

Studies on salt stress tolerance of citrus rootstock genotypes with arbuscular mycorrhizal fungi

A. A. MURKUTE¹, S. SHARMA¹, S. K. SINGH²

¹Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, India

²Division of Fruit and Horticultural Technology, Indian Agricultural Research Institute, New Delhi, India

ABSTRACT: Citrus is grouped under the salt sensitive crops. Mycorrhizal fungi, a symbiotic relationship between plant roots and beneficial fungi, are supposed to impart the stress tolerance in the host plants. The stress tolerance improved due to Arbuscular Mycorrhizal fungi (AM fungi) colonization can be attributed to enhanced mineral nutrition. In the present study the efforts are made to evaluate the effectiveness of AM fungi with two citrus genotypes under salt stress. Three-month-old seedlings of Karna Khatta (*Citrus Karna*) and Troyer Citrange (*Poncirus trifoliata* × *Citrus sinensis*) were inoculated with the indigenous soil based AM inocula (mixed strains). The salinity gradient was developed by frequent irrigation with NaCl (0, 50, 100, 150 mM w/v). The results indicated that all the physical parameters were affected with increasing salinity. The proline accumulation increased while the chlorophyll, calcium and magnesium contents decreased significantly with increasing salinity. In general, the decreased AM colonization did not show any significant effects under salt stress.

Keywords: *Citrus karna*; *Poncirus trifoliata* × *Citrus sinensis*; NaCl; mycorrhiza; proline; sugars; chlorophyll

Citrus is the third most important fruit crop in India with an estimated production of 4.39 mill t from 0.496 mill ha area during 2000–2001 (ANONYMOUS 2002). Generally raised by shield budding, citrus is grouped under highly salt sensitive crops. Rootstocks influence tree vigor, water relations, cold hardiness, mineral nutrition, hormonal balance and fruit yield and quality. The tree growth and fruit yield are impaired at a soil salinity of about 2dS/m without any concomitant expression of leaf symptoms (CERDA et al. 1990). Though citrus is a glycophyte, the differences in tolerance do exist among species (MASS 1993) and they need to be tested individually.

Mycorrhizal colonization is often thought to increase the ability of salt tolerance in certain plant species (HIRREL, GERDEMANN 1980; OJALA et al. 1983). The mycorrhized plants of Carrizo Citrange (*Citrus sinensis* L. × *Poncirus trifoliata* L.) had higher dry weights and lower concentrations of leaf proline, a stress indicator, than the non-mycorrhized plants, irrespective of salinity level (DUKE et al. 1986). However, detailed information on the role of Arbuscular Mycorrhizal fungi (AM fungi) with citrus is lacking. The selection of rootstock is done with respect to topography, where planting is to be done. Karna

Khatta (*Citrus karna*) and Troyer Citrange (*Citrus sinensis* × *Poncirus trifoliata*) are being used nowadays as popular rootstocks in India. This study was therefore undertaken to evaluate the effect of salinity stress on mycorrhiza inoculated Karna Khatta (*Citrus karna*) and Troyer Citrange (*Citrus sinensis* × *Poncirus trifoliata*).

MATERIALS AND METHODS

The mature fruits of Karna Khatta (*Citrus karna*) – KK and Troyer Citrange (*Citrus sinensis* × *Poncirus trifoliata*) – TC, were obtained from the Citrus Germplasm Block of the Division of Fruit and Horticultural Technology, Indian Agricultural Research Institute, New Delhi. Freshly extracted seeds were sown in the earthen pots having potting mixture as decomposed FYM, sand and soil (1:1:1). The pots were irrigated regularly. The three-month-old seedlings of about similar size, selected for the experiment were transferred into plastic containers (20 cm in depth and 20 cm in mouth diameter) having the same potting mixture composition. The pH of the substrate was 7.74 and the electrical conductivity (EC) was 0.126-mmhos/cm before the salt

treatment. The available phosphorus content in the substrate was 63.2 ppm. The soil based mycorrhizal inocula (*Glomus* sp., *Gigaspora* sp., spores) procured from the Division of Microbiology, I.A.R.I., New Delhi, were applied (ca. 300 spores/100 g soil) in the root zone (V1 – non-mycorrhized control, V2 – mycorrhized) while transplanting the seedlings. The salinity gradients were developed by regular irrigation with salt water with NaCl (S1 – control, S2 – 50, S3 – 100 and S4 – 150mM w/v).

The physical parameters viz. number of leaves, stem diameter and height of seedling were recorded 90 days after planting (90 DAP). The mean values of three replicates were given. The root colonization by Arbuscular Mycorrhizal fungi (AM fungi) was observed at the end of experiment i.e. 90 DAP. The percentage of root colonization was recorded using the method defined by PHILLIPS and HAYMAN (1970).

All the biochemical estimations were done at the end of the experiment, i.e. 90 DAP. The proline estimation was done using the shoot tip portions (stem and leaves) of fresh plant samples according to BATES et al. (1973), while the chlorophyll estimation was done by using only tender leaves according to BARNES et al. (1992). The total sugars and reducing sugars estimations were done by the Nelson-Somogyi method using oven dried samples as described by THIMMAIAH (2004).

The macro- and micronutrient analyses were done by using the oven dried whole plant samples. The seedlings were uprooted from pots and washed with dilute acid ($\text{HCl}^{\text{N}/10}$ N) and then rinsed thrice with distilled water. The excess water was wiped with tissue paper and then the seedlings were oven dried at 60°C for three days. The brittle and well-dried plant samples were crushed to a powder and 500 mg of

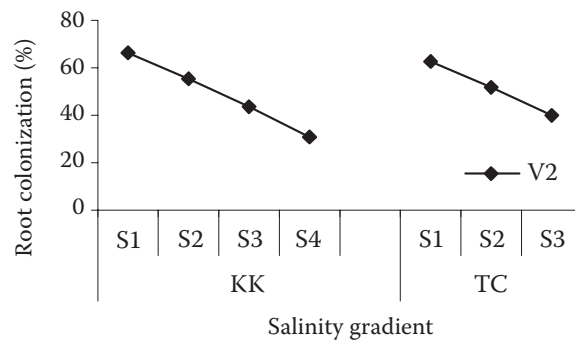


Fig. 1. Effect of salinity on AM root infection (%)
 KK – *C. karna*, TC – *P. trifoliata* × *C. sinensis*; V2 – with AM fungi; S1 – control, S2 – 50mM, S3 – 100mM, S4 – 150mM NaCl (w/v)

ground sample was digested with diacid solution (HNO_3 and HClO_4 , 9:4). This extract was used for the estimation of phosphorus, potassium, sodium, magnesium, calcium, manganese, copper, iron and zinc. The nitrogen estimation was done by micro Kjeldahl method. All the nutrient analyses were done as per the procedures given by BHARGAVA and RAGHUPATHI (1998).

The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following the methods of analysis of variance (ANOVA) and the means were separated using LSD at $p \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Mycorrhizal colonization

The mycorrhizal infection was observed at all the developed salt levels. With an increasing stress

Table 1. Effect of salinity on physical parameters of plants

	Salinity levels (NaCl mM)							
	Control		50mM		100mM		150mM	
	V1	V2	V1	V2	V1	V2	V1	V2
Plant height (cm)								
KK	14.93 ^a	14.83 ^a	12.26 ^b	12.33 ^b	10.63 ^c	10.50 ^c	9.56 ^d	9.30 ^d
TC	13.76 ^a	13.83 ^a	11.36 ^b	11.40 ^b	9.30 ^c	9.33 ^c	–	–
Stem diameter (mm)								
KK	2.43 ^a	2.42 ^a	1.94 ^b	1.94 ^b	1.61 ^c	1.64 ^c	1.48 ^d	1.48 ^d
TC	2.27 ^a	2.28 ^a	1.72	1.73	1.49 ^c	1.49 ^c	–	–
Number of leaves								
KK	10.66 ^a	10.66 ^a	10.33 ^{ab}	10.0 ^{abc}	9.00 ^{cd}	9.33 ^{bcd}	8.66 ^d	8.33 ^d
TC	11.33 ^a	11.66 ^a	10.33 ^b	10.33 ^b	8.33 ^c	8.00 ^c	–	–

Means followed by different letters varied significantly at $p \leq 0.05$ level

KK – *C. karna*, TC – *P. trifoliata* × *C. sinensis*; V1 – without AM fungi, V2 – with AM fungi

the root infection was decreased significantly from 66.8% to 31.3% in Karna Khatta and from 62.4% to 39.7% in Troyer Citrange (Fig. 1). The decline in colonization under stress could be caused by adverse conditions for sporulation and development of spores under unfavorable rhizosphere conditions (DUKE et al. 1986). The reduced colonization with the salt application was also reported by HIRREL and GERDEMANN (1980) and OJALA et al. (1983).

Physical parameters

All the physical parameters were highly adversely affected by the salinity treatments. The highest salinity level (150mM) proved lethal for the Troyer Citrange whereas Karna Khatta could survive with limited leaf toxicity symptoms (data not given). The plant height, stem diameter and number of leaves significantly decreased under salinity stress (Table 1). The AM inoculated plantlets responded similarly as the non-inoculated plantlets to an increasing salinity gradient and did not show any significant differences. Moreover, under non-stress conditions the mycorrhizal plants did not differ from non-inoculated plants for growth parameters in both the genotypes (Table 1).

The decrease in vegetative growth under salinity stress might have been caused by a water deficit

(EL-DESOUKY, ATAWIA 1998; TOZLU et al. 2000). However, the decline in the mycorrhizal activity due to salinity and the reverse osmosis could be the main reason of their non-significant response. The results related to the effect of AM fungi are also corroborated by GRAHAM and SYVERTSEN (1989).

Nutritional parameters

The nutrient uptake by both the genotypes was analyzed in two plant organs viz. the shoot and the root. The most noticeable features were an accumulation of nitrogen, phosphorus and potassium and a decrease in calcium and magnesium in the shoot (Table 2). The root phosphorus and potassium contents decreased, while calcium and magnesium remained unaltered in the increasing salinity field (Table 3). The phosphorus was found to respond positively to mycorrhization, but other nutrients showed a non-significant response to AM fungi under non-stress as well as under stress conditions.

A number of nitrogen-containing compounds, e.g. amides and amino acids, accumulate in plants subjected to stress (RABE 1990). The nitrogen accumulation under salinity stress was reported by ZEKRI (1993) in several citrus rootstocks.

The selective substitution of Na/K resulting in an accumulation of potassium is attributed as a chief

Table 2. Effect of salinity on macronutrients in shoot (%)

	Salinity levels (NaCl mM)							
	Control		50mM		100mM		150mM	
	V1	V2	V1	V2	V1	V2	V1	V2
<i>KK – C. karna</i>								
N	2.11 ^c	2.06 ^c	2.08 ^c	2.11 ^c	2.20 ^b	2.22 ^b	2.49 ^a	2.55 ^a
P	0.13 ^f	0.16 ^e	0.21 ^d	0.22 ^d	0.32 ^c	0.37 ^b	0.41 ^a	0.42 ^a
K	0.677 ^d	0.703 ^d	0.903 ^c	0.917 ^c	1.140 ^b	1.127 ^b	1.243 ^a	1.237 ^a
Ca	1.860 ^a	1.813 ^a	1.123 ^b	1.440 ^{ab}	1.027 ^b	1.047 ^{bc}	0.607 ^c	0.603 ^c
Mg	0.389 ^a	0.389 ^a	0.265 ^b	0.264 ^b	0.205 ^c	0.198 ^c	0.159 ^d	0.156 ^d
Na	0.357 ^d	0.353 ^d	0.760 ^c	0.767 ^c	1.004 ^b	1.023 ^b	1.842 ^a	1.817 ^a
Cl	0.287 ^f	0.387 ^e	1.283 ^d	1.267 ^d	1.900 ^c	2.000 ^b	2.330 ^a	2.297 ^a
<i>TC – P. trifoliata × C. sinensis</i>								
N	2.08 ^c	2.04 ^c	2.45 ^b	2.45 ^{ab}	2.50 ^{ab}	2.53 ^a	–	–
P	0.15 ^c	0.18 ^c	0.23 ^b	0.24 ^b	0.32 ^a	0.34 ^a	–	–
K	0.830 ^b	0.860 ^b	0.910 ^b	0.870 ^b	0.783 ^a	0.113 ^a	–	–
Ca	1.763 ^{ab}	1.850 ^a	1.423 ^b	1.023 ^c	0.837 ^c	0.797 ^c	–	–
Mg	0.353 ^a	0.355 ^a	0.308 ^b	0.310 ^b	0.254 ^c	0.248 ^c	–	–
Na	0.381 ^c	0.383 ^c	0.784 ^b	0.758 ^b	1.320 ^a	0.918 ^b	–	–
Cl	0.310 ^c	0.420 ^c	1.270 ^b	1.230 ^b	1.873 ^{ab}	2.047 ^a	–	–

Means followed by different letters varied significantly at $p \leq 0.05$ level

V1 – without AM fungi, V2 – with AM fungi

Table 3. Effect of salinity on macronutrients in root (%)

	Salinity levels (NaCl mM)							
	Control		50mM		100mM		150mM	
	V1	V2	V1	V2	V1	V2	V1	V2
<i>KK – C. karna</i>								
N	1.71 ^{cd}	1.66 ^d	1.73 ^c	1.73 ^c	2.07 ^b	2.06 ^b	2.21 ^a	2.20 ^a
P	0.382 ^b	0.395 ^a	0.312 ^d	0.325 ^c	0.219 ^f	0.236 ^e	0.144 ^h	0.164 ^g
K	1.129 ^a	1.137 ^a	1.040 ^b	1.027 ^b	0.803 ^c	0.187 ^c	0.603 ^d	0.577 ^d
Ca	0.550 ^{ab}	0.557 ^a	0.560 ^a	0.530 ^{ab}	0.547 ^{ab}	0.585 ^b	0.583 ^a	0.573 ^a
Mg	0.170 ^a	0.173 ^a	0.177 ^a	0.153 ^a	0.167 ^a	0.170 ^a	0.160 ^a	0.160 ^a
Na	0.036 ^d	0.036 ^d	0.115 ^c	0.115 ^c	0.204 ^b	0.207 ^{ab}	0.223 ^a	0.221 ^a
Cl	0.187 ^f	0.287 ^e	0.767 ^d	0.807 ^d	1.300 ^c	1.397 ^b	2.030 ^a	2.070 ^a
<i>TC – P. trifoliata × C. sinensis</i>								
N	1.78 ^c	1.74 ^c	1.95 ^b	1.95 ^b	2.00 ^{ab}	2.03 ^a	–	–
P	0.327 ^a	0.344 ^a	0.239 ^b	0.247 ^b	0.139 ^c	0.146 ^c	–	–
K	1.037 ^a	0.973 ^a	0.797 ^b	0.880 ^b	0.713 ^c	0.680 ^c	–	–
Ca	0.590 ^a	0.550 ^a	0.540 ^a	0.577 ^a	0.577 ^a	0.563 ^a	–	–
Mg	0.167 ^a	0.173 ^a	0.180 ^a	0.170 ^a	0.173 ^a	0.173 ^a	–	–
Na	0.034 ^c	0.036 ^c	0.116 ^b	0.115 ^b	0.213 ^a	0.213 ^a	–	–
Cl	0.210 ^d	0.320 ^d	0.870 ^c	0.830 ^c	1.447 ^b	1.633 ^a	–	–

Means followed by different letters varied significantly at $p \leq 0.05$ level

V1 – without AM fungi, V2 – with AM fungi

characteristic of salt tolerance (MARSCHNER 1995). The higher concentration of potassium is a response to balance the increasing concentration of chloride in the shoot (GRIEVE, WALKER 1983), however, the limited Na/K exchange and thus limited sequencing (WALKER, DOUGLAS 1983) is responsible for its decreased levels in roots (Table 3).

The increase in phosphorus increases the photosynthesis activity (MARSCHNER 1995), thereby balancing the losses occurred due to salinity. The increase in phosphorus with mycorrhization (Tables 2 and 3) is well known, however, its increase, decrease or unalteration with respect to salinity is yet to be examined properly (GRATTAN, GRIEVE 1992; TOZLU et al. 2000). Calcium has a key role in a prevention of cell membrane integrity, especially under stress caused by a higher pressure; magnesium is a key element for chlorophyll molecule. The model role of calcium in osmoregulation and K and Na selectivity is defined (ZEKRI 1993), but the decrease in both the elements (Tables 2 and 3) apart from chlorosis needs to be investigated further.

The sodium and chloride increased significantly in the shoots (Table 2) and the roots (Table 3), because NaCl salt was used to develop salinity gradient. Similar reports were documented by LEA-COX and SYVERTSEN (1993), TOZLU et al. (2000) and RUIZ et

al. (1999). Besides, with the use of high concentration of NaCl solution (> 0.20 MPa) as in the present study, the Na and Cl exclusion ability of some of the citrus rootstocks might be lost (ZEKRI 1987). Furthermore, AM fungi were found to increase the chloride irrespective of salinity. The increased chloride accumulation due to mycorrhization is caused by a carbon drain imposed by mycorrhizal hyphae on plants, which enhances the translocation of highly mobile anions like Cl from soil (BUWALDA et al. 1983; GRAHAM, SYVERTSEN 1989).

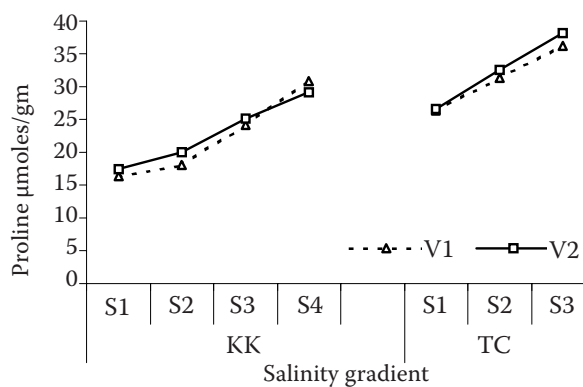


Fig. 2. Effect of salinity on shoot proline contents (fresh wt) KK – *C. karna*, TC – *P. trifoliata × C. sinensis*; V1 – without AM fungi, V2 – with AM fungi; S1 – control, S2 – 50mM, S3 – 100mM, S4 – 150mM NaCl (w/v)

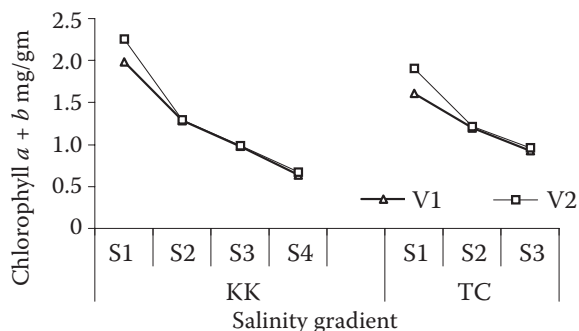


Fig. 3. Effect of salinity on total leaf chlorophyll (fresh wt) KK – *C. karna*, TC – *P. trifoliata* × *C. sinensis*; V1 – without AM fungi, V2 – with AM fungi; S1 – control, S2 – 50mM, S3 – 100mM, S4 – 150mM NaCl (w/v)

Biochemical parameters

The accumulation of proline varies together with the NaCl gradient (Fig. 2). Proline and various betaines can function as osmoprotectants and crytoprotectants, when accumulated in cells (NOLTE, HANSON 1997). DUKE et al. (1986) also reported a similar relationship with a non-significant increase of betaines in mycorrhized plants under stress.

Though a decrease in total chlorophyll contents was observed with increasing salinity, the mycorrhizal plants had higher chlorophyll as compared to non-mycorrhizal under non-stress conditions (Fig. 3). Chlorophyll decreased under stress due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments. Similarly, there was a decrease of the uptake of minerals needed for chlorophyll biosynthesis (EL-DESOUKY, ATAWIA 1998). However, the previous increase of chlorophyll in mycorrhizal plants could be due to the cytokinin-like substances secreted by fungi (MARKS, KOZŁOWSKI 1973), which enhance the chloroplast development.

Total sugars, increased as a response to stress (Fig. 4), could be seen as an osmotic adjustment to lower down the osmotic potential of plant cells (THANAA, NAWAR 1994). However, a positive effect of mycorrhization in sugar accumulation might be because of the hydrolysis of starch to sugars in the mycorrhiza-inoculated seedlings as cells are enveloped and occupied by the fungus (NEMEC 1981).

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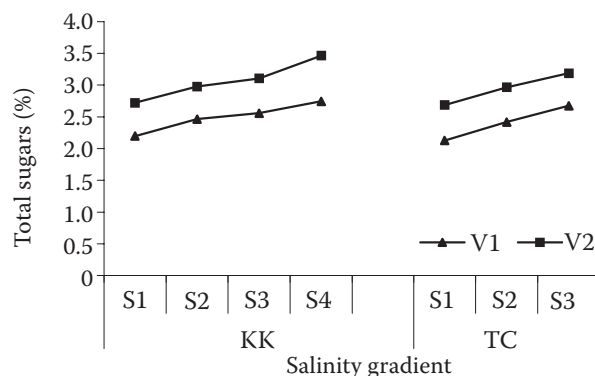


Fig. 4. Effect of salinity on total sugars (dry wt) KK – *C. karna*, TC – *P. trifoliata* × *C. sinensis*; V1 – without AM fungi, V2 – with AM fungi; S1 – control, S2 – 50mM, S3 – 100mM, S4 – 150mM NaCl (w/v)

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Studium tolerance genotypů citrusových podnoží inokulovaných arbuskulo-mycorhizními (AM) houbami k zasolení

ABSTRAKT: Citrus patří mezi plodiny citlivé k zasolení. Mycorrhizní houby, symbiotický vzájemný vztah mezi kořeny rostlin a prospěšnými houbami, přispívají k toleranci hostitelských rostlin vůči stresu. Kolonizace rostlin arbuskulo-vesikulárními (AM) houbami může zlepšit minerální výživu. V práci byla úspěšně stanovena účinnost AM hub u dvou genotypů citrusu ke stresu, způsobenému zasolením. Tři měsíce staré semenáče citroníku *Citrus karna* a křížence *Poncirus trifoliata* × *Citrus sinensis* byly inokulovány půdní směsí kmenů AM hub. Gradient salinity byl vyvolán opakovanou závlahou NaCl v koncentraci 0, 50, 100 a 150mM. Výsledky prokázaly, že všechny fyzikální

parametry byly ovlivněny vzrůstající salinitou – akumulace prolinu vzrostla, zatímco obsah chlorofylu, vápníku a hořčíku průkazně poklesl. Obecně se dá konstatovat, že nižší kolonizace AM houbami nevykázala žádný průkazný účinek při zatížení zasolením.

Klíčová slova: *Citrus karna*; *Poncirus trifoliata* × *Citrus sinensis*; NaCl; mykorhiza; prolin; cukry; chlorofyl

Corresponding author:

Ass. Prof. Dr. SATYAWATI SHARMA, Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110 016, India
tel.: + 91 112 659 1116, fax: + 91 112 659 1121, e-mail: satyawatis@hotmail.com
