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Optimal Concentration of Zinc Sulfate in Foliar Spray to Alleviate Salinity Stress in *Glycine soja*

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ABSTRACT

Zinc has previously been reported to alleviate salinity stress in plants. In this study, we monitored various biomass and chlorophyll fluorescence parameters to determine the optimum zinc sulfate concentration that can be used as foliar spray to alleviate salinity stress in *Glycine soja*. The plants subjected to a series of salinity levels (NaCl concentration of 0, 100, 200, and 300 mmol L^{-1}), applied via the nutrient solution, were sprayed with different concentrations of zinc sulfate (0, 5, 10, 15, 20, 25 µmol L^{-1}). The results showed that the biomass and chlorophyll fluorescence parameters of seedlings were significantly affected by salt stress ($P < 0.05$). However, zinc sulfate sprays helped the plants to cope with the stress condition. The zinc sulfate concentrations that helped *G. soja* to cope with the salinity stress of 100, 200, and 300 mmol L^{-1} were 15 to 20, 15 to 20, and 10 to 20 µmol L^{-1}, respectively. Lower zinc concentration was ineffective in alleviating stress and higher zinc concentration inhibited plant growth because of toxicological damage to plants. The zinc sulfate spray of 15 µmol L^{-1} was found to be the most appropriate at all salinity stress levels. The growth measurements such as true leaves part and dry weight of total seedlings were in agreement with the chlorophyll fluorescence parameters, indicating a visible enhancement of leaf photosynthetic activity at 10-20 µmol L^{-1} zinc concentrations.

Keywords: Biomass, Chlorophyll fluorescence parameters, Nutrients, Photosynthetic activity, Salt tolerance, Soybean.

INTRODUCTION

Soybean [*Glycine max* (L) Merr.] is the most important grain legume crop that is used as an oil and protein source throughout the world (Keyser and Li, 1992). Due to domestication and selective plant breeding, cultivated soybean has lost much of its genetic variability (Concibido *et al*., 2003). *Glycine soja* (wild soybean) is an annual self-pollinating plant and is believed to be ancestor of cultivated soybean (Liu *et al*., 2010). It is widely distributed throughout the world including the fareast of Russia, Korean peninsula, Japan, and China (Wang and Takahata, 2007). As an herbaceous plant with outcrossing rates of 2.4–19.0%, it has been utilized as an important genetic resource for improving soybean (Kiang *et al*., 1992, Fujita *et al*., 1997, Wang *et al*., 2008). In fact, QTLs for resistance to important pest and diseases as well as for enhanced yield have been successfully transferred from wild soybean to the cultivated species (Concibido *et al*., 2003).

Soil salinity is one of the major environmental factors that limit plant growth and yield in many areas of the world (Munns, 2002; Geilfus *et al*., 2010; Mantri *et al*., 2012). Plants respond to salinity stress with

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biochemical and physiological changes in order to maintain their photosynthetic activity and retain water despite the high external osmotic pressure (Ashraf and Harris, 2004, Du *et al*., 2010). Tavakkoli *et al.* (2010) have recently shown that the reduction in growth of faba bean under salinity stress is chiefly caused by Cl^- toxicity. It has been reported that Na^+ and Cl^- toxicity can be alleviated by supplementing the soil with zinc (Alpaslan *et al*., 1999; Tavallali *et al*., 2010; Aregheore, 2012). Zinc (Zn) is an essential nutrient for optimum growth of crops, and plays critical role in protecting vital cellular components such as chlorophyll by preventing their oxidation (Cherif *et al*., 2010; Cakmak, 2000; Zago and Oteiza, 2001). However, excessive zinc in plants could disrupt the metabolic processes such as transpiration and photosynthesis, which could result in reduced growth because of restricted root development and leaf chlorosis (Bonnet *et al*., 2000; Rout and Das, 2003; Lingua *et al*., 2008; Andrade *et al*., 2009; Wang *et al*., 2009). Leaf chlorosis is mainly due to excessive zinc that inhibits chlorophyll biosynthesis, and disrupts the electron transport of photosystem II (Chaloub *et al*., 2005, Dhir *et al*., 2008).

Different methods of zinc application have been used to investigate the importance of zinc in alleviating salinity stress condition. These include soil application of zinc sulfate (Verma and Neue, 1984), treatment of seedlings with zinc sulfate before transplanting (Alpaslan *et al*., 1999; Tzortzakis, 2010), and foliar spray of zinc sulfate (Yildirim *et al*., 2008). Whilst all studies have found zinc to relieve symptoms of salt stress, continuous soil application of large amounts of zinc sulfate is not recommended as it may result in zinc accumulation in the soil, which is undesirable. Further, treatment of seedlings with zinc sulfate can only help plants tolerate salt stress for a short period. In comparison, foliar application of zinc sulfate is more feasible as small amounts of zinc can be sprayed on the plants when required. Currently, there is no data on the optimal zinc concentration that can be used as a foliar spray in soybean to alleviate salinity stress. In one recent study, using hydroponic setting, 20 µmol L^{-1} zinc was shown to enhance the leaf chlorophyll and carotenoid content of *G. max* and *G. soja* seedlings under salt stress (Qu *et al*., 2009). Therefore, the objective of this study was to determine the optimum concentration of zinc that can be used in a foliar spray to alleviate salinity stress in *G. soja*. The assessment was supported by biometric observations and measurement of chlorophyll fluorescence.

MATERIALS AND METHODS

Plants Material and Growth Conditions

Seeds of *Glycine soja* were collected from the North Mountain area of Jinhua city (29°7′ N, 119°35′ E, 1,128 m in altitude, Zhejiang, China), air dried and stored in paper seed packets at room temperature (15–20°C). Mature seeds were sterilized with 10% hydrogen peroxide for 25 minutes and treated with 98% concentrated sulfuric acid to soften the seed coat. The seeds were subsequently washed with sterile distilled water and were sown in sand culture to germinate. The seedlings were transplanted into 15 cm diameter pots (five plants per pot) filled with sands. For the experiment, the pots were divided into four groups of six pots per group. The seedlings were watered with distilled water for a week. They were grown in a growth chamber at 25/20°C day/night temperature and 70/90% day/night relative humidity. Eight days after transplantation, sodium chloride (NaCl) was added to the irrigation water in different concentrations of 0, 100, 200, 300 mmol L^{-1} (four concentrations of NaCl; one for each group). At the same time, the six pots in each group were sprayed with different concentrations (0, 5, 10, 15, 20, 25 µmol L^{-1}) of zinc sulfate. All treatments were conducted in five biological replications.

Seedlings Biomass Measurement

The biomass was measured in separate segments as showed in Figure 1. Seedlings

were cut into different parts and fresh weight (FW) and dry weight (DW) of roots, cotyledon, and true leaves part (TLP), and total seedlings (TS) were recorded. The length and diameter of hypocotyls and epicotyls were also measured.

Chlorophyll Fluorescence Measurements

A MINIPAM (pulse-amplitude modulation) fluorometer (*WALZ*, Effeltrich, Germany) was used in this study as reported by Zheng *et al*. (2010) and Jiang *et al*. (2013). The samples were kept in the dark for 30 minutes before measuring *Fv/Fm*. Yield, *qP*, *NPQ*, and *ETR* of chlorophyll fluorescence were obtained over a range of *PAR* values from 0 to 1,800 µmol m^{-2} s^{-1} and light-adapted for nearly 10 minutes prior to measurements. The values were calculated with the Fluorescence Monitoring System (FMS) according to Genty *et al.* (1989). Measurements were replicated three times, using three plants each time.

Data Analysis

The experiments were conducted in a completely randomized block design with three replicates. The data was analyzed using SPSS version 17.0 (SPSS lnc., Chicago, USA). One-way ANOVA was performed on the mean±SD values at 95% significance. Duncan's multiple-range test was subsequently used and the differences between variables were considered significant set at $P< 0.05$.

RESULTS

Leaf Morphology

Leaf morphology characteristics under different NaCl stress were significantly affected by different concentrations of zinc (Figure 2). Under all salt treatments, the true leaves grown with zinc spray of 15 µmol L^{-1} were found to be healthier than leaves

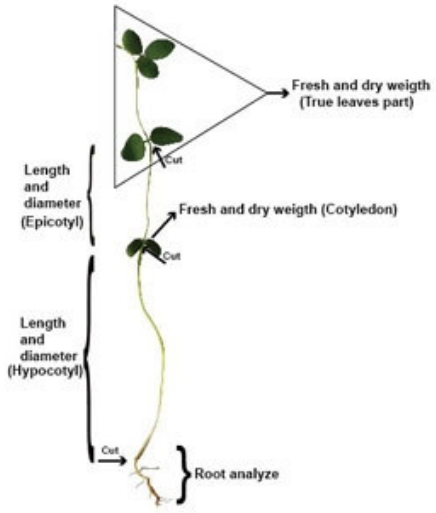

Figure 1. Schematic diagram of biomass measurement.

sprayed with other zinc concentrations. However, there was no significant difference in appearance of cotyledons. Under 300 mmol L^{-1} NaCl treatment, the leaves withered and dropped at zinc concentrations of 20 and 25 µmol L^{-1}, indicating toxic effects of high NaCl combined with high zinc concentration.

Effects of Zinc on Biomass of Seedlings

The biomass values of seedlings under different treatments are shown in (Table 1-2). The length or diameter of hypocotyls and epicotyls were significantly affected by zinc

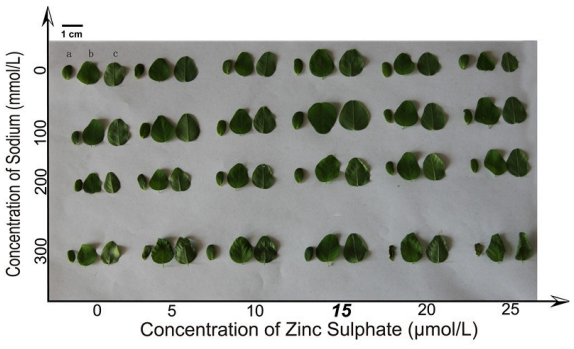

Figure 2. Leaves of *Glycine soja* seedlings sprayed with different zinc concentrations under various levels of NaCl stress. (a) Cotyledon; (b) Front surface of true leaf, (c) Back face of true leaf.

Table 1. Effects of zinc sprays on the seedling biomass under NaCl concentration of 0 and 100 mmol L^{-1} for 7 days.[a]

NaCl concentration	Plant part	Parameter	Zinc treatments (μmol L^{-1})				
			0	5	10	15	20	25
(0 mmol L^{-1})	Hypocotyl	Length[b]	88.01 ± 6.26 ab	87.26 ± 15.33 ab	**95.20 ± 7.34 a**	88.45 ± 4.95 ab	74.09 ± 9.32 b	87.33 ± 8.10 ab
		Diameter[b]	0.95 ± 0.18 a	1.03 ± 0.13 a	1.04 ± 0.11 a	1.05 ± 0.13 a	0.89 ± 0.18 a	0.84 ± 0.17 a
	Epicotyl	Length	69.61 ± 6.45 a	57.22 ± 10.16 a	52.89 ± 17.16 a	55.56 ± 13.37 a	54.74 ± 5.73 a	50.17 ± 10.13 a
		Diameter	0.55 ± 0.04 bc	0.59 ± 0.09 abc	**0.70 ± 0.09 a**	0.67 ± 0.06 ab	0.49 ± 0.05 cd	0.37 ± 0.10 d
	Cotyledon	*FW*[c]	27.67 ± 1.88 a	24.37 ± 13.44 a	27.17 ± 6.41 a	28.13 ± 9.27 a	21.63 ± 0.51 a	20.53 ± 5.51 a
		DW[c]	1.87 ± 0.25 c	2.53 ± 0.50 abc	2.60 ± 0.53 ab	**2.80 ± 0.20 a**	2.00 ± 0.36 bc	2.67 ± 0.15 ab
	TLP[d]	*FW*	28.03 ± 7.08 ab	28.40 ± 6.67 ab	22.73 ± 10.90 b	**35.90 ± 5.03 a**	37.57 ± 4.14 a	21.83 ± 2.35 b
		DW	3.13 ± 0.96 bc	3.47 ± 0.61 abc	4.47 ± 0.99 ab	**4.77 ± 0.87 a**	4.90 ± 1.00 a	2.87 ± 0.32 c
	Root	*FW*	35.33 ± 7.54 a	34.00 ± 6.41 a	36.07 ± 10.51 a	45.80 ± 22.06 a	25.07 ± 1.26 a	25.40 ± 3.35 a
		DW	2.47 ± 0.25 abc	2.67 ± 0.25 ab	2.13 ± 0.15 bc	**2.80 ± 0.20 a**	2.00 ± 0.61 c	2.00 ± 0.26 c
	TS[e]	*FW*	143.93 ± 32.43 a	142.50 ± 32.03 a	150.50 ± 44.46 a	152.47 ± 26.36 a	134.90 ± 13.64 a	125.90 ± 4.47 a
		DW	11.47 ± 0.32 bc	15.07 ± 1.86 a	**15.80 ± 0.10 a**	13.27 ± 1.97 ab	13.87 ± 1.29 ab	10.33 ± 2.06 c
(100 mmol L^{-1})	Hypocotyl	Length[b]	88.01 ± 6.26 ab	87.26 ± 15.33 ab	**95.20 ± 7.34 a**	88.45 ± 4.95 ab	74.09 ± 9.32 b	87.33 ± 8.10 ab
		Diameter[b]	0.95 ± 0.18 a	1.03 ± 0.13 a	1.04 ± 0.11 a	1.05 ± 0.13 a	0.89 ± 0.18 a	0.84 ± 0.17 a
	Epicotyl	Length	69.61 ± 6.45 a	57.22 ± 10.16 a	52.89 ± 17.16 a	55.56 ± 13.37 a	54.74 ± 5.73 a	50.17 ± 10.13 a
		Diameter	0.55 ± 0.04 bc	0.59 ± 0.09 abc	**0.70 ± 0.09 a**	0.67 ± 0.06 ab	0.49 ± 0.05 cd	0.37 ± 0.10 d
	Cotyledon	*FW*[c]	27.67 ± 1.88 a	24.37 ± 13.44 a	27.17 ± 6.41 a	28.13 ± 9.27 a	21.63 ± 0.51 a	20.53 ± 5.51 a
		DW[c]	1.87 ± 0.25 c	2.53 ± 0.50 abc	2.60 ± 0.53 ab	**2.80 ± 0.20 a**	2.00 ± 0.36 bc	2.67 ± 0.15 ab
	TLP[d]	*FW*	28.03 ± 7.08 ab	28.40 ± 6.67 ab	22.73 ± 10.90 b	**35.90 ± 5.03 a**	37.57 ± 4.14 a	21.83 ± 2.35 b
		DW	3.13 ± 0.96 bc	3.47 ± 0.61 abc	4.47 ± 0.99 ab	**4.77 ± 0.87 a**	4.90 ± 1.00 a	2.87 ± 0.32 c
	Root	*FW*	35.33 ± 7.54 a	34.00 ± 6.41 a	36.07 ± 10.51 a	45.80 ± 22.06 a	25.07 ± 1.26 a	25.40 ± 3.35 a
		DW	2.47 ± 0.25 abc	2.67 ± 0.25 ab	2.13 ± 0.15 bc	**2.80 ± 0.20 a**	2.00 ± 0.61 c	2.00 ± 0.26 c
	TS[e]	*FW*	143.93 ± 32.43 a	142.50 ± 32.03 a	150.50 ± 44.46 a	152.47 ± 26.36 a	134.90 ± 13.64 a	125.90 ± 4.47 a
		DW	11.47 ± 0.32 bc	15.07 ± 1.86 a	**15.80 ± 0.10 a**	13.27 ± 1.97 ab	13.87 ± 1.29 ab	10.33 ± 2.06 c

[a] Values represent mean±SD determined from three replicates. Means followed by the same letter within a row are not significantly different at P= 0.05 according to Duncan's multiple-range test. The maximum calculated values that were significantly high are in **bold** font; [b] Unit of length and diameter is millimeter (mm); [c] *FW* and *DW* stand for fresh weight and dry weight, respectively, in milligram (mg); [d] TLP stands for true leaves part, [e] TS stands for total seedlings.

Table 2. Effects of zinc sprays on the seedling biomass under NaCl concentration of 200 and 300 mmol L^{-1} for 7 days.[a]

NaCl concentration	Plant part	Parameter	Zinc treatments (µmol L^{-1})				
			0	5	10	15	20	25
(200 mmol L^{-1})	Hypocotyl	Length[b]	63.71 ± 20.09 a	71.09 ± 13.81 a	65.35 ± 6.15 a	74.49 ± 13.03 a	63.66 ± 5.27 a	69.40 ± 8.34 a
		Diameter[b]	0.75 ± 0.05 a	0.72 ± 0.11 a	0.71 ± 0.07 a	0.81 ± 0.06 a	0.71 ± 0.12 a	0.73 ± 0.26 a
	Epicotyl	Length	50.10 ± 6.01 ab	50.84 ± 9.26 ab	44.18 ± 6.22 b	**63.88 ± 11.87 a**	52.05 ± 7.68 ab	57.65 ± 4.94 ab
		Diameter	0.50 ± 0.11 a	0.50 ± 0.11 a	0.53 ± 0.12 a	0.62 ± 0.21 a	0.45 ± 0.07 a	0.42 ± 0.09 a
	Cotyledon	*FW*[c]	18.40 ± 7.79 a	19.80 ± 6.67 a	27.20 ± 12.63 a	32.87 ± 4.58 a	24.47 ± 3.75 a	25.50 ± 7.02 a
		DW[c]	2.20 ± 0.10 a	1.97 ± 0.15 a	2.33 ± 0.49 a	2.50 ± 0.46 a	2.43 ± 0.45 a	2.40 ± 0.17 a
	TLP[c]	*FW*	31.03 ± 16.35 ab	38.03 ± 17.64 ab	47.83 ± 8.21 ab	**52.03 ± 2.25 a**	38.43 ± 2.85 ab	28.90 ± 10.91 b
		DW	2.70 ± 0.53 d	5.60 ± 1.82 abc	7.13 ± 1.65 ab	**6.60 ± 0.20 a**	4.40 ± 0.78 cd	4.53 ± 0.93 bcd
	Root	*FW*	331.50 ± 9.00 b	34.77 ± 9.99 ab	38.07 ± 18.47 ab	56.13 ± 6.57 ab	37.13 ± 6.57 ab	33.57 ± 8.76 b
		DW	2.97 ± 0.65 ab	2.10 ± 0.10 b	**3.27 ± 0.64 a**	2.30 ± 0.10 b	2.43 ± 0.47 ab	2.87 ± 0.42 ab
	TS[c]	*FW*	138.60 ± 48.67 b	165.60 ± 24.25 ab	189.87 ± 69.10 ab	**223.43 ± 39.35 a**	163.80 ± 15.52 ab	148.97 ± 38.16 ab
		DW	12.63 ± 1.21 b	16.37 ± 3.01 ab	16.10 ± 3.35 ab	**17.10 ± 0.10 a**	13.03 ± 1.07 ab	14.23 ± 2.06 ab
(300 mmol L^{-1})	Hypocotyl	Length[b]	90.28 ± 14.04 a	74.50 ± 17.78 a	75.60 ± 6.97 a	76.13 ± 9.02 a	85.71 ± 28.14 a	61.68 ± 1.35 a
		Diameter[b]	0.73 ± 0.21 ab	0.54 ± 0.12 b	0.78 ± 0.00 ab	**0.91 ± 0.22 a**	0.71 ± 0.17 ab	0.61 ± 0.15 ab
	Epicotyl	Length	51.96 ± 15.52 a	53.12 ± 13.17 a	55.30 ± 7.84 a	56.22 ± 14.72 a	40.45 ± 11.09 a	56.79 ± 8.70 a
		Diameter	0.51 ± 0.10 a	0.51 ± 0.08 a	0.60 ± 0.18 a	**0.61 ± 0.08 a**	0.43 ± 0.08 ab	0.28 ± 0.10 b
	Cotyledon	*FW*[c]	20.70 ± 8.97 a	24.73 ± 8.46 a	17.33 ± 9.21 a	19.87 ± 4.50 a	9.63 ± 3.48 a	8.90 ± 9.01 a
		DW[c]	**2.70 ± 0.72 a**	2.33 ± 0.15 abc	1.80 ± 0.26 bc	1.67 ± 0.12 c	1.83 ± 0.15 bc	2.47 ± 0.31 ab
	TLP[c]	*FW*	9.53 ± 0.81 a	13.70 ± 7.82 a	17.07 ± 7.26 a	20.67 ± 5.37 a	12.97 ± 9.23 a	8.97 ± 8.94 a
		DW	1.00 ± 0.10 d	1.53 ± 0.38 cd	**11.63 ± 2.21 a**	4.20 ± 0.95 b	3.03 ± 0.15 bcd	3.70 ± 0.85 b
	Root	*FW*	27.40 ± 1.65 c	30.53 ± 8.10 bc	38.87 ± 9.11 abc	45.53 ± 1.22 ab	51.90 ± 13.99 a	**51.97 ± 6.79 a**
		DW	1.10 ± 0.20 a	1.20 ± 0.36 a	2.13 ± 0.15 a	1.77 ± 0.25 a	2.40 ± 0.36 a	3.33 ± 0.91 a
	TS[c]	*FW*	136.23 ± 12.79 ab	150.23 ± 45.25 ab	**172.60 ± 29.41 a**	132.70 ± 15.26 ab	133.63 ± 29.34 ab	114.80 ± 5.47 b
		DW	7.87 ± 0.70 d	16.50 ± 3.40 b	**20.99 ± 2.63 a**	13.67 ± 1.21 bc	11.93 ± 1.21 cd	15.80 ± 3.47 c

[a] Values represent mean±SD determined from three replicates. Means followed by the same letter within a row are not significantly different at $P= 0.05$ according to Duncan's multiple-range test. The maximum calculated values that were significantly high are in **bold** font; [b] Unit of length and diameter is millimeter (mm); [c] *FW* and *DW* stand for fresh weight and dry weight, respectively, in milligram (mg); [d] TLP stands for true leaves part, [e] TS stands for total seedlings.

concentration in the range of 10 to 15 µmol L^{-1} at all NaCl treatments ($P< 0.05$). Significant impacts on dry weight (DW) values of root and total seedlings (TS) were also found. Dry weight values of root (2.80 mg±0.20) and cotyledon (2.80 mg±0.20) under 15 µmol L^{-1} zinc concentration were significantly ($P< 0.05$) higher than other zinc treatments when seedlings grew in the absence of salt (0 mmol L^{-1}), while *DW* value of TS (15.80 mg±0.10) was significantly ($P< 0.05$) affected by zinc concentration of 10 µmol L^{-1}. Under 100 mmol L^{-1} NaCl concentration treatment, there was no difference in *DW* value of root in all zinc treatments. However, the *DW* value of TS (19.73 mg±1.52) showed significant differences ($P< 0.05$) in the 15 µmol L^{-1} zinc treatment (Table 1). In addition, both *DW* value of root (3.27 mg±0.64) and TS (17.10 mg±0.10) at NaCl concentration of 200 mmol L^{-1} were significantly influenced by zinc concentration of 15 µmol L^{-1}, whereas both fresh weight (FW) value and *DW* value of cotyledon were not significantly ($P> 0.05$) affected by different zinc treatments (Table 2). Under NaCl concentration of 300 mmol L^{-1} treatment, *DW* value of TS (20.99 mg±2.63) was significantly high under zinc concentration of 10 µmol L^{-1} (Table 2). Moreover, zinc concentration of 15 µmol L^{-1} also significantly ($P< 0.05$) affected fresh weight (FW) and *DW* value of true leaves part (TLP) at 0, 100, and 200 mmol L^{-1} NaCl treatments (Tables 1-2). (Table 2) shows that 10 µmol L^{-1} zinc concentration significantly ($P< 0.05$) affected *DW* value (11.63 mg±2.21) of TLP at 300 mmol L^{-1} NaCl stress.

Maximal Efficiency of PSII Photochemistry (Fv/Fm) Characteristics

Under all salt treatments, a single peak was observed for *Fv/Fm* ratio at different zinc concentrations (Figure 3; Table 3). The peak was found nearby the 15 µmol L^{-1} zinc concentration and the same trend was clearly

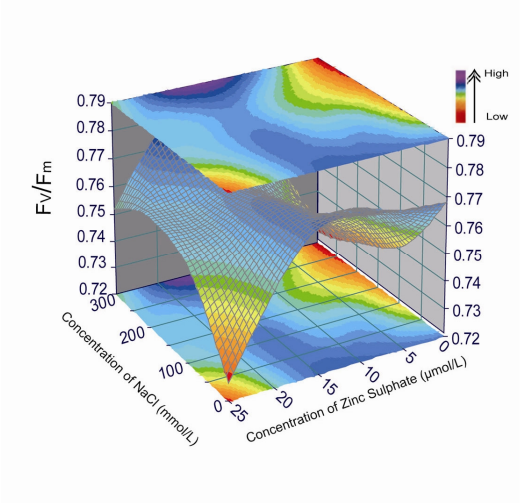

Figure 3. Three dimensional image of *Fv/Fm* response surface at different zinc and salt stress.

observed in 3D plot (Figure 3). High *Fv/Fm* ratio (purple) through the 15 µmol L^{-1} area under all different NaCl concentrations and low *Fv/Fm* ratio (chrysoidine and yellow) was found at both ends of the zinc concentrations (0 and 25 µmol L^{-1}).

Other Chlorophyll Fluorescence Measurements

In absence of salt (0 mmol L^{-1} NaCl), the non-photochemical quenching (NPQ) for 5 µmol L^{-1} zinc concentration was higher than all other treatments after the *PAR* increased to 600 µmol m^{-2} s^{-1}. However, in treatments less than 100 mmol L^{-1} NaCl concentrations, the *NPQ* for 15 µmol L^{-1} zinc concentrations established the highest position as the *PAR* increased above 1,200 µmol m^{-2} s^{-1}. In addition, we observed the highest *NPQ* value under 5 µmol L^{-1} zinc treatment in 200 mmol L^{-1} salt stress, and 20 µmol L^{-1} zinc treatment had the highest *NPQ* values under 300 mmol L^{-1} salt stress. The coefficient of non-photochemical quenching (qN) followed a similar pattern to *NPQ* (Figure 4).

Overall, there was a steady decline in the coefficient of photochemical quenching (qP) with increasing irradiance (Figure 5). In

Table 3. Effects of zinc supplement (sprays) on the *Fv/Fm* ratio in the leaves of *Glycine soja* under different NaCl stress treatments.[a]

Treatments	Fv/Fm ratio					
	Zinc concentration (µmol L^{-1})					
	0	5	10	15	20	25
0 mmol L^{-1} NaCl	0.767 ± 0.008 a	0.768 ± 0.008 a	**0.771 ± 0.005 a**	**0.771 ± 0.005 a**	0.753 ± 0.025 ab	0.726 ± 0.030 a
100 mmol L^{-1} NaCl	0.768 ± 0.023 a	0.758 ± 0.018 a	0.760 ± 0.006 a	**0.769 ± 0.010 a**	**0.769 ± 0.010 a**	0.754 ± 0.015 a
200 mmol L^{-1} NaCl	0.731 ± 0.001 d	0.745 ± 0.001 c	0.756 ± 0.007 b	**0.766 ± 0.004 a**	0.761 ± 0.005 ab	0.760 ± 0.006 ab
300 mmol L^{-1} NaCl	0.725 ± 0.004 c	0.754 ± 0.011 b	0.775 ± 0.003 ab	**0.782 ± 0.009 a**	0.769 ± 0.016 ab	0.752 ± 0.025 b

[a] Values represent means±SD determined from three replicates. Means followed by the same letter within a row are not significantly different at P= 0.05 according to Duncan's multiple-range test. The maximum calculated values are in **bold** font.

absence of salt, the highest *qP* value was found for 10 µmol L^{-1} zinc concentration. The zinc concentration required for the highest *qP* value increased with increasing salt stress. At 100 mmol L^{-1} salt concentration, the *qP* value was the highest at 15 µmol L^{-1} zinc treatment, whilst at 200 mmol L^{-1} salt level, the *qP* value was the highest at 20 µmol L^{-1} zinc concentration. However, zinc concentration required for the highest *qP* value decreased to 5 µmol L^{-1} when exposed to 300 mmol L^{-1} of salinity level (Figure 5). The changes of effective quantum yield of photochemical energy conversion in photosystem II (Φ_{PSII} or Yield, Y) was also determined for different zinc and salt concentrations. The Yield (Y) followed the same trend as *qP* (Figure 6).

The apparent electron transport rate (ETR) of photosystem II increased rapidly as the *PAR* increased for all different zinc and salt concentrations (Figure 7). Moreover, all *ETR* values peaked at the *PAR* of 600 µmol $m^{-2}s^{-1}$. The highest *ETR* values (42.03, 41.50, 37.53, 33.83) were found under 20, 15, 20, and 5 µmol L^{-1} of zinc in 0, 100, 200, and 300 mmol L^{-1} NaCl concentration salt treatments, respectively.

Characteristics of Chlorophyll Fluorescence Value under Different *PAR*

A maximum value of chlorophyll fluorescence parameter under all treatments could be identified under specific *PAR*. The highest *NPQ* and *qN* values were at the *PAR* of 1600 µmol $m^{-2}s^{-1}$, the highest *ETR* value was at 600 µmol $m^{-2}s^{-1}$, and the highest Yield value was at 0 µmol $m^{-2}s^{-1}$. In addition, the Pearson correlations between chlorophyll fluorescence parameters at these optimum *PAR* values and different salt treatments are shown in Table 4. There was a significant negative correlation (-0.594, P< 0.01) between *Fv/Fm* ratio and zinc concentration in the absence of salt, whereas the Fv/Fm ratio was positively correlated (0.805, P< 0.01) to increasing zinc concentration at 200 mmol L^{-1} salt stress.

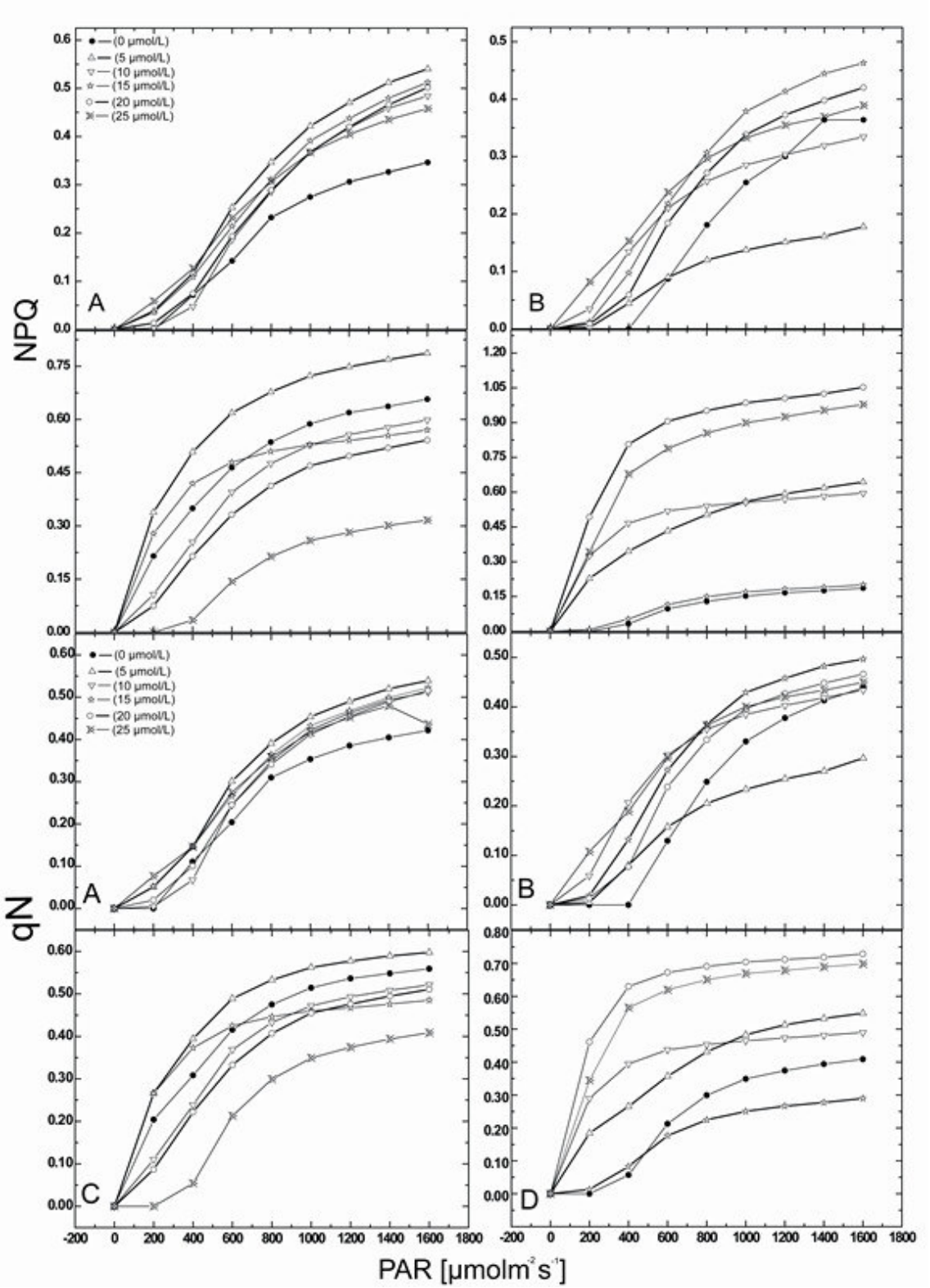

Figure 4. Changes in non-photochemical quenching (NPQ) and its coefficient (qN) in response to light irradiance at different levels of zinc treatments under the salt concentrations of 0 (A); 100 (B); (C), and 300 mmol L^{-1} (D).

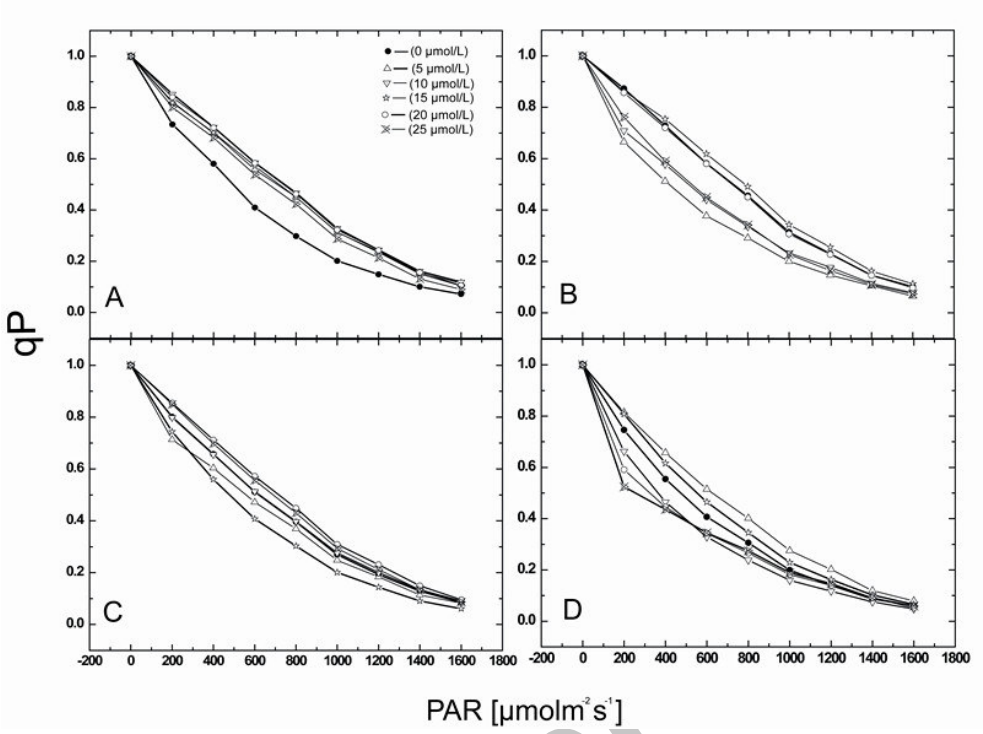

Figure 5. Changes in coefficient of photochemical quenching (qP) in response to light irradiance at different levels of zinc treatments under the salt concentrations of 0 (A); 100 (B); 200 (C), and 300 mmol L^{-1} (D).

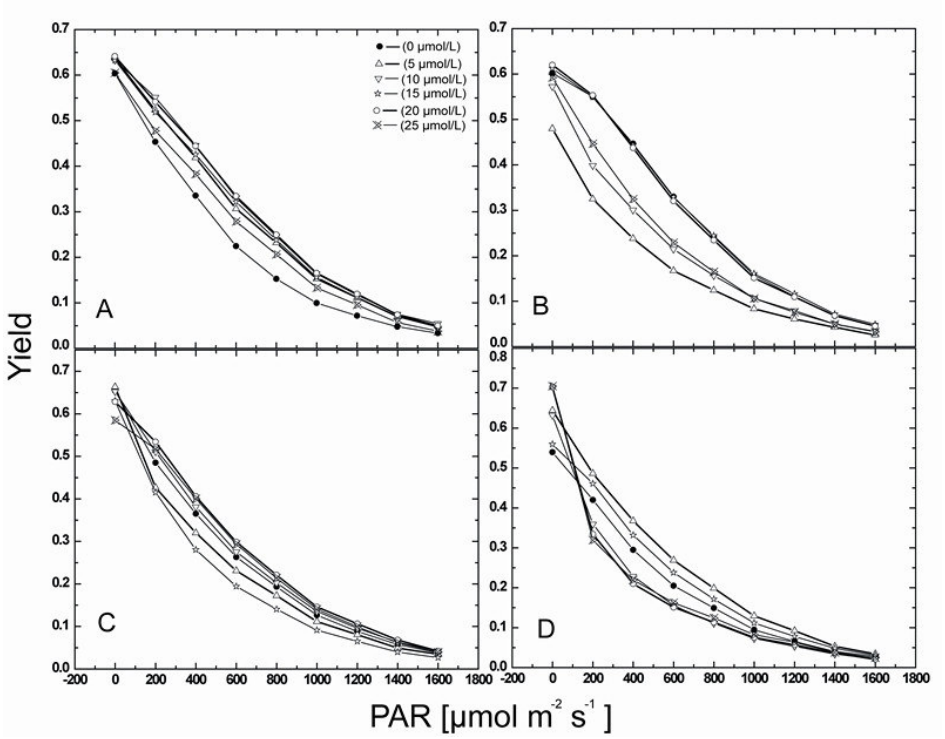

Figure 6. Changes in photochemical energy conversion (yield) in response to light irradiance at different levels of zinc treatments under the salt concentrations of 0 (A); 100 (B); 200 (C), and 300 mmol L^{-1} (D).

Table 4. The Pearson correlations between chlorophyll fluorescence parameters at optimum *PAR* values and different salt treatments.

Treatments	*Fv/Fm* ratio	*NPQ*	*qP*	*ETR*	Yield
		PAR of 1600 µmol $m^{-2}s^{-1}$	*PAR* of 200 µmol $m^{-2}s^{-1}$	*PAR* of 600 µmol $m^{-2}s^{-1}$	*PAR* of 0 µmol $m^{-2}s^{-1}$
0 mmol L^{-1} NaCl	-0.594 **	0.171	0.334	0.321	0.027
100 mmol L^{-1} NaCl	0.393	0.330	0.082	0.045	0.335
200 mmol L^{-1} NaCl	0.805 **	-0.347	0.312	0.230	-0.363
300 mmol L^{-1} NaCl	0.419	0.562 *	-0.577 *	-0.382	0.565 *

* $P< 0.05$, ** $P< 0.01$.

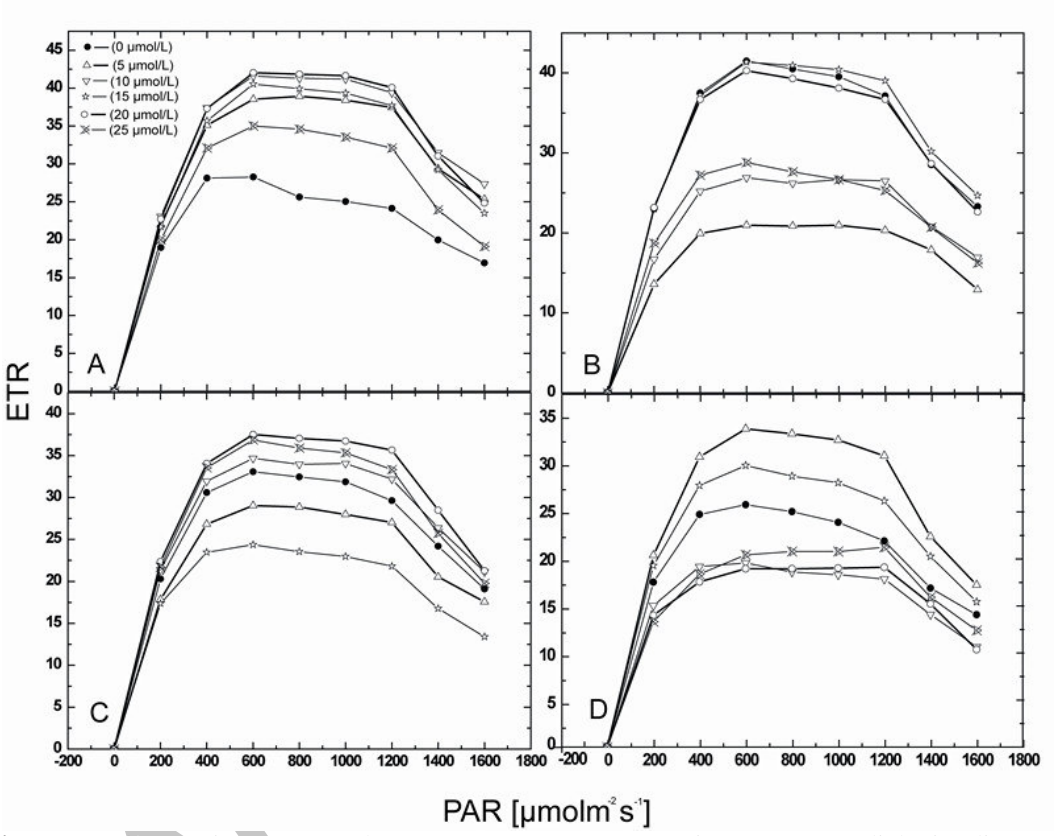

Figure 7. Changes in apparent electron transport rate (ETR) in response to light irradiance at different levels of zinc treatments under the salt concentrations of 0 (A); 100 (B); 200 (C), and 300 mmol L^{-1} (D).

Further, *NPQ* and Yield were significantly positively correlated (0.562 and 0.565, P< 0.05) with zinc concentration at 300 mmol L^{-1} salt stress, whilst the qP was negatively correlated (-0.577, P< 0.05) with zinc concentration at 300 mmol L^{-1} of salt stress.

The comparison of all chlorophyll fluorescence parameters at optimum *PAR* and different zinc and salt concentration is shown in Table 5. Although there was no significant difference (P> 0.05) in *NPQ* and *qN* values at different zinc concentrations under salt treatments of 0 and 200 mmol L^{-1}, a significant increase (P< 0.05) was observed for 15 µmol L^{-1} zinc at 100 mmol L^{-1} salt stress and 20 µmol L^{-1} zinc at 300 mmol L^{-1} salt stress. Similar results were obtained for Yield value at *PAR* of 0 µmol^{-2} s^{-1}. The Yield value of 5 µmol L^{-1} zinc concentration was significantly (P< 0.05) lower than the other zinc treatments under 100 mmol L^{-1} of salt stress. However, the

Table 5. Comparison of chlorophyll fluorescence parameters at optimum *PAR* and different concentrations of zinc and salt.[a]

Zinc concentration (µmol/L)	Chlorophyll fluorescence parameters			
	Concentration of NaCl (mmol L^{-1})			
(1) NPQ values at *PAR* of 1600 µmol $m^{-2}s^{-1}$				
	0	100	200	300
0	0.3463 a	0.3640 ab	0.6570 a	0.1863 b
5	**0.5403 a**	0.1780 b	**0.7880 a**	0.6433 ab
10	0.4840 a	0.3343 ab	0.5983 a	0.5960 ab
15	0.5123 a	**0.4630 a**	0.5703 a	0.2007 b
20	0.5017 a	0.4203 ab	0.5417 a	**1.0527 a**
25	0.4577 a	0.3890 ab	0.3160 a	0.9783 a
(2) *qN* values at *PAR* of 1600 µmol $m^{-2}s^{-1}$				
	0	100	200	300
0	0.4217 a	0.4387 a	0.5590 a	0.2827 b
5	**0.5393 a**	0.2967 a	**0.5977 a**	0.5487 a
10	0.5120 a	0.4340 a	0.5213 a	0.4900 ab
15	0.5220 a	**0.4967 a**	0.4853 a	0.2900 b
20	0.5157 a	0.4663 a	0.5107 a	**0.7287 a**
25	0.4990 a	0.4500 a	0.4087 a	0.6983 a
(3) *qP* values *at PAR* of 200 µmol $m^{-2}s^{-1}$				
	0	100	200	300
0	0.7333 b	**0.8730 a**	0.8013 a	0.7463 ab
5	0.8213 a	0.6653 b	0.7133 a	**0.8147 a**
10	**0.8520 a**	0.7090 b	0.7987 a	0.6627 abc
15	0.8177 a	0.8637 a	0.7443 a	0.8083 a
20	0.8403 a	0.8563 a	**0.8550 a**	0.5910 bc
25	0.8007 ab	0.7613 ab	0.8493 a	0.5240 c
(4) *ETR* values at *PAR* of 600 µmol $m^{-2}s^{-1}$				
	0	100	200	300
0	28.2667 b	**41.5000 a**	33.0667 a	25.7667 ab
5	38.5333 a	20.9667 b	29.0333 a	**33.8333 a**
10	41.6000 a	26.9000 b	34.6667 a	19.5667 b
15	40.5333 a	41.3667 a	24.3667 a	29.9333 ab
20	**42.0333 a**	40.3000 a	**37.5333 a**	18.9667 b
25	35.0000 ab	28.8000 ab	36.8667 a	20.4333 b
(5) Yield values at *PAR* of 0 µmol $m^{-2}s^{-1}$				
	0	100	200	300
0	0.6037 a	0.6023 a	0.6543 a	0.5397 b
5	0.6373 a	0.4797 b	**0.6633 a**	0.6440 ab
10	0.6330 a	0.5727 a	0.6527 a	0.6320 ab
15	0.6323 a	0.6123 a	0.6307 a	0.5593 b
20	**0.6413 a**	**0.6200 a**	0.6287 a	0.7027 a
25	0.6050 a	0.5920 a	0.5850 a	**0.7050 a**

[a] Values represent the mean of three variables. Values that are followed by different letters are significantly (P< 0.05) different from each other (Duncan's multiple-range test). Separate analysis was done for each column. The maximum calculated value is in **bold** font.

Yield value under zinc concentration of 25 µmol L^{-1} was significantly (P< 0.05) higher at 300 mmol L^{-1} of salt. Also, both *qP* and *ETR* values were not significantly (P> 0.05) affected at 200 mmol L^{-1} salt stress.

DISCUSSION

Salinity stress causes retardation in plant growth by decreasing length, fresh weight, and dry matter (Mantri *et al*., 2012). Water deficit caused due to high salt concentration results in abnormal change in plant morphology (Ghassemi-Golezani *et al*., 2009). In our experiments, 15 µmol L^{-1} zinc concentration was found to be the most ideal for use as a foliar spray to alleviate salinity stress in *G. soja*. For most plants, zinc is an essential component of enzymes and takes part in the synthesis of chlorophyll and other proteins (Vallee and Auld, 1990). In this study, significant differences were observed in the leaf morphology and biomass variables of seedlings sprayed with 10–15 µmol L^{-1} zinc under salt stress (Figure 2; Table 1). A significant increase in biomass, with respect to length or width of stem, true leaves part (TLP) and dry weight (DW) of total seedlings (TS) was observed by spraying the plants with 10-15 µmol L^{-1} zinc, thus indicating that proper concentration of zinc is required for dry matter accumulation and seedling growth. The positive effects of appropriate zinc concentration (10-20 µmol L^{-1}) on DW of seedling and especially newly-developed TLP under NaCl stress could be explained by the decrease in uptake of excess Na^+ and Cl^-. Also, zinc may help nutrient translocations from the aged cells to newborn cells (Rockenfeller and Madeo, 2008). Zinc may, therefore, play an important role in membrane permeability, phospholipids (P) accumulation, and scavenging free oxygen radicals. These results correlate well with the findings of Alpaslan *et al.* (1999) and Qu *et al.* (2009) who demonstrated that, in the salt affected areas, zinc application could alleviate possible Na^+ and Cl^- injury in plants.

Chlorophyll fluorescence is an important tool that has been extensively used to monitor plant health (Belkhodja *et al*., 1994; Jimenez *et al*., 1997; Sayed, 2003; Dai *et al*., 2009). The *Fv/Fm* ratio is frequently used as a stress indicator and depicts the potential yield of the photochemical reaction (Bjorkman and Demmig, 1987). Our experiment showed that the *Fv/Fm* ratio of *G. soja* was significantly affected by NaCl concentration of 200, 300 mmol L^{-1}. We also found that 15 µmol L^{-1} zinc spray was optimum for restoring the *Fv/Fm* ratio ($P< 0.05$). High *Fv/Fm* ratio represents maximal efficiency of PSII photochemistry (Lundmark *et al.*, 1998). Therefore, we conclude that salinity may reduce photosynthesis by indirect effect on the photosynthetic apparatus and the zinc concentration of 15 µmol L^{-1} could be optimal for stress relief.

The chlorophyll coefficients NPQ and qP represent the energy that cannot be utilized to transport photosynthetic electrons down to being dissipated harmlessly as heat energy from PSII antennae (Veres *et al*., 2006). Low *NPQ* value indicates that the plants efficiently utilized the energy absorbed by antenna pigments and effectively reduce the irradiance heat in PSII (Guo *et al*., 2006). The *ETR* value represents the relative quantity of electrons passing through PSII in steady-state photosynthesis (Tezara *et al*., 2003). Thus, *ETR* value reduction means loss of capture efficiency/ excitation of chlorophyll. The *qP* value is an indication of the proportion of PSII reaction centers that are open; high qP is beneficial to electron transport and PSII yield, a decrease in qP demonstrates an increase in susceptibility to metal inhibition (Mao *et al.*, 2007).

Further, under salt stress, the plants sprayed with 0 and 5 µmol L^{-1} zinc could not sustain growth. Zinc sprays of 10 to 20 µmol L^{-1} could restore the chlorophyll florescence parameter values under different salt concentration. These results are consistent with previous study where supplementation of irrigation water with low concentration of zinc was found to have protective effects against the salinity stress, whilst, high levels of zinc (25 µmol L^{-1}) combined with NaCl stress caused metal-induced inhibition of chlorophyll biosynthesis (Vallee and Auld,

1990; Qu *et al.* 2009). Excessive zinc also disrupts the chloroplast envelope and thylakoid membrane system, thus affecting photosynthesis of the plant (Jin *et al*., 2006).

In natural environment, plants are always subjected to a multitude of complex stresses among which salinity stress is frequently encountered (Mantri *et al*., 2010; Rang *et al*., 2011). Alleviating the salinity stress at the seedling stage is an ideal adaptation strategy for plants. Improvement of salt tolerance by addition of nutrients has been reported in rice (Matoh *et al*., 1986), wheat (Ahmad *et al*., 1992), tomato (Al-Aghabary *et al*., 2005), and cucumber (Zhu *et al*., 2004). Zinc is a vital component of some enzymes and is an important microelement required by plants. Our results reveal that salt toxicity in *Glycine soja* seedlings can be alleviated by foliar spray of zinc. The results are consistent with the previous findings of Qu *et al.* (2009), who added zinc to hydroponic media to alleviate salinity stress in soybean.

In conclusion, salinity stress significantly affected the growth of *Glycine soja* seedlings. However, foliar zinc sprays of 10–20 µmol L^{-1} concentration were found to alleviate the stress. Zinc supplementation at 15 µmol L^{-1} improved the biomass of the seedlings as revealed by the measurement of true leaves part (TLP), and dry weight (DW) of total seedlings (TS). The results correlate well with the plant chlorophyll fluorescence parameters. Thus, 15 µmol L^{-1} zinc concentration is considered to be the optimum zinc concentration for use as foliar sprays for alleviating salinity stress of *G. soja*. Our results will help to obtain better yields of *G. soja* under salt stress. Future study will determine if these results can be extended to its close relative, soybean.

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غلظت بهینه سولفات روی در برگپاشی برای کاهش تنش شوری در *Glycine soja*

و. جیانگ، ژ. ه. سان، ح. ل. ژو، ن. مانتری، و ح. ف. لو

چکیده

پژوهش های قبلی نشان داده است که عنصر روی تنش شوری را کم میکند. در پژوهش حاضر، به منظور تعیین غلظت بهینه سولفات روی در کودی که بر برگ ها پاشیده می شود تا اثر تنش شوری را کاهش دهد ، پارامترهای مختلف مربوط به زیست توده و فلورسنس کلروفیل در گیاه سویای Glycine soja اندازه گیری شد.نخست، بوته های گیاه از طریق محلول غذایی تحت تنش غلظت های مختلف کلرید سدیم (NaCl) برابر $0, 100, 200, \text{and } 300 \text{ mmol } L^{-1}$ قرار داده شدند و سپس غلظتهای مختلف سولفات روی برابر $0, 5, 10, 15, 20, 25 \text{ µmol } L^{-1}$ بر برگها پاشیده شد. نتایج نشان داد که زیست توده و پارامترهای فلورسنس کلروفیل گیاهچه ها به طور معنی داری($P < 0.05$) تحت تاثیر تنش شوری بودند. با این وجود، پاشیدن سولفات روی برگیاه در سازگاری با تنش شوری کمک کرد. غلظت هایی از سولفات روی که در سازگاری گیاه با شوری های ۱۰۰، ۲۰۰، و۳۰۰ میلی مول در لیتر به G. soja کمک کردند، به ترتیب برابر بود با ۱۵تا ۲۰، ۱۵تا ۲۰ و ۱۰ تا۲۰ میکرومول در لیتر. غلظت های کمتر سولفات روی در برابر تنش کارآیی نداشتند و غلظت های بیشتر با ایجاد مسمومیت باعث توقف رشد می شدند. در تمام سطوح تنش شوری، غلظت ۱۵ میکرومول در لیتر سولفات روی مناسب ترین تاثیر را داشت. اندازه گیری پارامترهای رشد مانند بخش برگ های واقعی و وزن خشک کل گیاهچه ها با پارامتر های فلوروسنس کلروفیل همآهنگ بود که این امر نشانگر بهبود فعالیت فتوسنتزی برگ در غلظت های ۱۰-۲۰ میکرومول در لیتر بود.