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The Effect of the Arbuscular Mycorrhizal (AM) Fungus *Glomus intraradices* on the Growth and Yield of Chilli (*Capsicum annuum* L.) Under Salinity Stress

G. Selvakumar and P. Thamizhiniyan

Department of Botany, Annamalai University, Annamalai Nagar-608 002 Tamilnadu, India

Abstract: The present investigation was aimed at determining the effects of arbuscular mycorrhiza on growth and yield of chilli plants grown under salinity condition. Seeds of chilli were sown in sand beds in size $5\times1m^2$ under shade net condition at Botanical Garden, Department of Botany, Annamalai University. Forty days old seedlings (DAS) were transplanted into pots (one seedling per pot) until the fruit ripening period. After transplanting, seedlings of chilli were treated with AM fungi, 25 mM NaCl, AM fungi with 25mM NaCl, 50 mM NaCl and AM fungi with 50 mM NaCl. Observations showed that whole plant dry weight (g plant^{-1}), plant height (cm), no. of primary branches per plant, fruit length (cm), fruit girth (cm), fruit yield (kg plant^{-1}) and root colonization (%) decreased with increasing dose of salinity. Among these treatments, AM fungi treated plants shows maximum yield when compared to control and others. It promotes salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition, producing plant growth hormones, improving rhizophoric and soil condition, altering the physiological and biochemical properties of the host. The overall results suggest that mycorrhizal colonization improves host plant mineral concentration and thereby alters fruit production and quality of fruits under salinity stress in chilli plants.

Key words: *Capsicum annuum* · Salinity stress · AM fungi · Mineral elements · Membrane Permeability

INTRODUCTION

Soil salinity is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi arid areas [1]. Saline soils occupy 7% of the earth's land surface [2] and increased salinization of arable land will result into 50% land loss by the middle of the 21st century [3]. At present out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares (5%) is affected by excess salt content [4].

The significance of soil salinity for agriculture yield is enormous as it affects the establishment, growth and development of plants leading to huge losses in productivity [5]. The direct effects of salt on plant growth may involve: (a) Reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought. To counteract this problem, plants must maintain lower internal osmotic potential in order to prevent water movement from roots into the soil [6]. (b) Toxicity of excessive Na^+ and Cl^- ions towards the cell that disrupts to the structure of enzyme and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis [7] and nutrient imbalance in the plant caused by nutrient uptake and or transport to the shoot leading to ion deficiencies [8].

AM fungi widely exist in salt affected soils and it is associated with the roots of 80% terrestrial plant species [9] including halophytes, hydrophytes and xerophytes. Root colonization by AM fungi involves a series of morpho-physiological and biochemical events that are regulated by the interaction of plants and fungus as well as by environmental factors. To some extent, these fungi have been considered as bioameliorators of saline soils [10].

Chili peppers that belong to the plant genus *Capsicum* (family Solanaceae) are among the most heavily consumed spices throughout the world. India is major producer, consumer and exporter of chilli contributing almost one fourth of the world production. During 2005-2006, 138,419Mt of chili worth $100 million were exported [11]. Chilli contributes about 33% the total spice export from India and accounts for about a 16% share of the world spice trade. In this context, the utilization of AM fungi may help to the growth and yield for managing under salinity in chilli plants.

Corresponding Author: P. Thamizhiniyan, Department of Botany, Annamalai University, Annamalai Nagar-608 002 Tamilnadu, India. Tel: 04144 238282, Fax: 04144 238080.

MATERIALS AND METHODS

Chilli Preparations: Chilli from var. PKM_1 "*Capsicum annuum*" was supplied by Vegetable and Fruit Research Station, Periyakulam, Tamilnadu, India. Chilli seeds were surface sterilized with 0.05% sodium hypochloride for 45min before sowing. The treated seeds including control were sown in sand beds in size 5×1 m^2 in a shade net condition at Botanical Garden, Department of Botany, Annamalai University, India.

Mycorrhizal Inoculation: Plants were inoculated with AM fungi *Glomus intraradices* obtained from the Agricultural Microbiology, Faculty of Agriculture, Annamalai University, India. The isolate was propagated on maize grown in a green house for 8 weeks on a perlite vermiculture medium. The colonized maize roots were used as an inoculum (10g fresh weight per pot containing approximately 1200 spores) was placed in the pot at 15cm depth, immediately prior to transplanting chilli seedling into the pots to facilitate fungal colonization on plant roots.

Sodium Chloride Treatments: Forty days old seedlings (DAS) were transplanted into pots (one seedling per pot). The experiment was designed in completely randomized block design (CRBD) with five replication. Seedlings of chilli were treated with AM fungi, 25 mM NaCl, AM fungi with 25mM NaCl, 50 mM NaCl and AM fungi with 50 mM NaCl.

Observation of the Chili Growth: The whole plant dry weight (g $plant^{-1}$), plant height (cm), no. of primary branches per plant, fruit length (cm), fruit girth (cm), fruit yield (kg $plant^{-1}$) and root colonization (%), chlorophyll and proline content were observed.

Estimation of Chlorophyll [12]: 0.5 mg of fresh leaf was ground in a mortar and pestle with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was restricted with 5 ml of 80 per cent acetone each time, until it become colorless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 and 663nm in spectrophotometer. The chlorophyll content was determined by using the following formulae.

Chlorophyll 'a' (mg/g fr. wt.) = (0.0127) × (OD663) - (0.00269) × (OD645)
Chlorophyll 'b' (mg/g fr. wt.) = (0.229) × (OD645) - (0.00488) × (OD663)
Total chlorophyll (mg/g fr. wt.) = (0.0202) × (OD645) - (0.00802) × (OD663)

Electrolyte Leakage: To resolve electrolyte leakage, fresh leaf samples (200 mg) were cut into 5 mm length and placed in test tubes containing 10 ml distilled deionized water. The tubes covered with plastic caps were placed in a water bath at a constant temperature of 32±8°C. After 2h the initial electrical conductivity of the medium (EC1) was measured using an electrical conductivity meter. The samples were autoclaved afterwards at 121±8°C for 20 min to kill the tissues completely and release all electrolytes. The samples were then cooled to 25±8°C and the final electrical conductivity (EC2) measured. The electrolyte leakage (EL) was estimated using the following formula:

EL = EC1/EC2 × 100 [13].

Proline Determination: Proline was determined by the following method [14]. Fresh leaf material (0.5g) was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and then this aqueous solution was filtered through whatmann's No. 2 filter paper and finally 2 ml of the filtrate solution mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1h at 100±8°C. The reaction mixture was extracted with 4ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature and the absorbance was read at 520nm using Shimadzu UV 1601 spectrophotometer. Appropriate proline standards were used for the calculation of proline in the sample.

Mycorrhizal Colonization: Root samples were washed well with 10% KOH solution and stained with 0.1% Trypan blue before estimation of mycorrhizal colonization. Arbuscular mycorrhizal colonization was estimated using a modified line intersect method [15], where a minimum of 100 line intersections per root sample, replicated three times, were scored for the presence of AM structures. These observations were made using the light microscopy to rate the degree of root infection by AMF in one plant per replicate (three plants per treatment). The percentage of AM infection was calculated from the following equation:

$$\text{Percentage of AM infection} = \frac{\text{Root length infected}}{\text{Root length observed}} \text{X } 100$$

Fruit Harvest, Dry Weight and Nutrient Analysis: Fruits were harvested weekly from the mid July to end of July for two weeks. The values for the fruit yields are the means of the fruit yield of six plants per replicate and given in grams per plant.

Three randomly selected plants per replicate were divided into leaves, stems and roots and dried in an oven at 70±8°C for two days to determine dry weights and elemental concentrations. Nutrient analyses were carried out on dry weight basis. Total N was determined in samples of 0.1 g dry weight using the Kjeldahl method. Ground samples were dry-ashed at 50±8°C for 6h, mixed with 2M hot Hcl filtered and then brought to a final volume of 50 ml with distilled water. Sodium and Potassium were determined in these sample solutions. Phosphorous was analysed by a vanadate molybdate method using a UV/Visible spectrophotometer (Shimadzu UV, 1601) and other elements were analyzed using an ICP (Inductive Coupled Plasma) following [16].

Statistical Analysis: Differences among treatments were analyzed for main effects (salinity and mycorrhizae) and their interaction by standard deviation (±SD).

RESULT AND DISCUSSION

Colonization Rates, Plant Growth and Fruit Yield: Microscopic assessment of AM colonization rates are shown in Table 1. confirmed that plants of the non-inoculation treatment were not colonized by AM fungi. The plants inoculated with isolate of mycorrhizae had colonization, percentage ranging from 66% to 10% in the roots of non-stressed and salt stressed plants, respectively. As is evident from (Table 1) the salinity, not only affects the host plant but also the AM fungi. It can hamper colonization capacity, spore germination and growth of hyphae of the fungus. The colonization rate declined with increasing NaCl level indicating that salinity suppressed the growth of AM fungi.

Salinity stress significantly reduced shoot, root and whole plant dry matter compared with control (Table 1). However, AM colonization significantly improved this parameter in the salt-stressed plants but they remained lower than the values for control plants in all cases. The reasons may be the non-availability of nutrients and the expenditure of energy to counter the toxic effects of NaCl. However, mycorrhization was found to increase the fitness of the host plant by enhancing its growth and biomass. Al-Karaki [17] observed a higher shoot and root dry weight, fresh fruit yield, weight and number in mycorrhizal tomato plants than in a non-mycorrhizal plant. Colla *et al.* [18] reported improved growth yield, water status, nutrient content and quality of fruits of *Cucurbita pepo* plants colonized by *Glomus intraradices* when exposed to salinity stress. In the present study, mycorrhizal chilli plants had higher leaf than non-mycorrhizal plants at both salinity treatments.

Fruit yield was significantly reduced by increasing salt concentration when compared to the control plants (Table 1). Similar effects of salinity in reducing yield for a range of the agricultural crops including tomato [19]. Mycorrhizal colonization significantly improved yield of salt-stressed tomato plants.

Chlorophyll, Proline Content and Electrolyte Leakage: Chlorophyll concentration was significantly reduced by salinity treatments (Table 2). The increasing salinity causes reduction in chlorophyll content [4] due to suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments [20] Reduction in the uptake of minerals like that Magnesium needed for chlorophyll biosynthesis also reduced the chlorophyll concentration in the leaf [21].

Table 1: Response of AM Fungi (*Glomus intraradices*) on growth, fruit and root colonization of chilli plant grown under different salinity stress

Treatment	Whole plant DW (g plant^{-1})	Plant height (cm)	No. of primary branches per plant	Fruit length (cm)	Fruit girth (cm)	Fruit yield (kg plant^{-1})	Root colonization (%)
Control	50.2±1.51	65.32±1.96	5.97±0.18	9.0±0.270	1.6±0.04	2.22±0.06	10±0.30
AM fungi	57.7±1.73	73.5±2.200	7.16±0.21	10.1±0.300	2.3±0.06	3.01±0.09	66±1.98
25mM NaCl	43.9±1.32	63.12±1.89	5.54±0.17	7.56±0.23	1.5±0.04	1.44±0.04	0.00
AM Fungi with 25 mM NaCl	53.1±1.59	66.03±1.98	6.72±0.20	8.73±0.26	1.9±0.05	1.29±0.03	24±0.72
50mM NaCl	26.3±0.79	42.89±1.29	4.32±0.13	6.22±0.19	1.0±0.03	1.11±0.03	0.00
AM Fungi with 50 mM NaCl	48.2±1.45	54.71±1.64	5.02±0.15	6.66±0.20	1.2±0.04	1.59±0.05	15±0.45

The values are mean ± SD for three samples in each group

Table 2: Chlorophyll contents, proline and electrolyte leakage of chilli plants grown at different salinity condition with or without arbuscular mycorrhizal (*Glomus intraradices*) inoculation

Treatment	Chlorophyll a (mg kg^{-1})	Chlorophyll b (mg kg^{-1})	Total Chlorophyll (mgkg^{-1})	Proline (µmolg^{-1})	Electrolyte Leakage
Control	1.33±0.039	0.857±0.025	2.08±0.06	1.39±0.04	16.14±0.48
AM fungi	1.34±0.041	0.881±0.026	2.39±0.07	1.26±0.03	16.16±0.48
25mM NaCl	1.12±0.033	0.728±0.021	1.75±0.05	2.35±0.07	31.17±0.94
AM Fungi with 25 mM NaCl	1.35±0.040	0.808±0.024	2.10±0.06	2.13±0.06	25.81±0.77
50 mM NaCl	1.05±0.031	0.631±0.018	1.54±0.04	3.14±0.09	42.14±1.26
AM Fungi with 50 mM NaCl	1.20±0.036	0.758±0.220	1.96±0.05	2.67±0.08	30.1±0.90

The values are mean ± SD for three samples in each group

Table 3: Mineral concentration (%) of Na, N, P and K in leaves of arbuscular fungi and non-arbuscular mycorrhizal (*Glomus intraradices*) chilli plants grown under different salinity stressed condition

Treatment	Sodium (Na)	Nitrogen (N)	Phosphorus (P)	Potassium (K)
Control	0.22±0.006	2.56±0.076	0.28±0.008	1.81±0.054
AM fungi	0.29±0.008	2.57±0.077	0.36±0.010	1.92±0.057
25mM NaCl	1.09±0.032	1.31±0.039	0.19±0.005	1.06±0.031
AM Fungi with 25 mM NaCl	1.02±0.030	1.99±0.059	0.27±0.008	1.33±0.039
50 mM NaCl	1.39±0.041	1.05±0.031	0.14±0.004	0.76±0.022
AM Fungi with 50 mM NaCl	1.16±0.034	1.46±0.043	0.22±0.006	1.12±0.033

The values are mean ± SD for three samples in each group

Hither, the mycorrhizal colonization significantly improved chlorophyll concentration but it did not significantly change chlorophyll concentration in non-stressed plants. This is because NaCl has an antagonistic effect on N absorption which is an essential component of the structure of chlorophyll molecule.

Proline concentration was significantly higher in the salt stressed plants than that in the non-treated plants (Table 2). Proline was significantly lower in mycorrhizal than in non-mycorrhizal plant at both salinity treatments except for non-stressed plants. Proline accumulation is thought to be an adaptive feature under salinity stress in AM [22] and non-AM [23] legumes. Under saline condition, many plants accumulate proline as a non-toxic and protective osmolyte to maintain osmotic balance under low water potentials [24] it also act as a reservoir of energy and nitrogen for utilization during salt stress.

Salt stress caused a significant increase in electrolyte leakage compared to that in the non-stressed plants (Table 2). Similar result was obtained by [25] for NaCl-sensitive rice varieties wherein high salt concentration increased membrane permeability. However, mycorrhizal inoculation significantly reduced the electrolyte leakage in the salt-stressed plants of chilli. These results confirms that the finding of a previous study in which it was shown that salt-stressed tomato plants inoculated with mycorrhizae had lower membrane permeability than non mycorrhizal plants [26].

Concentration of Mineral Elements: Concentration of Na^+ was significantly increased in the leaves and root of chilli plants in the presence of NaCl stress. These data closely match those reported by other workers for other crop species eg, tomato [27]. The AM fungi colonized plants have lower levels of Na^+ [28] compared to the non-mycorrhizal plants at the both salinity treatments, except for those in non-salt stressed plants (Table 3). Ruiz-Lozano *et al.* [2] found that AM can increase plant growth and uptake of nutrient, decrease yield losses of tomato under saline condition and hence improve salt tolerance of tomato.

Increasing NaCl levels reduced P concentration in non-inoculated plants (Table 3). It is known that salt-stress induces P deficiency in plants by reducing P uptake or translocation. In the present study, mycorrhizal inoculation promoted the growth of chilli plants under saline stress by increasing P accumulation in plant tissues. It improves P nutrition of plants under salinity stress and reduces the negative effects of Na^+ by maintaining vacuolar membrane integrity, which prevents this ion from interfering in growth metabolic pathways [29]. Concentrations of K^+ and N were decreased in the leaves of pepper in the presence of NaCl stress (Table 3). It has been earlier reported that K^+ concentration was lowered in plants by increasing NaCl concentration in nutrient solution or in soil, e.g., tomato [30].

CONCLUSION

Taken together, although salinity reduced mycorrhizal colonization, the dependency of chilli plants on mycorrhizal fungi was increased. This may be a sign showing the ecological importance of AM association for plant survival and growth under salinity stress. In the present study, mycorrhizal inoculation enhanced the growth and yield in chilli plants by reducing leaf Na^+ and increasing membrane stability and concentrations of essential inorganic nutrients such as N, P and K. Hither to, show that under saline conditions, chilli plants need mycorrhizae not only for acclimatization but also for continued nutrient uptake during the progressive growth stages. In view of these results, it is possible to recommend mycorrhizal inoculation to attain reasonable growth and yield of chilli under saline condition.

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