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Physio-biochemical responses of hybrid citrus rootstock progenies to NaCl induced salinity

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ABSTRACT

The study consisted 30 hybrids of Pummelo (P) × Troyer (T) with two check cultivars, viz. Attani-2 and Troyer citrange, were subjected to 100 mM NaCl treatment through irrigations till the appearance of foliar symptoms. Of the 30 hybrids, only 3 hybrids, viz. P × T-86, P × T-98 and P × T -102 showed higher photosynthetic rate (*A*) (6.22 -6.42 µmol m^2/s) than other hybrids. Furthermore, the level of O_2^- was lowest in Attani-2. The lowest Cl^- content was noticed in P × T-102 (0.03%) followed by P × T-98 (0.04%). Of the 30 hybrids (Pummelo × Troyer citrange), evaluated against 100 mM NaCl induced salinity, 3 hybrids P × T-86, P × T-98 and P × T-102 had very low scorching of leaves (32.88-33.15%), and were found tolerant as these hybrids expressed the low level of lipid peroxidation and Cl^- accumulation in leaves, besides maintaining the higher *A* and MSI than other hybrids and Troyer citrange.

Key words: Citrus hybrid, Lipid, Peroxidation, Proline, Salinity, Superoxide

Citrus is the most important fruit crop of India next to banana and mango, being grown worldwide. In India, citrus fruits occupy an area of 1078000 ha with the annual production of 11147000 tonnes, yielding very low productivity (10.34 t/ha) (Anonymous 2016) as against some small countries like Indonesia (35 t/ha). There is no precedence for the failure of citrus industry because of non-availability of scion varieties, but always because due to serious problems to be governed by the intended rootstocks, as it is the sole determinant to allow this crop in a particular growing conditions (Castle 2010).

Many abiotic (salinity, moisture stress, extreme temperature, chemical toxicity etc.) stress causing factors are there to be the reasons for low citrus productivity in India. Citrus is grown in tropical and subtropical climates, and require assured irrigation due to its peculiar fruit structure. So the sites affected with saline irrigation water or soils are the major limiting factors in the improvement of yield and the expansion of area under these fruits. In India, 29.56 lakh ha land in 16 states is affected due to salinity (www. cssri. org). Vast areas of land in the world and in India have become unfit for cultivation owing to excessive salt concentrations in soils, mostly chlorides and sulfates of sodium, calcium and magnesium (Singh *et al.* 2003). The situation may be further graved due to the effect of climate change on precipitation, evaporation, runoff and soil moisture storage (Paranychianakis and Chartzoulakis 2005).

Growth reduction and some physio-biochemical disturbances due to excessive concentrations of Cl^- and Na^+ in leaves are the main problems occur by salinity stress. Because of the relative importance of Cl^- toxicity in citrus, salinity tolerance of rootstocks is most often based on the ability of the root system to limit the transport of Cl^- to the leaves. Salt tolerance is usually one of the targeted traits in rootstock breeding programme (Herrero *et al.* 1996). The existence of large genetic diversity in citrus provides ample scope to develop hybrid rootstock (s) having resistance/ tolerance to multi-stresses. Keeping in view the severity of salinity stress, and protective mechanisms of these parents, the systematic citrus rootstock improvement programme using inter-generic crosses has been initiated by Indian Agricultural Research Institute, New Delhi to select salinity tolerant/resistant genotypes. Although assessment of salinity tolerance has to be considered in terms of yield, but being a very long time taken process, such type evaluation is very difficult in hybrid progenies (Raga *et al.* 2014). So the hybrids being obtained from the ongoing hybridization programme were tested against the NaCl to study the physio-biochemical reactions, and screening the citrus hybrid progenies based on selection criteria available.

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MATERIALS AND METHODS

The present study was conducted at the Division of Fruits & Horticultural Technology of Indian Agricultural Research Institute, New Delhi, wherein, the response of 30 Pummelo (P) × Troyer citrange (T) hybrid citrus rootstock seedlings was studied in relation to 100 mM NaCl induced salinity. Salt susceptible Troyer citrange (*C. sinensis* (L.) Osbeck ×*P. trifoliata* (L.) Raf.) and salt tolerant Attani-2 (*C. rugulosa* Hort. ex Tanaka, Acc. No. IC 285453) (Patel *et al.* 2011) were served as control treatments. Six month old hybrid seedlings were transplanted on October 27th, 2016 in the plastic pots (12") containing 8 kg sterilized mixture of soil (3 parts) and farm yard manure (1 part), and allowed to settle for 45 days, and irrigated with tap water. Urea (20 g), single super phosphate (15 g) and potassium sulphate (15 g) were applied to each pot, 1 month after transplanting. The seedlings were then irrigated to 70% of field capacity (FC) with water containing 100 mM NaCl. The hybrids were subjected to 8 applications of NaCl during October 10th-November 26th, 2016, after considering moisture loss, measured by direct weighing of the pots, during which the electrical conductivity ($EC_{1:2}$) was raised from 0.42 m/Sm to 4.17 mS/m. The seedlings were then maintained without saline treatment for 4 weeks, thereafter the final data were recorded.

The leaf gas exchange traits such as transpiration rate (E) and photosynthetic rate (A) were measured between 12. 00–14. 00 h, on four mature leaves using LCi-SD Ultra Compact Photosynthesis System (ADC Bio Scientific Ltd., Global House, Hoddesdon, UK) during 2nd week of December under the conditions of day temperature, 22°C; night temperature 12°C; relative humidity (RH) 72%. Membrane stability index (MSI) in terms of leakage of ions from the leaf was estimated as per the method of Sairam *et al.* (1997), using a conductivity meter (EC testr11+ Multi Range conductivity meter). The total superoxide radical (O_2^-) content was assayed according to the method described by Chaitanya and Naithani (1994). The lipid peroxidation product was estimated as per the method of Heath and Packer (1968). Free proline content in the leaves was determined by the method of Bates *et al.* (1973). The leaves were collected from the each seedling for the determination of leaf nutrients concentration. These leaves were washed in succession of tap water, 0.2% Teepol solution, 10 NHCl and double-distilled water. Thereafter, the leaves were separately packed in paper bags, and dried in a hot air oven at temperature of 70±2°C for two days. Total leaf potassium (K^+) and sodium (Na^+) content was estimated from diacid digested leaf samples using a microprocessor based flame photometer (Flame Photometer-128, Systronics, Ahmedabad) according to Jackson (1980). Calcium content in leaf samples was ascertained by atomic absorption spectrophotometer (Model- GBC, 904AA, GBC Scientific Equipment, Hampshire, Illinois,USA) as per the method of Jackson (1980). Chloride content in plant leaves was quantified by Mercury (II) thiocyanate method as suggested by Adriano and Doner (1982). After NaCl treatment, the data on leaf scorching was recorded at the end of experimentation. The percentage of leaf scorching was calculated using following formula:

$$\text{Leaf scorching} = \frac{\text{Number of leaver scorched}}{\text{Total number of leaves}} \times 100$$

The experiment was conducted in an Augmented Block Design. Data were analyzed using the SAS software Version 9.3 (SAS Institute, Inc., USA). P ≤ 0. 05 were considered significant. The adjusted means were separated using F test followed by DMRT. Cluster and principal component analysis (PCA) was also done using the statistical software SAS Version 9.3 (SAS Institute, Inc., USA.)

RESULTS AND DISCUSSION

The leaf gas exchange parameters, viz. photosynthesis rate (A) and transpiration rate (E) of hybrid progenies were significantly influenced by the application of 100 mM NaCl solution (Table 1). The highest *A* was exhibited by Attani- 2 (6.52 µmol/m²/s) significantly, which was closely followed by P × T-98, P × T-102, P ×T-86, P ×T-83, P × T-82, P ×T-81, P ×T-80, P × T-101 and P×T-73 without showing any significant difference. The lowest A was registered P × T- 93 (1. 46 µmol/m²/s), however it was similar statistically with P × T- 74, P × T- 75, P × T- 76, P × T- 77, P × T- 78, P × T- 79, P × T- 84, P × T- 88, P × T-89, P × T-91, P × T-92, P ×T-95, P × T-96, P × T-97, P × T- 99 and P × T- 100 hybrids and Troyer citrange. Attani-2, P × T- 98, P × T- 102, P × T- 86, P × T- 83, P × T- 82, P × T- 81, P × T- 80 and P × T- 101 tended to show the 230.96%, 225.89%, 218.27%, 215.74%, 188.83%, 181.22%, 173.60%, 162.44% and 156.35% higher A, than known susceptible Troyer citrange, respectively.

The highest E was noticed in P × T -95 (0.97 mol/m²/s), showing similarity statistically with P × T -75, P × T - 77, P × T -83 and P × T -89. Hybrid P × T -86 tended to exhibit the lowest E (0. 16 mol/m²/s), however, it was found statistically similar with P × T -98 (0.26 mol/m²/s) and P × T -102 (0.18 mol/m²/s¹). In comparison to Troyer citrange, the highest reduction in E due to salinity was recorded in P × T- 86 (80.16%) followed by P × T- 102 (77.73%), P × T- 98 (68.02%), P × T- 90 (59.51%), P × T- 94 (49.80%) and P × T -101(43.72%), while it was increased markedly in P × T- 75, P × T - 77 and P × T-95 (10.93-18.22%) and control treatment (Troyer citrange).

MSI values were significantly influenced by NaCl induced salinity in different hybrids (Table 2), and its highest value was recorded in P × T -98 (86.65%), however, it was found similar statically with P × T-102 (80.24%), P × T-99 (75.47%), P × T-96 (74.54%), P × T-89 (73.17%), P × T-86 (84.31%), P × T-84 (73.44%), and Attani-2 (80.74%). The lowest MSI was observed in P × T- 91 (42.25%) without any significant difference with P × T- 73, P × T- 74, P × T-75, P × T-76, P × T -77, P × T-79, P × T- 81, P × T- 82, P × T- 83, P × T- 85, P × T- 87, P × T- 88, P × T- 92, P × T-93, P × T-95, P × T-101 and Troyer citrange. In comparison to Troyer citrange (known susceptible), highest increase in MSI following NaCl application was noticed in P × T-98 (63.83%) followed by P × T-86 (59.40%), Attani-2 (52.64%), P × T-102 (51.70%) and P × T-99 (42.68%).

The level of O_2^- radical, a ROS, was significantly influenced in the hybrids, while subjected to the NaCl induced salinity stress (Table 2). Of 30 hybrids, the highest level of O_2^- was recorded in P × T-93 (0.048 Δ_{540}nM $m^{-1}g^{-1}$FW), however it showed similarity statistically with P × T-91. Attani -2 had the lowest level of O_2^- (0.01 Δ_{540} nM/m/g/FW) having non-significant difference with those of P × T-73, P × T-76, P × T-83, P × T-85, P × T-86, P × T-89, P × T-90, P × T-95, P × T-98, P × T-100 and P × T-102 hybrids. Per cent change in the level of O_2^- in various hybrids over known susceptible rootstock (Troyer citrange), indicated the reduction in majority of hybrids, registering its highest value in Attani-2 (-57.52%) followed by P × T-83 (-56.19%), P × T -86 and P × T-98 (-54.33% in each), P × T-102 (46.69%), P × T-76 (-45.06%), P × T- 89 (-44.98%), P × T-90 (-42.94%), P × T-85 (-42.43%), P × T-95 (-41.59%) and P × T- 73 (-41.46%). The level of O_2^- increased only in P × T-91 (8.21%), while calculated in reference to Troyer citrange.

Various citrus hybrids evaluated against NaCl (100mM) induced salinity stress showed the significant variation in the level of melanoldyhyde (Table 2). P × T-80 hybrid tended to have the highest level of melanoldyhyde (56.10 µg/g FW) and was found statistically at par with P × T-73, P × T-77, P × T-78 and P × T-88, however, it was Attain-2, which had the lowest content of melanoldyhyde (6. 84 µg/g FW) without showing any significant difference with those of P × T-85, P × T-86, P × T-95, P × T-98, P × T-99 and P × T-102 hybrids. Per cent change in the level of melanoldyhyde over Troyer citrange showed the wide variation ranging from -69.97% in Attani-2 to 146.32% in P × T- 80. Melanoldyhyde decreased drastically over Troyer citrange in Attani-2, P × T-86, P × T-98, P × T-99, and P T-102 (69.97 to 47.73%), while its level expressed the opposite trend in P × T-73, P × T-77, P × T-78, P × T-80 and P × T-88 (100.57 to 146.32%) over control Troyer citrange.

Various citrus hybrids showed the significant variation in the proline content due to application of NaCl solution (100 mM) (Table 2). The citrus hybrid P × T- 90 exhibited the highest proline content (48.76 mg/g FW), while it was lowest in P x T-86 (0.43 mg/g FW). The content of proline was found lower than Troyer citrange (control) in the majority of hybrids and Attani-2 (control) except P × T-73, P × T-88, P × T-90, P × T-95, P × T-97 and P × T-101, wherein it increased from 4.95 to 48.76% over control.

The data (Table 3) relating to leaf nutrient status (K^+, Ca^{+2}, Na^+ and Cl^-) clearly indicated that salinity stress (NaCl-100mM) could exert the significant influence on Cl^- in the leaves of various hybrids. Although highest K^+ (1.67%) and Ca^{+2} (6.82%) were recorded in P × T-98. The highest content of leaf Na was recorded in P × T-86 (0.17%). The leaf Cl^- content was highest in P × T-93 (0.42%) without having any significant differences with P × T- 84 (0.42%). The lowest Cl^- content was noticed in P × T-102 (0.03%) similar statistically showing been with P × T-98 (0.04%). The content of Cl^- was also found quite lower in P × T-86

Table 1 Effect of NaCl (100 mM) induced salinity stress on leaf gas exchange parameters of citrus rootstock hybrids

Hybrid	Photosynthetic rate (A) (µmol m²/S)	Transpiration rate (E) (mol m/S)
Attani-2 (control)	6.52^{a}	0.52^{mn}
Troyer (control)	1.97^{t}	0.82^{defg}
P × T-73	3.42^{hiklmnoprstuv}	0.73^{hijk}
P × T-74	3.42^{hiklmnoprstuv}	0.83^{cdefg}
P × T-75	3.72^{fghijklmnopqrstu}	0.94^{ab}
P × T-76	2.22^{oprstux}	0.76^{ghij}
P × T-77	2.71^{lmnoprstu}	0.91^{abc}
P × T-78	2.93^{lmnoprstu}	0.86^{bcdef}
P × T-79	3.32^{ilmnoprstuvw}	0.78^{efghi}
P × T-80	5.17^{abcdefghijk}	0.70^{ijk}
P × T-81	5.39^{abcdefghjk}	0.46^{no}
P × T-82	5.54^{abcdefgj}	0.68^{jk}
P × T-83	5.69^{abcdefghi}	0.89^{abcd}
P × T-84	2.36^{nrstux}	0.78^{fghij}
P × T-85	4.09^{ceghijklmnopqr}	0.44^{o}
P × T-86	6.22^{abdf}	0.16^{r}
P × T-87	4.44^{bcdefghijklmop}	0.50^{mno}
P × T-88	3.66^{ghijklmnoprst}	0.57^{lm}
P × T-89	3.02^{klmnoprstuw}	0.88^{abcde}
P × T-90	3.92^{eghijklmnoprs}	0.33^{pq}
P × T-91	3.29^{jklmnoprstuv}	0.49^{mno}
P × T-92	1.50^{u}	0.86^{bcdefg}
P × T-93	1.46^{stu}	0.88^{bcdef}
P × T-94	4.37^{defghijklmnoq}	0.41^{op}
P × T-95	1.70^{rstu}	0.97^{a}
P × T-96	2.00^{prstux}	0.83^{cdefgh}
P × T-97	1.47^{stu}	0.80^{defghi}
P × T-98	6.420^{abc}	0.26^{qr}
P × T-99	1.50^{stu}	0.64^{kl}
P × T-100	2.49^{mnoprstu}	0.57^{lm}
P × T-101	5.05^{abcdefghijkl}	0.46^{no}
P × T-102	6.27^{abcde}	0.18^{r}
LSD ($P \leq 0.05$)		
Control treatment Means	1.18	0.05
Test treatment in the same Block	2.04	0.08
Test treatment not in the same Block	2.50	0.10
Test treatment and a Control Treatment	1.86	0.07

P- Pummelo; T- Troyer

Table 2. Effect of NaCl (100 mM) induced salinity stress on membrane stability index (MSI) and biochemical constituents of citrus rootstock hybrids

Hybrid	MSI (%)	Super oxide radical (Δ_{540}nM/m/g FW)	Lipid peroxidation (µg/g FW)	Proline (mg/g FW)
Attani-2 (control)	80.74^{ab}	0.01^{l}	6.83^{r}	0.56^{u}
Troyer (control)	52.89^{jklm}	0.03^{bc}	22.77^{klmn}	1.21^{f}
P × T-73	$56.39^{efghijklm}$	0.02^{hijkl}	47.19^{abcd}	24.79^{c}
P × T-74	51.85^{ijklm}	0.02^{ghi}	30.87^{fghikl}	0.98^{l}
P × T-75	52.37^{ijklm}	0.03^{bcdf}	37.70^{cdefgh}	0.99^{kl}
P × T-76	$55.19^{ghijklmn}$	0.02^{hijkl}	$21.58^{iklmnopq}$	0.55^{u}
P × T-77	43.93^{lm}	0.03^{bcd}	46.35^{abcd}	0.82^{r}
P × T-78	$65.92^{bcdefghij}$	0.03^{bcdefg}	51.83^{ab}	0.87^{op}
P × T-79	52.31^{ijklm}	0.02^{hijk}	$27.25^{fghiklmn}$	0.92^{n}
P × T-80	$65.38^{cdefghij}$	0.02^{eghi}	56.09^{a}	1.16^{h}
P × T-81	51.36^{ijklm}	0.03^{cdefg}	36.48^{cdefgh}	0.87^{o}
P × T-82	46.44^{klm}	0.03^{bc}	$32.67^{efghijk}$	0.95^{m}
P × T-83	54.07^{ijklm}	0.01^{klm}	$23.87^{hiklmnop}$	1.20^{g}
P × T-84	$73.44^{abcdegh}$	0.02^{ghi}	$19.74^{klmnopq}$	1.03^{j}
P × T-85	42.70^{m}	0.02^{hijkl}	15.87^{nopqrs}	1.17^{h}
P × T-86	84.31^{abc}	0.01^{klm}	11.03^{qr}	0.43^{x}
P × T-87	$58.46^{efghijklm}$	0.02^{ghi}	31.41^{fghikl}	0.80^{s}
P × T-88	52.83^{ijklm}	0.02^{efghi}	45.67^{abce}	19.00^{d}
P × T-89	$73.16^{abcdefgh}$	0.02^{hijkl}	$27.74^{fghiklm}$	1.10^{i}
P × T-90	$63.78^{defghijkl}$	0.02^{hijkl}	33.67^{dfghij}	48.76^{a}
P × T-91	42.25^{m}	0.048^{ab}	$25.22^{ghiklmno}$	0.70^{t}
P × T-92	$56.84^{fijklmn}$	0.02^{ghij}	$27.74^{fghiklm}$	0.85^{pq}
P × T-93	$59.99^{efghijklm}$	0.04^{a}	15.25^{mopqrs}	0.86^{op}
P × T-94	$64.40^{cdefghijk}$	0.02^{ghik}	40.87^{bcdef}	1.20^{fg}
P × T-95	49.90^{jklm}	0.02^{hijkl}	$17.00^{lmnopqr}$	19.00^{d}
P × T-96	$74.54^{abcdefg}$	0.02^{fgh}	$34.87^{cdefghi}$	0.94^{m}
P × T-97	$64.40^{cdefghijk}$	0.02^{ghik}	17.64^{lmnopq}	45.45^{b}
P × T-98	86.65^{a}	0.01^{jlm}	10.29^{pqr}	0.47^{w}
P × T-99	75.47^{abcdef}	0.03^{bce}	11.90^{opqr}	0.84^{qr}
P × T-100	$70.86^{abcdefghi}$	0.02^{hijkl}	38.54^{bcdefg}	1.00^{k}
P × T-101	$55.60^{hijklmn}$	0.02^{dfgh}	27.25^{ghikln}	4.95^{e}
P × T-102	80.23^{abcd}	0.02^{ijkl}	7.19^{qr}	0.52^{v}
LSD (P≤0.05)				
Control treatment means	9.48	0.004	6.82	0.01
Test treatment in the same block	16.42	0.007	11.81	0.01
Test treatment not in the same block	20.11	0.008	14.47	0.02
Test treatment and a control treatment	15.00	0.006	10.78	0.01

P- Pummelo; T-Troyer

(0.09%) and Attani-2 (0.11%) then rest of other rootstocks tested during the course of experimentation.

The number of leaves got scorched significantly due to NaCl (100mM) induced salinity in different hybrids tested (Table 3). Three hybrids. viz. P × T-86, P x T-98 and P × T-102 proved similar statistically in respect of low scorching of leaves (32.88-33.15%) over other hybrids and Troyer citrange, whereas it was lowest in Attani-2 rootstock (26.69%). Some of the hybrids like, P × T-81, P × T-82, P × T-88, P × T-91, P × T-95 and P × T-96 proved worse affected due to salinity, showing the higher leaf scorching (86. 76-94.44%) even than proved susceptible rootstock i. e. Troyer citrange (86.13%). The percentage of leaf scorching in rest of the hybrids ranged between 53.86% in P × T-89 to 84.21% in P × T -80.

In cluster analysis done on the basis of physio-biochemical traits, the hybrid progenies of Pummelo × Troyer citrange, studied against NaCl induced salinity showed the clear separation into two clusters (Fig 1). In the first cluster, four hybrids (P × T-86, P × T-98 and P × T-102 and P × T-89) had the 37% similarity with known tolerant Attani-2. In first cluster, highest similarity was observed between P × T-98 and P × T-102. The second cluster was further divided into two sub-clusters at 60% similarity which was again divided in two sub-sub-clusters at 34% level of similarity.

In the present analysis, first five principal components having eigenvalue >1 were considered, which contributed 75% of total variability. In PC1, the most important factors were K/Cl ratio, Cl and leaf scorching, whereas K/Cl ratio had negative impact. Similarly, in PC2, Na and K/Na ratio were found to be the most important components. In PC3, hydrogen peroxide, MSI and transpiration rate (*E*) were identified as the most important components. In PC4, only proline showed the highest impact, while in PC5, MDA and MSI were found most significant components based on their weights (Table 4).

During the course of present study, Attani- 2, P × T-98, P × T-102, P × T-86, P × T-83, P × T-82, P × T-81, P × T-80, P × T-101 and P × T-73 rootstocks had higher photosynthetic rate (A) than other hybrids. P × T-86, P × T-98 and P × T-102 hybrids tended to exhibit the reduced transpiration rate (E) over others. Salt build up in citrus leaves tends to decrease A and E and carbohydrate accumulation (Garcia-Sanchez and Syvertsen 2006). The high tolerance to salinity as shown by some hybrids in the present study can be associated to the ability of keeping an active photosynthetic system at elevated saline conditions or may be linked to rapid reductions in canopy exchange parameters (Lopez-Climent *et al.* 2008).

In this study, the highest level of O_2^- was recorded in P × T-93; however it showed statistically similar with the level of O_2^- in P × T-91. Hybrids P × T-98, P × T-102, P × T-99, P × T-96, P × T-89, P × T-86, P × T-84, and Attani-2 showed the higher MSI value than other hybrids. The excess generation of ROS like O_2^- in the cytosol, chloroplasts, mitochondria, and the apoplastic space (Mittler 2002) cause phytotoxic reactions such as lipid peroxidation, protein degradation and DNA mutation (Pitzschke *et al.* 2006). However, in citrus trees, the antioxidant machinery appears to be sufficient to avoid high levels of damage caused by active oxygen species at moderate to low stress levels (Arbona *et al.* 2003). The higher MSI, observed in

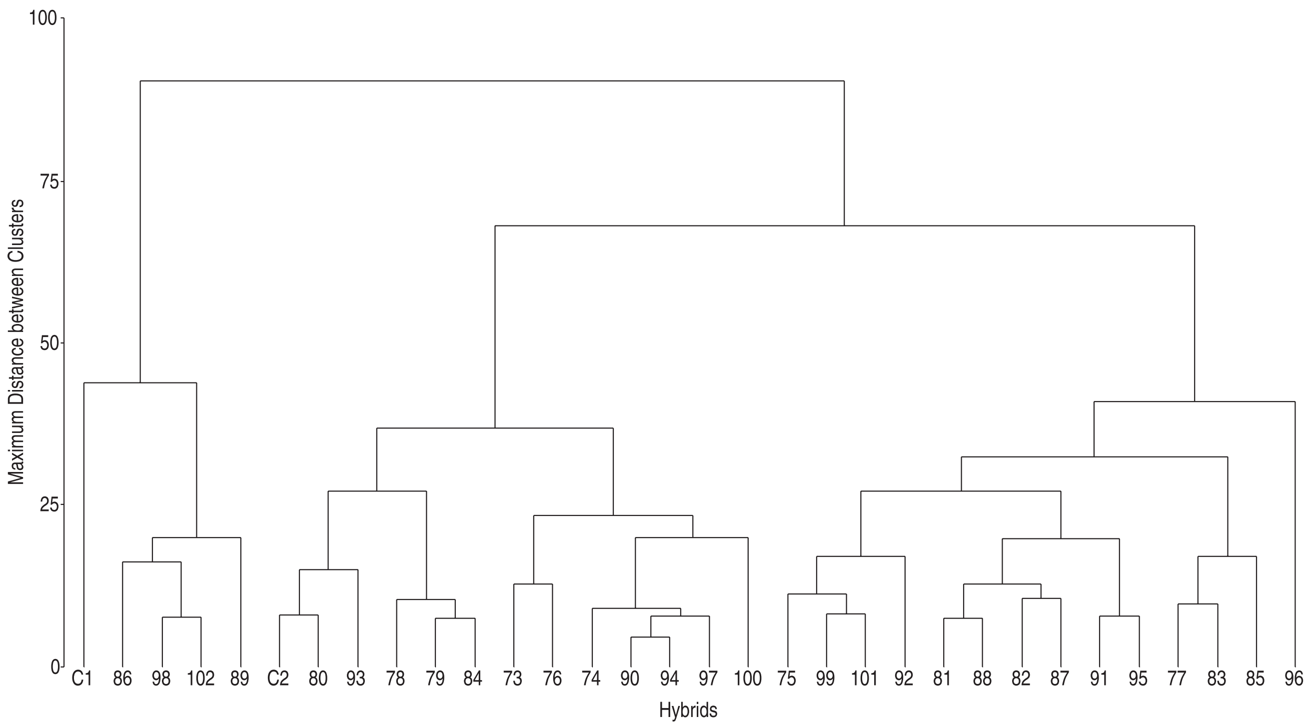

Fig 1 Cluster analysis showing similarity of citrus rootstock hybrids (Pumelo x Troyer citrange) based on physio-biochemical traits (C1- Attani-2; C2-Troyer citrange).

Table 3 Effect of NaCl (100 mM) induced salinity stress on nutrient content and leaf injury of citrus rootstock hybrids

Hybrid No.	K^+ (%)	Ca^{+2} (%)	Na^+ (%)	Cl^- (%)	Leaf scorching (%)
Attani-2 (control)	1.22^{a}	4.35^{a}	0.07^{a}	0.10^{o}	26.69^{q}
Troyer (control)	0.90^{a}	1.58^{a}	0.03^{a}	0.41^{b}	86.13^{c}
P × T-73	0.74^{a}	2.43^{a}	0.05^{a}	0.23^{k}	65.46^{lmn}
P × T-74	0.95^{a}	2.61^{a}	0.02^{a}	0.26^{hi}	76.06^{fghij}
P × T-75	1.39^{a}	3.33^{a}	0.09^{a}	0.28^{fg}	79.48^{efgh}
P × T-76	0.88^{a}	2.71^{a}	0.01^{a}	0.20^{l}	63.04^{n}
P × T-77	1.30^{a}	1.31^{a}	0.06^{a}	0.32^{de}	70.35^{jklm}
P × T-78	0.79^{a}	6.55^{a}	0.03^{a}	0.33^{de}	72.51^{ijk}
P × T-79	1.34^{a}	1.91^{a}	0.09^{a}	0.17^{mn}	65.99^{lmn}
P × T-80	1.60^{a}	1.71^{a}	0.02^{a}	0.33^{de}	84.21^{cde}
P × T-81	1.62^{a}	3.45^{a}	0.01^{a}	0.38^{c}	93.04^{ab}
P × T-82	0.96^{a}	3.73^{a}	0.07^{a}	0.25^{ij}	91.72^{ab}
P × T-83	0.72^{a}	2.91^{a}	0.05^{a}	0.37^{c}	71.13^{jkl}
P × T-84	0.61^{a}	0.28^{a}	0.04^{a}	0.42^{ab}	63.28^{mn}
P × T-85	0.88^{a}	0.47^{a}	0.13^{a}	0.31^{de}	68.15^{klmn}
P × T-86	1.53^{a}	5.71^{a}	0.17^{a}	0.09^{o}	33.15^{p}
P × T-87	1.54^{a}	0.55^{a}	0.08^{a}	0.23^{jk}	81.67^{cdef}
P × T-88	1.07^{a}	0.47^{a}	0.06^{a}	0.29^{fgh}	87.55^{abc}
P × T-89	1.43^{a}	0.63^{a}	0.15^{a}	0.17^{mn}	53.86^{o}
P × T-90	0.82^{a}	1.31^{a}	0.09^{a}	0.22^{k}	80.16^{defgh}
P × T-91	1.13^{a}	0.37^{a}	0.06^{a}	0.31^{ef}	87.55^{abc}
P × T-92	1.30^{a}	1.93^{a}	0.04^{a}	0.20l	78.24^{efghi}
P × T-93	0.8^{a}	2.50^{a}	0.13^{a}	0.43^{a}	83.28^{cde}
P × T-94	0.74^{a}	2.00^{a}	0.09^{a}	0.22^{k}	76.63^{fghij}
P × T-95	1.32^{a}	3.56^{a}	0.11^{a}	0.16^{n}	86.76^{bcd}
P × T-96	0.65^{a}	1.77^{a}	0.05^{a}	0.33^{d}	94.44^{a}
P × T-97	1.30^{a}	3.74^{a}	0.08^{a}	0.18^{lm}	74.38^{ghijk}
P × T-98	1.67^{a}	6.82^{a}	0.15^{a}	0.04^{p}	32.88^{p}
P × T-99	1.48^{a}	3.14^{a}	0.09^{a}	0.30^{f}	73.24^{hijk}
P × T-100	0.96^{a}	2.26^{a}	0.12^{a}	0.24^{jk}	64.38^{lmn}
P × T-101	1.04^{a}	2.72^{a}	0.09^{a}	0.27^{ghi}	79.92^{efg}
P × T-102	1.63^{a}	6.32^{a}	0.14^{a}	0.03^{p}	37.02^{p}
LSD ($P \leq 0.05$)					
Control treatment means	NS	NS	NS	0.01	3.40
Test treatment in the same block	NS	NS	NS	0.02	5.88
Test treatment not in the same block	NS	NS	NS	0.02	7.21
Test treatment and a control treatment	NS	NS	NS	0.01	5.37

P- Pummelo; T- Troyer

some rootstocks reflects the tolerance indicating the low damage of cell membrane due to salt stress, as it has also been reported in tolerant citrus rootstock genotypes during avoidance of salt stress (Patel *et al.* 2011).

Lipid peroxidation measured as malondialdehyde (MDA) content which is a toxic substance that causes cell injuries, and its content is often used as indicator of the extent of lipid peroxidation (Zhu *et al.* 2008). In this study the level of lipid peroxidation was quite lower in Attain-2, P × T-85, P × T-86, P × T-95, P × T-98, P × T-99 and P × T-102 hybrids than others. Excess ROS may initiate membrane lipid peroxidation, weaken membrane lipid unsaturation, trigger membrane protein polymerization, and result in an increase in membrane permeability (Chen 1991). Peroxidation of membrane lipids is an indication of membrane damage and leakage as a result of oxidative stress under salt stress conditions (Katsuhara *et al.* 2005).

In addition to its role as a cytoplasmic osmoticum, proline may function as a carbon and nitrogen source for post-stress recovery and growth (Fukutaku and Yamada 1984), a stabilizer for membranes, protein synthesis machinery (Kardpol and Rao 1985) and cytoplasmic enzymes (Paleg *et al.* 1984), a scavenger of free radicals (Smirnoff and Cumbes 1989), and as a sink for energy to regulate redox potential. The citrus hybrid P × T- 90 had the highest proline content, while P × T-86, P × T-98 and P × T-102 were among the low proline accumulators. Anjum (2008) reported more proline accumulation in the leaves

Table 4 Loadings of components (having λ<1) extracted through principal component analysis.

Trait	Loading				
	PC 1	PC 2	PC 3	PC 4	PC 5
LPO	0.18	-0.22	-0.09	-0.24	0.53
HO	0.12	0.40	0.47	0.27	-0.14
Proline	0.15	-0.07	0.15	0.60	0.08
Super oxide	0.31	0.12	-0.28	-0.18	0.27
K	-0.27	0.16	0.25	-0.36	0.24
Ca	-0.39	-0.03	-0.07	0.07	0.21
Na	-0.10	0.58	-0.09	-0.18	-0.03
Cl	0.42	0.06	-0.04	-0.01	0.14
K/Na	0.08	-0.55	0.30	0.001	-0.03
K/Cl	-0.44	-0.02	-0.04	-0.05	0.005
MSI	0.08	0.21	0.43	0.11	0.60
LS	0.40	0.04	-0.16	-0.16	-0.15
E	-0.01	0.19	-0.50	0.44	0.14
A	-0.23	-0.12	-0.21	0.28	0.31
Eigenvalue	4.35	2.15	1.53	1.44	1.06
Variance proportion	0.31	0.15	0.11	0.10	0.08
Cumulative variance	0.31	0.46	0.57	0.68	0.75

LPO-Lipid peroxidation; HO-Hydrogen peroxide; MSI-Membrane stability index; LS-Leaf scorching; E- Transpiration rate; A- Photosynthesis rate of salt sensitive Troyer citrange seedlings as compared to that of salt-tolerant Cleopatra mandarin, as also observed in the present study.

Following application of NaCl solution in the targeted hybrids, most of including Troyer citrange showed very high accumulation of Cl^- in their leaves except P × T-102 and P × T-98 hybrids, wherein very low accumulation of Cl^- was observed in the present study, while leaf Na^+, K^+ and Na^+ remain unaffected. Instead of individual concentration, the ratio of K^+/Cl^- proved more crucial for salinity tolerance as also observed in PC analysis. Troyer Citrange has been reported as a chloride accumulator and a salt sensitive rootstock (Bouchra *et al.* 2015). The citrus rootstocks tolerant to salts limit the translocation of the toxic ions like $Cl^–$ and Na^+ into the leaves, can acclimate to osmotic stress in the root zone by closing stomata and reducing leaf transpiration (Syvertsenand Smith 1983, Nieves *et al.* 1991). In this way, the water uptake by the roots and water loss by leaf transpiration is balanced as also observed in some hybrids in this study. In citrus, however, Na^+ is predominantly accumulated in root cortical cells, where substantial Na^+ is retained by epidermal cells. The pericycle also accumulates Na^+ in Swingle citrumelo roots (Gonzalez *et al.* 2012). This may be a critical mechanism in the relatively high Na^+ tolerance in Swingle citrumelo because the pericycle remains the last metabolic barrier before solutes enter the xylem stream to move toward leaves (Conn and Gilliham 2010). Some of the differences in Na regulation between *P. trifoliata* and *C. grandis* was due to differences in their anatomy. The phenomenon of exclusion of salts in citrus is rootstock specific. Citrange Carrizo (*C. sinensis*× *P. trifoliata*) and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) were Na^+ excluders, and highly sensitive to $Cl^–$(Gonzalez *et al.* 2012).

Irrigation water with an EC > 3 dS/m, is the critical level for citrus production (Garcia-Sanchez *et al.* 2002), as citrus trees are highly salt-sensitive (Storey and Walker 1999). In the present study, of the 30 hybrids, only 3 hybrids, *viz.* P × T-86, P × T-98 and P × T -102 proved statistically similar in respect of low scorching of leaves over other hybrids and Troyer citrange, whereas it was lowest in known salt tolerant rootstock i. e. Attani-2. These rootstocks also showed the close genetic similarity, as also indicated in the cluster analysis. Salt damages are usually displayed as leaf burn and defoliation, and are associated with accumulation of toxic levels of salts in leaf cells. Reactions against these toxicities depends on the rootstock used (Yesiloglu *et al.* 2015).

The phenomenon, named "Fine Root Turnover", is unique to *P. trifoliata*. It may be used as a genetic resource to improve *Citrus* for salinity tolerance through intergeneric hybridization since the F1 plants display responses intermediate to its parents leading to increased salinity tolerance. While leaves of *P. trifoliata* are tissues sensitive to salinity, root tissues are sensitive in *C. grandis* (Tozlu *et al.* 2002). Troyer citrange is a hybrid of Trifoliate orange and sweet orange, and the trait that restricts root to shoot transport of Cl^- and Na^+ in citrus has been shown to be heritable (Storey and Walker 1999). Furthermore, Tozlu *et al.* (2002) used $Cl^–$ tolerant varieties such as Cleopatra mandarin, Rangpur lime, Sunki mandarin and Shekwasha mandarin for breeding salt tolerant rootstocks, and concluded that the inheritance of salt tolerance is a quantitative trait and obtained some F1 seedlings which were as tolerant to salinity as Cleopatra mandarin. To avoid salt accumulation, *P. trifoliata* plants increase root dry mass production, while *C. grandis* plants increase leaf mass production. These traits appeared to be heritable, since F1 plants displayed responses intermediate to its parents leading to increased salinity tolerance (Tozlu *et al.* 2000b). In the present study, the behaviuor of the hybrids expressed the wide variation, even some of the hybrids performed better than their parents. The difference between the responses of the original parents and the heterotic F1 plant led to wide segregation in the BC1 progeny [(*C. grandis*) × (*C. grandis* × *P. trifoliata* selected F117–40)], in response to 16 weeks of saline treatment. Many traits showed transgressive segregation in both directions that may yield extreme values to breed salt hardy citrus. Some of the hybrids among the total hybrid progenies displayed less leaf damage and salt accumulation than best performing parents (Tozlu *et al.* 2000a,b). However, the microarray analysis revealed that responds to salt in citrus is a multigenic trait, but some genes probably exist that have a major impact on salt tolerance and or minimal accumulation (Cai *et al.* 1994).

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