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Full Length Research Paper

Role of mycorrhizal fungi in tolerance of wheat genotypes to salt stress

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Salinity is one of the main environmental constraints to crop productivity worldwide. The aim of the experiment was to study the role of mycorrhiza (*Glomus clarum* Nicol. & Schenck) in tolerance of wheat genotypes to salt stress in terms of growth, physiological and biochemical parameters. Wheat genotypes (cvs. Henta, Moaya and Samma) were grown at three levels of NaCl (0.75, 1.5 and 3 g kg^{-1} soil) with or without mycorrhiza. The growth and physio-biochemical characteristics of all genotypes decreased with increasing levels of salinity except concentration of reducing sugars, sodium and proline, and at 3 g of NaCl, only genotype 'Samma' survived and showed resistant against severe salinity. However, inoculation of mycorrhiza enhanced the growth and accumulation of nutrients, reducing sugars, total soluble carbohydrates, Chlorophyll (Chl) a and Chl b, carotene, proline and protein by reducing Na. The present study suggested that inoculation of fungi was effective in improving the tolerance of wheat genotypes by improving the accumulation of nutrients and soluble solutes that might be responsible for osmotic adjustment of plant to counteract oxidative damage generated by salinity.

Key words: Mycorrhiza, nutrients, osmolytes, salinity, *Triticum aestivum,* pigments, protein.

INTRODUCTION

Salinity is one of the major environmental factors limiting plant growth and crop productivity in arid and semiarid irrigated area (Szabolcs, 1989; Koca et al., 2007). The increasing salinization of arid and semiarid regions of the world is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2005). Many plant species especially crop species do not grow and tolerate salinity due to the accumulation of salts especially NaCl which compete with other nutrients and cause specific toxicity (Tester and Davenport, 2003). It is a menace to both agriculture and the soil body.

Nowadays, it has become a challenge for the scientist community to overcome the salinity problem by searching and developing salt tolerant plants through plant breeding and genetic engineering. However, taken approaches to fight against salt stress are successful but they are costly and beyond the economic means of developing nations (Cantrell and Linderman, 2001). In recent years, the use of biological methods as an inexpensive and practical way to alleviate soil stresses, including salinity, on plant growth in saline soils has received increased attention (Giri and Mukerji, 2004; Al-Karaki, 2006).

In rhizosphere, some beneficial bacteria and fungi are present and they improve plant performance under different environmental conditions. The symbiosis between plants and arbuscular mycorrhizal fungi (AMF) is one of the important ecological mutualisms (Remy et al., 1994). AMF are associated with the roots of over 80% of terrestrial plant families (van der Heijden et al., 1998). AMF plays a key role in the regulation of ionome and membrane

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transport proteins that control the ion homeostasis of the host plants (Ramos et al., 2011; Song and Kong, 2012). AMF is known to exist in saline soil, and participates in the plant growth and development, and also improves the plant tolerance against biotic and abiotic stress (Berta et al., 2005; Abdel-Fattah et al., 2010) by regulating the physiological and biochemical process of plants (Evelin et al., 2009; Fernanda et al., 2012).

Salinity is known to alter many physiological and biochemical activities, such as ion toxicity, mineral distribution, respiration rate, osmolytic synthesis, osmotic adjustment, seed germination, enzyme activities and photosynthesis (Marschner, 2002; Siddiqui et al., 2008, 2012; Al-Whaibi et al., 2012). AMF has a regulatory and stimulatory influence on protein, sucrose, glucose, proline and glycine-betaine (GB) synthesis; hence, these solutes may play a role in osmotic adjustment (Evelin et al., 2009) that helps plant to perform normally under salinity. Under salinity, AMF application increased accumulation of proline in *Vigna radiate* (Jindal et al., 1993) and in soybean (Sharifi et al., 2007). However, in contrast to the report above, Rabie and Almadini (2005) and Bhosala and Shinde (2011) reported that non-AMF plants accumulated more proline than AMF plants under abiotic stress. Many studies have demonstrated that AMF plays a pivotal role in improvement of tolerance of plant to abiotic stress by enhancing nutrient uptake, particularly of N and P and subsequent increased growth (Jeffries et al., 2003; Cho et al., 2006). However, in some cases salt tolerance was not related to P concentration (Ruiz-Lozano and Azcón, 2000). Copeman et al. (1996) suggested that inoculation with VAM fungi from non-saline soil enhanced shoot growth, while VAM fungi from saline soil suppressed shoot and root growth by increasing accumulation of Cl^- in leaf. Carbohydrates also constitute a major role in the adjustment of osmotic potential (Evelin et al., 2009). The increase in total soluble carbohydrates (SC) is found to be positively correlated with mycorrhization of the host plant as reported by Thomson et al. (1990), Porcel and Ruiz-Lozano (2004) and Al-Garni (2006). On the other hand, in some other reports, negative correlations were found between AMF colonization and total SC accumulation in host plants (Pearson and Schweiger, 1993; Sharifi et al., 2007). It is evident that there is no clear consensus regarding the mechanisms by which soluble solutes reduce salt stress. Also, AMF associated salt tolerant mechanisms of plant are still debatable and need to be confirmed. Proline regulates gene expression for osmotic adjustment (Iyer and Caplan, 1998). The strategy of osmotic adjustment varies from plant to plant, as well as from tissue to tissue (Shaddad et al., 1990; Siddiqui et al., 2009). In view of these reports, the experiment was aimed at testing if the inoculation with AMF can improve the tolerance of wheat genotypes to salinity by ameliorating the accumulation of solutes and mineral nutrient uptake, and by improving the growth performance of plants.

MATERIALS AND METHODS

Cultivation conditions and mycorrhizal inoculation

Experiment were carried out under glasshouse conditions using three wheat (*Triticum aestivum* L.) genotypes namely: Henta, Moaya and Samma obtained from a local market in Riyadh, Saudi Arabia. Healthy seeds were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with sterilized double distilled water (DDW) before sowing. The seeds were sown in plastic pots (25 cm diameter, 25 cm height) filled with sterilized soil brought from sandy soil of Dharama, west of Riyadh. The pots were kept in controlled greenhouse conditions at the Botany and Microbiology Department, College of Science, King Saud University, maintained at 32/19°C (day- night temperature), 63% (day relative humidity) and 55% (night relative humidity) with a 16-h light and 8-h dark lighting regime. Pots were irrigated every week with DDW (200 mL), and supplied with Hogland and Arnon's nutrient solution (Hogland and Arnon, 1950).

Mycorrhizal spores were isolated from the rhizosphere of plants growing naturally on saline soil at Algasab, northwest of Riyadh by using water sieving method of Gerdemann and Nilson (1963). Spores were identified according to the international basis of mycorrhizal classification (Gerdemann and Trappe, 1974; Fischer et al., 2004; Walker et al., 2007). The spores of *Glomus clarum* Nicol. & Schenck were replicated on the host plant *Sorghum valgare* var. sudanense and the spores and roots of the host plant were used as inoculum for *Triticum aestivum* genotypes. The fungal association with wheat plants was tested by the use of trypan blue according to the method of Philips and Hayman (1970).

Three levels of salinity were applied with mycorrhiza (M) or without mycorrhiza (NM). The pots were arranged in a simple randomized design with a single factor and ten replicates. The layout of treatments for each cultivar was: (i) 0.0 g NaCl kg^{-1} soil + without mycorrhiza (control), (ii) 0.0 g NaCl kg^{-1} soil + mycorrhiza, (iii) 0.75 g NaCl kg^{-1} soil + without mycorrhiza, (iv) 0.75 g NaCl kg^{-1} soil + mycorrhiza, (v) 1.5 g NaCl kg^{-1} soil + without mycorrhiza, (vi) 1.5 g NaCl kg^{-1} soil + mycorrhiza, (vii) 3.0 g NaCl kg^{-1} soil + without mycorrhiza and (viii) 3.0 g NaCl kg^{-1} soil + mycorrhiza. After 6 days of seedlings emergence, thinning was done and five healthy plants of uniform size were maintained in each pot. Treatments were started after 10 days of sowing, and the addition of NaCl solution to the pots was alternate days to attain the final concentration.

Plant growth and physiological parameters measurements

The plants were sampled to assess their growth characteristics [root fresh $plant^{-1}$ (RFW), root dry weight $plant^{-1}$ (RDW), shoot fresh weight weight $plant^{-1}$ (SFW), shoot dry weight $plant^{-1}$ (SDW), stem length $plant^{-1}$, number of leaves $plant^{-1}$ and leaf area $plant^{-1}$ and physio-biochemical attributes [content of chlorophylls: (Chl *a*) and Chl *b*), carotene, total soluble carbohydrates (TSC), reducing sugars, proline, protein content, and content of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg)]

The plant height was measured by using a meter scale after removal from the pots. The plants were then placed in oven run at 60°C for 48 h. These dried plants were weighed to record the plant dry weight. Leaf area was measured by leaf area meter (LI.COR-MODEL LI-3000).

The chlorophyll and carotenoids were extracted from fresh leaves of experimental plants using the acetone method based on Metzner et al. (1965). The Chl and carotenoids absorption in the extract were measured using Visible Spectrophotometer-LKB Biochrom 4050. Contents of the Chls and carotenoids were calculated from the following formula:

Table 1. Influence of mycorrhiza on fresh and dry weight of wheat cultivars under salinity. g of Nacl/kg soil, NM = no mycorrhiza, M = with mycorrhiza, and NS = did not survive. Same letters in each column show no statistical difference at P < 0.05 (Duncan multiple range test).

Treatment	Cultivar					
	Henta		Moaya		Samma	
	SFW plant^{-1} (g)	RFW plant^{-1} (g)	SFW plant^{-1} (g)	RFW plant^{-1} (g)	SFW plant^{-1} (g)	RFW plant^{-1} (g)
0.0 g NaCl + NM	1.72^{b}	0.29^{b}	1.23^{a}	0.27^{b}	1.35^{bc}	0.29^{ab}
0.0 g NaCl + M	2.27^{a}	0.54^{a}	1.56^{a}	0.52^{a}	1.65^{a}	0.48^{a}
0.75 g NaCl + NM	1.20^{d}	0.17^{d}	0.99^{bc}	0.19^{c}	1.20^{c}	0.19^{ab}
0.75 g NaCl + M	1.63^{c}	0.27^{b}	1.24^{ab}	0.30^{b}	1.42^{b}	0.31^{ab}
1.50 g NaCl + NM	0.72^{f}	0.14^{d}	0.72^{c}	0.10^{d}	0.74^{de}	0.10^{b}
1.50 g NaCl + M	0.99^{e}	0.21^{c}	0.79^{c}	0.18^{c}	0.79^{d}	0.13^{b}
3.00 g NaCl + NM	NS	NS	NS	NS	0.42^{f}	0.07^{b}
3.00 g NaCl + M	NS	NS	0.65^{c}	0.13^{cd}	0.61^{e}	0.10^{b}
	SDW plant^{-1} (g)	RDW plant^{-1} (g)	SDW plant^{-1} (g)	RDW plant^{-1} (g)	SDW plant^{-1} (g)	RDW plant^{-1} (g)
0.0 g NaCl + NM	0.61^{b}	0.19^{b}	0.63^{b}	0.18^{b}	0.71^{b}	0.18^{b}
0.0 g NaCl + M	0.71^{a}	0.35^{a}	0.81^{a}	0.30^{a}	0.89^{a}	0.31^{a}
0.75 g NaCl + NM	0.55^{b}	0.11^{cd}	0.45^{d}	0.12^{c}	0.56^{c}	0.12^{c}
0.75 g NaCl + M	0.61^{b}	0.18^{b}	0.57^{c}	0.19^{b}	0.71^{b}	0.21^{b}
1.50 g NaCl + NM	0.31^{d}	0.09^{c}	0.41^{de}	0.06^{d}	0.40^{d}	0.06^{d}
1.50 g NaCl + M	0.43^{c}	0.14^{c}	0.45^{d}	0.12^{c}	0.43^{d}	0.09^{cd}
3.00 g NaCl + NM	NS	NS	NS	NS	0.29^{e}	0.04^{d}
3.00 g NaCl + M	NS	NS	0.39^{e}	0.08^{cd}	0.33^{e}	0.07^{cd}

Chlorophyll (a) = $10.3 \times O.D_{663} - 0.918 \times O.D_{644}$ = μg/ml.
Chlorophyll (b) = $19.7 \times O.D_{664} - 3.87 \times O.D_{663}$ = μg/ml.

Carotenoids = $4.2 \times O.D_{452.5}$ – [0.0264 Chlorophyll (a) + 0.426 Chlorophyll (b)] = μg/ml

Proline content was determined by adopting the ninhydrin method of Bates et al. (1973) using spectrophotometer.

Lowry method (Lowry et al., 1951) was adopted for protein determination after preparation of plant tissue so that samples were free of lipids and pigments (Katerman and Eargle, 1970) using bovine serum albumin for the standard curve. Total SC concentration was estimated as described by the methods of Nelson (1944) and Somogy (1952) while reducing sugars were determined by the method of Bell (1955).

Plant content of some mineral elements such as Na^{+}, K^{+}, Ca^{++} and Mg^{++} were determined according to the Association of Official Analytical Chemistry methods (AOAC, 1984) using Atomic Absorption Spectrophotometer AA-675 Series. On the other hand, nitrogen content was estimated according to Kjeldhal method (Chapman and Pratt, 1961), and phosphorus content was determined following the methods of AOAC. (1956).

Statistical analysis

The data were analyzed statistically with SPSS-12 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's multiple-range test at p<0.05% level.

RESULTS

Under non-saline conditions, inoculation of mycorrhiza increased RFW, RDW, SFW, SDW, stem length, number of leaves and leaf area in all cultivars of wheat when compared with control (without mycorrhiza inocu-lation) (Tables 1 and 2). However, all growth parameters decreased with increasing level of salinity (0 to 3 g NaCl). The genotypes Henta and Moaya did not survive at 3 g of NaCl, except Samma. However, at 3 g of NaCl, genotypes Moaya and Samma survived when plants of both genotypes were inoculated with mycorrhiza. Under stress, inoculation of mycorrhiza significantly improved most of the growth characteristics of plants of all genotypes. However, RFW of Samma at all levels of NaCl, SDW of Henta at 0.75 g NaCl and Samma at 1.5 and 3 g of NaCl, RDW of Henta at 1.5 g of NaCl, leaf number of Henta and Moaya at 1.5 g NaCl and Moaya at 3 g NaCl, and leaf area of Moaya at 1.5 g and Samma at 0.75 were found statistically non-significant (Tables 1 and 2). Table 3 reveals that plants of all genotypes ino-culated with mycorrhiza exhibited reduced accumulation of reducing sugars in all cultivars of wheat. However, plants of all genotypes supplemen-ted with NaCl stress showed slightly enhanced accumulation of reducing sugars. The maximum accumulation was recorded in Samma at 3 g of NaCl as compared to the other geno-types. But a different pattern of crop response was observed when total SC was studied in mycorrhiza-treated plants in the presence of NaCl stress in all cultivars (Table 3). Under non-stress medium, mycorrhizal plants of all genotypes

Table 2. Influence of mycorrhiza on stem length, number of leaf and leaf area of wheat cultivars under salinity. g of Nacl/kg soil, NM = No mycorrhiza, M = with mycorrhiza, and NS = did not survive. Same letters in each column show no statistical difference at P < 0.05 (Duncan multiple range test).

Treatment	Cultivar		
	Henta	Moaya	Samma
	Stem length cm plant^{-1}		
0.0 g NaCl + NM	52.2^{ba}	48.3^{b}	45.8^{b}
0.0 g NaCl + M	59.3^{a}	54.1^{a}	51.4^{a}
0.75 g NaCl + NM	42.3^{d}	39.8^{d}	40.3^{c}
0.75 g NaCl + M	48.6^{c}	44.9^{c}	45.9^{b}
1.50 g NaCl + NM	18.3^{f}	20.3^{f}	23.6^{e}
1.50 g NaCl + M	23.2^{e}	24.7^{e}	27.7^{d}
3.00 g NaCl + NM	NS	NS	19.2^{f}
3.00 g NaCl + M	NS	17.8^{g}	23.1^{e}
Number of leaves plant^{-1}			
0.0 g NaCl + NM	4.6^{b}	4.3^{abc}	4.6^{ab}
0.0 g NaCl + M	5.3^{ab}	5.0^{a}	5.0^{a}
0.75 g NaCl + NM	5.3^{ab}	4.0^{bc}	4.3^{ab}
0.75 g NaCl + M	6.0^{a}	4.6^{ab}	5.3^{a}
1.50 g NaCl + NM	3.0^{c}	4.3^{abc}	3.6^{b}
1.50 g NaCl + M	3.0^{c}	4.3^{abc}	4.3^{ab}
3.00 g NaCl + NM	NS	NS	2.3^{c}
3.00 g NaCl + M	NS	3.6^{c}	2.6^{c}
Leaf area plant^{-1}			
0.0 g NaCl + NM	22.6^{c}	18.2^{c}	19.9^{b}
0.0 g NaCl + M	24.1^{b}	19.3^{b}	21.1^{a}
0.75 g NaCl + NM	24.1^{b}	19.5^{ab}	20.9^{a}
0.75 g NaCl + M	25.2^{a}	20.5^{a}	21.8^{a}
1.50 g NaCl + NM	15.1^{e}	14.1^{d}	11.2^{d}
1.50 g NaCl + M	17.2^{d}	14.6^{d}	14.9^{c}
3.00 g NaCl + NM	NS	NS	8.1^{e}
3.00 g NaCl + M	NS	10.1^{e}	11.2^{d}

Table 3. Influence of mycorrhiza on accumulation of reducing sugar and total soluble carbohydrate in leaf of wheat cultivars under salinity. g of Nacl/kg soil, NM = No mycorrhiza, M = with mycorrhiza, and NS = did not survive. Same letters in each column show no statistical difference at P < 0.05 (Duncan multiple range test)

Treatment	Cultivar		
	Henta	Moaya	Samma
	Reducing sugars (mg 100g^{-1} DW)		
0.0 g NaCl + NM	10.70^{d}	9.45^{e}	11.01^{c}
0.0 g NaCl + M	10.20^{e}	9.22^{f}	10.40^{d}
0.75 g NaCl + NM	11.30^{b}	9.90^{c}	11.80^{b}
0.75 g NaCl + M	10.90^{c}	9.60^{c}	10.00^{ab}
1.50 g NaCl + NM	11.49^{a}	10.40^{a}	12.00^{ab}
1.50 g NaCl + M	11.30^{b}	10.10^{b}	11.05^{c}
3.00 g NaCl + NM	NS	NS	12.10^{a}
3.00 g NaCl + M	NS	10.0^{bc}	10.90^{c}
	Total soluble carbohydrates (mg 100g^{-1} DW)		
0.0 g NaCl* + NM	15.71^{c}	14.05^{c}	15.11^{b}
0.0 g NaCl + M	16.19^{b}	14.21^{a}	15.27^{b}
0.75 g NaCl + NM	14.50^{e}	13.20^{d}	14.80^{c}
0.75 g NaCl + M	16.60^{a}	14.45^{b}	15.59^{a}
1.50 g NaCl + NM	14.19^{f}	13.00^{e}	14.00^{d}
1.50 g NaCl + M	14.90^{d}	14.24^{c}	15.30^{b}
3.00 g NaCl + NM	NS	NS	13.90^{d}
3.00 g NaCl + M	NS	14.20^{c}	14.90^{c}

exhibited higher value for total SC as compared to the controls. However, effects of mycorrhiza equalled by control, gave maximum value for total SC in Samma, under stress conditions. The degree of efficiency of mycorrhiza inoculation in alleviating the adverse effect of salt stress, and the accumulation of total SC in leaves of all genotypes was found to be high.

Under non-stress conditions, the content of N, P, K, Ca and Mg was recorded higher in mycorrhiza-inoculated-plants of all genotypes than the respective non-inoculated plants, except Na content in all genotypes (Table 4). However, these nutrients decreased with increasing levels of NaCl treatments in all cultivars, except Na content. Under stress conditions, inoculation was found to be effective in improving leaf- N, P, K, Ca and Mg concentration in all genotypes. On the other hand, under stress, low content of Na was recorded at all salinity levels when plants of all genotypes were inoculated with mycorrhiza.

Figures 1 and 2 reveal that the content of Chl *a* and carotene was higher, except Chl *b*, in all cultivars under non-stress conditions. However, synthesis of plant pigments in all cultivars was found to be decreased with increasing levels of NaCl. On the other hand, application of mycorrhiza enhanced a significant increase in the plant pigments in all the genotypes under stress. Under NaCl stress, genotypes Moaya and Samma were found to be contain higher accumulation of pigments than Henta, especially at 3.0 g NaCl.

Under normal conditions, proline and protein accumulation were observed to be high in plants of all genotypes inoculated with mycorrhiza (Figure 3). An increase in proline accumulation was recorded with increasing levels of salinity in all genotypes. But a different pattern was observed with protein content in all genotypes under salt stress. The content of protein decreased with increasing levels of salinity. However, a similar trend has been observed in this study with respect to the accumulation of proline and protein in all genotypes. In plants of all genotypes under stress, increased proline and protein concentration was observed when they were inoculated with mycorrhiza, but Henta cultivar survived at high dose of NaCl (3.0 g).

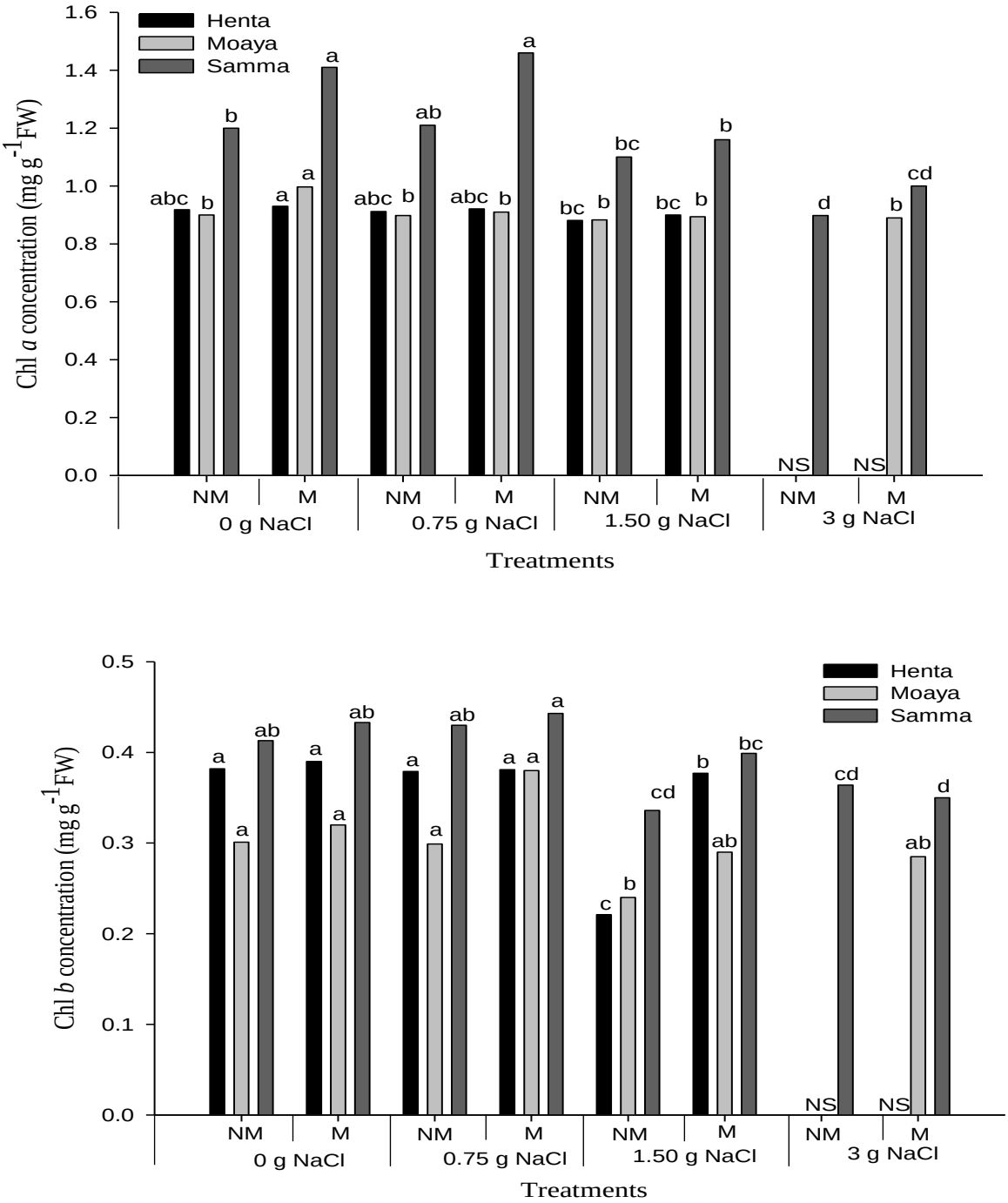

Figure 1. Influence of mycorrhiza on content of Chl *a* and Chl *b* in leaf of wheat cultivars under salinity. Bars followed by the same letters show no statistical difference at $P < 0.05$ (Duncan multiple range test). Average of four determinations are presented with bars indicating SE.

DISCUSSION

It is well established that salt stress inhibits plant growth. In the present experiment, RFW, SFW, RDW, SDW, stem length and number of leaves decreased with increasing levels of NaCl over the control (Tables 1 and 2). At high dose of NaCl, genotypes Henta and Moaya did not survive except Samma. It may be due to the toxic effects of NaCl by accumulating more salt (Afroz et al., 2005; Siddiqui et al., 2009). However, plants inoculated with *G. clarum* showed enhanced growth attributes in all cultivars under both saline and non-saline conditions, while inoculation of mycorrhiza increased SFW, RFW, SDW, RDW, stem length and leaf area of Moaya and SFW, RDW, stem

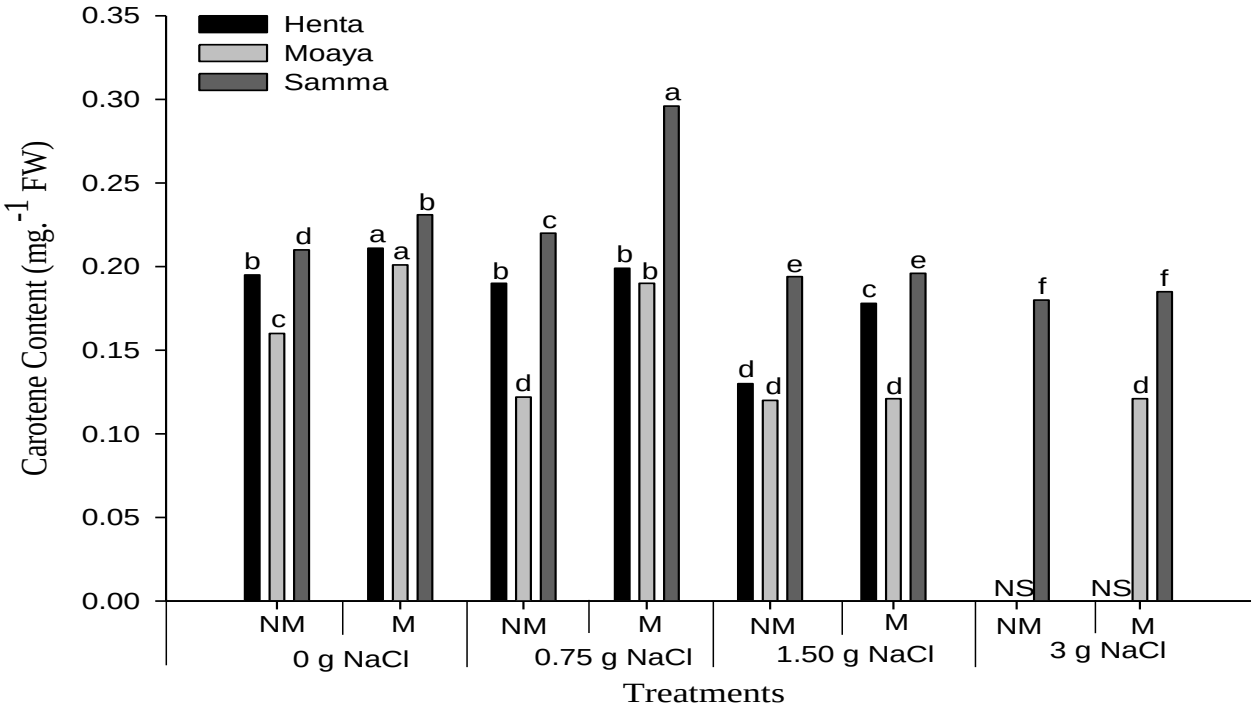

Figure 2. Influence of mycorrhiza on content of carotene in leaf of wheat cultivars under salinity. Bars followed by the same letters show no statistical difference at $P < 0.05$ (Duncan multiple range test). Average of four determinations are presented with bars indicating SE.

length and leaf area of Samma even under severe saline conditions. Therefore, we may postulate that inoculation of *G. clarum* isolated from saline soil can differentially suppress the inhibitory effects of salinity and also varietal differences in plant growth responds to *G. clarum* under stress may be dependent on genotypic differences in rates of nutrients uptake, transport, accumulation and distribution within the plant (Siddiqui et al., 2009).

The mechanism of osmotic adjustment plays a vital role in the protection of plant against stress particularly against the salinity (Siddiqui et al., 2008). Plants accumulate soluble sugars that participate actively in the osmotic adjustment when they are under stress (Evelin et al., 2009). In this study, reducing sugars increased with increasing level of salinity (Table 3), but TSC decreased with increasing levels of salinity in plant of all genotypes (Table 3). However, both parameters increased in plants of all genotypes when inoculation was applied to the host plants. The increase in TSC is found to be positively correlated with mycorrhization of the host plant and this result coincides with earlier studies of Thomson et al. (1990). Conversely, the decrease in reducing sugars was found to be negatively correlated with mycorrhizal treatment. Pearson and Schweiger (1993) reported a reduction in carbohydrate concentration with an increase in the percentage of root colonization. These differences among the cultivars could be ascribed to the variation in the genetic make-up of cultivars. Naureen and Naqvi (2010) reported that accumulation of reducing sugars varied among the wheat genotypes under salt stress. Under stress, increase in accumulation of reducing sugars and TSC may be due to the nutrients uptake induced by the inoculation of mycorrhiza (Table 4).

Salt stress disturbs the regulation of ion homeostasis of the host plants (Niu et al., 1995; Siddiqui et al., 2009, 2012). All genotypes showed similar trends for the nutrients content in leaf (Table 4). Under stress, a decrease in the content of N, P, K, Ca and Mg was recorded in all cultivars under NaCl stress, while a quantum of enhancement of these nutrients was higher in inoculated-plants of all genotypes (Table 4). However, interestingly, mycorrhizal plant exhibited reduced Na accumulation. These results strengthen the findings of Garg and Manchanda (2009).

The increased accumulation of these nutrients with the inoculation of *G. clarum* has been important factors for increasing plant growth because they are important components of many metabolically active compounds and play a crucial role in several physiological and biological functions (Marschner, 2002). Siddiqui et al. (2012) reported that accumulation of nutrients improved the tolerance of plant by inducing the many enzymes associated with nutrients assimilation and antioxidant enzymes. Experiment with wheat genotypes indicates that salt tolerance is associated with enhanced accumulation of nutrients by the inoculation of mycorrhiza.

Salt stress suppressed the synthesis of photosynthetic

Table 4. Influence of mycorrhiza on content of N, P, K, Na, Ca and Mg in leaf of wheat cultivars under salinity. g of Nacl/kg soil, NM = No mycorrhiza, M = with mycorrhiza, and NS = did not survive. Same letters in each column show no statistical difference at P < 0.05 (Duncan multiple range test).

Treatment	Cultivar								
	Henta			Moaya			Samma		
	N content	P content	K content	N content	P content	K content	N content	P content	K content
0.0 g NaCl + NM	18.1^{b}	4.1^{c}	10.0^{b}	15.7^{b}	3.9^{d}	9.6^{a}	19.1^{de}	5.1^{de}	7.1^{b}
0.0 g NaCl + M	20.9^{a}	5.2^{b}	11.1^{a}	16.0^{b}	4.8^{a}	9.8^{a}	19.8^{cd}	5.9^{bc}	8.6^{a}
0.75 g NaCl + NM	17.1^{b}	4.2^{c}	6.1^{d}	15.1^{b}	4.1^{cd}	7.6^{b}	22.2^{b}	5.4^{cd}	5.9^{cd}
0.75 g NaCl + M	18.3^{b}	5.7^{ab}	6.9^{c}	18.1^{a}	5.3^{b}	8.1^{b}	24.4^{a}	6.2^{ab}	6.6^{c}
1.50 g NaCl + NM	15.8^{c}	3.1^{d}	4.1^{f}	13.8^{c}	3.2^{e}	5.4^{c}	18.1^{e}	5.8^{bc}	4.5^{c}
1.50 g NaCl + M	17.3^{b}	6.2^{a}	5.1^{e}	15.3^{b}	4.6^{c}	5.9^{c}	21.0^{bc}	6.7^{a}	5.1^{cd}
3.00 g NaCl + NM	NS	NS	NS	NS	NS	NS	15.9^{f}	3.8^{f}	4.0^{e}
3.00 g NaCl + M	NS	NS	NS	13.7^{c}	4.1^{cd}	4.1^{d}	16.5^{f}	4.6^{e}	4.6^{e}
	Na content	**Ca contant**	**Mg content**	**Na content**	**Ca contant**	**Mg content**	**Na content**	**Ca content**	**Mg content**
0.0 g NaCl + NM	2.9^{d}	6.3^{a}	2.5^{ab}	3.2^{f}	5.9^{bc}	1.9^{ab}	5.3^{f}	7.0^{a}	3.1^{c}
0.0 g NaCl + M	2.6^{d}	7.0^{a}	3.1^{a}	3.2^{f}	6.2^{ab}	2.2^{a}	4.9^{f}	6.8^{a}	3.6^{ab}
0.75 g NaCl + NM	4.1^{c}	6.4^{a}	2.4^{ab}	5.6^{d}	6.8^{ab}	1.7^{b}	6.8^{d}	7.1^{a}	3.2^{bc}
0.75 g NaCl + M	3.7^{c}	6.9^{a}	2,9^{a}	4.9^{e}	7.2^{a}	2.0^{ab}	6.2^{e}	7.6^{a}	3.9^{a}
1.50 g NaCl + NM	7.3^{a}	3.1^{c}	1.1^{c}	7.3^{b}	4.3^{d}	1.1^{c}	8.6^{b}	3.1^{c}	2.1^{d}
1.50 g NaCl + M	6.2^{b}	5.1^{b}	2.0^{b}	6.5^{c}	5.1^{cd}	1.6^{b}	7.9^{c}	4.9^{b}	2.9^{c}
3.00 g NaCl + NM	NS	NS	NS	NS	NS	NS	9.7^{a}	1.2^{e}	1.8^{d}
3.00 g NaCl + M	NS	NS	NS	7.8^{a}	2.6^{e}	1.5^{b}	8.4^{b}	2.1^{d}	2.2^{c}

pigments in plants of all genotypes (Figures 1 and 2). The inhibition of plant pigments content might be due to instability of protein complexes and destruction of chlorophyll by enhanced activity of chlorophyllase, a Chl degrading enzyme, under salt stress (Reddy and Vora, 1986). These results strongly agreed with the findings of Siddiqui et al. (2009, 2010). Interestingly, content of pigments (Chl *a*, Chl *b* and carotene) increased when mycorrhiza was inoculated to the host plants of all wheat genotypes under stress and non-stress conditions (Figures 1 and 2). The improvement of photosynthetic pigments might be due to the stimulation of plant by colonization and an inhibition of Na transport towards the plants leaves, and led to better functioning of photosynthetic apparatus (Rabie and Almadini, 2005). Borde et al. (2010) reported that the highest chlorophyll was found in inoculated plants as compared to non-inoculated plant. These results indicated that mycorrhiza alleviated the adverse effect of salinity by increasing pigments that enhanced the photosynthetic efficiency leading to improvement in values for growth parameters of wheat genotypes.

In the present study, plants of wheat genotypes under stress conditions exhibited increased proline accumulation that further increased by the application of mycorrhiza and thus could improve tolerance of wheat genotypes to salt stress by maintaining the osmotic balance and reducing the free radicals damage induced by osmotic stress (Jain et al., 2001; Garg and Manchanda, 2009). It has been demonstrated that proline serves as a storage sink for carbon and nitrogen and a free-radical scavenger, stabilizes subcellular structures (membranes and proteins) and buffers cellular redox potential under stress (Bohnert and Jensen, 1996; Chen and Murata, 2002) and that the level of accumulated solute is correlated with the degree

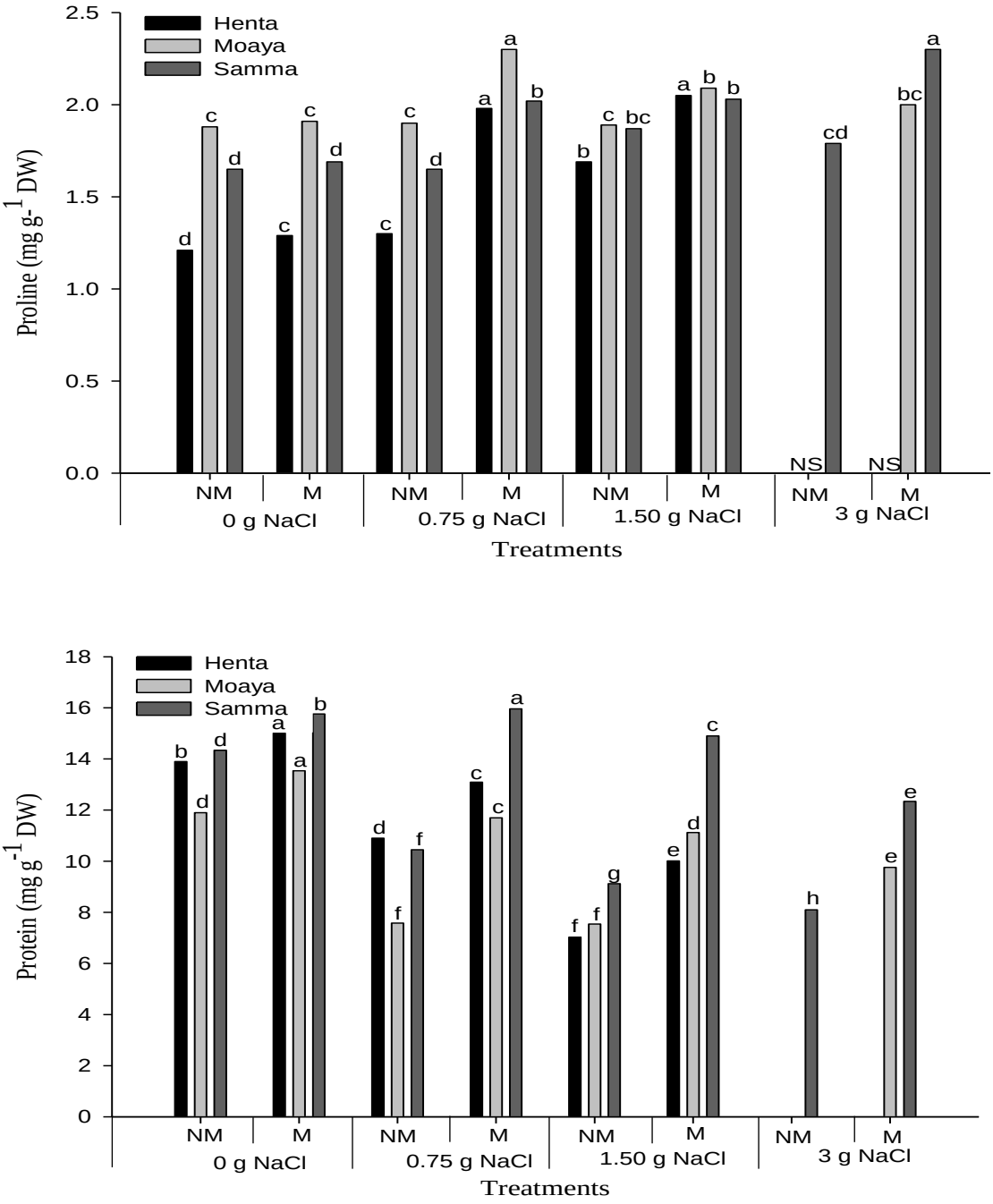

Figure 3. Influence of mycorrhiza on content of proline and protein in leaf of wheat cultivars under salinity. Bars followed by the same letters show no statistical difference at $P < 0.05$ (Duncan multiple range test). Average of four determinations are presented with bars indicating SE.

of salt tolerance (Garg and Manchanda, 2009). Elevated level of proline in inoculated-plants may be due to the accumulation of N and other nutrients. The regulation of biosynthesis of proline is very closely related to the nitrogen assimilation (Siddiqui et al., 2010). Also,Neuberg et al. (2010) found that a marked increase in proline content was recorded in plant after nitrogen treatment. Proline may act as an N source in the cell under stress conditions, where the accumulation of this nitrogenous compound could be utilized as a form of stored N (Dandekar and Uratsu, 1988). In the present experiment, protein content decreased with increasing levels of salinity, while inoculation of mycorrhiza increased the content of protein in all cultivars (Figure 3). This result corroborates the finding of Parida et al. (2004). Thus, the results suggested that application of salt stress on wheat genotypes at different levels exhi-

bited an increase in proline pool by decreasing protein, which facilitated the mode of adjustment to Sali-nity stress (Parida et al., 2004). Fukutoku and Yamada (1981) suggested that some *de novo* synthesis of proline occurs under stress and that the N source for this proline synthesis may be protein. The improvement of protein content by the inoculation of mycorrhiza might be due to accumulation of nutrients that are constituent of several metabolically active compounds (Marschner, 2002). The change in protein content in NaCl fed plants of all genotypes could be responsible for the plants performing normally under stress conditions by changing biological adaptation process.

Conclusion

From the results, it can be concluded that improved growth performance of all genotypes in terms of RFW, RDW, SFW, SDW, plant height, leaf number and leaf area was accompanied by increased nutrients accumulation in plants inoculated with mycorrhiza under stress and non-stress conditions. Mycorrhizal plants showed reduced accumulation of Na and enhanced content of N, P, K, Ca and Mg than non-mycorrhizal plants. The parallel increase in the content of nutrients, photosynthetic pigments, reducing sugars, TSC, proline and protein in inoculated plants might be responsible for plants counteracting oxidative damage generated by salinity. Thus, the present study provides a highly cost-effective and environmental friendly approach to overcome the adverse effect of salinity.

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