphate buffer (pH 7.0). Test tubes were incubated at room temperature in dark for 1 h. Absorbance was read at 390 nm against reagent blank. The amount of H_2O_2 was calculated by preparing standard curve of 50–200 µmol of H_2O_2. H_2O_2 was expressed as µmole g^{-1} FW.

Extraction and estimation of lipid peroxidation

The concentration of lipid peroxide products was determined in the shoot tissues in terms of thiobarbituric acid reactive substances (TBARS) content according to the method of Heath and Packer (1968). 0.5 g of fresh tissues was homogenized in 0.1% TCA and mixed with 5 ml of TBA solution containing 0.5% (w/v) TBA in 20% TCA. The mixture was heated at 90 °C for 30 min, cooled on ice and centrifuged at 10,000 g for 15 min. The color was measured at A_{532} nm and A_{600} nm. An extinction coefficient 155 mM^{-1} cm^{-1} was used to quantify lipid peroxide content and expressed as µmole g^{-1}FW.

Extraction and estimation of proline

Proline was estimated according to the method of Bates et al. (1973). Five hundred milligrams of shoot tissue was homogenized with 3% sulfosalicylic acid, and the contents were centrifuged at 10,000*g*. A volume of 2 ml of glacial acetic acid and 2 ml of acid ninhydrin was added to 2 ml of tissue homogenate and incubated for 1 h in boiling water bath followed by cooling in ice bath. About 4 ml of toluene was then added and mixed vigorously. The chromophore containing toluene was aspirated from aqueous phase, and the absorbance was measured at 575 nm.

Extraction and estimation of DPPH radical scavenging activity

2,2 Diphenyl-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois (1958). The

mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance for the sample (A sample) was measured using the UV spectrophotometer at 517 nm against ethanol blank. A negative control (A control) was taken after adding DPPH solution to 0.2 ml of the respective extraction solvent. The percent of DPPH discolouration of the sample was calculated according to the equation:

$$\%\ \text{discolouration} = \left[1-(\text{A sample}/\text{A control})\right] \times 100$$

Enzyme assay for proline metabolism

Shoot tissue (100 mg) was homogenized in prechilled pestle and mortar in the extraction medium containing 100 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 10 mM 2-mercaptoethanol, 1% (w/v) polyvinylpolypyrrolidone, 5 mM $MgCl_2$ and 0.6 M KCl. The homogenate was centrifuged at 12,000 x g for 20 min at 4 °C; the resulting supernatant was kept at 20 °C and used for enzymatic assays. NADH-dependent glutamate dehydrogenase (NADH-GDH) activity was assayed according to the method of Akihiro et al. (2008). The assay mixture comprised of 50 mM $(NH_4)_2SO_4$, 13 mM α-ketoglutarate, 0.25 mM NADPH and 1 mM $CaCl_2$ in 100 mM Tris–HCl buffer (pH 8). Absorbance was read at 340 nm, and GDH activity was expressed as µmole $min^{-1}mg^{-1}FW$-. The pyrroline-5-carboxylate synthetase (P5CS) activity was estimated as described by Silva-Ortega et al. (2008). The reaction mixture (3 ml) contained 100 mM Tris–HCl buffer (pH 7.2), 25 mM $MgCl_2$, 75 mM sodium glutamate, 5 mM ATP and 0.2 ml of enzyme extract. The reaction was initiated by the addition of 0.4 mM NADPH. The activity was measured as the rate of consumption of NADPH monitored by the decrease in absorbance at 340 nm. Proline dehydrogenase (PDH) activity was examined by monitoring the $NADP^+$ reduction at 340 nm in 0.15 M Na_2CO_3 buffer (pH 10.3) containing 15 mM L-proline and 1.5 mM $NADP^+$ (Lutts et al. 1999).

Statistical analysis

Data were statistically analyzed by multifactor ANOVA (CPCS1). Values are presented as a mean ± SD ($n = 3$). Significant differences were observed at C.D (5%).

Results

Effect of exogenous ABA on length and biomass

Exposure of wheat seedlings to water and salt stress significantly reduced shoot lengths (30–41%) and dry weights of all the five cultivars (Gladius, Drysdale, Kharchia, PBW 175 and PBW 660) analyzed (Table 1). However, ABA treatment significantly improved shoot length and dry weight under control and stress conditions. Maximum increase in shoot length and dry weight was observed in Gladius followed by Drysdale, PBW 175 and PBW 660 under ABA in water stress while Kharchia exhibited maximum increase under ABA in salt stress.

Effect of exogenous ABA on TBARS, H_2O_2 and DPPH

A significant increase in the membrane stability parameter in terms of TBARS, and H_2O_2 contents was observed in five wheat genotypes under control conditions. However, exogenous ABA supply led to significant decline in TBARS and H_2O_2 content under water and salt stress conditions (Table 2). Major decrease in TBARS and H_2O_2 was observed in PBW 175 under ABA in water stress while in Kharchia under ABA in salt stress condition. DPPH radical scavenging activity was found to increase by 7–9% in Drysdale under ABA in water stress conditions while Kharchia exhibited 4% increase under ABA in salt stress condition (Table 2).

Effect of ABA supply on Proline Content and its metabolizing enzymes

Water and salt stress conditions along with ABA treatment induced proline accumulation and increase in activities of synthesizing enzymes, i.e., GDH and P5CS as compared to control seedlings. Maximum increase in proline, GDH and P5CS was observed in Gladius and Drysdale under water stress conditions, while in Kharchia under ABA in salt stress (Table 3). In contrast, PDH activity was found to decrease about 9–39% in shoots under water stress while 6–31% under salt stress conditions in all five genotypes (Table 3). The maximum decrease was reported in Gladius and Drysdale in water stress while in Kharchia under salt stress condition. ABA treatment had negligible effect on PDH activity under all stress conditions.

Discussion

It is well documented from the growth and biomass observations that the application of ABA caused elongation and increased biomass production in shoots of Gladius and Drysdale (drought tolerant) under water stress and in Kharchia (salt tolerant) under salt stress conditions, indicating the role of ABA in stress tolerance. These results are in agreement with previous studies where ABA alleviated osmotic stress in wheat seedlings (Sakhabutdinova et al. 2003; Marcińska et al. 2013).

Table 1 Effect of exogenous ABA application on growth and biomass

Parameters	Treatment	Genotypes					
		PBW 660	PBW 175	Kharchia	Gladius	Drysdale	C.D value (5%)
Shoot length (cm)	C	8.60 ± 0.91	6.70 ± 1.06	8.20 ± 0.77	9.60 ± 0.49	6.40 ± 0.77	A = 0.44
	C + ABA	4.70 ± 0.84	3.90 ± 0.77	5.70 ± 0.42	6.65 ± 0.63	3.90 ± 0.56	B = 0.56
	W.D	5.10 ± 1.34	4.60 ± 0.42	6.35 ± 0.77	11.10 ± 0.28	8.55 ± 0.77	AB = 1.2
	W.D + ABA	5.50 ± 0.28	5.90 ± 0.42	6.30 ± 0.42	13.25 ± 0.35	10.65 ± 0.49	
	W.W	5.00 ± 0.49	4.55 ± 0.77	5.60 ± 0.56	10.75 ± 0.35	9.05 ± 0.35	
	W.W + ABA	4.10 ± 1.20	6.90 ± 0.70	5.85 ± 0.21	12.90 ± 0.07	12.10 ± 0.14	
	S	2.50 ± 0.77	4.45 ± 0.49	11.65 ± 0.49	7.55 ± 0.77	4.65 ± 0.49	
	S + ABA	1.60 ± 0.35	3.65 ± 0.49	13.75 ± 0.21	7.00 ± 0.28	4.15 ± 0.21	
Shoot fresh weight (mg)	C	56.50 ± 0.006	46.50 ± 0.007	52.00 ± 0.004	80.50 ± 0.009	57.50 ± 0.006	A = NS
	C + ABA	36.50 ± 0.003	25.50 ± 0.004	38.00 ± 0.004	44.00 ± 0.005	39.50 ± 0.004	B = 0.005
	W.D	43.50 ± 0.007	27.00 ± 0.005	44.50 ± 0.006	84.00 ± 0.007	64.00 ± 0.005	AB = 0.013
	W.D + ABA	40.00 ± 0.009	31.50 ± 0.003	40.00 ± 0.004	89.50 ± 0.007	67.00 ± 0.007	
	W.W	46.00 ± 0.004	25.50 ± 0.006	43.00 ± 0.009	81.00 ± 0.004	66.50 ± 0.004	
	W.W + ABA	38.00 ± 0.008	29.00 ± 0.002	39.00 ± 0.007	84.70 ± 0.008	73.50 ± 0.013	
	S	32.50 ± 0.003	25.00 ± 0.005	66.50 ± 0.007	58.50 ± 0.009	48.00 ± 0.004	
	S + ABA	25.00 ± 0.005	26.00 ± 0.007	79.00 ± 0.002	57.00 ± 0.002	48.50 ± 0.007	
Shoot dry weight (mg)	C	7.20 ± 0.70	5.00 ± 0.14	8.80 ± 0.98	10.70 ± 0.42	10.50 ± 2.12	A = NS
	C + ABA	4.55 ± 0.63	3.30 ± 0.56	7.65 ± 0.77	8.30 ± 0.56	6.55 ± 0.77	B = NS
	W.D	4.85 ± 0.35	4.10 ± 0.28	7.10 ± 0.28	11.00 ± 1.41	12.05 ± 1.48	AB = 2.4
	W.D + ABA	3.90 ± 0.28	5.40 ± 1.10	6.85 ± 1.48	12.85 ± 2.47	14.65 ± 0.49	
	W.W	4.79 ± 0.57	3.69 ± 0.69	6.05 ± 0.21	11.10 ± 2.68	11.80 ± 1.55	
	W.W + ABA	4.33 ± 1.58	5.80 ± 0.56	5.82 ± 0.82	13.83 ± 1.64	13.00 ± 2.54	
	S	3.88 ± 0.30	3.39 ± 0.71	9.25 ± 1.06	9.05 ± 1.34	9.15 ± 0.91	
	S + ABA	3.79 ± 1.13	3.25 ± 0.49	11.55 ± 2.19	8.90 ± 0.28	8.95 ± 0.07	

Lipid peroxidation is considered as an indicator of stress which is measured as TBA in the form of thiobarbituric acid reactive substances (TBARS) (Jain et al. 2001). However, in order to authenticate this test, another test, namely DPPH, was included that measures antioxidant potential of seedlings. The result of two parameters antagonistically affected the seedling growth of all genotypes by increasing membrane damage in form of TBARS and scavenging ROS, i.e., H_2O_2 via DPPH activity under stress conditions. Similar reports of stress-induced elevated levels of TBARS and DPPH have been observed in wheat seedlings under water and salt stress conditions (Kaur and Asthir 2019). In contrast to this, exogenous application of ABA resulted in concomitant increase in DPPH radical scavenging activity in Gladius, Drysdale and Kharchia under stress conditions, along with reduced lipid peroxidation and H_2O_2 content, depicting potential of these genotypes to overcome adverse conditions. Quan et al. (2008) reported the role of H_2O_2 in direct relationship with membrane damage as it has long life and more permeable to membrane and Kaur et al. (2014) correlated increasing DPPH scavenging activity under water deficiency has been positively with stress tolerance.

Proline emerged as powerful osmolyte as it supplies energy for growth and reduces the lipid peroxidation linked membrane damage (Chun et al. 2018). Our results indicated that exogenous supply of ABA upregulated proline accumulation to a larger extent as compared to control in Gladius, Drysdale and Kharchia by generating precursor, i.e., glutamate via increased activity of glutamate dehydrogenase and P5C via pyrroline-5-carboxylate synthetase under stress conditions as follows: α-ketoglutarate

Table 2 Effect of exogenous ABA application on DPPH, membrane stability and H_2O_2

Parameters	Treatment	Genotypes					
		PBW 660	PBW 175	Kharchia	Gladius	Drysdale	C.D value (5%)
DPPH scavenging activity (%)	C	33.25 ± 1.20	29.9 ± 1.13	56.25 ± 1.20	55.15 ± 0.77	50.9 ± 0.84	A = 1.15
	C + ABA	33.61 ± 0.70	30.8 ± 1.13	56.45 ± 1.20	54.15 ± 1.34	51.25 ± 1.34	B = 1.46
	W.D	33.45 ± 2.05	31.75 ± 1.76	61.05 ± 2.89	70.2 ± 3.11	62.35 ± 2.75	AB = 3.27
	W.D + ABA	33.8 ± 1.27	33.2 ± 1.13	60.95 ± 1.62	77.8 ± 1.83	69.1 ± 1.55	
	W.W	31.00 ± 1.41	31.75 ± 1.76	65.2 ± 1.69	72.95 ± 1.90	64.95 ± 1.34	
	W.W + ABA	31.55 ± 0.63	33.75 ± 1.62	65.4 ± 1.55	79.25 ± 1.76	73.85 ± 1.62	
	S	33.05 ± 1.48	33.25 ± 0.91	80.2 ± 1.83	56.1 ± 1.83	58.5 ± 0.84	
	S + ABA	33.2 ± 1.69	32.25 ± 1.76	83.9 ± 1.69	56.5 ± 0.84	58.8 ± 1.97	
TBARS (µmole/g FW)	C	8.14 ± 0.23	6.98 ± 0.35	7.59 ± 0.73	6.70 ± 0.56	8.40 ± 0.69	A = 0.733
	C + ABA	9.45 ± 0.31	6.71 ± 0.33	7.97 ± 0.57	6.91 ± 0.38	8.34 ± 0.31	B = 0.927
	W.D	11.15 ± 1.20	10.7 ± 1.27	8.8 ± 1.27	5.16 ± 0.42	6.91 ± 0.56	AB = 2.07
	W.D + ABA	9.84 ± 1.47	8.4 ± 0.98	7.55 ± 0.77	4.03 ± 1.32	4.75 ± 0.91	
	W.W	11.28 ± 0.70	10.88 ± 1.71	9.1 ± 1.56	6.15 ± 1.20	6.1 ± 1.41	
	W.W + ABA	10.23 ± 0.46	7.29 ± 1.54	8.91 ± 0.85	4.45 ± 0.91	4.20 ± 1.55	
	S	13.69 ± 0.86	12.5 ± 1.83	6.05 ± 1.20	9.05 ± 0.49	9.35 ± 1.90	
	S + ABA	12.22 ± 0.47	12.73 ± 0.24	4.43 ± 1.08	9.09 ± 0.84	9.06 ± 0.86	
H_2O_2 (µmole/g FW)	C	6.97 ± 0.028	9.25 ± 0.05	13.56 ± 0.07	10.93 ± 0.05	11.50 ± 0.47	A = 0.378
	C + ABA	7.63 ± 0.65	9.31 ± 0.04	12.57 ± 0.04	11.01 ± 0.10	12.44 ± 0.34	B = NS
	W.D	5.93 ± 0.02	7.42 ± 0.04	11.76 ± 0.23	8.38 ± 0.04	9.85 ± 0.06	AB = 1.06
	W.D + ABA	5.74 ± 0.04	3.85 ± 0.07	11.10 ± 0.14	5.09 ± 0.15	7.45 ± 0.09	
	W.W	5.81 ± 0.09	6.37 ± 0.05	10.85 ± 0.10	7.45 ± 0.09	8.26 ± 0.06	
	W.W + ABA	5.69 ± 0.05	3.03 ± 0.06	11.06 ± 0.05	4.10 ± 0.14	5.75 ± 0.21	
	S	7.98 ± 0.04	7.07 ± 0.08	8.91 ± 0.04	6.08 ± 0.09	4.32 ± 0.15	
	S + ABA	7.28 ± 0.09	7.17 ± 0.14	6.82 ± 0.10	5.85 ± 0.35	4.25 ± 0.21	

→ glutamate → P5C → proline. This elucidation is in agreement with previous investigation carried by Stewart (1980), where ABA mediated stimulation of proline synthesis through glutamic acid via glutamate dehydrogenase. The results of our investigation were further strengthened by a research finding where exogenous ABA induced salt tolerance in rice by overexpressing P5CS (Sripinyowanich et al. 2013). On the other hand, the proline-degrading enzyme, i.e., PDH, was found nonresponsive to ABA application in all genotypes under stress conditions, which may be correlated with a previous reports submitted by Dallmier and Stewart (1992). Thus, PDH activity may not be regulated by exogenous ABA, as signal other than ABA may be responsible for its regulation and it is still to be elucidated.

In crux, the mechanism of ABA in ameliorating salt stress in Kharchia (salt tolerant) and water deficit in Gladius and Drysdale (drought tolerant) led to suggest the involvement of DPPH radical scavenging activity along with proline content as a reliable measure. The substantial enhancement in shoot fresh weight under stress conditions strengthen the role of proline vis-à-vis its synthesizing enzyme particularly P5CS activity under ABA supply.

Table 3 Effect of exogenous ABA application on proline and its metabolizing enzymes

Parameters	Treatments	Genotypes					
		PBW 660	PBW 175	Kharchia	Gladius	Drysdale	C.D Value
Proline (µg/g FW)	C	4.23 ± 0.40	4.94 ± 0.47	7.64 ± 0.24	6.72 ± 0.57	7.27 ± 0.75	A = 0.71
	C + ABA	4.05 ± 0.36	4.86 ± 0.57	7.34 ± 0.55	6.67 ± 0.95	7.19 ± 0.84	B = 0.90
	W.D	4.38 ± 0.41	5.04 ± 0.72	7.79 ± 0.84	19.94 ± 0.23	18.17 ± 0.38	AB = 2.02
	W.D + ABA	4.48 ± 0.27	6.19 ± 0.49	7.68 ± 0.41	22.03 ± 0.67	20.72 ± 0.61	
	W.W	4.44 ± 0.63	5.10 ± 1.25	7.77 ± 1.01	19.83 ± 0.73	18.07 ± 1.44	
	W.W + ABA	4.53 ± 1.52	6.37 ± 1.49	7.73 ± 0.91	21.99 ± 1.36	19.62 ± 1.10	
	S	4.20 ± 0.87	4.88 ± 0.64	19.20 ± 1.44	7.05 ± 1.31	7.33 ± 1.63	
	S + ABA	4.21 ± 0.77	4.89 ± 0.42	22.33 ± 1.61	7.11 ± 1.31	7.47 ± 1.60	
GDH (µmole $min^{-1}mg^{-1}FW$)	C	0.29 ± 0.05	0.21 ± 0.08	0.53 ± 0.11	0.66 ± 0.09	0.58 ± 0.06	A = 0.352
	C + ABA	0.27 ± 0.05	0.22 ± 0.06	0.56 ± 0.08	0.57 ± 0.10	0.54 ± 0.06	B = 0.446
	W.D	0.31 ± 0.05	0.37 ± 0.12	0.75 ± 0.25	3.20 ± 0.60	2.73 ± 1.06	AB = 0.998
	W.D + ABA	0.33 ± 0.07	0.41 ± 0.10	0.81 ± 0.32	4.33 ± 0.90	3.85 ± 1.59	
	W.W	0.31 ± 0.03	0.33 ± 0.15	0.79 ± 0.38	3.31 ± 1.15	2.81 ± 0.88	
	W.W + ABA	0.32 ± 0.07	0.35 ± 0.14	0.83 ± 0.46	4.84 ± 0.89	4.05 ± 0.94	
	S	0.25 ± 0.09	0.23 ± 0.09	2.23 ± 0.35	0.70 ± 0.03	0.53 ± 0.06	
	S + ABA	0.26 ± 0.09	0.25 ± 0.10	3.54 ± 0.46	0.75 ± 0.04	0.54 ± 0.06	
P5CS (µmole $min^{-1}mg^{-1}FW$)	C	4.27 ± 1.01	3.87 ± 0.84	9.85 ± 0.72	8.27 ± 1.24	5.62 ± 0.65	A = 0.577
	C + ABA	4.32 ± 0.64	3.93 ± 0.77	9.67 ± 0.55	8.57 ± 1.49	5.80 ± 0.74	B = 0.730
	W.D	6.14 ± 0.37	5.01 ± 0.95	11.22 ± 0.78	11.18 ± 0.99	10.89 ± 0.31	AB = 1.63
	W.D + ABA	6.07 ± 0.57	6.64 ± 0.94	11.39 ± 0.75	16.30 ± 1.05	14.05 ± 0.31	
	W.W	6.21 ± 0.73	5.35 ± 0.76	11.08 ± 1.04	11.07 ± 0.84	10.52 ± 0.76	
	W.W + ABA	6.08 ± 0.69	6.85 ± 0.87	11.26 ± 0.77	15.32 ± 0.65	13.17 ± 0.79	
	S	6.29 ± 0.77	6.12 ± 0.35	14.15 ± 0.36	11.00 ± 0.94	6.89 ± 0.72	
	S + ABA	6.40 ± 0.72	6.21 ± 0.59	19.75 ± 1.22	10.57 ± 0.76	7.08 ± 0.63	
PDH (µmole $min^{-1}mg^{-1}FW$)	C	0.098 ± 0.07	0.122 ± 0.06	0.452 ± 0.09	0.439 ± 0.09	0.343 ± 0.06	A = 0.044
	C + ABA	0.104 ± 0.05	0.113 ± 0.05	0.441 ± 0.06	0.428 ± 0.10	0.338 ± 0.05	B = 0.055
	W.D	0.059 ± 0.02	0.082 ± 0.01	0.410 ± 0.01	0.374 ± 0.05	0.235 ± 0.07	AB = 0.124
	W.D + ABA	0.067 ± 0.03	0.093 ± 0.01	0.401 ± 0.03	0.368 ± 0.08	0.226 ± 0.10	
	W.W	0.047 ± 0.03	0.101 ± 0.03	0.391 ± 0.02	0.372 ± 0.06	0.231 ± 0.02	
	W.W + ABA	0.053 ± 0.02	0.111 ± 0.02	0.382 ± 0.05	0.365 ± 0.07	0.229 ± 0.06	
	S	0.079 ± 0.02	0.104 ± 0.04	0.218 ± 0.11	0.299 ± 0.04	0.322 ± 0.07	
	S + ABA	0.084 ± 0.03	0.114 ± 0.06	0.210 ± 0.09	0.282 ± 0.02	0.331 ± 0.04	

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Compliance with ethical standards

Conflict of interest The author and coauthors have no conflict of interest.

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