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# Response of mycorrhizal and nonmycorrhizal *Arachis hypogaea* to NaCl and acid stress

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**Abstract** The response of peanut to salt (NaCl) and acid (HCl) stress was studied in association with *Glomus caledonium,* an arbuscular mycorrhizal (AM) fungus. The plants were exposed to salt stress by irrigation on alternate days with 1% or 5% NaCl solutions, or with 0.1 N HCl to induce acid stress. Plant yield almost tripled in mycorrhizal plants compared with nonmycorrhizal control plants. AM inoculation significantly increased plant yield and biomass at 1% NaCl, while at 5% NaCl AM was less effective in alleviating salt stress. Percentage AM colonization was also lowest at 5% NaCl. AM inoculation was found to promote the establishment of peanut plants under acid stress conditions.

**Key words** *Glomus caledonium* · Peanut · Acid · Salt · Stress

## Introduction

Arbuscular mycorrhizal (AM) fungi have profound effects on host plants, resulting in greater water usage, enhanced resistance to drought and diseases, elevated rates of photosynthesis and improved rates of growth under both normal and stress conditions (Rosendahl and Rosendahl 1991; Bethlenfalvay and Linderman 1992). Improved growth of onion and bell pepper in saline soils was reported after inoculation with AM fungi (Hirrel and Gerdemann 1980). Increased concentrations of NaCl in the soil solution reduced the growth and increased the diameter of hyphae arising from spores of *Gigaspora decipiens* (Juniper and Abbott 1992). Mycorrhizae were not observed where soil Na exceeded 3131 μg/g soil, but in well-drained playas with relatively low Na contents (10–32 μg/g and 275–625 μg/g) and diverse plant cover, spore numbers and infection levels were high and not correlated with soil Na concentration (Juniper and Abbott 1993). AM fungi vary with respect to their tolerance of soil temperature, pH, moisture, low fertility, salinity and toxic metals (Menge 1983; Kucey and Diab 1984; Bethlenfalvay and Franson 1989; Kothari et al. 1991; Griffioen et al. 1994).

The commercial interest in AM fungi has primarily focussed on enhanced nutrient uptake by agricultural and horticultural crops. Few studies have been directed towards the role of AM in salt and acid tolerance of plants (Hirrel and Gerdemann 1980; Dodd et al. 1990; Estaun 1991; Juniper and Abbott 1991; Kothari et al. 1991) and these mostly concern the growth of plants in saline and acid soils. In the present investigation, the effects of acid (HCl) and salt (NaCl) in the presence of AM on the growth of peanut plants are presented.

Quite large land areas have unfavourable acidic and saline soil conditions (Tata and Wadhawani 1992). Crops grown in acidic soil suffer from phosphorus deficiency, while in saline and sodic soils drainage is poor and soluble salt accumulates on the surface of the soil; plant growth is affected adversely. Such situations are ideal for attempting to apply mycorrhizal technology to improve farming efficiency. Acidic, saline and sodic soil conditions are not suitable for peanut plants, and adaptation of peanut cultivars is difficult as, for all practical purposes, the crop is 100% inbred (Tata and Wadhawani 1992). Hence the present studies were undertaken in an attempt to improve the survival and growth of peanut plants in acidic and saline soil conditions using AM fungi.

## Materials and methods

### *Plant material*

Ten-day-old seedlings of peanut (*Arachis hypogaea* cv JL 24) raised in moist chambers in sterile petri plates were transplanted into earthernware pots containing steam-sterilized soil (121 °C and 15 lb/in.$^2$ for 1 h. Soil pH was 8.4, available N 100.4 kg/ha, P 12.8 kg/ha, K 275.5 kg/ha, $CaCO_3$ 9.0 kg/ha, organic carbon 0.15% and electrical conductivity 0.11 mS/cm.

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**Table 1** Percentage root colonization and number of arbuscular mycorrhizal (AM) fungal spores isolated (in each case ± standard deviation)

| Days after inoculation | Treatment | % Colonization | | Spores (per 10 g soil) |
|---|---|---|---|---|
| | | No. of segments | Intensity | |
| 30 | Control | – | – | 13 ± 0.96 |
| | Control + HCl | – | – | 5 ± 1.14 |
| | Control + NaCl (1%) | – | – | 7 ± 1.27 |
| | Control + NaCl (5%) | – | – | 4 ± 1.58 |
| | + AM | 33 ± 0.88 | 41 ± 1.18 | 7 ± 1.24 |
| | AM + HCl | 28 ± 1.40 | 37 ± 2.27 | 12 ± 2.12 |
| | AM + NaCl (1%) | 27 ± 1.28 | 39 ± 1.86 | 11 ± 1.86 |
| | AM + NaCl (5%) | 18 ± 1.13 | 23 ± 2.27 | 9 ± 1.14 |
| 60 | Control | – | – | 10 ± 2.23 |
| | Control + HCl | – | – | 3 ± 0.00 |
| | Control + NaCl (1%) | – | – | 4 ± 1.14 |
| | Control + NaCl (5%) | – | – | 4 ± 0.85 |
| | + AM | 68 ± 1.23 | 79 ± 1.58 | 11 ± 0.57 |
| | AM + HCl | 53 ± 1.08 | 73 ± 1.98 | 7 ± 1.27 |
| | AM + NaCl (1%) | 56 ± 1.71 | 71 ± 2.19 | 8 ± 1.37 |
| | AM + NaCl (5%) | 32 ± 1.05 | 41 ± 1.63 | 6 ± 1.54 |
| 90 | Control | – | – | 9 ± 2.13 |
| | Control + HCl | – | – | – |
| | Control + NaCl (1%) | 8 ± 1.09 | 18 ± 1.25 | 6 ± 0.23 |
| | Control + NaCl (5%) | – | – | – |
| | + AM | 86 ± 1.86 | 92 ± 0.58 | 28 ± 0.34 |
| | AM + HCl | 73 ± 1.08 | 88 ± 1.85 | 13 ± 1.31 |
| | AM + NaCl (1%) | 82 ± 1.55 | 93 ± 0.20 | 18 ± 1.24 |
| | AM + NaCl (5%) | 33 ± 1.61 | 40 ± 1.32 | 12 ± 1.57 |
| 120 | Control | 3 ± 1.20 | 7 ± 1.90 | 3 ± 1.93 |
| | Control + HCl | – | – | – |
| | Control + NaCl (1%) | 10 ± 1.83 | 21 ± 1.76 | 7 ± 1.69 |
| | Control + NaCl (5%) | – | – | – |
| | + AM | 100 ± 0.00 | 100 ± 0.00 | 39 ± 1.62 |
| | AM + HCl | 82 ± 1.88 | 93 ± 0.57 | 21 ± 1.28 |
| | AM + NaCl (1%) | 90 ± 1.33 | 100 ± 0.00 | 27 ± 1.74 |
| | AM + NaCl (5%) | 35 ± 1.66 | 42 ± 0.03 | 15 ± 1.29 |

*AM inoculum*

*Glomus caledonium* Nicolson and Gerdemann (Gerdemann and Trappe 1974) was used as the AM inoculum. Spores were isolated from soil of the National Chemical Laboratory garden (Pune) by the wet sieving and decanting technique (Gerdemann and Nicolson 1963). The inocula were multiplied in pot cultures with *Trigonella* spp. for 2 months in sterilized soil. Ten-day-old peanut seedlings were inoculated with 5 g of soil containing spores and mycelia of *G. caledonium* and infected root fragments of *Trigonella* spp. during planting into pots containing 3 kg of sterilized soil. The plants were grown in a glasshouse (20–25 °C) under natural light.

*Experiment 1*

The purpose of this experiment was to determine the effect of salt stress on both AM-infected and uninfected peanut plants. Preinoculated plants were watered with 100 ml NaCl solution (1% or 5%) every second day for 3 months. Plants watered with tap water served as controls. Six sets of treatments each with 20 replicates were maintained. These were: (1) AM + 1% NaCl, (2) 1% NaCl only, (3) AM + 5% NaCl, (4) 5% NaCl only, (5) AM only, (6) control without NaCl or AM. All plants were watered every 3rd day with normal tap water to meet the normal water requirement.

*Experiment 2*

This experiment was set up to determine the effect of acid (HCl) on inoculated and uninoculated peanut plants. Plants were watered with 50 ml of 0.1 N HCl every 2nd day for 3 months. After acid treatment, the pH of the soil varied between 4.5 and 5.5. Plants watered with tap water served as controls. For comparison, two treatments with 20 replicates each were maintained and studied; (1) control + HCl, (2) control + AM + HCl. Plants were watered with tap water every 3rd day. The control and control + AM treatments served as common controls for both acid and saline treatments.

The growth of peanut plants in different treatments was evaluated using the following parameters: (1) plant height, (2) number, size and weight of nodules per plant, (3) number, size and weight of pods per plant. Data were collected at regular intervals of 30 days.

*Root colonization*

Parts of the root system of each treatment were cleared and stained according to Phillips and Hayman (1970) to determine percentage root segments colonized and the intensity of root colonization. Spores were isolated from soil by wet sieving and decanting (Gerdemann and Nicolson 1963) and their number estimated.

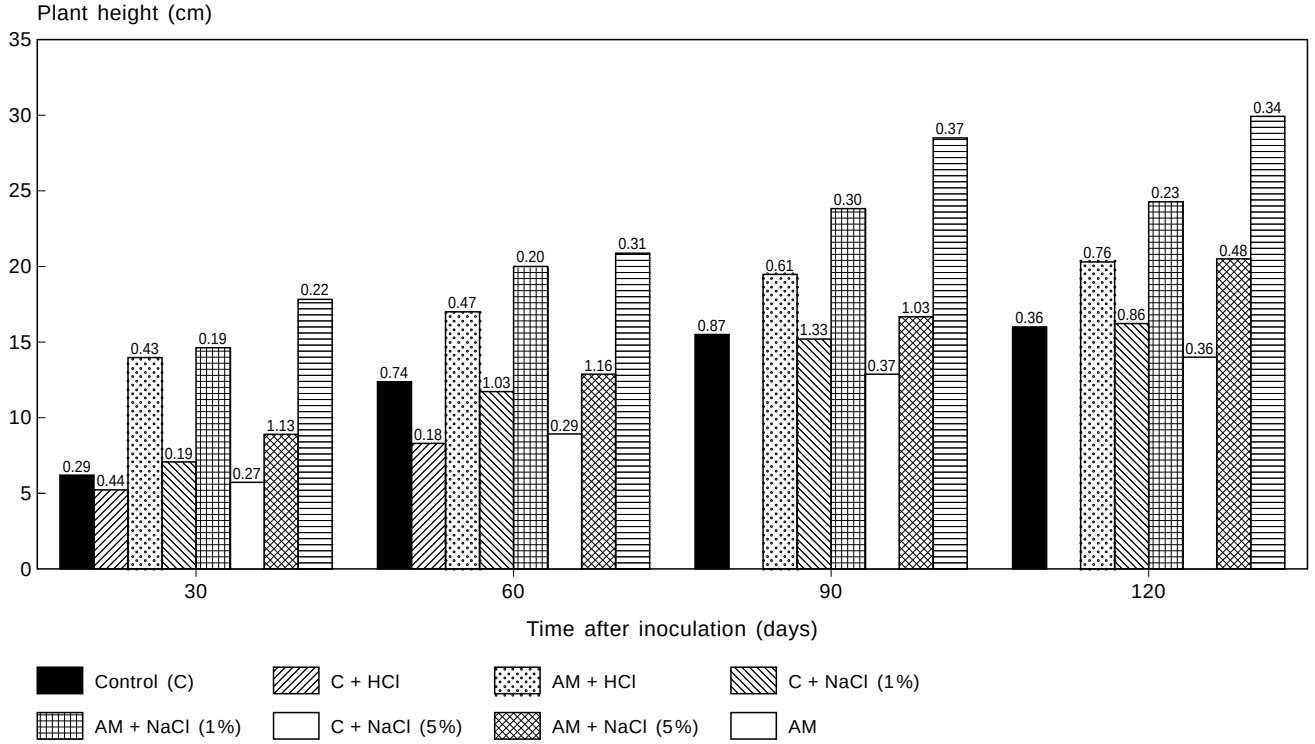


**Fig. 1** Change in height of peanut (*Arachis hypogea*) plants with time after inoculation with the arbuscular mycorrhizal fungus *Glomus caledonium.* The value of one standard deviation is shown numerically at the top of each column

## Results and discussion

Characteristic morphological structures of AM were observed in the roots of peanut after inoculation with *G. caledonium.* Extraradical hyphae were associated with the roots, and arbuscules and vesicles were observed in cortical cells.

The percentage of AM infection, number of spores isolated, and the number of nodules and pods per plant are given in Tables 1, 2 and 3, respectively. Very slight infection was observed after 90 and 120 days in uninoculated control plants (Table 1). This was due to the fact that roots penetrated through the base of pots and became infected by external indigenous AM fungal spores in the glasshouse bed. Thirty days after planting, the growth of uninoculated plants in all treatments was drastically reduced compared with mycorrhizal plants (Fig. 1), and those receiving 0.1 N HCl died after 60 days. Uninoculated peanut plants were not able to grow in acidic conditions, while plants in the AM + HCl treatment were not only able to tolerate acidic conditions but grew well up to 120 days, though less than untreated AM plants (Fig. 1). AM plants showed almost a threefold increase in yield in terms of pod formation over uninoculated controls (18.3 and 6.8, respectively). The yield in terms of pod weight per plant was also significantly higher for plants with AM than respective control plants (Table 3). Nodule formation was also found to be affected in the presence of *G. caledonium,* with increases in number, size and weight (Table 2). Flowering was earlier in untreated AM plants, i.e. 35 days after inoculation compared with 45 days for uninoculated controls and AM + HCl plants.

Legumes have long been recognized to be either sensitive or only moderately resistant to salinity (Bajaj 1990). Peanut plants in our studies were able to tolerate moderate saline conditions (1% NaCl), and plant growth was only affected when NaCl was applied at 5%. Plants treated with 1% NaCl grew better than plants treated with 5% NaCl upto 120 days after inoculation (Fig. 1). The number of pods produced per control plant, and the weight and size of pods, were lower in plants receiving 5% NaCl than 1% NaCl or untreated plants (Table 3). Similarly, nodule formation was affected by the higher concentration of NaCl (Table 2). The presence of the AM fungus in root tissue certainly had a favourable effect on nodule formation, as the number and weight of nodules were more than double in all treatments with *G. caledonium* compared with their uninoculated control plants (Table 2). The size of nodules was also greatest in AM-inoculated plants, and flowering was earlier by 8–10 days in all plants with AM than the uninoculated controls. After 90 days growth, control plants treated with 1% NaCl had 8% AM infection, while plants watered with 5% NaCl showed no infection, even after 120 days (Table 1). AM plants receiving 1% NaCl gave almost double the yield over uninoculated plants and AM colonization was unaffected (Tables 1, 3). At the higher concen-

**Table 2** Influence of AM inoculation on root nodulation

| Treatment | No. of nodules per plant | Nodule weight (mg/plant) | Nodule diameter (mm) |
|---|---|---|---|
| Control | 59 ± 1.86 | 421 ± 2.03 | 1–2 ± 0.37 |
| Control + HCl | – | – | – |
| Control + NaCl (1%) | 41 ± 1.33 | 404 ± 3.54 | 1 ± 0.35 |
| Control + NaCl (5%) | 20 ± 2.04 | 231 ± 3.87 | 1 ± 0.34 |
| + AM | 115 ± 4.16 | 921 ± 4.13 | 3–4 ± 0.20 |
| AM + HCl | 61 ± 2.06 | 430 ± 3.13 | 1–2 ± 0.61 |
| AM + NaCl (1%) | 86 ± 2.66 | 619 ± 4.21 | 2–3 ± 0.48 |
| AM + NaCl (5%) | 51 ± 2.19 | 418 ± 2.41 | 2 ± 0.00 |

**Table 3** Influence of AM inoculation on pod formation

| Treatment | No. of pods per plant | Pod weight (mg/plant) | Pod length (cm) |
|---|---|---|---|
| Control | 6.8 ± 0.67 | 8.18 ± 0.68 | 2.10 ± 0.31 |
| Control + HCl | – | – | – |
| Control + NaCl (1%) | 5.4 ± 0.48 | 6.80 ± 0.86 | 2.22 ± 0.20 |
| Control + NaCl (5%) | 3.0 ± 0.69 | 3.80 ± 1.13 | 1.92 ± 0.21 |
| + AM | 18.3 ± 2.79 | 23.30 ± 1.18 | 2.80 ± 0.62 |
| AM + HCl | 5.8 ± 0.68 | 6.90 ± 1.03 | 1.90 ± 0.34 |
| AM + NaCl (1%) | 8.7 ± 0.63 | 13.20 ± 1.14 | 2.50 ± 0.60 |
| AM + NaCl (5%) | 5.2 ± 0.75 | 7.15 ± 0.76 | 2.10 ± 0.36 |

tration of NaCl (5%), AM colonization was found to be only 35%. In spite of this, *G. caledonium* had a positive effect on peanut plant growth compared with control plants in the 5% NaCl treatment, where pod and nodule formation was higher than for the equivalent control plants (Tables 2, 3). The present investigation clearly shows that formation of arbuscular mycorrhiza is reduced by an increase in salt concentration, but that plant growth is still improved by the symbiosis as compared with nonmycorrhizal plants growing under similar salt conditions.

In conclusion, the presence of *G. caledonium* inside the roots of peanut increased the salt and acid tolerance of plants. The physiological mechanisms for the observed tolerance remain unclear. One possible explanation is that the presence of an AM fungus alters the osmotic balance in root tissues, since AM fungi have been found to influence the composition of amino acids and carbohydrates in host plants growing under salt-stress conditions (Rosendahl and Rosendahl 1991). Avoidance of P deficiency by AM formation is certainly an important component in the adaptation of plants to acid mineral soils (Marschner 1991). Berta et al. (1990) demonstrated morphogenetic changes in root systems with AM formation, with decreasing meristematic activity leading to an increase in the number of adventitious roots and greater branching of the roots. Such modifications could be important for water relations in AM plants, which in turn may be relevant to salt tolerance.

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