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Influence of Arbuscular Mycorrhizal (AM) Fungi and Salinity on Seedling Growth, Solute Accumulation, and Mycorrhizal Dependency of *Jatropha curcas* L.

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Abstract Production of *Jatropha curcas* as a biodiesel feedstock on marginal lands is growing rapidly. Biomass production on these lands is limited. Hence, the objective of this study was to evaluate the effect of arbuscular mycorrhiza (AM) fungi and salinity (0.1, 0.2, 0.3, 0.4, and 0.5% NaCl) on (1) seedling growth, leaf relative water content (RWC), lipid peroxidation, solute accumulation (proline and sugars), and photosynthetic pigments (Chl *a* and *b*) of *Jatropha*; (2) mycorrhizal colonization (%) and mycorrhizal dependency (MD) of *Jatropha*; and (3) glomalin content (Bradford reactive soil protein) in soil. Increased soil salinity significantly ($P < 0.05$) decreased AM root colonization ($r^2 = 0.98$) of AM-inoculated plants and decreased survival ($r^2 = 0.93$) and growth (shoot length, $r^2 = 0.89$; tap root length, $r^2 = 0.93$; shoot diameter, $r^2 = 0.99$; shoot dry weight, $r^2 = 0.92$; and root dry weight, $r^2 = 0.92$) of non-AM-inoculated *Jatropha*. Under salt stress, AM-inoculated *Jatropha* plants had greater dry weight of shoots and roots, better leaf water status, less leaf membrane damage (low lipid peroxidation activity), higher solute (proline and sugars), and higher leaf chlorophyll concentrations than non-AM-inoculated plants. The mycorrhizal dependency (MD) of *Jatropha* increased from 12.13 to 20.84% with salinity (0–0.4% NaCl). Root AM colonization (%) and glomalin content in soil were negatively correlated with salinity ($P < 0.05$, $r = -0.95$). We conclude that inoculation with AM fungi lessens the deleterious effect of salt stress on seedling growth parameters under salt levels up to 0.5% NaCl (electrical conductivity of 7.2 dS m^{-1}). Inoculation of *Jatropha* seedlings with AM fungi can promote the establishment of *Jatropha* under NaCl-induced stress.

Keywords Jatropha curcas · Malondialdehyde content · Leaf relative water content · Mycorrhizal dependency · Bradford reactive soil protein · Photosynthetic pigments

Introduction

Salinity can render arable land unproductive and is one of the most widespread agricultural problems in arid and semiarid regions of the world (Ashraf and Foolad 2007). Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the middle of the 21st century (Wang and others 2003). It is well known that crop productivity is low in saline soil, mainly due to nutrient imbalances, reduced uptake of nutrients including P, and ion toxicity because of high Na^+ and Cl^- concentrations (Jacoby 1994; Marschner 1995; Cornillon and Palloix 1997; Adiku and others 2001; Juniper and Abbott 2006; Miransari and Smith 2007). As a consequence, salt stress affects all aspects of plant physiology, including growth, photosynthesis, protein synthesis, energy, and lipid metabolism (Ramoliya and others 2004).

Recently, the use of arbuscular mycorrhizal (AM) fungi as a practical way to alleviate soil stresses on plant growth

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has received increased attention (Al-Karaki 2006; Sannazzaro and others 2006; Miransari and others 2007, 2008). AM fungi are important soil organisms, fundamental for plant nutrition and soil fertility (Smith and Read 1997) and represent a living bridge for the translocation of nutrients from the soil to plant roots and of carbon from plant roots to the soil (Zhu and Miller 2003).

Many researchers have reported that AM fungi can enhance the ability of plants to cope with salt stress (Tian and others 2004; Rabie 2005; Jahromi and others 2008) by improving plant nutrient uptake (Cantrell and Linderman 2001; Asghari and others 2005) and ion balance (Zandavalli and others 2004; Giri and others 2007), protecting enzyme activity (Giri and Mukerji 2004; Rabie and Almadini 2005), and facilitating water uptake (Berta and others 1990; Ruiz-Lozano and Azcón 1995). However, there have been very few attempts to study the influence of AM inoculation on photosynthesis and water status under salt stress. A few reports indicate that AM colonization can enhance relative water content in zucchini leaves (Colla and others 2008), water potential and photosynthesis of maize plants (Feng and others 2000; Sheng and others 2008), and chlorophyll concentration in the leaves of several plant species, that is, *Sesbania aegyptiaca*, *Sesbania grandiflora*, and *Lotus glaber* (Giri and Mukerji 2004; Sannazzaro and others 2006; Colla and others 2008).

Recent discoveries suggest that glomalin, a glycoprotein (Wright and others 1998) produced in copious amounts by AM fungal hyphae and related to soil aggregate stability (Rillig 2004), can influence soil carbon storage indirectly by stabilizing soil aggregates (Zhu and Miller 2003) and soil stability. Soil aggregate stability is one of the most important properties controlling plant growth in arid and semiarid environments by controlling soil-plant water status. To better understand salinity tolerance of AM plants, it appears important to include glomalin-related soil protein (GRSP) in studies on AM effects on soil improvement.

Jatropha curcas is currently being promoted by the Indian Government for protection of the environment and for fulfilling future energy requirements (Kumar and Sharma 2008) by planting this species on marginal lands. These marginal sites are limited in use for plant production due to salinity and low soil moisture. Hence, the objective of this work was to evaluate the efficacy of AM fungi in altering various biochemical parameters (leaf relative water content, lipid peroxidation, soluble sugar, proline, and photosynthetic pigments) and mycorrhizal dependency (%) of *Jatropha* when plants are grown in saline soil conditions. We also evaluated whether AM colonization (%) in *Jatropha* and glomalin content in soil surrounding roots of *Jatropha* were influenced by different amounts of NaCl in the soil.

Materials and Methods

Test Plant

Jatropha curcas L., a multipurpose small tree or large shrub (Euphorbiaceae), was used as a test plant. Seeds of *Jatropha* were obtained from C.C.S. H.A.U, Regional Research Station Bawal, Haryana, India.

Field Site and Soil Analysis

The experiment was conducted between August and October 2007 at Micromodel, an experimental site at IIT Delhi, India (28.38 N, 77.12E). The soil used in the experiment was loamy with electrical conductivity (EC) of 0.18 dS m^{-1}, pH 7.26, organic carbon (C, %) 1.73, total nitrogen (N, %) 0.51, 11.3 mg kg^{-1} available phosphorus (P), and 54.3 mg kg^{-1} potassium (K). Soil EC and pH were determined with an EC meter and a digital pH meter (Scientific Systems, New Delhi, India). Total organic C and N were determined by combustion using a CHN analyzer Vario Max CN (Elementar, Hanau, Germany), available P was assessed using Olsen's method (Olsen and others 1954), and K was determined using an ammonium acetate assay (Hanway and Heidel 1952).

Arbuscular Mycorrhizal Inoculum Production

AM fungi were collected from trap cultures (rhizospheric soil of *Ricinus communis* L.) maintained at Micromodel IIT Delhi, India. The inoculum contained a consortium of species, the most dominant of which were *Glomus mosseae*, *Glomus microcarpum*, *Glomus fasciculatum*, *Glomus intraradices*, *Gigaspora margarita*, and *Gigaspora heterogama*. Identification was done using keys (Trappe 1982; Schenck and Perez 1990) based on color, size, surface, number of spore wall layers, hyphae, and hyphal attachments. Propagule infectivity was tested according to the method of Sharma and others (1996).

Experimental Design

The experiment was a 6 × 2 × 4 complete factorial design comprising six salinity treatments (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) and two inoculation treatments (inoculation with AM consortium plus a noninoculated treatment) with four replicates for each treatment. Containers were arranged in a randomized manner. The addition of NaCl (0–5 g NaCl per kg of soil) to the soil increased initial soil EC values from 0.18 (0%, NaCl) to 4.6, 5.6, 6.3, 6.8, and 7.2 dS m^{-1} at 0.1, 0.2, 0.3, 0.4, and 0.5%, NaCl, respectively. These initial soil EC values were used as target values for each salinity treatment during the experiment. For the experiment, 120

earthen containers (25 cm mouth diameter × 30 cm height) and soil (collected from Micromodel, IIT Delhi) were fumigated by using 1% (v/v) formalin for 24 h under airtight plastic sheets, and the fumigant was allowed to dissipate for 1 week. Each container was filled with 4 kg of soil. Seeds of *Jatropha* plants were surface-sterilized with 7% calcium hypochlorite for 20 min, washed with distilled water, and sown in the soil at a depth of approximately 3-5 cm (2 seeds/container). Soil-based inoculum (containing 90–100 infectious AM fungal spores in 50 g soil) for mycorrhizal treatment was added to the appropriate containers when seeds were sown. Noninoculated treatments received the same amount of autoclaved inoculum (to obtain the same soil texture) together with a 10-ml aliquot of an inoculum filtrate. Seedlings were thinned to one seedling per container 15 days after sowing.

Containers were placed outside at an average daily temperature of 24°C (min) and 36°C (max). Soil EC was measured at weekly intervals and NaCl solutions were added to containers as needed to maintain soils at initial target EC values recorded for each salinity treatment. Plants were watered three times a week and any leachate from runoff was collected using trays under the containers and added back to soil.

Plant Growth Measurement and Biochemical Analysis

Seedling survival was recorded after 2 weeks of seed germination at all the salt levels in all treatments. Four plants per inoculation × salinity treatment combination were harvested 60 days after sowing the seeds for measurements of shoot height, tap root length, and shoot diameter (5 cm above soil surface), and leaf subsamples were taken before harvesting for relative water content (RWC) and biochemical analysis. Roots were rinsed to remove the soil particles and subsamples were saved for assessment of AM fungal root colonization. Roots and shoots were separated and root and shoot biomasses were determined after oven-drying at 70°C until they reached constant weight.

Mycorrhizal infection was estimated by the method of Phillips and Hayman (1970). Randomly sampled roots were cleared and stained with trypan blue. Thirty root segments (0.5 cm long) from each treatment were mounted in 50% glycerol and examined under a compound light microscope. Root pieces that contained even a single hyphae or one or more vesicles or arbuscules were considered infected. The percent root colonization was calculated as the proportion (%) of infected roots out of the total number of roots assessed.

Roots from AM or non-AM *Jatropha* grown with 0 and 0.3% NaCl-mixed soil were taken and scanned (Epson Scanner, V500, high 2400 dpi resolution, Japan) to visualize the effect of salt injury on the root surface.

The mycorrhizal dependency (MD) or response to mycorrhizal colonization was calculated for plants in each salinity treatment by using the following formula (Plenchette and others 1983):

$$\text{MD}\ (\%\ ) = \frac{\text{dry weight of mycorrhizal plant} - \text{average dry weight of noninoculated plant}}{\text{dry weight of mycorrhizal plant}} \times 100$$

Glomalin-related soil protein (GRSP; Bradford reactive soil protein; Rillig 2004) was extracted from soil subsamples from AM-inoculated plants as total glomalin (TG) using the method of Wright and Upadhyaya (1998) at the end of the experiment. Protein content was determined by Bradford assay (Sigma-Aldrich, Inc., St. Louis, MO, USA) with bovine serum albumin as the standard. GRSP analyses were carried out on soil from four replicate pots.

Leaf relative water content (RWC) of the second or third youngest fully expanded leaf from the top of the plant at the end of experiment was used to assess the relative tolerance of mycorrhizal and nonmycorrhizal plants to salinity using the following equations (Schonfeld and others 1988):

$$\text{RWC}(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where FW is leaf fresh weight, DW is leaf dry weight after 24 h drying at 70°C, and TW is leaf turgid weight after submergence in distilled H_2O for 4 h.

Lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid-reactive substances (TBARS) in fresh leaves of *Jatropha* (Heath and Packer 1968).

Chlorophyll Determination

Leaf chlorophyll concentration (Chl *a* and *b*) was measured on the second fully expanded leaf of *Jatropha*. Fresh tissue

(1.0 g) was cut into small segments, extracted with 90% acetone, and read using a UV/visible spectrophotometer at 663, 645, and 750 nm. Absorbance at 750 nm was subtracted from the absorbance at the other two wavelengths to correct for any turbidity in the extract before chlorophyll concentrations were calculated using the following formulae (Strain and Svec 1966):

$$\text{Chl } a\left(\text{mg ml}^{-1}\right) = 11.64 \times (\text{A663}) - 2.16 \times (\text{A645})$$

$$\text{Chl } b\left(\text{mg ml}^{-1}\right) = 20.97 \times (\text{A645}) - 2.16 \times (\text{A663})$$

A663 and A645 represent absorbance values read at 663- and 645-nm wavelengths, respectively.

Proline concentration was determined in fresh leaves using calibration according to Bates and others (1973) and expressed as μmol g^{-1} FW of leaf.

The amount of total soluble sugar was estimated in fresh leaf material using the Anthrone method as given by Thimmaiah (2004). All spectrophotometric analyses were conducted on a PerkinElmer Lambda 25 UV/VIS spectrophotometer (PerkinElmer, Waltham, MA, USA).

Statistical Methods

All data were statistically analyzed using analysis of variance (ANOVA) and least significant difference (LSD) using the SPSS Windows version 10.0 (SPSS, Inc., Chicago, IL, USA). Duncan's multiple-range tests were performed at $P < 0.05$ on each of the significant variables measured. Relationships between AM colonization, glomalin content, and NaCl concentration and between soil EC and NaCl concentration were assessed using Pearson's correlation coefficient (r) at $P < 0.05$. Linear regression analysis was applied to show the effects of increased salinity on plant growth parameters.

Results

Effect of AM Fungi and Salinity on Soil ECs and Seedlings Growth

The ANOVA analysis showed that treatment and salinity affected most growth-related parameters (Tables 1 and 2). Most seedling variables were affected by increased salt levels in the soil. The EC values of the soil solution gradually increased ($r^2 = 0.77$ in non-AM-inoculated pots) from 0.2 to 7.2 dS m^{-1} with increased salt concentration (0–0.5% NaCl), but no significant differences in EC were observed between AM and noninoculated soils at the same salt concentration, even after 50 days of plant growth (Fig. 1). The pH (7.5–7.7) of the soil solution was unaffected by NaCl concentration and inoculation with AM. Increased salinity concentration reduced percentage survival ($r^2 = 0.93$), shoot length ($r^2 = 0.89$), root length ($r^2 = 0.93$), and shoot diameter ($r^2 = 0.99$) of noninoculated *Jatropha* (Table 1). However, AM seedlings had significantly greater survival (%), shoot length, root length, and shoot diameter than noninoculated seedlings, although increment in length with respect to shoot and root was found to be insignificant at 0.5% NaCl concentration (Table 1). Inoculation of *Jatropha* with AM increased percentage survival at high salinity (0.3, 0.4, and 0.5% NaCl) compared to survival of noninoculated plants.

Table 1 Effects of inoculation with AM fungi on root colonization by AM fungi, plant survival, shoot length, root length, and shoot diameter of 60-day-old *Jatropha curcas* grown in soils amended with different rates of NaCl

Salinity NaCl (%)	AM status	AMF root colonization (%)	Survival (%)	Shoot length (cm)	Tap root length (cm)	Shoot diameter (cm)
0	Non AM	0	95 **a**	30.15 **a**	8.74 **bc**	0.72 **b**
	AM	60.66 **a**	100 **a**	33.02 **b**	9.65 **a**	0.78 **a**
0.1	Non AM	0	93.33 **a**	23.27 **c**	8.56 **bc**	0.65 **cd**
	AM	56.66 **a**	100 **a**	28.35 **d**	9.09 **ab**	0.70 **b**
0.2	Non AM	0	81.66 **b**	17.60 **f**	7.09 **d**	0.63 **de**
	AM	48.33 **b**	95 **a**	20.98 **e**	8.26 **c**	0.70 **b**
0.3	Non AM	0	66.66 **c**	17.42 **f**	5.84 **ef**	0.59 **ef**
	AM	41.66 **c**	81.66 **b**	18.36 **f**	7.26 **d**	0.63 **de**
0.4	Non AM	0	51.66 **d**	14.63 **g**	5.33 **fg**	0.55 **fgh**
	AM	38.33 **c**	71.66 **c**	17.78 **f**	6.20 **e**	0.56 **fg**
0.5	Non AM	0	44.44 **d**	12.73 **gh**	4.70 **g**	0.50 **h**
	AM	29.33 **d**	65 **c**	13.61 **g**	5.26 **fg**	0.53 **gh**

AM arbuscular mycorrhizal, Non AM, noninoculated; AM, inoculated

The same letter within each column indicates no significant difference among treatments ($P < 0.05$) as determined by Duncan's multiple-range test. Value are means of $n = 4$

Table 2 Effects of AM fungi on shoot, root dry weight, mycorrhizal dependency, and glomalin[a] concentration in soil of 60-day-old *Jatropha curcas* grown in soils amended with different rates of NaCl

NaCl (%)	AM status	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Mycorrhizal dependency (%)	BRSP (mg g^{-1})
0	Non AM	1.71 **b**	0.63 **b**		
	AM	1.94 **a**	0.72 **a**	12.13 **a**	1.75 **a**
0.1	Non AM	1.27 **de**	0.47 **de**		
	AM	1.51 **c**	0.56 **c**	15.58 **b**	1.58 **b**
0.2	Non AM	1.11 **fg**	0.41 **fg**		
	AM	1.36 **d**	0.50 **d**	18.67 **c**	1.51 **b**
0.3	Non AM	0.98 **gh**	0.36 **gh**		
	AM	1.24 **def**	0.46 **def**	20.53 **d**	1.41 **c**
0.4	Non AM	0.91 **hi**	0.33 **hi**		
	AM	1.15 **ef**	0.42 **ef**	20.84 **d**	1.30 **d**
0.5	Non AM	0.70 **j**	0.26 **j**		
	AM	0.85 **i**	0.31 **i**	17.82 **c**	1.29 **d**

The same letter within each column indicates no significant difference among treatments ($P < 0.05$) as determined by Duncan's multiple-range test. Value are means of $n = 4$

AM arbuscular mycorrhizal; Non AM, noninoculated; AM, inoculated

[a] *BRSP* Bradford-reactive soil protein

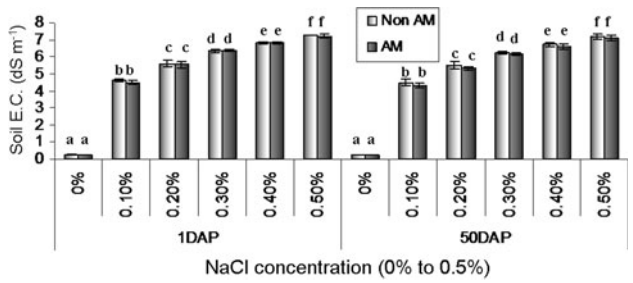

Fig. 1 Effect of arbuscular mycorrhizal (*AM*) fungi on electrical conductivity (*EC*) of soil from containers containing *Jatropha curcas* 1 day after planting (*DAP*) and 50 DAP and grown in soils amended with different rates of NaCl. Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple-range test. Values are mean ± SE ($n = 4$). NonAM, noninoculated; AM, inoculated with fungi

Increased concentrations of NaCl decreased shoot ($r^2 = 0.92$) and root ($r^2 = 0.92$) dry weights and inoculation with AM reduced the impact of salinity on plant biomass (Table 2).

Effect of AM Fungi and Salinity on Root Colonization, Root Development, Mycorrhizal Dependency (MD), and Glomalin Content

Noninoculated plants had no AM colonization. Colonization of roots of seedlings inoculated with AM ranged from approximately 30 to 61% (Table 1). Root colonization of AM-inoculated plants was greatest on plants grown with less than 0.3% NaCl and lowest for plants grown with 0.5% NaCl. There was a significant negative relationship observed between AM fungal root colonization and salinity ($r = -0.959$, $P < 0.01$). The MD of *Jatropha* increased from 12.13 to 20.84% between 0% NaCl and 0.4% NaCl. The MD of *Jatropha* grown with 0.5% NaCl (17.82%) was lower than that for plants grown with 0.4% NaCl and higher than that for the plants grown with less than 0.2% NaCl (Table 2).

Increased NaCl concentrations in the soil decreased root system size (tap root length and root biomass), and plants inoculated with AM fungi had larger root systems than noninoculated plants (Tables 1 and 2). Visual observations using scanned photographs of roots of *Jatropha* seedlings grown at 0 and 0.3% NaCl also indicated that AM-inoculated *Jatropha* had greater root development than noninoculated plants (Fig. 2a–c), these findings have been supported by data pertaining to root length and root dry weight (Tables 1 and 2).

BRSP content was greatest in the rhizospheric soils collected from AM-inoculated *Jatropha* seedlings grown with 0–0.5% NaCl concentrations and BRSP decreased with increased NaCl concentration ($P < 0.05$, $r^2 = 0.95$) (Table 2).

Effect of AM Fungi and Salinity on Leaf RWC and MDA Content

The leaf RWC decreased with increasing concentration of NaCl, and the lowest value recorded in non-inoculated *Jatropha* grown with 0.5% NaCl. Relative water content in the leaves was significantly higher in AM-inoculated than

Fig. 2 Scanned photographs of roots of *Jatropha* plants grown in soils amended with different rates of NaCl. **a** 0% NaCl and noninoculated. **b** 0.3% NaCl and noninoculated. **c** 0.3% NaCl and AMF-inoculated

in noninoculated *Jatropha* plants in all treatments with NaCl (Fig. 3a).

MDA content increased significantly ($P < 0.05$) with increased salinity. Plants inoculated with AM had lower

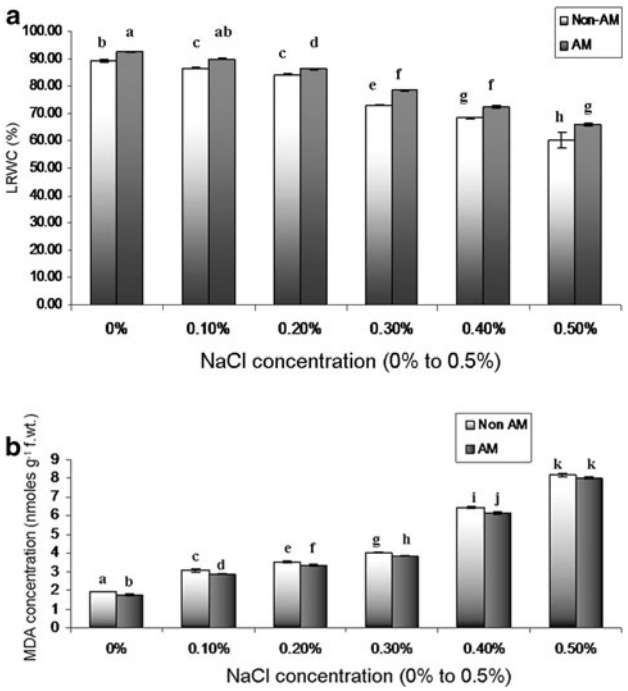

Fig. 3 Effects of inoculation with arbuscular mycorrhizal (*AM*) fungi on **a** leaf relative water content (*LRWC*) and **b** malondialdehyde (*MDA*) content in leaves of 60-day-old *Jatropha curcas* grown in soils amended with different rates of NaCl. Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple-range test. Values are mean ± SE ($n = 4$). NonAM, noninoculated; AM, inoculated with fungi

MDA concentrations than noninoculated plants, except when plants were grown with 0.5% NaCl (Fig. 3b).

Effect of AM Fungi and Salinity on Photosynthetic Pigments (Chl *a* and *b*)

Plants inoculated with AM had higher concentrations of Chl *a* and Chl *b* when grown with significantly 0, 0.1, 0.2, and 0.3% NaCl (Fig. 4a, b). Chlorophyll concentrations in the leaves of both AM-inoculated and noninoculated plants decreased as soil salinity increased and no difference was observed between noninoculated and AM-inoculated plants above 0.3% NaCl.

Effect of AM Fungi and Salinity on Total Soluble Sugars and Proline Content

Plants inoculated with AM had significantly higher concentrations of soluble sugars than noninoculated plants at all NaCl treatments (Fig. 4c), and in the case of proline, AM-inoculated plants had higher concentrations when grown with NaCl concentrations above 0.2% (Fig. 4d).

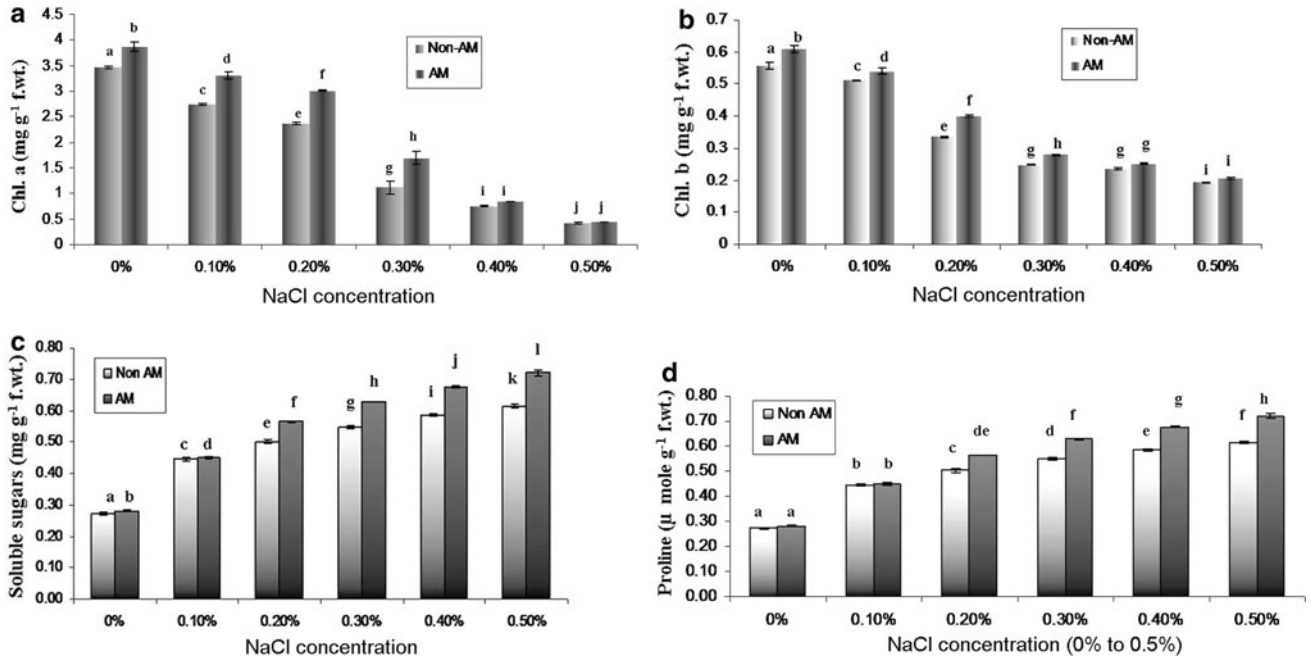

Fig. 4 Effects of inoculation with arbuscular mycorrhizal (*AM*) fungi on **a** Chl. *a*, **b** Chl. *b*, **c** soluble sugar, and **d** proline content in leaves of 60-day-old *Jatropha curcas* grown in soils amended with different rates of NaCl. Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple-range test. Values are mean ± SE ($n = 4$). NonAM, noninoculated; AM, inoculated with fungi

Discussion

Mycorrhizal symbiosis is a key component in helping plants to cope with adverse environmental conditions (Augé and others 1992). Understanding plant responses at the seedling stage is particularly important for elucidating the mechanism of salt tolerance, sensitivity, and survival in plants (Mayer and Poljakoff-Mayber 1963). Seedling establishment is often thought to be one of the stages most sensitive to salinity (Jones and Jones 1989). Our results showed that the AM fungal colonization rate decreased with increasing salinity. This is in agreement with the results of other researchers (Ojala and others 1983; Poss and others 1985; Johnson-Green and others 1995; McMillen and others 1998; Zuccarini and Okurowska 2008; Kumar and others 2009), but not with those of Levy and others (1983) and Hartmond and others (1987).

Soil EC was not influenced by AM even after 50 days of plant growth, and although high soil EC decreased AM colonization, the MD of *Jatropha* plants increased in soil containing up to 0.4% NaCl. The decrease in MD at 0.5% NaCl (Table 2) could be due to the inhibitory effect of NaCl on AM fungal root colonization or the effect on the spore density in the soil (Abbott and Robson 1991). Our results indicate that the symbiotic association between mycorrhizal fungi and *Jatropha* plants was strengthened in the saline environment once the association was established and there may be ecological importance of AM association for plant survival and growth of plants under salinity stress. This is consistent with previous findings of Tian and others (2004). *Jatropha* seedlings inoculated with AM had greater survival (%), shoot length, shoot diameter, and shoot, root, and plant dry weights than non-AM seedlings (Tables 1 and 2), indicating that AM plants grew better than non-AM plants under saline conditions.

The beneficial effects of AM on growth may be related to mycorrhiza-mediated effects on water absorption, nutrient uptake, and increased photosynthetic activity under salinity stress (Mukerji and Chamol 2003; Al-Karaki 2006; Miransari and others 2008). Mycorrhizal plants develop a more efficient carbon-use root system, which is more effective with the AM fungus assisting nutrient absorption (Schellenbaum and others 1991). Plants, then, would convert higher amounts of photosynthates into root development to increase their absorptive capacity (Wang and Cao 2004; Neocleous and Vasilakakis 2007).

Our results also support those by Giri and others (2003), Hartmond and others (1987), Dixon and others (1993), Giri and Mukerji (1999), Johnson-Green and others (2001), and Al-Karaki and others (2001) who reported AM fungi alleviated the adverse effect of NaCl in soil on *Acacia auriculiformis*, *Citrus*, *Leucaena leucocephala* and *prosopis juliflora*, *Sesbania* spp., and *puccinellia nuttallina* and tomato, respectively. The chlorophyll concentration in leaves is an important physiological index representing the degree of photosynthesis in plants. Mycorrhizal fungi

enhanced the chlorophyll concentration of *Jatropha* leaves, similar to that found in other reports (Giri and Mukerji 2004; Sannazzaro and others 2006; Colla and others 2008; Sheng and others 2008). Moreover, the AM plants under saline conditions had greener leaves than non-AM plants, suggesting that salt interfered with chlorophyll synthesis. Cantrell and Linderman (2001) reported a similar response in AM-inoculated lettuce and onion plants. Mycorrhizal inoculation also enhances phosphorus and magnesium uptake and reduces sodium concentrations in the plant; this in turn helps in increasing the chlorophyll content and improves the overall performance of mycorrhizal plants (Giri and others 2002).

Plant roots are the key structure in contact with soil; therefore, the abiotic stresses (for example, salt, alkaline, drought) in the soil environment can directly injure the roots. Scanned photographs of roots of AM-inoculated *Jatropha* seedlings at 0.3% NaCl showed greater root development than noninoculated seedlings (Fig. 2a–c). These findings are supported by data pertaining to root length and root dry weight (Tables 1 and 2). Such AM-mediated modifications in root morphology and biomass may be important for nutrient uptake and water balance in the host plant under salinity stress (Giri and others 2003). Moreover, incremental growth may also be regulated by improved nutrient supply (Ojala and others 1983). These results indicate that at low levels of salinity AM-inoculated *Jatropha* plants performed better than non-AM-inoculated plants, but at high NaCl concentrations growth of *Jatropha* plants was retarded, probably because of salinity-induced osmotic/and or toxic effect.

Glomalin, a glycoprotein produced by AM fungi, is tightly correlated with soil aggregate stability and could influence soil carbon storage indirectly by stabilizing soil aggregates. Increased soil aggregation can improve soil structure and soil moisture (Rillig and others 2002). Glomalin was quantified in the soil to establish a correlation between salinity and glomalin content. The data pertaining to glomalin content in the soil (AM-inoculated normal soil) showed a higher amount of glomalin and the glomalin content decreased with increasing salinity levels ($P < 0.05$, $r = 0.95$), which in turn can potentially result in poor soil structure (Rillig 2004) and low plant drought resistance.

In our study AM-inoculated *Jatropha* plants maintained higher leaf RWC under saline conditions (Fig. 3a). This is consistent with the findings of others (Feng and others 2000; Colla and others 2008). Peroxidation of membrane lipids is an indication of membrane damage and leakage under salt stress conditions and it can be estimated by measuring MDA concentration in leaves (Porcel and Ruiz-Lozano 2004). Lipid peroxidation or membrane damage in AM-colonized *Jatropha* was significantly lower (displayed lower cell membrane damage or higher salt tolerance) than in noninoculated plants at NaCl concentrations less than 0.5% (Fig. 3b). Lower MDA concentrations in the leaves of AM-inoculated plants may have been due to activation of antioxidative enzymes [superoxide dismutase (SOD) and catalase (CAT)] by AM fungi invasion (Ruiz-Lozano 2003; Gara 2004; Wu and others 2006). Our results are consistent with the findings of He and others (2007) who reported that lower MDA concentration in the leaves of AM-inoculated tomato plants compared to that in noninoculated plants is associated with lower cell membrane damage or higher salt tolerance.

Solute accumulation is a sensitive physiological index of plants responding to salt and other stresses (Peng and others 2008). For plants to survive under salt stress conditions, adjustment of leaf osmotic potential is very important and it requires intracellular osmotic balance. Under salt stress, plants accumulate some organic solutes (proline, soluble sugars, and so on) and inorganic ions to maintain higher osmotic adjustment (Yang and others 2009). It appears that the presence of the AM fungi in the roots may modify the osmotic potential of the leaves as they have been shown to influence the composition of carbohydrates (Augé and others 1987) and the level of proline (Ruiz-Lozano and Azcon 1995). Therefore, better growth of AM-inoculated *Jatropha* compared to noninoculated plants when exposed to salinity may be a result of increased soluble sugars and proline in the leaves of AM-inoculated plants. Furthermore, greater concentrations of soluble sugars and proline in leaves may have enabled AM-inoculated plants to maintain higher leaf water potential during stress and kept plants protected against oxidative stress. The combined effect of the AM fungi consortium on various growth parameters increased plant tolerance to salinity.

Conclusions

Under salt stress AM-inoculated plants had higher biomass, but dry weights were greater for AM plants under no salt stress. Our results indicate that *Jatropha* plants need mycorrhizae not only for acclimatization but also for seedling survival and successful growth when grown in saline soils. During the production of *Jatropha,* AM inoculation in the nursery before transplanting in the field may be useful for improving the growth of plants in soils with up to 0.5% NaCl (EC of 7.2 dS m^{-1}). This study also supports the positive impact of AM and glomalin contents on soil health, which are useful parameters for the assessment of biological soil fertility in sustainable agriculture.

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