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ORIGINAL PAPER

Promotion of Mineral Contents and Antioxidant Compounds in Water Spinach Using Foliar Paclobutrazol and Salt Elicitors

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Abstract

Green vegetables are important food resources to feed the world population. Water spinach (*Ipomoea aquatica* Forsk; Convolvulaceae) is one such food resource that can grow rapidly in a short-cultivation period and by simple cultivation practices. The objective of this study was to stimulate antioxidant compounds, mineral nutrients, physiological adaptation, and overall growth performances in two cultivars of water spinach (wild type, WT; Chia Tai, CT) using paclobutrazol (PBZ) and NaCl salt elicitors. Six-week-old seedlings of WT and CT water spinach were foliar-sprayed with 0 (control), 17, and 51 M PBZ for 1 week, followed by 0 (control) and 100 mM NaCl (salt elicitor) for 7 days. The growth characteristics and physiological and biochemical changes were measured for all scenarios during the period of application. Plant height in WT was found to be sensitive to PBZ (decline by 16.5% under 17 M PBZ and 46.6% under 51 M PBZ as compared to the control scenario). This in turn led to a 50% shorter shoot length when compared to the control scenario, and the 100-mM NaCl salt treatment significantly inhibited shoot length, whereas it was not affected in CT. The number of leaves and shoot fresh weight were maximal in WT with 51 M PBZ and 0 mM NaCl at 14.7 leaves plant^{-1} and 9.97 g plant^{-1}, respectively. On the contrary, these parameters in the 0-µM PBZ and 100-mM NaCl scenarios decreased by 40.9% and 57.4%, respectively, over the control scenario. A significant reduction in Na^+ and Ca^{2+} was found in PBZ-pretreated plants exposed to NaCl elicitor. Interestingly, Na^+ enrichment in the leaf tissues of 51- µM PBZ-treated plants under salt stress decreased by 40% over 0 M PBZ, leading to sustained chlorophyll pigments, photon yield of PSII (Φ_{PSII}), net photosynthetic rate (P_n), and plant biomass. Moreover, total soluble sugar (TSS), ascorbic acid (AsA), and tocopherol contents in the leaf tissues were enhanced in the NaCl-treated cultivar CT as compared to certain degrees of PBZ foliar applications. In summary, reduced plant height and NaCl toxicity in water spinach using PBZ were observed. In addition, osmotic adjustment compounds and non-enzymatic antioxidants were regulated as major defense responses to retain the photosynthetic abilities and growth performances. Accumulation of AsA and tocopherol in the green leaf vegetable of water spinach was found to be regulated by a combination of PBZ pretreatment and NaCl elicitor.

Keywords Ascorbic acid · Calcium · Chlorophyll content · Chlorophyll *a* fluorescence · Photosynthetic abilities · Sodium chloride · Tocopherol

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1 Introduction

Water spinach (*Ipomoea aquatica* Forsk; Convolvulaceae) is a common aquatic plant that grows as both an aquatic (floating over a freshwater surface) and terrestrial plant (Mandal et al. 2008). In China, water spinach was reported to be used as a green leafy vegetable in China Dynasty during 290–307 A.D. (Edie and Ho 1969; Austin 2007). The micronutrients present as 87 mg g^{-1} DW thiamine (vitamin B1), 1.37 mg g^{-1} AsA (ascorbic acid or vitamin C), 0.6 mg g^{-1} nicotinic acid, 120 g g^{-1} riboflavin and carotenoid (provitamin A), and the mineral content included 41.4 mg g^{-1} potassium (K), 31 mg g^{-1} magnesium (Mg), 5 mg g^{-1} sodium (Na), 2 mg g^{-1} calcium (Ca), and 1.7 mg g^{-1} zinc (Zn) per 100 g of water spinach (Mandal et al. 2008). In addition, major non-enzymatic antioxidants, α-tocopherol (vitamin E; 2.6 mg per 100 g) and AsA (50 mg per 100 g) have also been reported in the leaf tissues of water spinach (Ching and Mohamed 2001; Ismail and Fun 2003) that function as free radical-scavenging antioxidant compounds (Huang et al. 2005; Prasad et al. 2005). Recently, the microgreen vegetable in water spinach is a good candidate for mineral and phytochemical composition (Yadav et al. 2019). Microgreen production requires a large number of seeds with a high germination rate (> 80%; Ebert and Wu 2019). Therefore, the high density (biomass), uniformity plants, and best quality (enriched nutrients and phytochemical compounds) of water spinach in the mature stage still need further investigation.

In higher plants, NaCl salt elicitor is known to effectively promote secondary metabolites (Giri and Zaheer 2016) such as anthraquinone (alizarin and purpurin) in *Rubia tinctorum* (Aşcı et al. 2018); solasodine in *Solanum nigrum* (Bhat et al. 2008; Šutković et al. 2011); solamargine and solasonine in *S. incanum* (Al-Sinani and Eltayeb 2015); α-tocopherol in *Carthamus tinctorius* (Chavan et al. 2011); scopolamine in *Datura metel* (Ajungla et al. 2009); reserpine in *Rauvolfia tetraphylla* (Anitha and Kumari 2006); and salvianolic acid A, salvianolic acid B, and rosmarinic acid in *Salvia miltiorrhiza* (Yu et al. 2019). Low exposure to NaCl (50 mM) can promote the production of α-tocopherol, anthocyanins, and calcium mineral content in water spinach (Kitayama et al. 2019). Moreover, a combination of chemical elicitors such as NaCl and paclobutrazol (PBZ) can further affect the production of secondary metabolites as seen in *Ocimum basilicum* (Keramati et al. 2016) and *Oryza sativa* cv. KDML105 (Jasmine rice; Detpitthayanan et al. 2019). Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl)-pentan-3-ol] is an anti-gibberellic member of triazole family, which causes growth retardation in higher plants (Berova and Zlatev 2000; Desta and Amare 2021) and ameliorates abiotic stress effects (Soumya et al. 2017; Chandra and Roychoudhury 2020). The basic knowledge on the regulation of secondary metabolites in water spinach using chemical elicitors, NaCl and PBZ, is still lacking. The objective of this investigation was to promote vitamin E (tocopherol), vitamin C (AsA), calcium (Ca^{2+}), total soluble sugar (TSS), physiological adaptation, and overall growth performances in two cultivars of water spinach using PBZ pretreatment and NaCl salt elicitor.

2 Materials and Methods

2.1 Plant Materials and PBZ/NaCl Treatments

Seeds of Chia Tai (CT; green stem variety) and wild type (WT; red stem variety) of water spinach were sown in the plastic bags (4 × 4 × 5 cm in W × L × H) containing 1 kg garden soil (EC = 2.69 dS m^{-1}; pH = 5.7; total organic carbon = 12.26%; available N = 0.30 mg kg^{-1}; available P = 578 mg kg^{-1}; available K = 3,073 mg kg^{-1}), a silt–clay soil class and cultivated under greenhouse conditions (80–90% relative humidity, 28–32 °C air temperature, 800–1000 mol m^{-2} s^{-1} photosynthetic photon flux density), according to Kitayama et al. (2019). Slow-releasing fertilizer (13–13-13 NPK) 10 g $plant^{-1}$ was supplied by mixing into the soil substrate before the transplanting process. Three seeds were sown in each bag containing soil substrate, abnormal seedlings were thinned, and then the uniform plants in terms of size and number of leaves were selected as plant material. Six-week-old uniform seedlings with 4–5 true leaves (5.0 ± 0.5 cm in plant height) were foliar-sprayed only one time with 0 (control), 17, and 51 M paclobutrazol (PBZ) foliar application (50 mL $plant^{-1}$) and then cultivated in the greenhouse for 1 week. Subsequently, water spinach seedlings pretreated by PBZ were subjected to 0 (control) and 100 mM NaCl (chemical elicitation) by flood-irrigation method for 10–15 min daily and were then cultivated in a greenhouse for 7 days. Overall growth performances, photosynthetic abilities, soluble sugar, tocopherol, ascorbic acid, Na^+, and Ca^{2+} were measured in the treated plants.

2.2 Morphological Measurements

Plant height, number of leaves, leaf length, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight in each treatment were measured. Shoot and root were separated and dried in a hot air oven at 80 °C for 72 h, and subsequently transferred to a desiccant chamber until room temperature is achieved for dry weight determination.

2.3 Physiological Analysis

Photosynthetic pigments including chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), and total chlorophyll (TC) in the leaf tissues of water spinach were determined according to the method suggested by Shabala et al. (1998). One hundred milligrams of fresh leaf tissues was chopped, transferred to a glass vial containing 10 mL of 99.5% acetone, and then blended using a homogenizer. The glass vials were capped and sealed with Parafilm® to prevent evaporation, and then stored at 4 °C for 48 h. Chl_a and Chl_b were measured at 662 nm and 644 nm, respectively, using a UV–VIS spectrophotometer (HACH DR/4000; Model 48,000, HACH Company, Loveland, Colorado, USA) against acetone (99.5%) as a blank.

Chlorophyll a fluorescence emission from the adaxial surface of a second fully expanded leaf was detected by a fluorescence monitoring system (FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode (Loggini et al. 1999). A target leaf, kept in the dark for at least 30 min, was initially exposed to the modulated measuring beam of far-red light (LED source) with a typical peak at a wavelength of 735 nm. Original (F_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light (1500 µmol m^{-2} s^{-1} PPFD) and calculated using FMS software for Windows®. The variable fluorescence yield (F_v) was calculated using the equation: $F_v = F_m - F_0$. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated as the maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φ_{PSII}) in the light was calculated as: $\Phi_{PSII} = (F_m' - F)/F_m'$ after 45 s of illumination when a steady state was achieved (Maxwell and Johnson 2000).

Net photosynthetic rate (P_n; µmol m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s; mmol m^{-2} s^{-1}) of a second fully expanded leaf of water spinach were measured using a portable photosynthesis system (LI 6400XT, LI-COR, Lincoln, NE, USA) according to the method suggested by Cha-um et al. (2006). The air-flow rate of the IRGA chamber was fixed at 500 µmol s^{-1}, and the chamber temperature was set at 27 °C. The light intensity was adjusted to 1000 µmol m^{-2} s^{-1} PPFD of 6400-02B red-blue LED light source.

2.4 Biochemical Assays

Soluble sugars (sucrose, glucose, and fructose) in the stem and leaf tissues were assayed following the method of Karkacier et al. (2003). γ- and α-Tocopherol in the stem and leaf tissues of water spinach were extracted and analyzed using the HPLC system (Fig. S1) following the method of Annunziata et al. (2012). Ascorbic acid in the stem and leaf tissues of water spinach was detected by the HPLC system (Fig. S2) according to the method suggested by Radulescu et al. (2013). Na^+ and Ca^{2+} in root, stem, and leaf tissues were determined according to a protocol adopted from Tanaka et al. (1999) and Hossain et al. (2006).

2.5 Experiment Layout and Statistical Analysis

The experiment was designed as $3 \times 2 \times 2$ factorials in a completely randomized design (CRD) with 5 replications ($n = 5$) of each treatment. The mean values were compared using Tukey's HSD and analyzed by SPSS statistics software version 11.5. Pearson's correlation coefficient was used for the validation of physiological, morphological, and yield traits.

3 Results

3.1 Growth Performances

In cultivar (cv.) CT, the dark green color of leaves was observed with an increase in PBZ concentration; however, plants under 100 mM NaCl for 7 days demonstrated a yellow color of leaves (Fig. 1). In contrast, the dark green color in the leaves of cv. WT was retained in each treatment (Fig. 1). Plant height in cv. CT was unchanged in all treatments (Fig. 2a and b) but, in cv. WT, declined by 16.5% under 17 M PBZ treatment and 46.6% under 51 M PBZ treatment over the control (0 M PBZ treatment). The plant height further dropped by 33.7% in 0-µM PBZ plants and 37.4% in 17-µM PBZ-pretreated plants when exposed to 100 mM NaCl (Fig. 2a) compared to control (0 mM NaCl). In addition, plant height in the 51-µM PBZ-pretreated WT plant was the shortest (58.07 cm) and did not vary significantly (43.50 cm) when subjected to 100 mM NaCl (Fig. 2a). In this case, the water spinach cvs. CT and WT were identified as PBZ unresponsive and responsive cultivars, respectively (Fig. 2c). The number of leaves in cv. CT was unaffected, while it was significantly dropped by 40.9% in 0-µM PBZ plants in cv. WT, when exposed to 100 mM NaCl (Table 1). Leaf length in cv. CT was significantly increased by 142% in 0-µM PBZ-pretreated plants, and in contrast, it was unchanged in cv. WT when exposed to 100 mM NaCl (Table 1). Root fresh weight in the cv. CT 0-µM PBZ treatment was sensitive to salt treatment, leading to a 34.5% decrease over the control, whereas it remained unaffected in cv. WT (Table 1). Shoot fresh weight in cv. CT 0-µM PBZ-pretreated plants and exposed to 100 mM NaCl sharply declined by 57.4% over the control (Table 1).

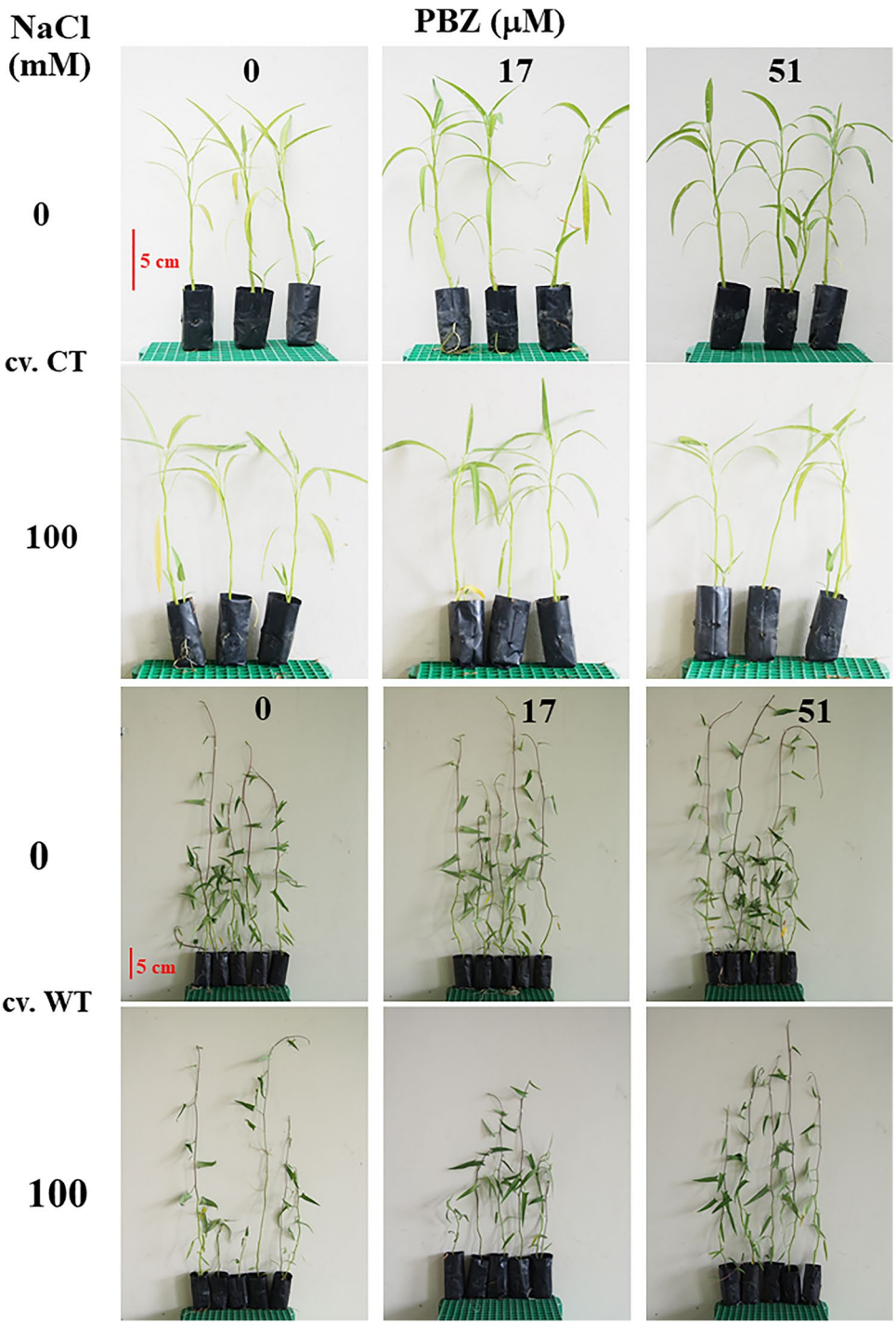

Fig. 1 Plant morphological characteristics of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) and wild type (WT cultivar, red stem) pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days

3.2 Na^+ and Ca^{2+} Enrichment

Na^+ content in the leaf, stem, and root tissues of water spinach significantly increased (root > stem > leaf) within 7 days of 100 mM NaCl salt treatment in both cvs. CT and WT, especially in the 0-μM PBZ treatment, when compared with untreated plants (0 mM NaCl) (Fig. 3a - c). However, Na^+ levels in the PBZ-pretreated plants increased less rapidly compared to PBZ-untreated plants (Fig. 3c). Na^+ contents in the stem and leaf tissues of both water spinach cultivars under 100 mM NaCl were significantly low in 51-μM PBZ-pretreated plants (Fig. 3a, b). On the other hand, Ca^{2+} contents in the leaf tissues of water spinach cv. CT were nearly the same in all the treatments, whereas it was found to increase in cv. WT pretreated with 17 and 51 M PBZ and subsequently exposed to 100 mM NaCl (Fig. 3d). In stem

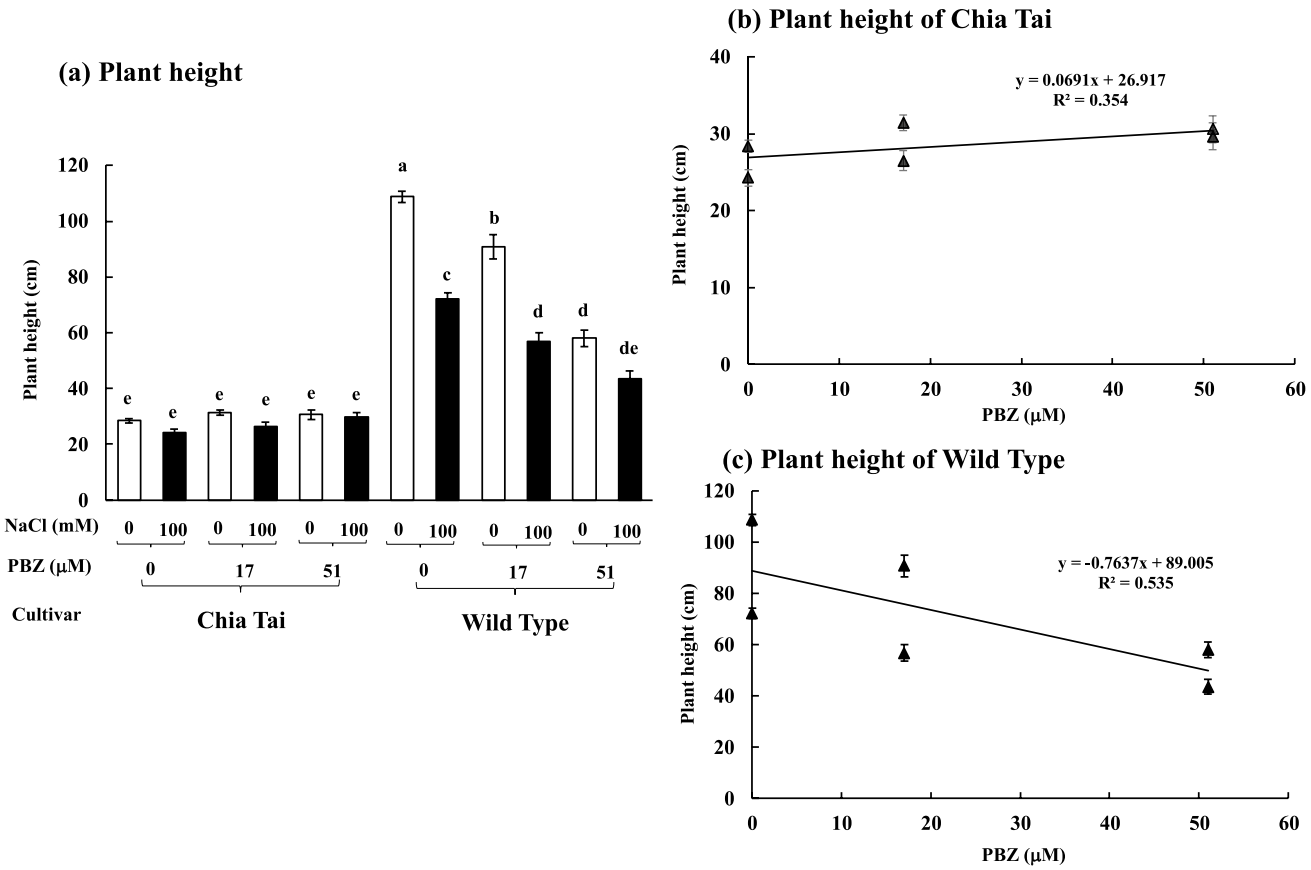

Fig. 2 Plant height of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) and wild type (WT cultivar, red stem) pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days (**a**), the relationship between PBZ concentrations and plant height of CT (**b**), and WT (**c**). Data presented as mean ± SE ($n = 5$). Different letters in each column show significant differences at $p \leq 0.01$, according to Tukey's HSD

Table 1 Number of leaves (NL), leaf length (LL), root fresh weight (RTFW), and shoot fresh weight (STFW) of two water spinach (*Ipomoea aquatica*) cultivars, Chia Tai or 'CT' and Wild Type or 'WT' seedlings, pretreated with 0, 17, and 51 M paclobutrazol (PBZ) and subsequently exposed to 0 and 200 mM NaCl for 7 days. Data in each column is represented as ± SE ($n = 5$)

Cultivar	PBZ (μM)	NaCl (mM)	NL	LL (cm)	RTFW (g)	STFW (g)
	0	0	8.0 ± 1.0bc	10.6 ± 1.1bc	4.29 ± 0.26ab	7.16 ± 1.02b
		100	6.0 ± 1.0c	15.0 ± 0.8a (+142%)	2.81 ± 0.53c (−34.5%)	5.06 ± 1.14bc
	17	0	8.7 ± 0.3bc	9.7 ± 0.5c	4.75 ± 0.65ab	7.19 ± 1.09b
CT		100	7.3 ± 1.7bc	13.8 ± 1.9ab (+142%)	3.95 ± 0.91bc	5.07 ± 0.71bc
	51	0	9.3 ± 1.2b	9.3 ± 1.3c	5.95 ± 0.39a	8.48 ± 0.48ab
		100	11.7 ± 0.9ab	9.0 ± 0.6c	5.27 ± 0.58ab	9.00 ± 0.88a
	0	0	11.0 ± 1.8ab	7.6 ± 0.3c	3.76 ± 0.22bc	7.91 ± 0.76b
		100	6.5 ± 0.9c (−40.9%)	7.7 ± 0.5c	2.46 ± 0.20c	3.37 ± 0.34c (−57.4%)
	17	0	14.0 ± 1.6a	7.8 ± 0.4c	3.67 ± 0.29bc	8.98 ± 0.65ab
WT		100	9.0 ± 1.6b (−35.7%)	6.9 ± 0.5c	2.93 ± 0.24c	6.50 ± 0.68b
	51	0	14.7 ± 0.7a	7.7 ± 0.7c	3.08 ± 0.42bc	9.97 ± 0.95a
		100	11.7 ± 1.5ab	6.5 ± 0.6c	2.98 ± 0.11c	7.24 ± 1.41b (−27.4%)

Different letters in a column represent significant differences according to Tukey's HSD test at $p \leq 0.05$

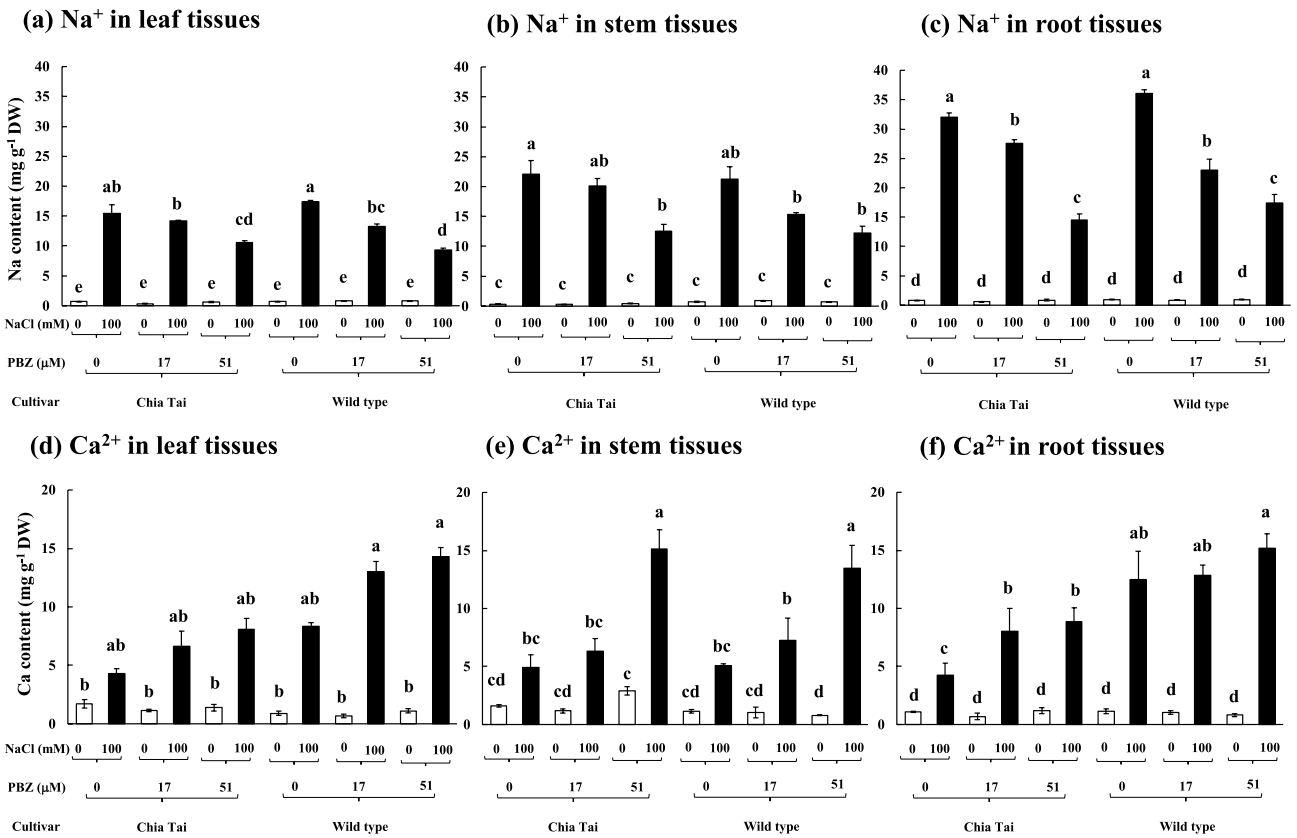

Fig. 3 Na^+ in leaf (**a**), stem (**b**), and root tissues (**c**), $Ca.^{2+}$ in leaf (**d**), stem (**e**), and root tissues (**f**) of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) and wild type (WT cultivar, red stem) pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days (**a**), the relationship between PBZ concentrations and plant height of CT (**b**) and WT (**c**). Data presented as mean ± SE ($n = 5$). Different letters in each column show significant differences at $p \leq 0.01$, according to Tukey's HSD

tissues, Ca^{2+} contents in both cvs. CT and WT pretreated with 51 M PBZ and subjected to 100 mM NaCl increased (Fig. 3e). Interestingly, Ca^{2+} in the root tissues of two water spinach cultivars increased with the PBZ and NaCl application rate (Fig. 3f).

3.3 Photosynthetic Abilities and Relationships

Chl_a, Chl_b, and TC content in water spinach cv. CT was sensitive to 100 mM NaCl, leading to their degradation by 22.1%, 50%, and 36% over the control, respectively. The pigments in NaCl-treated plants of cvs. CT and WT were sustained when pretreated with 51 M PBZ (Table 2). In cv. WT, Chl_b and TC levels in NaCl-treated plants in the 0-μM PBZ application sharply reduced by 27.3% and 20.9% over the control, respectively. This led to a diminished quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) by 11.0% and 15% over the control, respectively (Table 2). In cv. CT, the net photosynthetic rate (P_n) in NaCl-treated plants in the 0-μM PBZ application was decreased by 41.1% over the control and it was improved by 17- and 51-μM PBZ foliar applications (Table 3). P_n, g_s, and E in cv. WT in each treatment were greater than in cv. CT (Table 3).

A negative correlation between Na^+ in roots and root fresh weight of cv. CT (Fig. 4a; $R^2 = 0.534$), Na^+ in roots and root fresh weight of cv. WT (Fig. 4b; $R^2 = 0.981$), Na^+ in shoots and shoot fresh weight of cv. CT (Fig. 4c; $R^2 = 0.387$), and Na^+ in shoots and shoot fresh weight of cv. WT (Fig. 4d; $R^2 = 0.832$) was demonstrated. Moreover, a negative relationship between Na^+ in leaves and TC content was found in cv. CT (Fig. 5a; $R^2 = 0.720$), whereas it was independent in cv. WT (Fig. 5b). In contrast, a positive connection between TC content and Φ_{PSII} was demonstrated in cv. CT (Fig. 5c; $R^2 = 0.666$), while it was not observed in cv. WT (Fig. 5d). Moreover, positive relations between Chl_a content and F_v/F_m in cvs. CT (Fig. 6a; $R^2 = 0.512$) and WT (Fig. 6b; $R^2 = 0.509$) as well as Φ_{PSII} and P_n in cvs. CT (Fig. 6c; $R^2 = 0.809$) and WT (Fig. 6d; $R^2 = 0.272$) were observed.

Table 2 Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC), the maximum quantum yield of PSII (F_v/F_m), and photon yield of PSII (Φ_{PSII}) two water spinach (*Ipomoea aquatica*) cultivars, Chia Tai or 'CT' and Wild Type or 'WT' seedlings, pretreated with 0, 17, and 51 M paclobutrazol (PBZ) and subsequently exposed to 0 and 200 mM NaCl for 7 days. Data in each column is represented as ± SE ($n = 5$)

Cultivar	PBZ (μM)	NaCl(mM)	Chl_a (μg g^{-1} FW)	Chl_b (μg g^{-1} FW)	TC (μg g^{-1} FW)	F_v/F_m	Φ_{PSII}
	0	0	57.1 ± 1.3a	57.0 ± 3.0c	114.1 ± 2.5c	0.772 ± 0.022ab	0.644 ± 0.039ab
		100	44.5 ± 0.2c (− 22.1%)	28.5 ± 1.8d (− 50.0%)	73.0 ± 1.8d (− 36.0%)	0.722 ± 0.021b	0.575 ± 0.005bc
	17	0	57.2 ± 1.7a	73.1 ± 5.7b	130.3 ± 6.7bc	0.752 ± 0.021ab	0.669 ± 0.016a
CT		100	49.1 ± 6.2bc (− 14.2%)	36.3 ± 5.1d (− 50.3%)	85.4 ± 8.5d (− 34.5%)	0.725 ± 0.028b	0.616 ± 0.005b (− 7.9%)
	51	0	54.2 ± 3.2ab	56.0 ± 2.2c	110.2 ± 3.1c	0.791 ± 0.013a	0.678 ± 0.013a
		100	54.2 ± 2.6ab	58.2 ± 3.5c	112.4 ± 4.9c	0.743 ± 0.021ab	0.616 ± 0.005b (− 9.1%)
	0	0	58.3 ± 0.9a	96.8 ± 2.0a	155.1 ± 1.8a	0.800 ± 0.009a	0.645 ± 0.015ab
		100	52.3 ± 1.3ab	70.41 ± 1.8bc (− 27.3%)	122.7 ± 2.5c (− 20.9%)	0.712 ± 0.022b (− 11.0%)	0.548 ± 0.022c (− 15.0%)
	17	0	56.7 ± 1.3a	78.0 ± 2.7b	134.7 ± 2.4bc	0.783 ± 0.016ab	0.654 ± 0.015a
WT		100	58.2 ± 2.3a	83.9 ± 1.7ab	142.1 ± 2.2ab	0.758 ± 0.016ab	0.570 ± 0.020bc (− 12.8%)
	51	0	55.5 ± 0.2ab	76.1 ± 2.9b	131.6 ± 3.0bc	0.775 ± 0.021ab	0.690 ± 0.016a
		100	55.8 ± 3.8ab	81.3 ± 2.1ab	137.1 ± 3.2ab	0.797 ± 0.014a	0.548 ± 0.022c (− 20.6%)

Different letters in a column represent significant differences according to Tukey's HSD test at $p \leq 0.05$

Table 3 Net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of two water spinach (*Ipomoea aquatica*) cultivars, Chia Tai or 'CT' and Wild Type or 'WT' seedlings, pretreated with 0, 17, and 51 M paclobutrazol (PBZ) and subsequently exposed to 0 and 200 mM NaCl for 7 days. Data in each column is represented as ± SE ($n = 5$)

Cultivar	PBZ (μM)	NaCl (mM)	P_n (μmol m^{-2} s^{-1})	g_s (mmol H_2O m^{-2} s^{-1})	E (mol H_2O m^{-2} s^{-1})
	0	0	3.24 ± 0.36c	0.04 ± 0.005bc	0.27 ± 0.04bc
		100	1.91 ± 0.52d (− 41.1%)	0.01 ± 0.005c	0.05 ± 0.01c
	17	0	3.29 ± 0.16c	0.01 ± 0.001c	0.27 ± 0.01bc
CT		100	2.81 ± 0.16 cd	0.02 ± 0.001c	0.10 ± 0.01c
	51	0	4.77 ± 0.48c	0.02 ± 0.001c	0.35 ± 0.07bc
		100	2.47 ± 0.27 cd	0.01 ± 0.002c	0.19 ± 0.05bc
	0	0	13.00 ± 0.87ab	0.11 ± 0.02ab	2.08 ± 0.30ab
		100	11.48 ± 1.49b	0.11 ± 0.04ab	2.22 ± 0.59ab
	17	0	11.04 ± 1.03b	0.14 ± 0.02ab	2.52 ± 0.30ab
WT		100	13.00 ± 1.14ab	0.11 ± 0.02ab	2.12 ± 0.64ab
	51	0	16.40 ± 1.94a	0.23 ± 0.04a	4.10 ± 0.71a
		100	13.95 ± 1.79ab	0.12 ± 0.04ab	2.09 ± 0.35ab

Different letters in a column represent significant differences according to Tukey's HSD test at $p \leq 0.05$

3.4 Soluble Sugar, AsA, and Tocopherol

Sucrose, glucose, and fructose contents in the leaf tissues of water spinach cv. CT grown under salt elicitor were increased by 908%, 1,623%, and 232%, respectively, over the control, but were subsequently reduced by PBZ foliar spray (Table 4). In the stem tissues, glucose was found to be enriched, while sucrose was declined by 35.3% and 51.9% over the control in 17- and 51-μM PBZ-pretreated plants, respectively, when plants were exposed to 100 mM NaCl (Table 4). In cv. WT, sucrose, and glucose in the leaf tissues of water spinach grown under salt elicitor decreased by 71.8% and 63.7% over the control, respectively, but these were significantly increased by PBZ pretreatment. In contrast, sucrose and fructose were elevated by 190% and 200% over the control, respectively, in 51-μM PBZ-pretreated plants exposed to NaCl treatment (Table 4). Total soluble sugar (TSS) in the leaf tissues of water spinach cv. CT

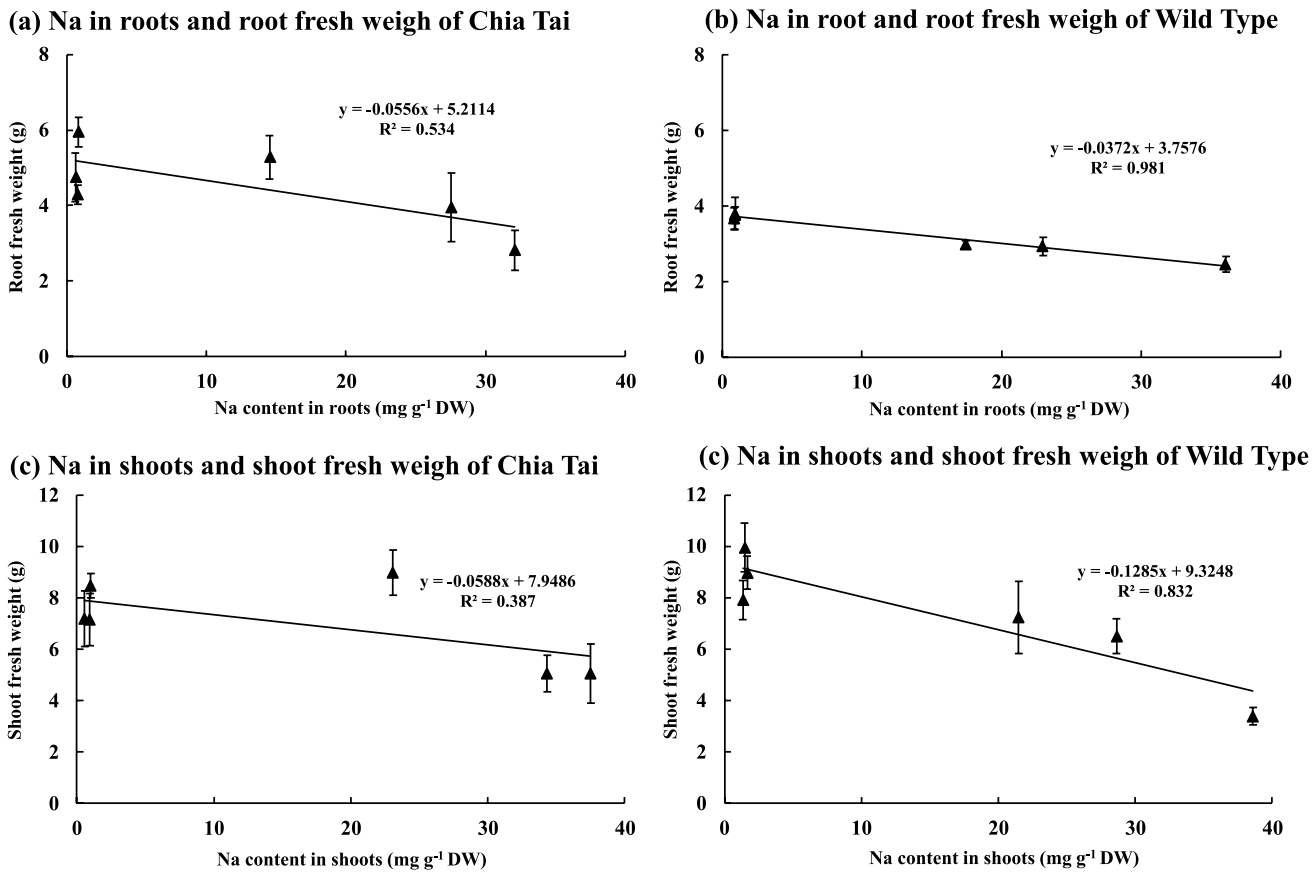

Fig. 4 Relationships between Na^+ in roots and root fresh weight of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) (**a**) and wild type (WT cultivar, red stem) (**b**), $Na.^+$ in shoots and shoot fresh weight of CT (**c**) and WT (**d**), pre-treated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days. Data presented as mean ± SE ($n = 5$)

was very low when not exposed to NaCl, but subsequently increased by 840%, 260%, and 470% over the control when pretreated with 0, 17, and 51 M PBZ, respectively, together with 100 mM NaCl (Fig. 7a). On the other hand, TSS declined in cv. WT plants in the 0-μ PBZ pretreatment and increased by 170% over the control in 51-μM PBZ-pretreated plants when exposed to 100 mM NaCl (Fig. 7a). TSS in stem tissues of water spinach cv. CT pretreated with 0 and 17 M PBZ and grown under salt elicitor were increased by 360% and 140%, respectively, over the control. In addition, TSS in stem tissues of cv. WT in was stable in all the treatments (Fig. 7d).

AsA in the leaf tissues of cv. CT grown under 100 mM NaCl was increased by 110%, 120%, and 110% over the control with 0-, 17-, and 51-μM PBZ pretreatments, respectively (Fig. 7b). In contrast, AsA in the stem tissues of cv. CT grown under 100 mM NaCl was significantly declined by 13.0%, 20.7%, and 8.3% over the control, in relation to 0-, 17-, and 51-μM PBZ pretreatments, respectively (Fig. 7e). In cv. WT, AsA in the leaf tissues of plants in 0-μM PBZ pretreatment was significantly declined when exposed to salt treatment and then it was steady in PBZ-treated plants (Fig. 7b). Moreover, AsA in the stem tissues of plants pretreated with 0- and 17-μM PBZ plants grown under 100 mM NaCl was increased by 130% over the control, while it was dropped by 17.5% over the control in 51-μM PBZ-treated plants (Fig. 7e). Tocopherol content was in the order leaf tissues > stem tissues, and it was higher in cv. WT as compared to cv. CT (Fig. 7c and d). Tocopherol in leaf tissues of cv. CT-pretreated with 0 and 17 M PBZ grown under 100 mM NaCl was increased by 120% and 130% over the control, respectively. In addition, tocopherol in both the cultivars treated with 51 M PBZ and 100 mM NaCl was promoted by 120% over the control (0 mM NaCl) (Fig. 7c). Degradation of tocopherol in stem tissues of two water spinach cultivars by salt elicitor was observed, except in the CT plants pretreated with 51 M PBZ (Fig. 7f). In the leaf tissues of cv. CT, γ-tocopherol was enriched by salt elicitor, especially in plants without PBZ pretreatment (174% over the control) and in plants with 17-μM PBZ pretreatment (183% over the control), and α-tocopherol was upregulated by salt elicitor (119% over the control) in plants pretreated with 51 M

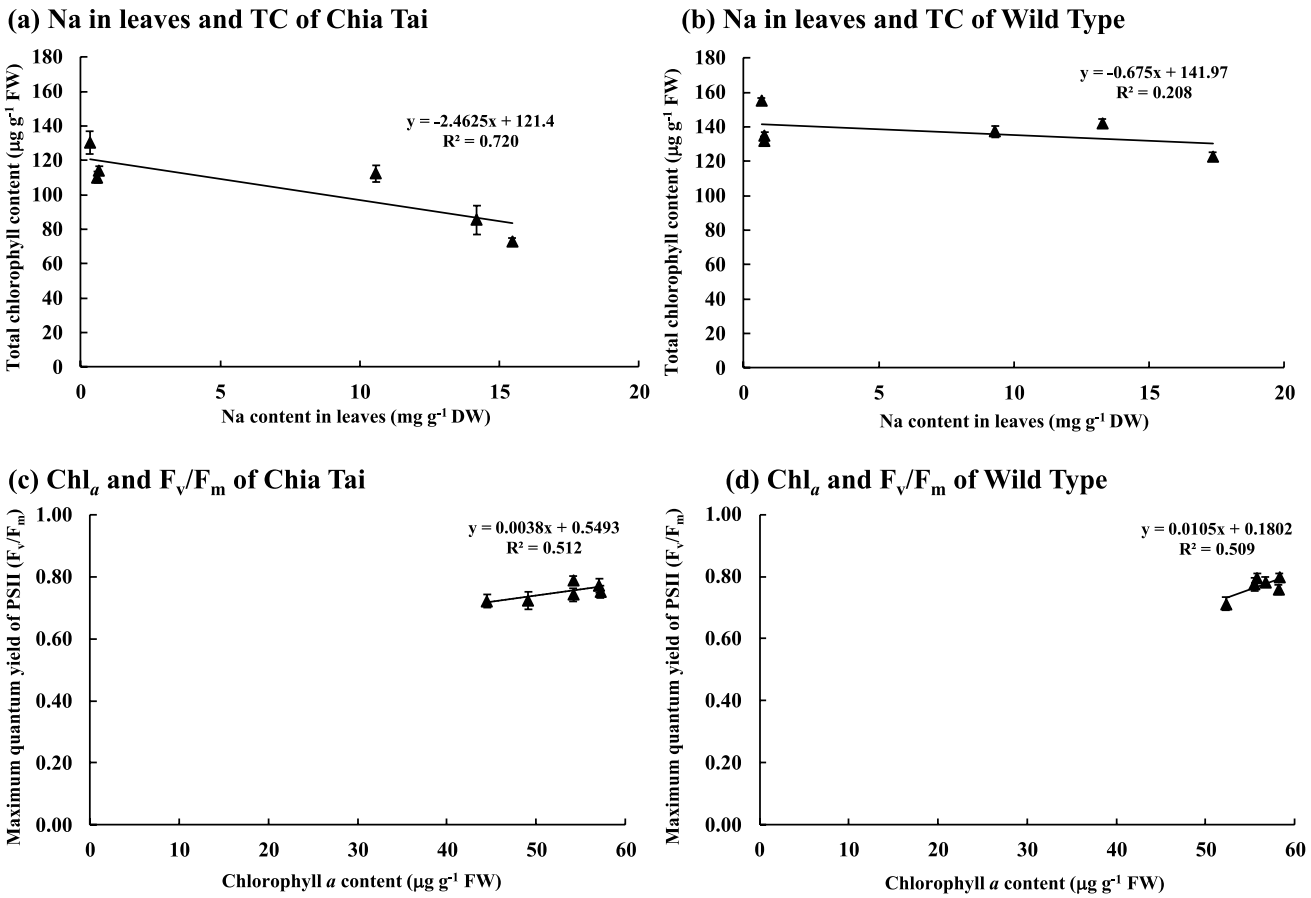

Fig. 5 Relationships between $Na.^+$ in leaves and total chlorophyll content of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) (**a**) and wild type (WT cultivar, red stem) (**b**), chlorophyll a content and maximum quantum yield of PSII (F_v/F_m) of CT (**c**) and WT (**d**), pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days. Data presented as mean ± SE ($n = 5$)

PBZ (Table 1). Likewise, γ-tocopherol in the stem tissues was enriched by salt elicitor (187% over the control; 51 M PBZ) (Table 5). In contrast, γ-tocopherol in NaCl-treated stem tissues of cv. WT pretreated with 0 and 17 M PBZ declined by 21.8% and 22.8% over the control, respectively, (Table 5). However, α-tocopherol was increased by 124% over the control in plants pretreated with 17 M PBZ and by 119% over the control in plants pretreated with 51 M PBZ. In stem tissues, only γ-tocopherol in NaCl-treated stem tissues of cv. WT pretreated plants with 51 M PBZ declined by 61.7% over the control, whereas it was maintained in other treatments.

4 Discussion

In general, the mode of action of PBZ is well-known as an anti-gibberellin plant growth regulator (GA-biosynthesis inhibitor), and therefore, it leads to a slow rate of shoot elongation (Kang et al. 1985; Yim et al. 1997; Forghani et al. 2018). However, the shoot height retardation rate depends on the cultivar, degree of PBZ dose, and their interaction (Lee et al. 2015; Lyons et al. 2018). In NaCl-treated plants, overall growth performances were retained by PBZ pretreatment (Hajihashemi et al. 2007; Khunpon et al. 2017). Therefore, plant height was significantly decreased with the treatment of PBZ and NaCl in sweet sorghum (Forghani et al. 2018). The reduction in Na^+ content in each organ of salt-treated plants using PBZ application is still unclear. Likewise, low Na^+ accumulation was observed in the root, stem, and leaf tissues of salt-treated peach (*Prunus persica* cv. Nemaguard) pretreated with 1000–2000 mg L^{-1} PBZ (El-Khashab et al. 1997). Similar results have also been observed in mango (*Mangifera indica*; Kishor et al. 2009), quinoa (*Chenopodium quinoa*; Waqas et al. 2019), oleander (*Nerium oleander*; Bañón et al. 2005), and wheat (*Triticum aestivum*; Hajihashemi et al. 2007). A low Na^+ level in root and leaf tissues of salt-treated plants and Kinnow (*Citrus nobilis* × *C. deliciosa*) budded on Jatti Khatti (*C. jambhiri*) with 100 mg L^{-1} PBZ pretreatment was detected, leading

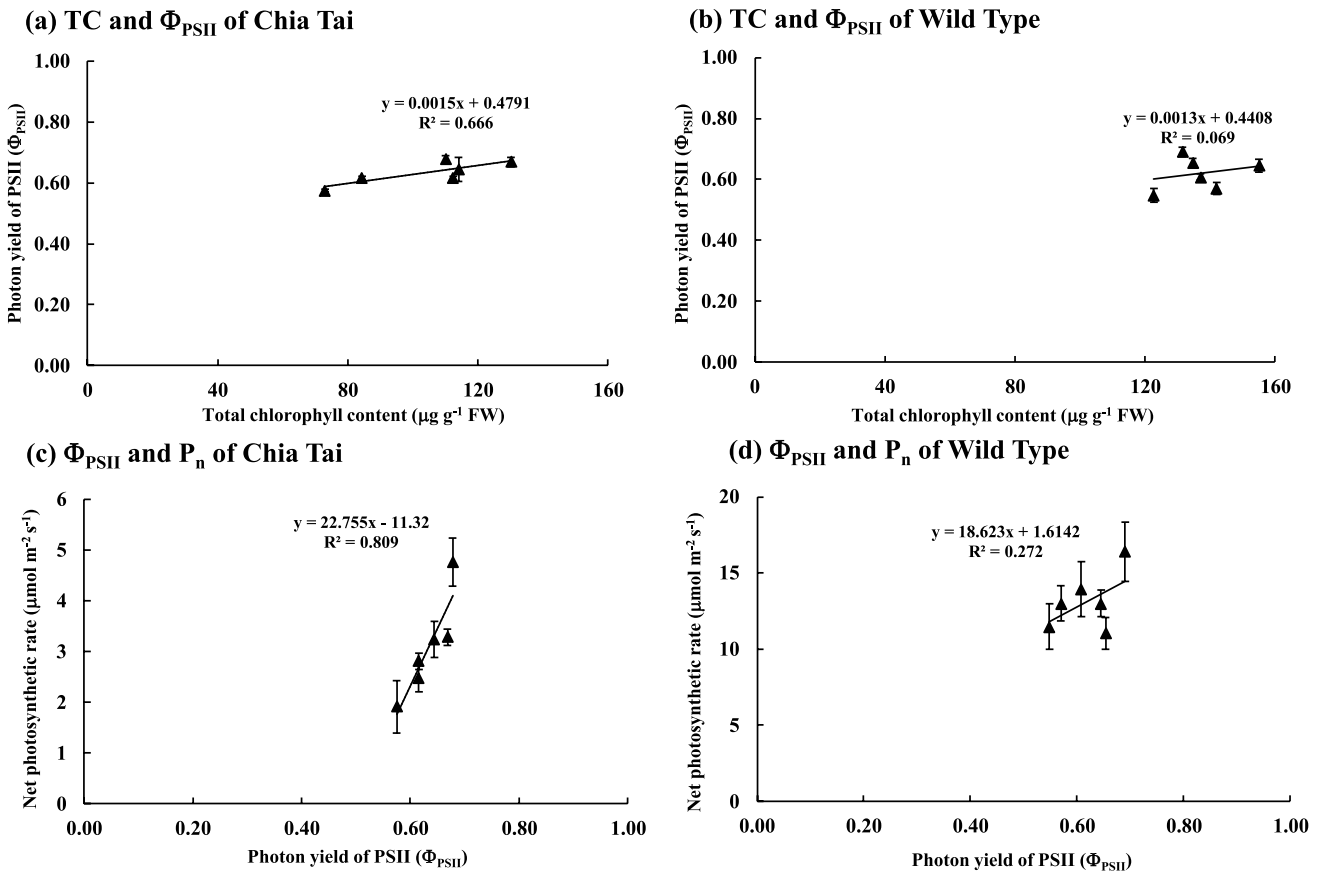

Fig. 6 Relationships between total chlorophyll content and photon yield of PSII (Φ_{PSII}) of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) (**a**) and wild type (WT cultivar, red stem) (**b**), Φ_{PSII} and net photosynthetic rate (P_n) of CT (**c**) and WT (**d**), pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days. Data presented as mean ± SE ($n = 5$)

to less toxicity in the plants as identified by high membrane stability index (MSI) (Kakade et al. 2014). In addition, Ca^{2+} enrichment has been observed with PBZ and salt treatment in water spinach. Previously, a Ca^{2+} level in water spinach was found to be alleviated by NaCl elicitor (Kitayama et al. 2019) and significantly regulated by PBZ (Kitayama et al. 2018). Moreover, Ca^{2+} in the leaf tissues of salt-treated citrus rootstock (*Citrus karna*) significantly declined, whereas it increased with the PBZ pretreatment (Sharma et al. 2011).

Photosynthetic abilities in NaCl-treated plants are good parameters to identify the plant's ability to regulate stress and resultant toxic effects. Upon PBZ pretreatment, Chl_a, Chl_b, and TC in salt-treated plants were maintained as compared to PBZ-untreated plants (Kishor et al. 2009; Khunpon et al. 2017; Forghani et al. 2018). In two Chinese bayberry genotypes (*Myrica rubra* cvs. Wangdao and Shenhong), Chl_a, Chl_b, and TC in pretreated plants exposed to NaCl treatment were sustained (Hu et al. 2017). Similarly, degradation of chlorophyll pigment and stomatal function [g_s and CO_2 assimilation (A)] was prevented in PBZ-pretreated citrus rootstock plants (*Citrus karna*) grown under salt treatment (Sharma et al. 2011). In addition, P_n, g_s, and E in salt-treated quinoa were significantly improved by PBZ pretreatment (Waqas et al. 2019). Previously, it has been reported that the degradation of Chl_b and TC levels in two water spinach cultivars under salt treatment for 14 days was prevented by pretreatment (Kitayama et al. 2018).

Previously, Na^+ enrichment in the leaf tissues of salt-treated plants damaged photosynthetic pigments, especially Chl_b and TC, and consequently negative effects on overall photosynthetic abilities have been observed (Kitayama et al. 2019). A positive relationship between TC ($Chl_a + Chl_b$) and P_n in two Chinese bayberry genotypes, Wangdao ($R^2 = 0.94$) and Shenhong ($R^2 = 0.85$), pretreated with PBZ and subsequently exposed to NaCl treatment was observed (Hu et al. 2017). Alternatively, malondialdehyde (MDA) content, a member of membrane damage compounds, was negatively related to chlorophyll stability (in Chinese bayberry; Hu et al. 2017), and TSS to maintain the water use efficiency at the cellular level was established with high regression coefficient (Kitayama et al. 2019).

Table 4 Sucrose (Suc), glucose (Gluc), and fructose (Fruc) in leaves and stems of two water spinach (*Ipomoea aquatica*) cultivars, Chia Tai or 'CT' and wild type or 'WT' seedlings, pretreated with 0, 17, and 51 M paclobutrazol (PBZ) and subsequently exposed to 0 and 200 mM NaCl for 7 days. Data in each column is represented as ± SE ($n = 5$) 0963 8757 246

Cultivar	PBZ (μM)	NaCl (mM)	Leaves (mg g^{-1} DW)			Stems (mg g^{-1} DW)		
			Suc	Gluc	Fruc	Suc	Gluc	Fruc
CT	0	0	3.35 ± 0.69f	4.59 ± 0.38d	6.37 ± 1.34c	11.42 ± 0.97e	2.30 ± 0.49d	2.32 ± 0.78b
		100	30.42 ± 1.60a (+908%)	74.49 ± 3.83a (+1623%)	14.76 ± 1.41b (+232%)	15.51 ± 1.44de	7.88 ± 0.29bc (+343%)	6.04 ± 0.68ab
	17	0	6.15 ± 0.24de	6.94 ± 0.35d	9.30 ± 0.32c	67.65 ± 2.02a	10.00 ± 0.92a	10.85 ± 2.76a
		100	12.00 ± 0.69c (+195%)	28.69 ± 1.16c (+413%)	17.90 ± 2.11b (+193%)	43.75 ± 0.68bc (−35.3%)	11.43 ± 0.46a	6.10 ± 0.14ab
	51	0	6.27 ± 0.17de	4.96 ± 0.28d	7.37 ± 0.62c	48.87 ± 2.73ab	6.04 ± 0.78c	2.94 ± 0.45b
		100	20.61 ± 0.53b (+329%)	52.96 ± 0.33b (+1068%)	15.08 ± 0.88b (+205%)	23.50 ± 2.56de (−51.9%)	9.58 ± 0.85a (+159%)	4.76 ± 0.42b
WT	0	0	6.62 ± 0.81de	29.24 ± 1.39c	11.98 ± 2.11bc	21.53 ± 1.61de	1.85 ± 0.10d	4.18 ± 0.17b
		100	1.87 ± 0.11 g (−71.8%)	10.63 ± 1.59d (−63.7%)	16.08 ± 0.92b	12.60 ± 0.59e	2.09 ± 0.37d	3.27 ± 0.51b
	17	0	3.82 ± 0.38f	25.14 ± 0.86c	8.96 ± 0.82c	24.26 ± 2.75de	1.99 ± 0.22d	8.46 ± 0.46ab
		100	2.25 ± 0.20 g (−41.1%)	8.62 ± 1.18d (−65.7%)	11.01 ± 0.56bc	16.82 ± 0.97de	2.04 ± 0.53d	3.89 ± 1.33b
	51	0	4.93 ± 0.46ef	7.68 ± 0.49d	13.21 ± 1.5bc	36.42 ± 1.73 cd	1.11 ± 0.10d	3.85 ± 0.12b
		100	9.35 ± 0.80 cd (+190%)	8.57 ± 1.13d	26.47 ± 0.80a (+200%)	24.77 ± 2.64de	2.03 ± 0.48d	3.42 ± 0.41b

Different letters in a column represent significant differences according to Tukey's HSD test at $p \leq 0.05$

In general, TSS in *Catharanthus roseus* and total carbohydrate in *Hyoscyamus muticus* grown under salt treatment was significantly lower compared to the degree of salt concentrations and developmental stages (Fatima et al. 2015; Abdelrazik et al. 2019). Regulation of TSS enrichment in leaf tissues (sucrose and fructose) of water spinach using NaCl elicitor has been previously confirmed, and it may play a key role in major osmotic adjustment when plants are subjected to salt treatment (Kitayama et al. 2019). However, the sugar content in NaCl-treated plants was reduced when PBZ was applied as a foliar spray. In strawberry fruit, total soluble solid (% Brix) was promoted by PBZ and it was still retained in plants subjected to a 5–10-mM NaCl elicitor (Jamalian et al. 2008, 2009). In addition, reducing sugars and water-soluble carbohydrates in root and flag leaf tissues were alleviated by increasing the PBZ application and salt concentration, thereby playing a role of major osmolyte to control cellular water potential (Hajihashemi et al. 2009).

AsA in the root and leaf tissues of *Catharanthus roseus* was regulated by NaCl elicitor and PBZ treatment to function as a non-enzymatic antioxidant (Jaleel et al. 2007). Moreover, a peak of AsA was observed in the strawberry fruit (58 mg per 100-ml juice) by mild NaCl and PBZ elicitors (Jamalian et al. 2008, 2009). Under salt treatment, AsA in tomatoes with overexpression of dehydroascorbate reductase (DHAR) genes was increased (Li et al. 2012). In addition, AsA in cotton was promoted by NaCl elicitor (Kumari et al. 2013). Alternatively, exogenous AsA foliar application is a way to increase endogenous AsA levels (Farouk 2011) and pistachio (Bastam et al. 2013). In water spinach, α-tocopherol was found to be enriched by NaCl elicitor (Kitayama et al. 2019). Tocopherol-deficient mutants (*vte1* and *vte4*) of *Arabidopsis* showed a low endogenous α-tocopherol, leading to a salt-sensitive response (Ellouzi et al. 2013). Exogenous α-tocopherol application in soybean strongly improved biomass even exposed to NaCl conditions (Mostafa et al. 2015; Sereflioglu et al. 2017).

5 Conclusion

Plant height retardation and less NaCl toxicity in water spinach cv. WT using PBZ and NaCl elicitors were investigated. A low amount of Na^+ in PBZ-treated plants under NaCl treatment was observed, leading to less toxicity in the root, stem, and leaf tissues of salt-treated water spinach. Photosynthetic pigment stabilization, PSII function, and net photosynthetic rate in PBZ-pretreated plants under NaCl treatment were stabilized using TSS as major osmolyte along with AsA, and tocopherol functioning as non-enzymatic antioxidants in PBZ-pretreated plants under salt treatment was demonstrated, which leads to better growth performance (Fig. 8). Moreover, enrichment of mineral nutrient and phytochemical compounds using

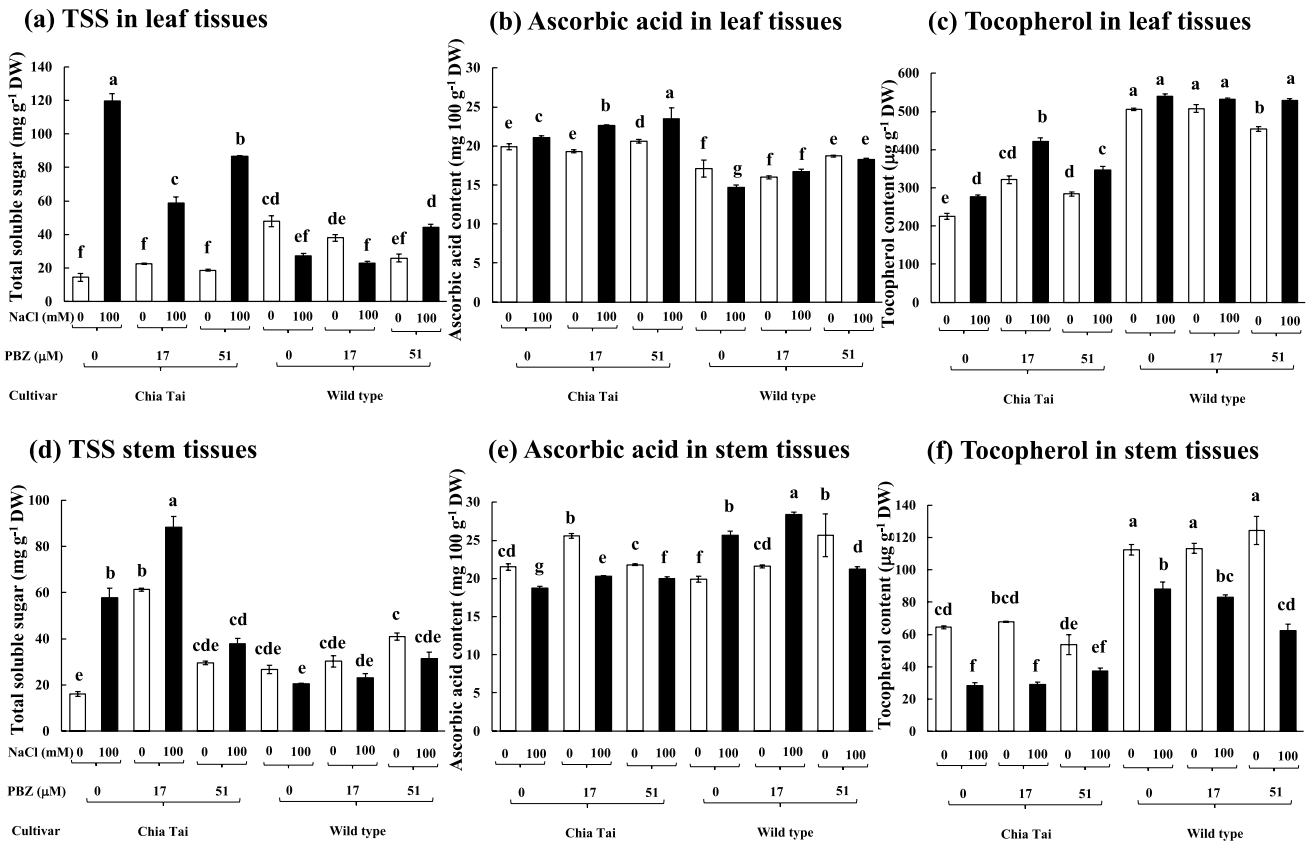

Fig. 7 Total soluble sugar (TSS) in leaf tissues (**a**), ascorbic acid in leaf tissues (**b**), tocopherol in leaf tissues (**c**), total soluble sugar (TSS) in stem tissues (**d**), ascorbic acid in stem tissues (**e**), tocopherol in stem tissues (**f**) of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) and wild type (WT cultivar, red stem) pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 0 and 100 mM NaCl for 7 days. Data presented as mean ± SE ($n = 5$). Different letters in each column show significant differences at $p \leq 0.01$, according to Tukey's HSD

Table 5 γ-Tocopherol and α-tocopherol of two water spinach (*Ipomoea aquatica*) cultivars, Chia Tai or 'CT' and wild type or 'WT' seedlings, pretreated with 0, 17, and 51 M paclobutrazol (PBZ) and subsequently exposed to 0 and 200 mM NaCl for 7 days. Data in each column is represented as ± SE ($n = 5$)

Cultivar	PBZ (μM)	NaCl (mM)	Leaves (μg g^{-1} DW) γ-Tocopherol	α-Tocopherol	Stems (μg g^{-1} DW) γ-Tocopherol	α-Tocopherol
	0	0	64.1 ± 6.9d	161.5 ± 1.3f	17.1 ± 1.8de	47.4 ± 1.2bcd
		100	111.6 ± 9.9c (+174%)	165.8 ± 8.9f	7.0 ± 2.6e	21.2 ± 2.6f (−55.3%)
	17	0	102.1 ± 2.1c	219.4 ± 8.2e	31.1 ± 2.3bc	36.7 ± 1.8cde
CT		100	186.3 ± 10.5ab (+183%)	235.7 ± 9.6e	9.8 ± 2.1e (−68.5%)	19.2 ± 0.8f
	51	0	99.9 ± 4.4c	184.3 ± 5.9f	12.0 ± 2.3e	25.2 ± 0.6ef
		100	127.3 ± 11.3c	219.7 ± 9.5e (+119%)	22.4 ± 6.1 cd (+187%)	31.2 ± 2.4def
	0	0	214.9 ± 5.0a	324.3 ± 3.2bc	42.8 ± 3.3b	69.8 ± 0.3a
		100	168.0 ± 3.0b (−21.8%)	337.0 ± 3.6b	33.6 ± 5.4bc	54.6 ± 1.8abc
	17	0	210.4 ± 2.6a	297.4 ± 7.5 cd	35.1 ± 2.8bc	48.7 ± 2.5bcd
WT		100	162.5 ± 6.2b (−22.8%)	368.7 ± 7.0a (+124%)	35.7 ± 4.1bc	47.4 ± 2.7bcd
	51	0	173.3 ± 0.8b	281.2 ± 6.8d	58.7 ± 2.3a	65.8 ± 2.9ab
		100	194.2 ± 1.7ab	334.2 ± 4.0b (+119%)	22.5 ± 3.2 cd (−61.7%)	40.0 ± 1.4bcd

Different letters in a column represent significant differences according to Tukey's HSD test at $p \leq 0.05$

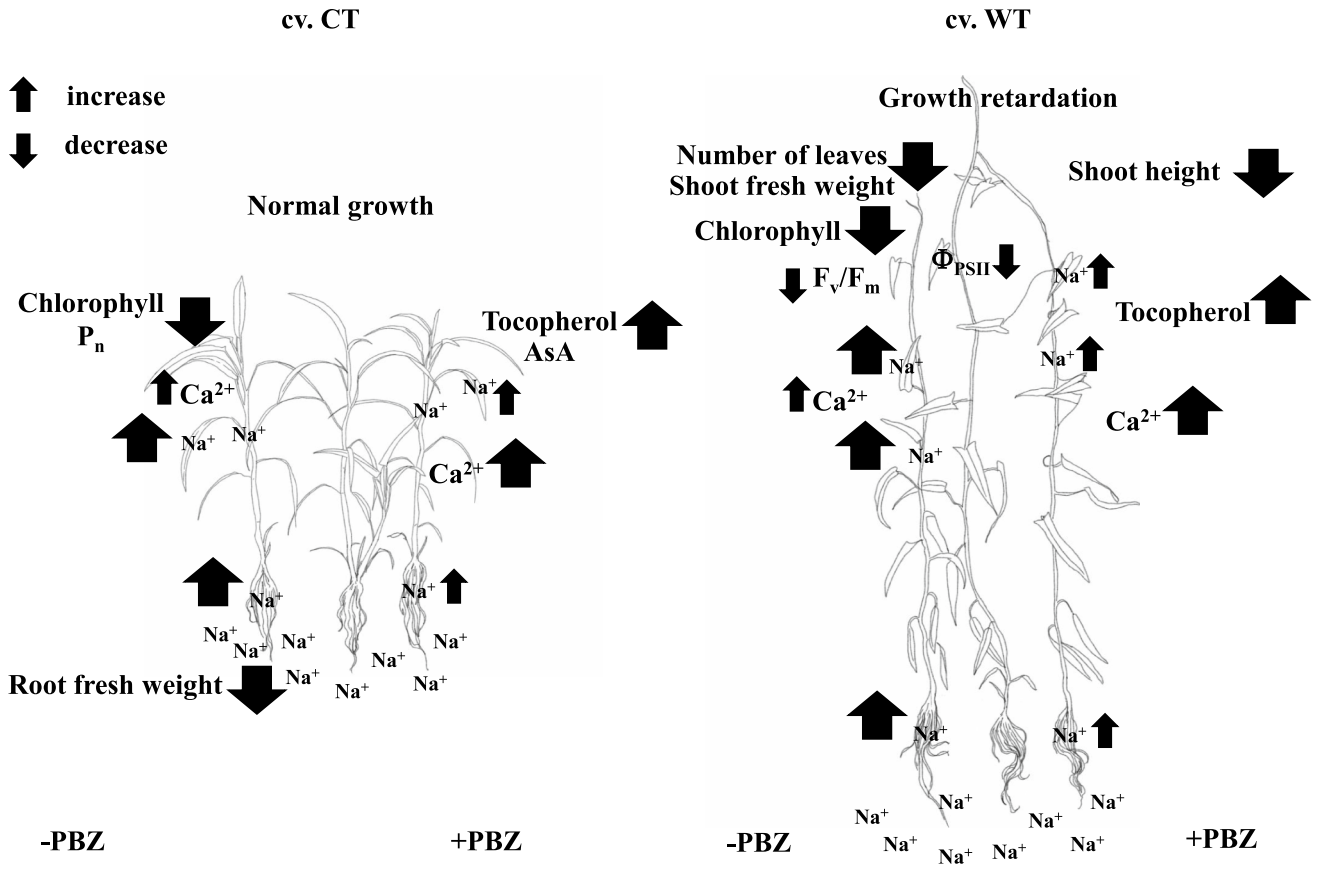

Fig. 8 A summary of evident morphological, physio-morphological, and biochemical traits of water spinach (*Ipomoea aquatica*) cvs. Chia Tai (CT, commercial cultivar; green stem) and wild type (WT cultivar, red stem) pretreated with 0, 17, and 51 M paclobutrazol (PBZ) for 7 days, and subsequently subjected to 100 mM NaCl for 7 days

PBZ and NaCl elicitors may further be implemented for large-scale production in the future.

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Author Contribution SC and MT conceived the project and designed the experiment. MK and RT performed all the experimental works, analyzed the data, and wrote the first draft of the paper. SC, TS, and KC performed the critical revision of the data, analyzed the data, and wrote the revised manuscript. SC and RT helped in writing the manuscript. SC, MT, and SKH contributed to the editing and proofreading of the final manuscript draft.

Declarations

Conflict of Interest The authors declare no competing interests.

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