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PROLINE TREATMENT INDUCES SALT STRESS TOLERANCE IN MAIZE (*ZEA MAYS* L. CV. SAFAID AFGOI)

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Abstract

A pot experiment was performed to assess the effect of proline as seed treatment on maize (*Zea mays* L.) under salt stress. Seeds of maize cultivar (cv.) Safaid Afgoi were soaked in different proline solutions (0.0, 0.5, 1.0, 1.5, and 2.0 mM) for 12 hours. Salt treatment (0 and 75 mM NaCl) was applied to one week old maize seedlings. Data was taken of 21 day old maize plants for the determination of different growth and physiochemical parameters. Salt stress inhibited most of the growth attributes and decreased ratio of chlorophyll *a* to chl. *b*, while increased chl. *b* and total soluble proteins and malondialdehyde (MDA) contents in maize plants. Seed treatment with proline increased shoot length significantly and chlorophyll *a* non-significantly, while all other attributes remained unaffected of maize plants under salt stress. In conclusion, proline show differential response in increasing growth by regulating different physiochemical parameters not only in different plant species but also under diverse environmental conditions.

Key words: Chlorophyll contents, Hydrogen peroxide, Maize, Proline, Soluble proteins.

Introduction

Soil salinity adversely affects crop plants throughout the world (Alagoz & Toorchi, 2018). However, plants respond to saline stress by modulating inherent mechanism to the external environment (Khan *et al.*, 2014). A strong defense system against free radicals and biosynthesis of key metabolites are the important mechanisms involved in the protection of plants from abiotic stresses (Fahramand *et al.*, 2014).

Pre-sowing seed treatment is a controlled hydration technique that allows synchronized seed germination with reduced seedling emergence time and high metabolites accumulation for osmotic adjustment (Burgass & Powell, 1984; Bradford, 1986; Bray *et al.*, 1989; Farooq *et al.*, 2006, 2009). Seed priming involves seed treatment with a variety of priming agents like seed soaking in water (hydro-priming) (Arif *et al.*, 2014), hormone priming (Atalou *et al.*, 2014), soaking in PGRs (Perveen *et al.*, 2010, 2011, 2012a, 2012b; Ratnakar & Rai, 2014) or some osmotic solution (Arif *et al.*, 2014). Pre-soaking seed treatment with gibberllic acid, (GA_3), benzyl adenine (BA), 6-furfuryladenine (kinetin) increased protein and chlorophyll contents, while decreased stress metabolites such as sugars and proline in the leaves of *Trigonella foenum-graecum* L. var. PEB (Ratnakar & Rai, 2014). Seed priming has been reported to increase growth, chlorophyll, protein and proline contents in safflower (*Carthamus tinctorius* L. cv. Safola) (Aymen *et al.*, 2014) and wheat plants under salt stress (Abbasdokhta & Edalatpisheh, 2013). However, growth and seedling establishment depend upon seed soaking duration and concentration of priming agents (Carlos & Cantliffe, 1992; Hardegree, 1998).

Compatible osmolytes such as proline has been thought to accumulate in plants under stressful environment (Kalsoom *et al*., 2016). Proline induces salt stress tolerance in crop plants by regulating gas exchange characteristics (Ali *et al.*, 2007), chlorophyll flourescence (Deivanai *et al.*, 2011), enhance antioxidant defense system (Ben Rejeb *et al.*, 2014), protect protein turnover machinery and up-regulates stress protective proteins (Ismail, 2014). Proline concentration has been shown to prove effective in inducing salt stress tolerance in various studies. For example, 60 mmol proline proved effective in sunflower (*Helianthus annuus* L.) (Khan *et al.*, 2014), 50 mg L^{-1} in wheat (Ismail, 2014), 5 mM in rice (Hasanuzzaman *et al.*, 2014) and 100 mM in wheat (Talat *et al.*, 2013). Pre-sowing seed treatment with different concentration of proline particularly 10 mM increased salinity stress tolerance in tomato (Kaur & Gupta, 2018). However, effect of proline on different genotypes of same species may vary significantly (Garg, 2003).

Maize is a cereal food crop for human intake and widely used as feed for livestock. Inspite of its wide use throughout the world maize is sensitive to salt stress (Parvaiz, 2014). It has been reported that seed treatment with some chemicals can improve abiotic stress tolerance in maize plants (Anosheh *et al.*, 2011). Due to protective functions against abiotic stresses proline has been used exogenously as foliar spray, however, there is little work published about proline application as pre-sowing seed treatment under salt stress. So, the prime objective of current study will be to explore the role of proline as seed treatment in changing various growth and physiochemical attributes such as photosynthetic pigments, relative membrane permeability (%), attributes of oxidative stress (H_2O_2 and MDA contents), free proline, total soluble proteins and nitrate reductase activity of maize plants under normal (0 mM NaCl) and salt stress environment (75mM NaCl).

Materials and Methods

An experiment was carried out to explore the role of proline on maize under salt stress. Seeds of maize cultivar "Safaid Afgoi'' were collected from Maize and Millets Research Institute, Yusafwala, Sahiwal (Pakistan). Sterilized (in 5 % sodium hypochlorite solution) maize seeds were soaked in 0, 0.5, 1.0, 1.5 and 2.0 mM levels of proline (Mol. Wt. 115) solutions for 12 hours and dried under shade to bring seeds to original weight. Proline-treated seeds were then sown in sand filled plastic pots. Thinning was performed to one week old seedling to maintain three plants per pot. Salt (75 mM NaCl) treatment was applied to one week old plants by gradually increasing salt level in an aliquot of 25 mM NaCl until final volume 75 mM was achieved. Data of various growth and physiochemical parameters was collected of 21-day-old maize plants. Two

plants were carefully uprooted and measured shoot and root lengths (cm) and shoot and root fresh weights (g) after washing roots with distilled water. The same plants were air-dried under shade for one week and then oven-dried at 72°C for 48 hours and root and shoot dry weights (g) measured. Carleton & Foote (1965) protocol was used for the measurement of total leaf area per plant (cm^2) using formulae maximum leaf length × leaf width multiplied by a correction factor (0.75).

Determination of membrane permeability (MP %): To 0.5 g finely chopped fresh leaves added 10 ml of distilled water. Electrical conductivity (EC_o) was recorded with the EC meter. Then samples were placed at room temperature for overnight and EC_1 was noted. Next day autoclaved for one hour and EC_2 was measured. Formulae for MP (%) was RMP (%) = $(EC_1\text{-}EC_o/EC_2\text{-}EC_o) \times 100$

Determination of biochemical parameters: The chlorophyll content in leaf tissues were determined by Arnon (1949) method, H_2O_2 contents by Velikova *et al.*, (2000) method, MDA contents by Carmak and Horst (1991) method, free proline contents by Bates *et al.*, (1973) method, protein contents by Bradford (1976) method and Nitrate reductase activity was determined by following the method of (Jaworski, 1971).

Statistical analysis

Analysis of variance (ANOVA) was performed by using computer software (Co-STAT).

Results

Fresh and dry biomass of maize plants (cultivar Safaid Afgoi) were decreased under 75 mM NaCl stress. Proline application as seed treatment did not significantly change fresh and dry weights of shoots (Fig. 1; Table 1). Application of salt stress significantly decreased fresh weight of root, while dry weight of root remained unchanged. Seed treatment with proline did not alter fresh and dry matter of maize plants (Fig. 1; Table 1). Salt stress markedly decreased shoot length of maize plants, while pre-sowing seed treatment with proline significantly increased shoot length and this increase was consistent with the increasing level of proline (Fig. 1; Table 1). Application of both salt stress and seed treatment with proline did not change root length of maize plants. Total leaf area per plant decreased under salt stress, while seed treatment with proline did not show any positive effect on total leaf area $plant^{-1}$ (Fig. 1; Table 1).

Application of salt stress and pre-sowing seed treatment with proline did not change chlorophyll *a* contents (Fig. 1; Table 1), however, chlorophyll *b* contents significantly increased under salt stress, while remained unaffected under different proline concentrations (Fig. 2; Table 1). Chlorophyll *a/b* ratio was significantly increased under salt stress but remained unchanged under pre-sowing seed treatment with different levels (Fig. 2; Table 1).

Membrane permeability (%) and contents of hydrogen peroxide (H_2O_2) did not change under saline conditions of maize plants. Effect of proline as seed treatment was non-significant on both these attributes. Malondialdehyde (MDA) contents significantly increased under salt stress, however, exogenous application of proline as seed treatment did not change MDA content significantly (Fig. 2; Table 1).

Free proline contents and nitrate reductase activity did not change either under salt stress (75 mM NaCl) or seed treatment with different proline concentrations. However, salt stress of 75 mM NaCl significantly increased total soluble protein contents but exogenous proline as seed treatment did not alter protein contents significantly (Fig. 2; Table 1).

Table 1. Statistical analysis of different parameters of growth, chlorophyll contents, permeability of membrane (%), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), free proline, total soluble proteins and nitrate reductase activity (NRA) of maize plants seeds of which treated with proline under salt stress.

Source of variation	df	Shoot f. wt.	Shoot dry wt.	Root f. wt.	Root dry wt.
Salinity (S)	1	0.779*	0.0206**	2.7*	0.0057ns
Proline (P)	4	0.297ns	0.0025ns	0.1351ns	0.0033ns
S x P	4	0.0101ns	0.0012ns	0.1722ns	0.00085ns
Error	20	0.112	0.0018	0.4407	0.005
Source of variation	**df**	**Shoot length**	**Root length (cm)**	**leaf area/plant(cm^2)**	**Chlorophyll *a***
Salinity (S)	1	30.40**	12.67ns	3715.4*	0.0097ns
Proline (P)	4	11.77**	8.31ns	1831.76ns	0.0011ns
S x P	4	0.867ns	3.72ns	424.87ns	0.0015ns
Error	20	2.234	4.935ns	655.30	0.0025
Source of variation	**df**	**Chlorophyll *b***	**Chl. *a/b* ratio**	**RMP (%)**	**H_2O_2**
Salinity (S)	1	0.043*	3.033*	19.80ns	0.024ns
Proline (P)	4	0.004ns	0.463ns	86.42ns	0.224ns
S x P	4	0.0174ns	1.113ns	36.97ns	0.2291
Error	20	0.0089	0.565	73.51	0.2944
Source of variation	**df**	**MDA**	**Proline**	**Total proteins**	**NRA**
Salinity (S)	1	45.76**	6.13ns	4.805***	1.7118ns
Proline (P)	4	8.26ns	0.58ns	0.224ns	1.129ns
S x P	4	3.407ns	0.35ns	0.430ns	0.633ns
Error	20	3.597	2.45	0.228	1.286

Ns = non-significant; df = degrees of freedom; 0.05, 0.01 and 0.001 are shown by *, ** and *** respectively

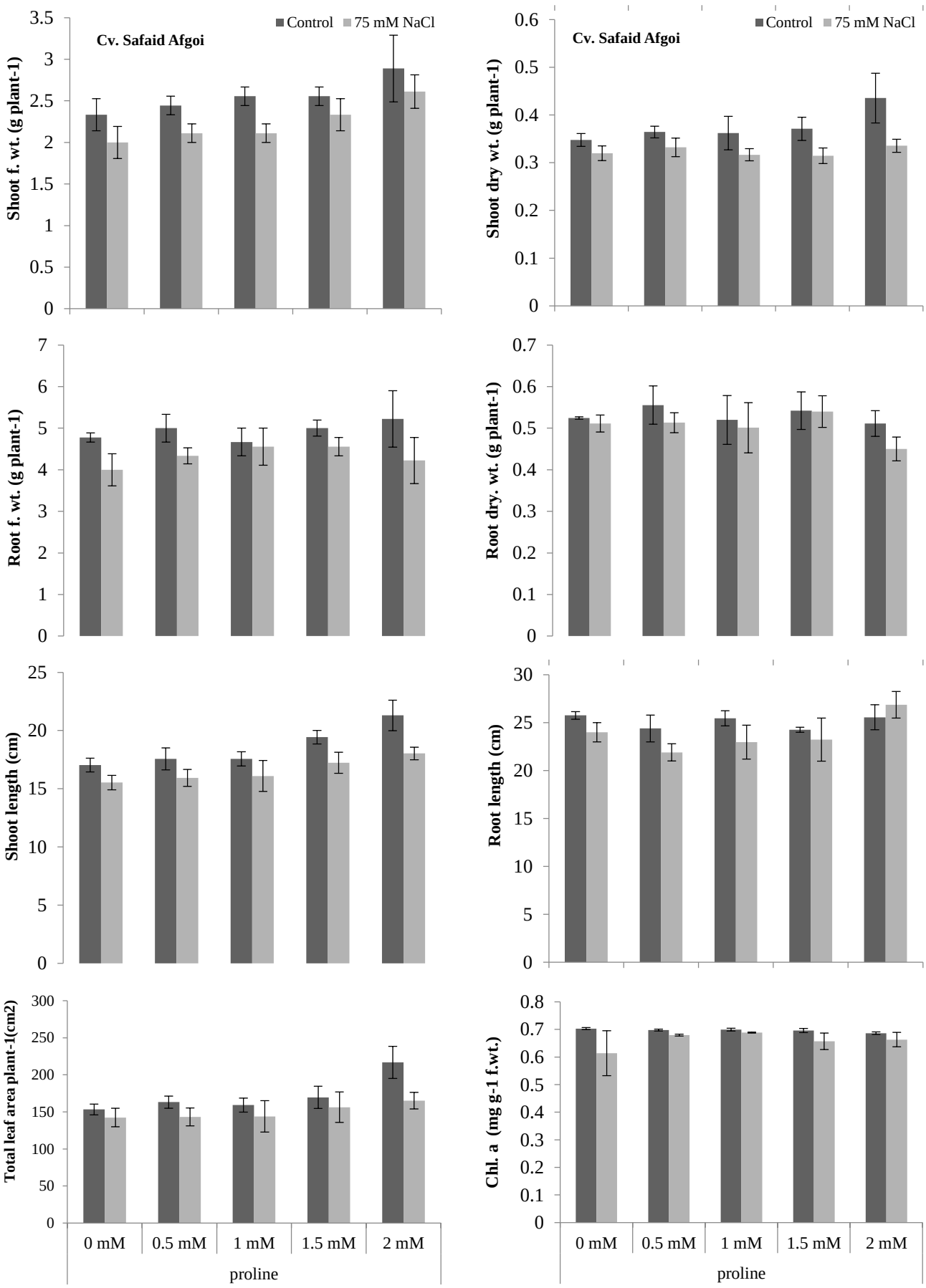

Fig. 1. Shoot and root fresh and dry weights, shoot and root length, total leaf area per plant and chlorophyll *a* contents of maize (*Zea mays* L.) plants foliarly-applied with different proline levels under salt-stressed and non-stressed conditions.

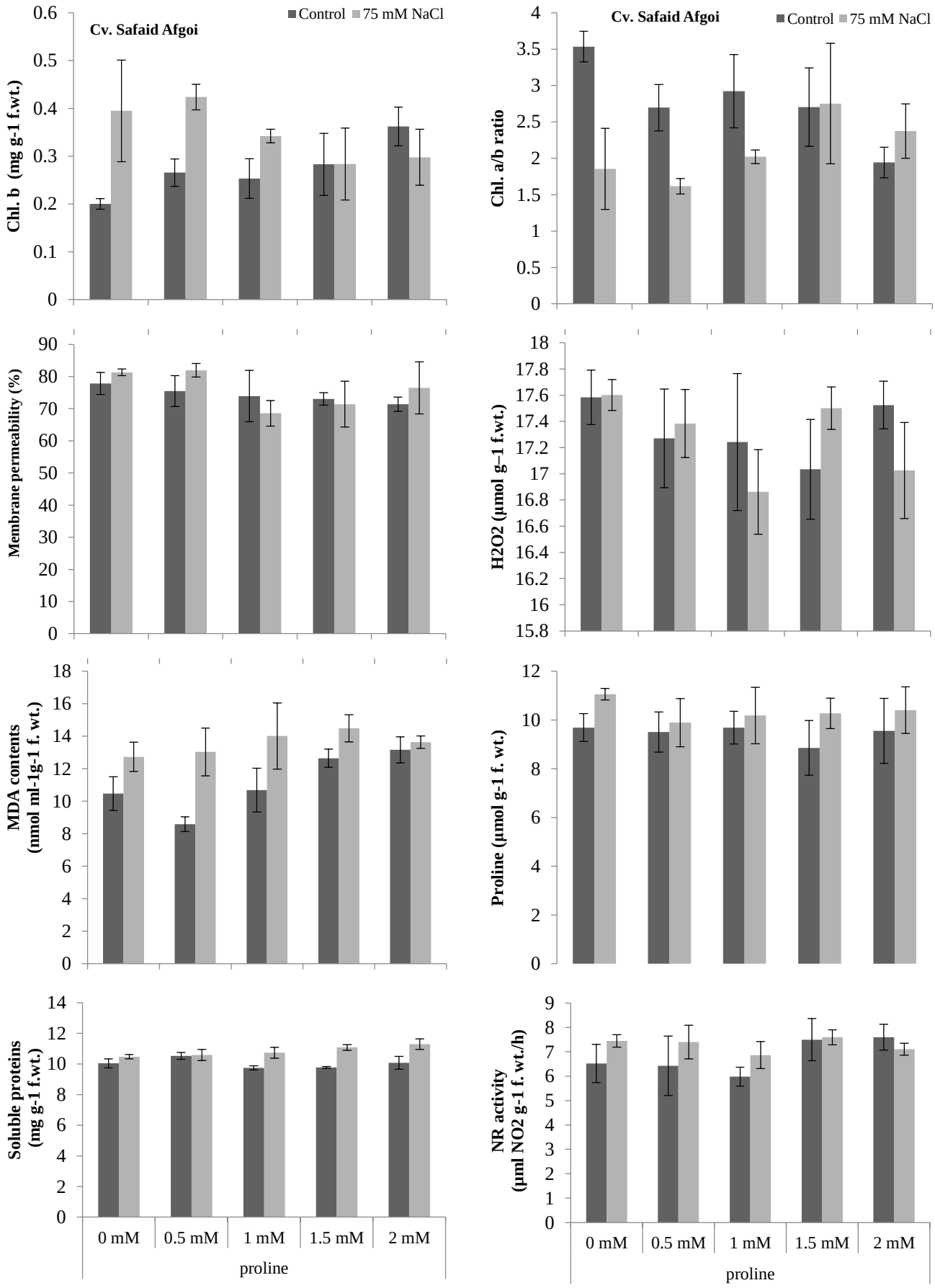

Fig. 2. Photosynthetic pigments, membrane permeability (%), H_2O_2, MDA, total soluble proteins and nitrate reductase activity of maize (*Zea mays* L.) plants foliarly-applied with different proline levels under salt-stressed and non-stressed conditions.

Discussion

Seed priming with some plant growth regulators (PGRs) can improve crops performance under saline conditions (Ratnakar & Rai, 2014; Meriem *et al.,* 2014). This technique has gained popularity in improving early seedling emergence, seedling establishment, growth, flowering and metabolites accumulation (Bakht *et al.,* 2011; Anosheh *et al.,* 2011; Arif *et al.,* 2014). However, effectiveness of proline treatment depends upon the plant developmental stage (Ochoa-Alfaro *et al.,* 2008), plant species, treatment level and mode of proline application on salt stressed plants (Amzalleg, 2002). For example, pre-sowing seed treatment with 1 mM proline promoted germination but could not increase germination percentage under higher concentration of salt (75 mM NaCl) in *Opuntia streptacantha* (Ochoa-Alfaro *et al.,* 2008). Pre-sowing seed treatment with 0.1 mM proline has been reported to ameliorate salt stress effects in perennial halophyte shrub (*Allenrolfea occidentalis*) (Gul & Khan, 2008) and 1 mM in rice (Deivanai *et al.,* 2011). Pre-sowing seed treatment with 1 mM proline ameliorated adverse effects of drought stress in mustard (*Brassica juncea* L.) (Hossain *et al.,* 2014). In another report by Khan and Unger (1997) 0.1 mM proline alleviated the effect of 25 mM NaCl stress than higher level of 75 and 125 mM NaCl in *Zygophyllum simplex* plants. On the other hand, it has also been reported that higher concentration of proline (100 mM) proved more effective in reducing salt stress effects than lower concentrations (50 mM) in wheat (Talat *et al.,* 2013). However, Deivanai *et al.,* (2011) was of the view that higher concentration of proline (10 mM) could not reduce negative effects of salinity stress in rice. In another report 10 mM proline did not improve germination under 100 mM NaCl stress in *Kosteletzkya virginica* (Poljakoff-Mayber *et al.,* 1994).

In this study, pre-sowing seed treatment with proline significantly increased shoot length of maize plants, while other growth attributes were not altered. Deivanai *et al.,* (2011) reported that seed treatment with 1 mM proline could increase root length in salt stressed rice plants. In another study, pre-sowing seed treatment with proline significantly improved growth and yield components, however, did not alter shoot and root Ca^{+2} and K^{+} and root Na^{+} contents significantly in wheat cultivars under drought stress (Kamran *et al*., 2009).

Proline application has been reported to increase chlorophyll contents in wheat (Talat *et al.,* 2013), rice (Deivanai *et al.,* 2011) and beans (Aggarwal *et al.,* 2011). However, chlorophyll '*a*' and '*b*' contents and chl. *a/b* ratio did not change significantly by seed treatment with proline in this study.

Exogenous application of proline reduced membrane damages by decreasing products of oxidative stress such as H_2O_2 and MDA (Huang *et al.,* 2009; Noujan & Theerakulpist, 2012). Recently, it has been reported that proline cannot scavenge reactive oxygen species such as superoxide, singlet oxygen, nitric oxide, nitrogen dioxide and peroxynitrite (Signorelli *et al*., 2013, 2016). However, proline accumulation appears to be a symptom of salt-susceptibility rather than stress tolerance in barley plants (Chen *et al*., 2007). In this study, seed treatment with proline did not change RMP (%), H_2O_2 and MDA contents significantly.

Proline accumulation in plant tissues maintains cell osmotic pressure (Zadebagheri *et al.,* 2014) leading to reduced oxidative stress, high photosynthetic capability and improved tolerance to saline conditions (Sona *et al.,* 2013; Ghahremani *et al.,* 2014; Gurmani *et al.,* 2014; Iqbal *et al.,* 2014). In this study, free proline contents were not altered by seed treatment with proline. Proline accumulation varies not only from species to species but also among plant organs (Nathalie & Christian, 2008). Seed treatment with different proline concentrations did not change total soluble protein contents of 3-week-old maize plants in the current study. Deivanai *et al.,* (2011) reported that different proline levels cause a remarkable reduction in protein content in rice plants. Nitrate reductase activity did not change under seed treatment with different proline concentrations in the present study. There are reports which show that proline treatment failed to improve germination in *Salicornia rubra*, *Atriplex rosea*, *Salsola iberica* and *Sarcobatus vermiculatus* (Gul *et al.,* 2000; Khan *et al*., 2004; Khan & Gul, 2006). Similarly, seed treatment with proline did not change dry weight, MDA contents and superoxide dismutase activity significantly in wheat seedlings under cadmium stress (Konotop *et al*., 2017).

In conclusion, salt stress of 75 mM NaCl adversely affected growth and decreased shoot fresh and dry weight, root fresh weight and total leaf area per plant, while increased malondialdehyde and total soluble protein contents of maize plants. However, seed treatment with proline increased shoot length under salt stress, while all other growth and physiochemical attributes remained unaffected under salt stress or non-stress conditions. Of various proline concentrations, 2 mM level seemed more effective in reducing the inhibitory effect of salt stress on maize plants. In this study, increase in shoot length might be due to some metabolic changes not clearly studied yet. Moreover, it can also be concluded that proline show differential response in increasing growth by regulating different physiochemical parameters not only in different plant species but also under diverse environmental conditions.

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