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Evaluation Effects of Mycorrhizal Fungi (AM) and Nano Zinc Oxide on Seed Yield and Dry Matter Remobilization of Wheat (*Triticum aestivum* L.) under Salinity Stress

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ABSTRACT

This research was carried out to assessment agro physiological traits of bread wheat affected salinity stress, Nano zinc oxide and arbuscular mycorrhiza (AM) fungi under greenhouse condition via factorial experiment based on randomized complete blocks design with three replications. Experimental factors included salinity stress in three levels [no-salt (S_0) or control, salinity 40 (S_1), and 80 (S_2) mM NaCl], Arbuscular mycorrhizal fungi at two level [no application (M_0), application of arbuscular mycorrhiza (M_1)] and nano zinc oxide at three levels [without nano zinc oxide as control (Zn_0), application of 0.4 (Zn_1) and 0.8 (Zn_2) $g.lit^{-1}$]. Result of analysis of variance showed effect of soil salinity, AM fungi and nano zinc oxide on dry matter remobilization from stem and shoots, contribution of remobilization from shoots to seed, stem reserve contribution in seed yield, chlorophyll index and seed yield was significant at 1% probability level. Also interaction effect of treatments on measured traits was not significant but effect of salinity, AM fungi and nano zinc oxide on seed yield was significant at 5% probability level. Mean comparison result revealed salinity stress decreased seed yield and chlorophyll index of wheat. But dry matter remobilization from shoots increased. The highest dry matter (0.198, 0.195 and 0.194 g per plant) and stem reserves remobilization to seeds (0.177, 0.175 and 0.177 g per plant) were observed in the highest salinity level and no application of AM fungi and nano zinc oxide, respectively. The results indicated that the highest (0.44 g per plant) seed yield was obtained from plants under low salinity level, AMF and 0.8 $g.lit^{-1}$ nano zinc oxide. Generally, it was concluded that AM fungi and nano zinc oxide can be as a proper tool for increasing wheat yield under salinity condition.

Key words: *Agro-Physiological traits, Chlorophyll index, Nutrition.*

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INTRODUCTION

Wheat is an important source of energy and protein. Yield of wheat depends mainly on the processes of starch synthesis and accumulation (Pan *et al*., 2007). In the seed filling period, dry-matter remobilization from source organs to sink organs is an important factor influencing the final wheat yield. In cereals, such as wheat and barley, seed filling is sustained by current photosynthesis of the upper parts of the plant, such as the flag and the ear (Tambussi *et al*., 2007) and by redistribution of assimilates stored in the stem (Ehdaie *et al*., 2008). The relationship between decrease in stem weight from anthesis to maturity and seed yield indicates that the smallest decreases in stem weight were associated with the highest yields. This decrease in stem weight during seed filling support the proposal that stored assimilates was used during seed filling (Flood *et al*., 1995). The rate of seed starch accumulation and seed weight are affected by photosynthetic assimilation, dry matter accumulation, dry matter remobilization and starch accumulation capacity on one side and growing conditions (Przulj and Momcilovic, 2003). In conditions when assimilates from current photosynthesis become unavailable due to stress such as drought, salinity and high temperature, seed filling depends to a high extent on remobilized resources (Przulj and Momcilovic, 2003). The availability of stored assimilate that can be remobilised for seed filling may act as a buffer to seed yield against suboptimal growth conditions, (Bidinger *et al.,* 1977). Wheat is moderately tolerant to salinity, and species variations in salinity tolerance have been reported (Ali *et al*., 2007). Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas (Abdel Latef and Chaoxing, 2011). It adversely affects the growth of most agricultural crops through its influence on certain aspects of plant metabolism such as osmotic adjustment (Bernstein, 1963), reduce chlorophyll content, photosynthetic rate and the stomatal conductance, imbalance in the synthesis of endogenous plant growth regulators (PGR) (Iqbal and Ashraf, 2013). Ionic imbalance is considered as one of the main effect of salinity stress in many plants (Zheng *et al*., 2008; Iqbal and Ashraf, 2013). The ionic balance has a key role in photosynthesis and other metabolic activities of the cell (Zheng *et al*., 2008). Several strategies have been developed in order to decrease the toxic effects caused by high salinity on plant growth. Among them the usage of arbuscular mycorrhizal (AM) fungi as a biologically based strategy to alleviate the adverse impact induced by salt is of such alternatives. Many researchers have reported that AMF could enhance the ability of plants to cope with salinity (Talaat and Shawky, 2011; Abdel-Fattah and Asrar, 2012). AM fungi symbiosis promote salinity tolerance by utilizing various mechanisms, such as defending roots against soil-borne pathogens, improving rhizospheric and soil conditions, modifying microbial communities, enhancing antioxidant enzymes activity, maintaining membrane integrity, stimulating plant growth regulators, enhancing plant nutrient acquisition, physiological changes (leaf gas exchange rate, photosynthetic efficiency, relative permeability, water status and abscissic acid accumulation) (Sheng *et al*., 2008; Talaat and Shawky, 2011; Abdel-Fattah and Asrar, 2012). Kadian *et al*. (2013) reported that chlorophyll activity is restored in mycorrhizal plants grown under salt stress due to increased activity of specific enzymes required for its biosynthesis. The demand for nanotechnology-based products has been increasing in recent years. Generally, nano materials refer to a colloidal particulate system, with size ranging from 10 to 1000 nm, possessing unique properties, such as size dependent qualities, high surface-to-volume ratio, and promising optical properties. It was possible that the nano materials aggregates were smaller than the pore size of the plant leaf cell, and thus some micro-sized aggregates pass through the plant cells (Al-Halafi, 2014). Among the micronutrients, Zn affects the susceptibility of plants via drought and salinity stresses (Sharma *et al*., 2009). A number of researchers described the key role of ZnO nano materials for in crop growth (Wang et al., 2004) involving processes of photosynthesis, nitrogen assimilation, respiration and activation of other biochemical and physiological processes and hence their importance in obtaining greater yields (Zozi *et al*., 2012). Better understanding of wheat physiological responses under salinity may help in programs which the objective is to improve the seed yield under salinity levels. Considering the above facts, the present study was conducted to evaluate the effect of different salinity level, application of ZnO nano materials and Mycorrhizal fungi on agro-physiological traits.

MATERIALS AND METHODS

Greenhouse and Treatments Information

This research was carried out to assessment agro physiological traits of bread wheat affected salinity stress, Nano zinc oxide and arbuscular mycorrhiza (AM) fungi under greenhouse condition via factorial experiment based on randomized complete blocks design with three replications along 2015 year. Experimental factors included salinity stress in three levels [no-salt (S_0) or control, salinity 40 (S_1), and 80 (S_2) mM NaCl], AM fungi at two level [no application (M_0), application of arbuscular mycorrhiza (M_1)] and nano zinc oxide at three levels [without nano zinc oxide as control (Zn_0), application of 0.4 (Zn_1) and 0.8 (Zn_2) g.lit^{-1}].

Greenhouse Management

Arbuscular mycorrhiza (AM) fungi were purchased from the Zist Fanavar Turan institute and soils were treated based on the manufacturer's protocol 10 g of inoculums per 1 kg soil, each pot containing approximately 790 spores. The soil was silty loam, with pH about 6.9. Air temperature ranged from 22 to 27 °C during the day and 18 to 21°C during the night. Humidity ranged from 60 to 65%. The wheat cultivar "Attila 4" was used in the experiment. Optimal density of cultivar "Attila 4" is 400 seeds.m^{-2}, so 40 seeds were sown in each pot with 4 cm deep, filled approximately with 20 kg of above-mentioned soil. The pots were immediately irrigated after planting. Salt stress treatments were applied two weeks after planting. Foliar application ZnO nano was done in two growth stages (4-6 leaf stage and before booting stage).

Measured Traits

Dry matter and stem reserves mobilization to seed yield assay: Dry matter and stem reserves mobilization to seed yield were evaluated as follows (Inoue *et al*., 2004):

Equ. 1. Dry matter remobilization to seed (g per plant) = maximum shoot dry matter after anthesis (g per plant) – shoot dry matter (except seeds) in maturity (g per plant)

Equ. 2. Dry matter contribution of assimilates to seed (%) = [remobilization/ seed yield] ×100

Equ. 3. Stem reserves remobilization to seed yield (g per plant) = maximum stem dry matter after anthesis (g per plant) – stem dry matter in maturity (g per plant)

Equ. 4. Stem reserve contribution to seed yield (%) = [stem dry matter remobilization/ seed yield] × 100.

Chlorophyll content: The fully developed flag leaf of the main tillers was randomly selected from five plants of each plot for determination of chlorophyll content. The chlorophyll content of leaves was determined with a SPAD-502 (Konica Minolta Sensing, Osaka, Japan) (Jifon *et al*., 2005).

Seed yield per plant: In order to measure seed yield per plant, 10 plants of each pot randomly were harvested.

Statistical Analysis

The analysis of variance was done by SAS software and the means were compared with using least significant difference test (LSD) at 5% probability level.

RESULT AND DISCUSSION

Result of ANOVA showed effect of soil salinity, AM fungi and nano zinc oxide on dry matter remobilization from stem and shoots, contribution of remobilization from shoots to seed, stem reserve contribution in seed yield, chlorophyll index and seed yield was significant at 1% probability level. Also interaction effect of treatment on measured traits was not significant but effect of salinity, AM fungi and nano zinc oxide on seed yield was significant at 5% probability level (Table 1).

Dry matter and stem reserves mobilization to seed yield

In the seed filling period, dry-matter remobilization from source organs to sink organs is an important factor influencing the final winter wheat yield. The results showed that salinity levels decreased the chlorophyll index and led to more mobilization of dry matter and stem reserves assimilates to seed (Table 2). El-Tayeb, (2005) reported that reduced chlorophyll contents under stress is attributed to increased activity of chlorophyllase causing degradation of pigments and hence resulting in reduced photosynthesis and hence plant growth and biomass production. In contrast, nano zinc oxide foliar application and inoculation of AM fungi slowed senescence and retarded such mobilization. These decreases in stem weight during seed filling support the proposal that stored assimilate was used during seed filling. Furthermore, in wheat this reliance on stored assimilate is increased with rapidly developing salinity severity. In 80 mM salinity, chlorophyll index was reduced but dry matter and stem reserves remobilization to seed were significantly increased (Table 2). It seems that the increase of chlorophyll index due to current photosynthesis in plants that were grown under nano zinc oxide and mycorrhizae treatments decreased mobilization of dry matter and stem reserves to seed yield. The highest dry matter (0.198, 0.195 and 0.194 g per plant) and stem reserves remobilization to seeds (0.177, 0.175 and 0.177 g per plant) were observed in the highest salinity level (S_3) and no application of AM fungi and nano zinc oxide, respectively (Table 2). The stress tolerance efficiency of cereals is not only dependent on the assimilation of dry matter and stem reserves but also on the effective partitioning of these reserves to the seeds. The contributions become greater when plants are grown under salinity stress than under non- salinity treatment. The highest of dry matter from shoots and stem reserves contribution to seed yield (32.09 % and 26.72 % respectively) was observed in salinity of

80 mM. The lowest of them (16.45 and 13.33 % respectively) was obtained at no-salinity (Table 2). Yang *et al*. (2006) reported that enhanced remobilization and increased seed filling duration are mainly attributed to elevated ABA levels in the stems and seeds when subjected to stress condition. On the other hand, there were a reduction about 47.45% and 50% in contribution of remobilization from shoots to seed and stem reserve contribution in seed yield, respectively in application of nano zinc oxide as Zn_2 and inoculation of AM fungi as M_1 in comparison with related controls (Table 2). In many respects this is surprising, considering that there was much early speculation concerning a role of growth regulator in mediating plant responses to drought and high salinity (Wang *et al*., 2009).

Table 1. Summary result of analysis of variance of measured parameters

S.O.V	df	Dry matter remobilization from shoots	Dry matter remobilization from stem	Contribution of remobilization from shoots to seed	Stem reserve contribution in seed yield	Chlorophyll index	Seed yield
Replication	2	0.0045^{ns}	0.0004^{**}	18.10^{**}	9.65^{**}	51.30^{*}	0.036^{ns}
Soil salinity (S)	2	0.088^{**}	0.003^{**}	1150.57^{**}	719.57^{**}	236^{**}	0.163^{**}
Nano zinc oxide (Zn)	2	0.06^{**}	0.001^{**}	588.97^{**}	386.23^{**}	163.5^{**}	0.166^{**}
Mycorrhiza (M)	1	0.08^{**}	0.0003^{**}	87.98^{**}	33.35^{**}	224.89^{**}	0.107^{**}
S × Zn	4	0.001^{ns}	0.00001^{ns}	7.09^{ns}	4.79^{ns}	13.452^{ns}	0.028^{ns}
S × M	2	0.0006^{ns}	0.00003^{ns}	0.84^{ns}	0.658^{ns}	2.16^{ns}	0.034^{ns}
Zn × M	2	0.001^{ns}	0.00007^{ns}	4.52^{ns}	2.30^{ns}	3.61^{ns}	0.050^{ns}
S × Zn × M	4	0.0009^{ns}	0.000005^{ns}	3.720^{ns}	1.96^{ns}	2.58^{ns}	0.033^{*}
Error	34	0.0008	0.000005	0.98	0.59	10.25	0.020
CV(%)	-	10.71	7.76	15.13	12.11	9.8	2.03

ns, * and ** show not significant and significant differences at 5% and 1% probability level, respectively.

Chlorophyll index

Nano zinc oxide, mycorrhiza and saline condition had strong effects on leaf chlorophyll content. Soil salinity drastically lowered leaf chlorophyll content (Table 2). However, nano zinc oxide and mycorrhizal inoculation considerably increased the pigment content (Table 2). Salinity causes a reduction in chlorophyll content (Table 2), which is the essential component of the chlorophyll structure (Kadian *et al*., 2013), suppressing the activity of specific enzymes required for the synthesis of photosynthetic pigments (Murkute *et al*., 2006) and decreased uptake of nutrients (e.g., Zu) needed for chlorophyll biosynthesis (Sheng *et al*., 2008; Abdel Latef and Chaoxing 2011). The highest activity of chlorophyll index (60.57, 60.16 and 58.76) was observed in salinity of S_1, application mycorrhizae as M_1, nano zinc oxide as Zn_2 respectively (Table 2). A decrease in chlorophyll index of wheat under salinity stress (Table 3) would be a typical symptom of oxidative stress (Reddy *et al*., 2004). Higher contents of chlorophyll pigments in AMF-inoculated plants contribute to the greater photosynthetic activity leading to maintained growth. Our results of enhanced chlorophyll contents in AMF-colonized plants are similar to support of the findings of Hajiboland *et al*. (2010).

Table 2. Means comparison of measured traits affected by salinity, nano zinc oxide and mycorrhizal fungi

Treatment	Dry matter remobilization from shoots (g per plant)	Dry matter remobilization from stem (g per plant)	Contribution of remobilization from shoots to seed (%)	Stem reserve contribution in seed yield (%)	Chlorophyll index
Salinity Stress					
S_1	0.185^c	0.170^c	16.45^c	13.33^c	60.57^a
S_2	0.192^b	0.174^b	23.09^b	19.26^b	55.17^b
S_3	0.198^a	0.177^a	32.09^a	26.72^a	54.4^b
Nano zinc oxide					
Zn_0	0.195^a	0.175^a	28.81^a	23.91^a	54.57^b
Zn_1	0.191^b	0.171^b	23.54^b	19.45^b	55.41^b
Zn_2	0.188^c	0.165^c	19.54^c	15.95^c	60.16^a
Mycorrhizal fungi					
M_0	0.194^a	0.177^a	24.75^a	20.89^a	54.67^b
M_1	0.190^b	0.173^b	22.18^b	18.65^b	58.76^a

*Means with similar letters in each column are not significantly different via LSD test at 5% probability level.
S_0, S_1 and S_2: no-salt (control), salinity 40 and 80 mM NaCl, respectively.
M_0 and M_1: no-application of mycorrhiza and application mycorrhiza, respectively.
Zn_0, Zn_1 and Zn_2: without nano zinc oxide as control, application of 0.4 and 0.8 $g.lit^{-1}$, respectively.

Enhancement in the chlorophyll pigments due to AMF is because of enhanced mineral uptake especially magnesium, an important component of chlorophyll (Sheng *et al*., 2008). Kadian *et al*. (2013) also reported that chlorophyll activity is restored in mycorrhizal plants grown due to increased activity of the specific enzymes required for its biosynthesis. Balashouri and Prameeladevi, (1995) reported that the increased chlorophyll content was obviously due to zinc at low level act as a structural and catalytic components of proteins, enzymes and as cofactors for normal development of the pigment biosynthesis. Sharma *et al*. (1994) reported added zinc enhanced the growth of cabbage and improved the chlorophyll content and photosynthetic activity in the leaves. Zarrouk *et al*. (2005) indicated a positive correlation of Zn concentrations with leaf chlorophyll.

Seed yield

Interaction effect between salinity, mycorrhizae and nano zinc oxide showed that the highest seed yield (0.44 g per plant) was obtained at no salinity condition, use of mycorrhizae and nano zinc oxide as M_1Zn_2. But the minimum of seed yield (0.27 g per plant) observed in salinity of 80 mM and Zn_0M_0 (Table 3). Salt stress affects plant metabolism, which results in decreased growth and yields. Lower chlorophyll index due to salt stress resulted in higher dry matter and stem reserves mobilization to seed yield, which might have been a major reason for seed yield reduction in wheat. The relationship between decrease in the stem weight from anthesis to the maturity and seed yield indicates the smallest decreases in stem weight at each of the sites were associated with the highest yields. This decrease in stem weight during seed filling support the proposal that stored assimilates was used during seed filling. Rhizosphere the micro organisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and, consequently, enhance yield both directly and indirectly (Dimkpa *et al*., 2009).

Table 3. Means comparison interaction effect of different salinity level, mycorrhiza fungi and nano zinc oxide on Seed yield

Treatment		Seed yield (g per plant)	
Salinity	**Nano zinc oxide**	**M_0**	**M_1**
S_1	Zn_0	0.34^{*bd}	0.36^{ab}
	Zn_1	0.36^{cd}	0.40^{bc}
	Zn_2	0.45^{ab}	0.44^{a}
S_2	Zn_0	0.32^{f-j}	0.35^{f-i}
	Zn_1	0.37^{f-i}	0.38^{cde}
	Zn_2	0.40^{d-g}	0.43^{def}
S_3	Zn_0	0.27^{c-f}	0.34^{j}
	Zn_1	0.35^{j}	0.35^{g-j}
	Zn_2	0.39^{d-g}	0.41^{cd}

*Means with similar letters in each column are not significantly different via LSD test at 5% probability level.

S_0, S_1 and S_2: no-salt (control), salinity 40 and 80 mM NaCl, respectively.

M_0 and M_1: no-application of mycorrhiza and application mycorrhiza, respectively.

Zn_0, Zn_1 and Zn_2: without nano zinc oxide as control, application of 0.4 and 0.8 $g.lit^{-1}$, respectively.

Azcón and Barea (2010) has been proposed co-inoculation with bio fertilizer as an efficient procedure to increase plant growth. Vivas *et al*. (2003) suggested there are synergistic effects on plant growth when mycorrhiza is inoculated, particularly under growth limited conditions. Gobarah *et al*. (2006) reported foliar application with zinc levels had a significant effect on growth, yield and its components as well as seed quality under salinity conditions. Significant increases in seed yield with foliar Zn application have been reported in other crops such as rice (Cakmak, 2008), triticale (Cakmak *et al*., 1997), common beans (Teixeira *et al*., 2004) and maize (Potarzycki and Grzebisz, 2009).

CONCLUSION

Salinity reduced yield and chlorophyll but dry matter remobilization from shoots increased. Use of mycorrhizae and nano zinc oxide can be recommended to improve production under salinity condition.

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