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# Evaluation Effects of Symbiosis of Mychorrhiza on Yield Components and Some Physiological Parameters of Barley Genotypes Under Salinity Stress

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**Abstract:** This research was conducted to study the effects of AMF (Arbuscular Mycorrhiza Fungi) on yield and root in relation with salinity tolerance index and mycorrhizal dependence on genotypes of barley. To this order, in green houstrial, 4 genotypes of barley (hull less and hull barley) harvested in lisimetry pools in Gorgan. Each plant species was either mycorrhizal fungus, *Glomus intraradicaes* or non-mycorrhizal and irrigated with 3 NaCl levels (0, 8 and 16 dS $m^{-1}$). Data was analyzed with two factor (RCBD). Also, interaction between mycorrhiza and different genotypes of barley were studied. Analysis of variance indicated that at salinity levels and mycorrhiza inoculation, ratio shoot to root, spike weight and length, yield, number of seed, mycorrhizal dependencies and tolerance indices, were significant ($p<0.05$). Correlation coefficients among the traits showed that most of them had positive correlated with each other in this survey. There was no significant correlation among to root length with, ratio shoot to root, root weight, spike length, spike weight and seed number and root weight with ratio shoot to root ($p<0.01$ and 0.05). Single regression has done with Enter method that yield as dependent variable showed 92% variation of yield explained with WDSp/Yield and 64% with WDSp and 73% with RW. Also in arrangement variables such as WDSp/Yield, WSp and RW entered to regression equation stepwise method so that 98% variation of yield proved. Results showed that the inoculation of AMF affected on chlorophyll contents. In EC = 8 dS $m^{-1}$, mycorrhizal dependency increased. High tolerance index was in EC = 8 dS $m^{-1}$. Yield of barley with AMF increased at all salinity levels. The AMF adaptation has shown by this study may explain to increase the salinity tolerance of genotypes barley under salinity conditions.

**Key words:** Barley, mycorrhiza, salinity, colonisation

## INTRODUCTION

Stalinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years (Wang *et al*., 2003). Many studies have demonstrated that AMF protected the host plants inoculation with AMF improves growth of plants under variety of salinity stress condition (Tian *et al*., 2004). Recently Rabie and Almadini (2005) suggested that Arbuscular Mycorrhizal Fungi (AMF) protected the host plants against the determinate effects of salt. To some extent, these AMF have been considered as bio-ameliorators of saline soils (Yano Melo *et al*., 2003).

Under diverse stress conditions, more than 80% of all higher plants are colonized by AMF. These fungi exploit water and mineral salts from soils more effectively than plant roots and transfer them to host (Bothe *et al*., 2006). The symbiotic association between AMF and plant roots is in nature (Uhlmann *et al*., 2006).

Vesicles are hyphal swelling in the root cortex that contains lipids and cytoplasm. In principle, symbiosis can be achieved from only one propagule that germinates and infects a host root but it may take a long time for the AMF to Spread to a significant portion of the root system under such conditions (Tiwari and Adholeya, 2005). Also mycorrhizal root colonization occurred, whether cultivars were salt stressed or non stressed, but the extent of AMF root was higher in AMF inoculated than uninoculated plants. In generally the salt tolerant cultivar had higher AMF root colonization than the salt sensitive cultivar (Paradis *et al*., 2001). The reduced plant growth under highly saline soil is mainly attributed to the negative effect of the high osmotic potential of the soil solution of the highly saline soils which tend to reduce the nutrient and water uptake as well as reduce the plant root growth

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(Munir *et al*., 2003). The objective of this research was to evaluate, salinity tolerance index, mycorrhizal density, colonization and yield compartments of barley genotypes under salinity conditions.

## MATERIALS AND METHODS

This study was conducted in agricultural and natural resources research center of Golestan province Gorgan (Iran) during 2005-2006 periods. Data was performed on two factor (RCBD) over three pools of lisimetry. The factors were; (a) four barley genotypes, $G_1$ = HB7. MOLA /SHIRI/ARUPO*2/JET… $G_2$ = SAHRA (L. B. IRAN), $G_3$ = ELDO/BERMEJO/5/CM67 B/CENTENO/…and $G_4$ = 46951105/VEA/32TH/3/ALGER/CERES36211. SAHRA. In this experiment ($G_2$) was a common barley and the others genotypes were promising lines of hulless, (b), two levels of AMF, AMF0 = non-inoculation and $AMF_1$ = inoculation with *Glomus intraradicaes* and (C): the pools of lisimetry were three levels of salinity stress $S_1$ = 0, $S_2$ = 8 and $S_3$ = 16 dS $m^{-1}$. Each genotype seeds put in peteridishes and placed under 20±2°C temperature. There was planted in separately pools. AMF inoculums were placed in the furrow below. The barley seeds covered with soil on the time of planting. No AMF inoculums were added to the $M_0$ plots.

**Harvest and analysis:** Immediately after harvest, parts of the root systems of non-AM and AM genotypes of barley were washed carefully in 4°C water to remove the adhering soil particles. In order to study percentage of colonization, the roots were cleared and stained. Fragments were cut into approximately 1 cm pieces. Philips and Hayman (1970) procedure in which roots cleared for 50 min in a 10% KOH solution at 90°C in an autoclave rinsed with water, acidified in 10% HCl solution for 10 min and stained with glycerol trypan blue solution (0.05%) at 90°C for 20 min. The root pieces were washed thoroughly with water before distaining overnight then mounted on a microscope slide percentage of root length colonized by mycorrhizal fungi was estimated from entry points were counted at 45-100 magnification and expressed as percentage of root length colonized and colonized root length (Carvalho *et al*., 2004).

**Analytic methods:** At the end of the experiment, dry matter was measured. At each salinity level the Mycorrhizal Dependency (MD) of the plants was calculated according to Gedemann (1975) as:

$$MD = \frac{\text{DW AM plant particular level of salinity}}{\text{DW non AM plant particular level of salinity}} \times 100$$

Tolerance indices (Ti) of AM and non-AM barley genotypes were determined according to Shetty *et al*. (1995) as:

$$Ti = \frac{\text{DW plant at salinity level}}{\text{DW plant at control level}} \times 100$$

In order to evaluate the role of mycorrhiza symbiosis in relation of reducing effects of stress, parameters of yield were calculated.

Roots collected separately. Samples were carefully washed with tap water and then demonized water. After oven drying at 70±2°C for 48 h. The dry matter of spike, root and seed were done. Statistical analyses for obtained data were done by using of the MSTATC program (Michigan state university, East Lansing, Mich, USA).

Duncan's test was used to compare treatments means when the analysis of variances indicated significance. Person's correlation coefficients were calculated t-values to describe the relationship between them (Gomez) Then, to delete effect of worthless variables, on yield of barley, in regression model, we used from stage regression.

## RESULTS AND DISCUSSION

**Plant growth and shoot to root ratio:** Analysis of variance indicated that high salinity caused low shoot and root in barley (Table 1).

Interaction of mycorrhizal colonization in salinity level (EC = 8 dS $m^{-1}$) effected Shoot barley compared ($p<0.05$) with non-mycorrhizal, but had no effect in root growth. Among of different genotype was studied, HBL11 (hull-less) enhancement root and shoot growth. Mycorrizal colonization induced in morphological or physiological changes in the roots (Atkinson *et al*., 1994) with subsequent changes in mycorrhizospher and effects on uptake mineral nutrients and promotes conditions conductive to plants (Chen *et al*., 2004).

Non mycorrhizal hyper accumulator species that translocation metals efficiently to the shoots may be useful for phytoextraction of ions from the soil, but mycorrhizal plants minimize toxicity ion translocation to the shoots, may be useful for phytostablization of contaminated sites (Leyva *et al*., 2002).

Root dry weights of barley in salinity levels were higher in *G. intraradicaes* Plants than non-inoculated plant. Among interaction genotypes of barley and AMF inoculation had not significantly different, but sahra. Var (hull) showed highest root dry weight (Table 1). The positive effects of AMF on yield and root weight were enhanced by addition to hyphal compartment

Table 1: Analysis of variance among different genotypes of barley

| Treatments | df | Yield | Root dry weight | Spike weight | Shoot/ root | Colonisation |
|---|---|---|---|---|---|---|
| Salinity | 2 | 0.139 | 44.185 | 8.46** | 0.424** | 984.979 |
| R(Salinity) | 12 | 0.079 | 26.338 | 0.46 | 0.050 | 439.966 |
| G | 3 | 0.278** | 47.904 | 7.51** | 0.304** | 308.287 |
| M | 1 | 0.786** | 11.255 | 8.74** | 0.672** | 6429.591** |
| Salinity×G | 6 | 0.208** | 16.983 | 1.39** | 0.058 | 834.719 |
| G×M | 3 | 0.279** | 120.779 | 0.94** | 0.223** | 156.533 |
| Salinity×M | 2 | 0.008 | 23.269 | 3.70** | 0.049 | 213.445 |
| Salinity×G×M | 6 | 0.166* | 53.560 | 1.53** | 0.097 | 369.658 |

*: $p<0.05$; **: $p<0.01$

(Ruiz-Lozano and Azcon, 1995). The upper root compartment. contained the mycorrhizal plants in salinity conditions related to ability. Penetration AMF hyphae, that symbiosis with roots, but not only by roots. The solutes, which participate in osmotic Adjustment, are inorganic ions or uncharged organic compounds, like praline or glycine betaine, as well as carbohydrates (Ruiz-Lozano, 2003).

**Yield:** Yield of barley was also affected by an interaction of salinity and AMF treatments and increased with *Glomus intraradicaes* than without inoculums in EC = 8 and 16 dS $m^{-1}$ levels. Yield was generally higher in the AMF inoculated plants than in the non-inoculated plants. Also, there was a significant genotype×mycorrhiza inoculums interaction. These evidence improved $G_1$ with *G. intraradicaes* had significantly low yield (Table 1). At salinity levels and mycorrhiza inoculation, in barley genotypes, length and weight of spike, number of seed and seed yield improved significantly.

**Percentage of colonization:** The colonization of barley roots with *Glomus intraradicaes* was significant ($p<0.05$) (Table 1). Analysis variance percentage root colonized did not differ significantly between sites, but in EC16 dS $m^{-1}$ without AMF comparison average of data decreased difference significantly in compare of with it. Interaction salinity×mycorrhiza indicated that Colonization of roots with *G. intraradicaes* was higher than without inoculums.

Colonization of plant roots by some AMF is reduced in the presence of sodium chloride (NaCl), probably due to a direct effect of NaCl on the fungi (Juniper and Abbott, 2006) and these depend on carbohydrate Supply (Ulrich *et al*., 2001). In generally, the percentage colonized in glycophytes significantly decreased with increasing soil salinity, barley with mycorrhizal colonization in high soil salinity (Aliasgharzadeh *et al*., 2001).

**Mycorrhiza Dependence (MD):** Evidence from the data in Fig. 1 indicates that mycorrhizal dependencies for plant dry mass decreased by raising salinity, but this ratio, was highest in 8 dS $m^{-1}$. A comparison between types of

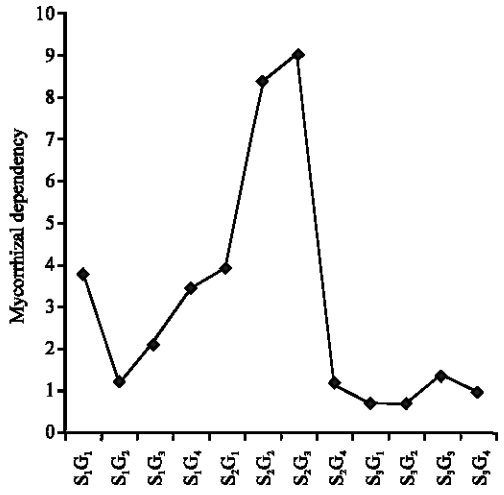


Fig. 1: Effect of salinity levels on mycorrhizal dependency of the plants

barley genotypes and salinity area's indicated that $G_3$ was significantly high in EC = 8 dS $m^{-1}$ (Fig. 1). A similar effectiveness of AMF for different plant species was reported by Dixon *et al*. (1997) in slain soil, the MD of *S. aegyptiaca* and *S. grandiflora* increased with the age of the plants our results show that, under salt stress conditions. Plants need mycorrhiza not only for acclimatization but also for continued nutrient uptake during progressive growth stages (Giri and Mukerji, 2004).

**Tolerance index:** Tolerance indices of barley genotypes with *G. intraradicaes* were significant (in EC = 8, 16 dS $m^{-1}$). Among different genotypes of barley in lisimetry pool of EC = 8 dS $m^{-1}$, hb17 had high significant tolerance indexes, but in EC = 16 dS $m^{-1}$ hb7 increased significantly (Fig. 2). As previously mentioned, mycorrhizal protection against salinity stress caused. It perhaps one of the most important mechanisms by which the AM symbiosis increases the tolerance of host plants against salinity (Rabie and Almadini, 2005).

**Correlation and regression:** Correlation coefficients among the traits showed that most of them had positive correlated with each other in this survey. There was no significant correlation among to root length with, ratio shoot to root, root weight, spike length, spike weight and seed number and root weight with ratio shoot to root ($p<0.01$ and 0.05) (Table 2).

In order to show regression equation between variables such as yield, spike dry weight/Yield, spike and root dry Weight we used single regression with Enter method. So that yield was recorded as dependent variable and others as independent variable. All of them regression model were significant ($\alpha<0.01$) (Table 3, 4).

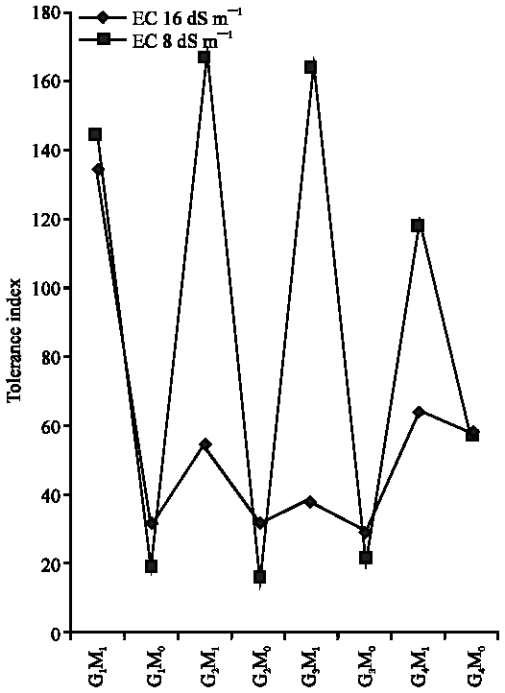


Fig. 2: Effect of salinity levels on salinity tolerance of AM and non-AM plant

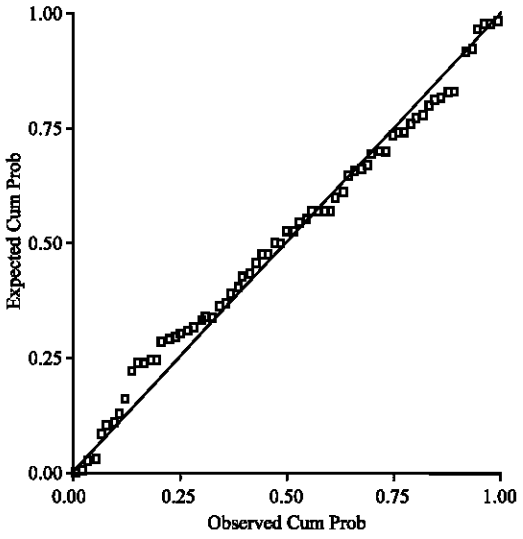


Fig. 3: Normal p-p plot of regression standardized Residua

$$SQRT\ (1/Yeild) = 0.929-0.194\ WDSp/Y+\varepsilon_i$$
$$ADJR^2 = 0.92$$
$$Yield = 1.066\ WSp+ \varepsilon_i$$
$$ADJR^2 = 0.64$$
$$Log\ (1/Yeild) = -0.203\ WRoot +\varepsilon$$
$$ADJR^2 = 0.73$$

Table 2: Correlation coefficients among studied traits

| Traits | Root length | Root weight | Spike length | Spike weight | Seed No. | Shoot/ root | Plant height |
|---|---|---|---|---|---|---|---|
| Root length | 1 | 0.035 | 0.039 | 0.039 | 0.005 | 0.053 | 0.210* |
| Root weight | | 1 | 0.394** | 0.435** | 0.264** | 0.083 | 0.472** |
| Spike length | | | 1 | 0.671** | 0.644** | 0.614** | 0.806** |
| Spike weight | | | | 1 | 0.804** | 0.862** | 0.748** |
| Seed No. | | | | | 1 | 0.810** | 0.597** |
| Shoot/Root | | | | | | 1 | 0.677** |
| Plant height | | | | | | | 1 |

Table 3: Analysis variance in regression model between different variables

| Durbin watson | Adjusted $R^2$ | $R^2$ | Sig | F | Mean square | df | Sum of squares | Model |
|---|---|---|---|---|---|---|---|---|
| 1.983 | 0.982 | 0.983 | 0.000 | 949.57 | 11.486* | 4 | 45.945 | 4 |

Table 4: Coeffections of dependent variables

| | | Unstandardized coefficients | Standardized coefficients |
|---|---|---|---|
| Model 4 | β | Std. Error | β |
| (Constant) | -0.574 | 0.075 | |
| WDSPY | 1.012 | 0.021 | 0.922 |
| WDSP | 0.429 | 0.076 | 0.103 |
| WSP | 0.089 | 0.026 | 0.073 |
| WROOT | 0.058 | 0.026 | 0.039 |

In arrangement showed that 92, 64 and 73% yield variations proved. With aim to remove worthless variables, results of multiple regression between yield and other variable that they had significant correlation with yield (Table 4). Stepwise regression showed, in arrangement variables of WDSp/yield, Spike and root dry weight entered to regression model and they were significant ($\alpha<0.01$) (Table 3 and Fig. 3)

$$Yield = 0.0574 + 1.012\ (WDSp/y) + 0.429\ (WDSp) + 0.089\ WSp + 0.058\ (Wroot)$$
$$With\ ADJR^2 = 0.98$$

Equation Showed that 98% variation of yield, proved with these variables. Results of coefficients and β standardized, determined that the most important variable on yield was ratio spike dry weight to yield.

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