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# Tolerance mechanism in hybrid citrus rootstock progenies against *Phytophthora nicotianae* Breda de Haan

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*Phytophthora* species is the major threat for world citrus industry in general, and for India, in particular due to commercial use of susceptible rootstocks. The resistant gene possessed by *Poncirus* genus may be of immense use, if transferred in a well acclimatized citrus species which can have good impact on fruit size of scion varieties. Being a soil borne problem, development of resistant/tolerant rootstock(s) is the most eco-friendly solution to combat with this deadly disease. The present study was conducted during 2016 to understand the tolerance mechanism in the intergeneric hybrids of citrus rootstocks against *Phytophthora nicotianae*. The materials of study consisted of 30 hybrids, ten each of Pummelo (P) × Troyer (T), Pummelo (P) × Sacaton (S) and Pummelo (P) × Trifoliate orange (TO) were tested against the inoculation of *P. nicotianae*, taking Pummelo, Troyer and Citrumelo as control treatments. Of the total hybrid progenies, only six hybrids (P × TO-103, P × TO- 112, P × S-117, P × S-119, P × T-125 and P × T-130) expressed resistance against *P. nicotianae* on the basis of lesion length (nil or <2.5 cm). Of the tested hybrids, P x S-117 had the highest photosynthetic rate (*A*) (8.12 µmol $m^{-1}s^{-1}$) followed by P × TO-112 and P × S-119. Excised leaf water loss (ELWL) was lowest in P × S -119 (7.47%) without having significant diference with Troyer citrange, and rest of the resistant hybrids. The highest relative leaf water content (RWC) was registered in P × T-125 (84.47%), which was similar statistically with P × T-119, P × T-103 and P × T-112 (77.59-83.42%) hybrids. Hybrid P × S -117 tended to show the highest total chlorophyll content (12.14 mg $g^{-1}$FW), followed by P × TO-112 and P × T-127. P × S-117 expressed the lowest level of hydrogen peroxide (100.72 mM $mg^{-1}$ FW) without having any significant difference with those of P × TO-110, P × T-127 and P × T-130 hybrids. The lipid peroxidation was highest in P × T-132 (25.99 µmol $g^{-1}$ FW), while its lowest accumulation was in P × S-119 (6.44 µmol $g^{-1}$ FW) with statistically similar to P × T-130 hybrid. The highest content of glycine betain was noticed in P × TO-103, P × TO-117 and P × TO-130 (0.33 mg $g^{-1}$ FW in each). Of the total hybrid progenies, highest accumulation of leaf N was found in P × T-125 (2.74%) followed by P × S -119 and P × T-130. All six resistant hybrids excelled over other hybrids in respect of leaf $K^{+}$ content. The content of $Ca^{+2}$ was highest in the leaves of P × S-117 (4.90%) having similarity statistically with P × T-125 (4.87%). The resistance of identified hybrids (P × TO-103, P × TO- 112, P × S-117, P × S-119, P × T-125 and P × T-130) against *P. nicotianae* was also evidenced by low ROS generation and ELWL, with high RWC and leaf nutrient status over other hybrids. Among the various physicochemical characters studied, only *A*, ELWL and N were found to have significant but inverse relationship with lesion length caused by *Phytophthora* inoculation.

**Keywords:** Citrus gummosis, Gum lesions, Lipid peroxidation, Pummelo, Sacaton, Trifoliate orange, Troyer

In India, citrus is the most important group of fruit crops next to banana and mango, contributing 13.7% (1192 thousand tones) production share of total fruit production[1]. Andhra Pradesh, Telangana, Madhya Pradesh, Punjab, Maharashtra and North Eastern states are leading citrus producing regions of India. Sweet orange, mandarin, lemon/limes and grapefruit are the principal citrus fruit crops grown commercially in India. Despite of favourable soil and climatic conditions, the productivity of citrus fruits in India is very low (10.30 MT $ha^{-1}$) as against 35.60 MT $ha^{-1}$ in Indonesia. Various biotic factors (viruses, fungal and bacterial diseases) are also responsible for low citrus productivity in India. Of these, *Phytophthora* species caused diseases are considered one of the most serious soil borne threats, causing considerable losses (10-30%) in all the citrus growing regions of the world[2,3]. In California, the largest citrus producer in the United States, *Phytophthora* caused diseases account for $12.9 million annual loss to citrus industry[4].

*Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur), the causal agent of citrus gummosis disease, has caused great damage to citrus orchards throughout the world. While chemical and horticultural measures do not guarantee the preventive or curative control of citrus gummosis, the use of

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resistant rootstocks is the most reliable management strategy against the disease. With the objective of developing citrus rootstocks resistant to gummosis and to better elucidate the Phytophthora-citrus pathosystem, citrus breeding programs is in progress worldwide, mostly employing directed crosses[5].

Genus *Poncirus* is a valuable gene resource because this possesses genes endowed with many important traits of commercial importance, not discovered in *Citrus*. For example, *Poncirus* has been reported to possess the resistance against CTV, *Phytophthora* caused root rot, nematode, and tolerance to low temperature[6]. *P. trifoliata* has been proved to be a good parent to develop the populations of several adaptable rootstocks. Most of these populations have been derived from the crosses with *Poncirus*, partly because of high interest in its exclusive genes, attempts at comparison of the related genomes, and the great advantage of the dominant trifoliate leaf trait of *Poncirus* over the monofoliate of *Citrus*, which helps to identify the zygotic hybrids morphologically from nucellar seedlings, whose recovery is difficult in crosses within the polyembryonic citrus species.

Another citrus species i.e. *Citrus grandis* Osbeck tends to increase leaf biomass, hence may have good impact on fruit growth of scion variety, if used as rootstock. Although, pummelo is quite seedy, but produces seeds containing embryos of zygotic origin, and therefore, not amenable to standard nursery production. In addition, pummelo has also been reported to have superior combining ability for intergeneric crosses involving *P. trifoliata* inheritance and other desirable traits useful to citrus breeders[8]. The proportion of live to dead plants, recorded at 11months postinoculation of *P. nicotianae*, showed that the Sarawak (Pummelo) × Bower mandarin performed significantly better than other rootstocks[7]. The pummelo is regarded as resistant to *P. nicotianae*[9], however, it does not present nucellar embryony, producing only monoembryonic zygotic seed; therefore, it should be expected that the many of its offspring would also be monoembryonic. However, there is still chance of obtaining hybrids that have some degree of polyembryony, such as sour orange, which is an offspring of the same kind of cross. If any of the tested hybrid shows high resistance to *Phytophthora* and is monoembryonic, propagation by tissue culture would be an option[8]. On these basis, pummelo was selected as a first seed parent for its improvement through hybridization.

Keeping in view the severity of *P. nicotianae*, and opportunity to combine more than one trait in one genotype, the systematic citrus rootstock improvement programme using inter-generic and inter-specific crosses is under progress in the Division of Fruits & Horticultural Technology, Indian Agricultural Research Institute, New Delhi to develop *Phytophthora* tolerant/resistant rootstock(s). Hence, the hybrids obtained from the ongoing rootstock improvement programme were tested against the artificial inoculation of *P. nicotianae* during the course of present experimentation.

## Materials and Methods

### Experimental site

The present study was undertaken in the Division of Fruits & Horticultural Technology at ICAR-Indian Agricultural Research Institute, New Delhi, located at 77º12' E; 28º40' N; 228.6 m asl. May and June were the hottest months (highest temperature ranging from 41-44ºC), while December and January were the coolest months (minimum temperatures ranging from 3-7ºC).

### Planting materials

The response of 30 hybrids, 10 each of Red Fleshed pummelo (*Citrus grandis*) x Troyer citrange (*C. sinensis* × *Poncirus trifoliata*), Red Fleshed pummelo (*C. grandis*) × Sacaton citrumelo (*P. trifoliata* × *C. paradisi*) and Red Fleshed pummelo (*C. grandis*) × Webster Trifoliate orange (*P. trifoliata*) were studied against the artificial inoculation of *P. nicotianae*. Pummelo, Troyer citrange and citrumelo plants were used as control plants. The hybrid fruits were harvested in the month of December, 2015. The seeds were extracted, washed with tap water and sown in the nursery beds just after fruit harvesting. Six month old hybrid seedlings were transplanted in the plastic pots (12 inches) containing 8 kg sterilized mixture of soil (3 parts) and farm yard manure (1 part), allowed to settle for 45 days, and irrigated with tap water. Each pot was supplied with urea (20 g), single super phosphate (15 g) and potassium sulphate (15 g) one month after transplanting.

### Fungal inoculation

Inoculation of oomycetes was done by slit method[10]. Pure culture of *P. nicotianae* was procured

from Central Citrus Research Institute, Nagpur (India), and maintained on Potato Dextrose Agar medium at 4°C for further use. The stem was inoculated with the culture of *P. nicotianae*. The incision (3.0 mm long and 0.2-0.5 mm deep) was made with a sterile scalpel into the bark of stem of 7 months old seedlings. Agar disks (3.0 mm diameter) were cut from an active culture of oomycete, and the disk were held in place by wet strips of cheesecloth wrapped around each stem and sealed with cello tape to keep the inoculum moist. The inoculated plants were then incubated in polyethylene sheet chamber at 24°C and 90-95% relative humidity for one week. The data on various aspects were recorded 45 days after inoculation.

**Pathological reaction**

Resistance of targeted hybrids was evaluated by comparing the lesion length on stem and with a known degree of resistance to *P. nicotianae*[11].

**Physiological response**

Photosynthetic rate (*A*) was measured on four mature leaves using LCi-SD Ultra Compact Photosynthesis System (ADC Bio Scientific Ltd., Global House, Hoddesdon, UK) during 2nd week of December. Apex fully developed leaves were clamped to the leaf chamber, and the observations were recorded when RH and *Ci* (internal $CO_2$ concentration) reached a stable value. Measurements were performed between 12.00-14.00 h. Observations on *A* were recorded under the conditions of day temperature 23°C; night temperature 10°C; relative humidity (RH) 60%.

Relative water content (RWC) was estimated by gravimetric method[12]. The leaves were collected in the morning, sealed in polyethylene bags, placed in ice box and brought to the laboratory for analysis. Fresh weight (FW) of the leaf sample (0.1 g) was taken, and dipped in petriplates containing 100 mL of distilled water for 4 h at 20°C. Thereafter, the turgid weight (TW) of the leaf sample was measured after the removal of excess water of leaf discs with blotting paper. The sample was packed in butter paper bag and oven dried at 65°C for 48 h, and finally, dry weight (DW) of same sample was recorded. RWC was calculated using the formula as

RWC (%) = (FW - DW)/(TW - DW) × 100.

For excised leaf water loss (ELWL) measurements, the fully-developed leaves were collected and sealed in polyethylene bag from the each seedling, 4 days after irrigation, thereafter placed in an ice box, and brought to the laboratory. The fresh weight (FW) of each leaf was recorded, and kept at room temperature for 2 h, and thereafter, the weight of the wilted leaf samples (WW) was recorded. ELWL was then calculated using the given formula as ELWL (%) = (FW – WW) / FW × 100.

**Biochemical response**

The leaf chlorophyll content was measured by non-maceration method using DMSO[13]. The finely chopped leaf sample of about 100 mg was placed in test tube, and added with 10 mL DMSO. The test tubes were covered with aluminium foil and kept in an oven at 65°C for 4 h. Thereafter, the absorbance of chlorophyll solution was recorded on Spectro UV-VIS Double Beam Pc Ver 3.3 Labomed, Inc. at 645 and 663 nm against DMSO as blank. The content of total chlorophyll was estimated as per the formula[14], and expressed as (mg $g^{-1}$ FW).

$$\text{Total chlorophyll (a + b)} = \frac{20.2\,A_{645} + 8.02\,A_{663} \times V}{1000 \times W}$$

[A= Absorbance, W= Weight of the sample, V= Volume of DMSO in mL]

Hydrogen peroxide ($H_2O_2$) was estimated by forming titanium-hydro peroxide complex which absorbs at 415 nm[15]. The leaf sample (1.0 g) was ground with the help of liquid nitrogen to fine powder, and added with 10 mL cooled acetone in a cold room at 10°C. Mixture was filtered with Whatman No. 1 filter paper followed by the addition of 4 mL titanium reagent and 5 mL ammonium solution to precipitate the titanium-hydro peroxide complex. Reaction mixture was centrifuged at 10000 ×g for 10 min in a refrigerated centrifuge (Hermle Z 323K). Precipitate was dissolved in 10 mL of 2M $H_2SO_4$ and then recentrifuged. Supernatant was read at 415 nm against reagent blank in UV-visible spectrophotometer (Spectro UV-VIS Double Beam PC Ver 3.3 Labomed, Inc). Concentration of $H_2O_2$ was calculated using a standard curve made from known concentrations of $H_2O_2$.

The lipid peroxidation product was estimated as per the method suggested by Heath & Packer[16]. Initially, fresh leaf sample (0.5 g) was crushed in 0.1% TCA using pestle and mortar. At 15000 ×g for 15 min, the homogenate was centrifuged. For the estimation of thiobarbituric acid reactive substance (TBARS), supernatant was used. In separate tube 1ml of supernatant and 4.0 mL of 0.5 per cent of TBA in

20 per cent TCA was mixed. Then this mixture was heated at 95°C for 30 min in electric oven followed by cooling in ice bath. Again this mixture was centrifuged at 10000 ×g for 10 min. Supernatant was taken and read at 532 nm (specific value) and 600 nm (non-specific values) with UV-visible spectrophotometer (Spectro UV-VIS Double Beam PC Ver 3.3 Labomed, Inc). Finally, extinction coefficient value 155 $mM^{-1}$ $cm^{-1}$ was used to calculate the content of TBARS content.

Glycine betaine was estimated in dried leaf powder as percentage using method of Grieve & Grattan[17]. Thawed extract was diluted 1:1 with 2N sulphuric acid. Aliquot (0.5 mL) was measured in test tube and cooled in ice water for 1 h. Cold potassium iodide-iodine reagent (0.2 mL) was added, then the mixture was mixed gently with vortex mixture, and stored at 0-4°C for 16 h. Thereafter, sample was transferred to centrifuge tubes, and centrifuged (Hermle Z 323K) at 10000 ×g for 15 min at 0°C. The separation of supernatant was done carefully with the help of 1 mL micropipette. As the solubility of the periodite complexes in the acid reaction mixture increased markedly with temperature, so cold condition was maintained until the periodite complex was separated from acid media. The periodite crystals were dissolved in 9 mL of 1,2-dichloro ethane (reagent grade). Vigorous vortex mixing was done to influence complete solubility in developing solvent. After 2.0-2.5 h, the absorbance was read at 365nm with UV-visible spectrophotometer (Spectro UV-VIS Double Beam PC Ver 3.3 Labomed, Inc). Reference standards of glycine-betaine (50-200 μg $mL^{-1}$) were prepared in 2N sulphuric acid, and the procedure for sample estimation was followed.

**Tissue nutrient analysis**

The leaf nitrogen content was determined using Digestion Block method[18]. For this, finely ground leaf sample (0.5 g) taken from each hybrid rootstocks was taken in digestion tube. A pinch of catalyst mixture ($K_2SO_4$:$CuSO_4$ in the ratio of 10:1), and concentrated $H_2SO_4$ (10 mL) were added. Then digestion tubes were attached to digestion system for heating about 385°C till the disappearance of black or brown colour. The digestion tubes were then taken to distillation unit which was set up to perform the various steps like dilution, addition of alkali, steam generation and titration. About 20 mL of 4% boric acid containing mixed indicator in a 250 mL conical flask was placed under ammonia- receiving tube of the distillation assembly, and distillation was ran for 6.0 min. Then the parrot green colour distillate was titrated against 0.1N $H_2SO_4$ solution to light pink endpoint. Nitrogen in leaf samples was determined using following formula:

$$N\ (\%) = [(T\text{-}B) \times N \text{ of } H_2SO_4 \times 1.4007] / S$$

where, T = Volume of standard $H_2SO_4$ taken for sample; B = Volume of standard $H_2SO_4$ taken for blank; N = Normality of acid; S = Weight of plant sample taken

Ground leaf samples were digested in nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) mixture in the ratio of 4:1 (i.e. diacid mixture). The ground leaf sample (0.5 g) was taken in 100 mL capacity conical flask; thereafter 10.0 mL of diacid mixture was added. This solution was kept overnight for pre-digestion after putting a funnel on each flask. The next day, conical flask containing predigested leaf sample in diacid mixture was digested in the digestion chamber at a temperature of 100°C for the initial 1 h. The temperature was increased to 200°C for 2-3 h till the volume reduced to 1-2 mL, and the solution turned colourless with the disappearance of white dense fumes from the digesting sample. The digested sample was then diluted and filtered through Whatman No.1 filter paper in series. Thereafter, double distilled water was added to make final volume 50 mL. This diluted material was used to estimate the potassium and calcium. Total potassium content in leaves was estimated using a micro-processor based flame photometer (Flame Photometer-128, Systronics, Ahmedabad) as per the method of Jackson[19]. The calcium content was estimated by atomic absorption spectrophotometer (Model- GBC, 904AA, GBC Scientific Equipment, Hampshire, Illinois, USA).

**Statistical analysis**

The experiment was conducted in an Augmented Block Design. Data were analyzed using the SAS software Version 9.3 (SAS Institute, Inc., USA). P values ≤0.05 were considered as significant. The adjusted means were separated using F test followed by DMRT. For studying relationship of various physio-chemical variables with lesion length, the coefficient of determination was calculated using regression analysis approach.

## Results

### Pathological reaction

The reaction of citrus rootstock inoculated with *Phytophthora nicotianae* was studied, and targeted hybrids were categorized on the bases of lesion length (Table 1 & Plate 1). Of the 30 hybrids, two hybrids *viz.,* P × TO- 112 and P × T- 130 were found absolutely free from the any sign of *P. nicotianae* caused lesion. Besides, P × TO- 103, P × S- 117, P × S- 119 and P × T- 125 hybrids expressed only single and very small lesion development (ranged between 0.6 to 1.5 mm ). Hence, of the total number of the hybrids tested, only two hybrids each of Pummelo × Troyer citrange (P × T- 125 and P × T- 130), Pummelo x Sacaton (P × S- 117 and P × S- 119) and Pummelo × Trifoliate orange (P × TO- 103 and P × TO- 112) were found resistant against *P. nicotianae*. The number of lesions and gum flow length in susceptible hybrids ranged from 1-5 and 3.70-14.65 cm, respectively.

Table 1 — Degree of resistance of citrus hybrids determined by artificial *Phytophthora nicotianae* inoculation

| No. of Hybrids | No. of lesions | Average lesion length (mm) | Average gum flow length (cm) | Degree of resistance |
|---|---|---|---|---|
| Citrumelo (control) | 1 | 2.20 | 2.50 | Resistant |
| Pummelo (control) | 4 | 8.30 | 7.85 | Susceptible |
| Troyer (control) | - | - | - | Resistant |
| P × TO-103 | 1 | 1.50 | 2.80 | Resistant |
| P × TO-104 | 4 | 7.50 | 9.75 | Susceptible |
| P × TO-105 | 2 | 7.85 | 7.90 | Susceptible |
| P × TO-106 | 4 | 6.45 | 7.10 | Susceptible |
| P × TO-107 | 1 | 4.50 | 3.80 | Susceptible |
| P × TO-108 | 5 | 9.85 | 7.45 | Susceptible |
| P × TO-109 | 1 | 2.90 | 3.70 | Susceptible |
| P ×TO-110 | 3 | 10.55 | 8.10 | Susceptible |
| P × TO-111 | 3 | 13.30 | 11.20 | Susceptible |
| P × TO-112 | - | - | - | Resistant |
| P ×S-113 | 1 | 6.00 | 3.80 | Susceptible |
| P × S-114 | 4 | 9.40 | 7.00 | Susceptible |
| P × S-115 | 3 | 5.20 | 7.35 | Susceptible |
| P × S-116 | 2 | 11.75 | 10.30 | Susceptible |
| P × S-117 | 1 | 0.60 | 2.40 | Resistant |
| P × S-118 | 1 | 2.90 | 3.20 | Susceptible |
| P × S-119 | 1 | 1.20 | 1.20 | Resistant |
| P × S-120 | 2 | 8.75 | 10.6 | Susceptible |
| P × S-121 | 1 | 5.80 | 4.50 | Susceptible |
| P × S-122 | 5 | 9.65 | 12.75 | Susceptible |
| P × T-123 | 3 | 11.05 | 8.00 | Susceptible |
| P × T-124 | 5 | 9.00 | 12.40 | Susceptible |
| P × T-125 | 1 | 0.80 | 2.50 | Resistant |
| P × T-126 | 1 | 4.50 | 4.75 | Susceptible |
| P × T-127 | 2 | 7.95 | 9.30 | Susceptible |
| P ×T-128 | 3 | 6.00 | 7.40 | Susceptible |
| P × T-129 | 2 | 7.85 | 8.00 | Susceptible |
| P × T-130 | - | - | - | Resistant |
| P × T-131 | 2 | 7.10 | 8.75 | Susceptible |
| P × T-132 | 5 | 13.95 | 14.65 | Susceptible |

[C, Citrumelo; P, Pummelo; TO, Trifoliate Orange; S, Sacaton; and T, Troyer Citrange]

### Physiological response

*P. nicotianae* inoculation exerted the significant effect on *A*, ELWL and RWC of hybrid progenies of citrus rootstocks (Table 2). P × S- 117 hybrid exhibited significantly highest *A* (8.12 µmol $m^{-1}s^{-1}$), which was followed by P × TO- 112 (7.64 µmol $m^{-1}s^{-1}$) and P × S-119 (7.32 µmol $m^{-1}s^{-1}$). The lowest *A* was recorded in P × TO- 110 (3.23 µmol $m^{-1}s^{-1}$). Over pummelo, highest per cent increase in *A* was observed in P × T-117 (106.09%) followed by P ×T-112 (93.91%), P × S-119 (85.69%), P ×TO-127 (78.23%), Troyer (78.17%) and P × TO-104 (76.14%) rootstocks. Even Citrumelo showed 57.87% higher *A* than pummelo.

Among various hybrid progenies, P × T- 128 hybrid tended to show the highest ELWL (42. 15%) without exhibiting any significant difference with P × TO-105, P × TO-106, P × TO-109, P × S-113, P × S- 114, P × S -116, P × S-121, P × T-131 and P × T- 132 hybrids. The value of ELWL was lowest in P × S -119 (7.47%) having similarity statistically with Troyer citrange, P × TO- 103, P × TO-112, P × S-117, P × T-125, and P × T- 130 hybrids. A large variation (increase or decrease) in ELWL over pummelo expressed the highest change in P × S-119

Plate 1 — Hybrids showing *Phytophthora* caused lesion development

Table 2 — Photosynthetic rate (*A*), excised leaf water loss (ELWL) and relative water content (RWC) of *Phytophthora nicotianae* inoculated progenies of citrus hybrids (adjusted mean)

| Hybrid No. | *A* (μmol $m^{-2}$ $S^{-1}$) | ELWL (%) | RWC (%) |
|---|---|---|---|
| Citrumelo (control) | $6.22^{g}$ | $19.63^{h}$ | $67.72^{ef}$ |
| Pummelo (control) | $3.94^{s}$ | $29.82^{def}$ | $54.12^{lm}$ |
| Troyer (control) | $7.02^{d}$ | $13.2^{j}$ | $74.25^{cd}$ |
| P × TO-103 | $6.94^{e}$ | $10.23^{j}$ | $77.59^{abc}$ |
| P × TO-104 | $4.93^{k}$ | $24.62^{fgh}$ | $60.07^{ghijkl}$ |
| P × TO-105 | $4.22^{p}$ | $35.93^{abcd}$ | $54.41^{klm}$ |
| P × TO-106 | $3.47^{u}$ | $42.17^{ab}$ | $58.99^{ghijkl}$ |
| P x TO-107 | $5.35^{I}$ | $22.27^{gh}$ | $57.27^{hijklm}$ |
| P × TO-108 | $3.77^{t}$ | $31.30^{cdef}$ | $64.40^{fghi}$ |
| P × TO-109 | $4.59^{n}$ | $41.31^{ab}$ | $49.97^{m}$ |
| P × TO-110 | $3.23^{v}$ | $21.28^{gh}$ | $52.94^{klm}$ |
| P × TO-111 | $4.73^{m}$ | $27.66^{efg}$ | $63.57^{fghij}$ |
| P × TO-112 | $7.64^{b}$ | $9.97^{j}$ | $80.11^{abc}$ |
| P × S-113 | $5.11^{j}$ | $35.19^{abcde}$ | $54.54^{klm}$ |
| P × S-114 | $4.26^{p}$ | $34.15^{abcde}$ | $59.25^{ghijkl}$ |
| P × S-115 | $3.98^{rs}$ | $22.81^{gh}$ | $65.29^{efgh}$ |
| P × S-116 | $4.29^{p}$ | $39.02^{abc}$ | $55.71^{jklm}$ |
| P × S-117 | $8.12^{a}$ | $10.09^{j}$ | $77.69^{abc}$ |
| P × S-118 | $4.45^{o}$ | $22.51^{gh}$ | $65.29^{efgh}$ |
| P × S-119 | $7.32^{c}$ | $7.47^{j}$ | $83.42^{ab}$ |
| P × S-120 | $3.75^{t}$ | $25.61^{fgh}$ | $56.95^{ijklm}$ |
| P × S-121 | $4.72^{m}$ | $39.45^{abc}$ | $66.13^{efg}$ |
| P × S-122 | $5.96^{h}$ | $20.03^{ghi}$ | $63.66^{fghi}$ |
| P × T-123 | $3.41^{u}$ | $24.99^{fgh}$ | $59.96^{ghijkl}$ |
| P × T-124 | $4.25^{p}$ | $23.58^{fgh}$ | $61.07^{ghijk}$ |
| P × T-125 | $4.13^{q}$ | $11.31^{j}$ | $84.47^{a}$ |
| P × T-126 | $3.75^{t}$ | $34.07^{bcde}$ | $64.66^{fghi}$ |
| P × T-127 | $7.03^{d}$ | $28.27^{defg}$ | $76.06^{bcd}$ |
| P ×T-128 | $4.04^{r}$ | $42.15^{a}$ | $73.25^{cde}$ |
| P × T-129 | $5.10^{j}$ | $24.63^{fgh}$ | $68.96^{def}$ |
| P × T-130 | $6.69^{f}$ | $12.57^{ij}$ | $79.09^{abc}$ |
| P × T-131 | $4.83^{l}$ | $41.23^{ab}$ | $65.51^{fgh}$ |
| P × T-132 | $3.92^{s}$ | $37.136^{abc}$ | $59.96^{ghijkl}$ |
| LSD *(P≤0.05)* | | | |
| Control treatment means | 0.04 | 4.17 | 4.26 |
| Test treatment in same block | 0.07 | 7.22 | 7.39 |
| Test treatment not in same block | 0.08 | 8.34 | 8.53 |
| Test and a control treatment | 0.06 | 6.37 | 6.52 |

[C, Citrumelo; P, Pummelo; TO, Trifoliate Orange; S, Sacaton; and T, Troyer Citrange]

(–74.95%) followed by P × TO- 112 (–66.57%), P × S-117 (–66.16%), P × TO-103 (–65.69%), P × T-125 (–62.07%), P × T-130 (–57.85%) and Troyer citrange (–55.73%).

The content of ELWL and RWC exhibited inverse relationship during the course of present study. The highest RWC was recorded in leaves of P × T -125 (84.47%), which was statistically at par with P x T-119 (83.42 %), P × T- 103 (77.59 %) and P × T- 112 (80.11%). P × T -109 had the lowest RWC (49.97%) having similarity statistically with pummelo, P × TO-105, P × TO- 107, P × TO- 110, P × TO- 113, P × TO- 116, and P × S-120. The per cent change in the RWC over pummelo was positive in majority of the hybrids except P × TO-109 and P × TO-110 (-7.67 to -2.18 %). Citrus hybrids *viz.,* P × TO-103, P × S-119, P × TO-125 and P × TO-130 showed the highest increase in RWC (43.37 to 56.08 %) then rest of the hybrids tested over pummelo rootstock seedlings.

**Biochemical response**

The data relating to the concentration of total chlorophyll, hydrogen peroxide ($H_2O_2$), lipid peroxidation and glycine betain (GB) in the leaves of citrus hybrids as influenced significantly by the inoculation of *P. nicotianae* are given in Table 3. The total chlorophyll content was significantly highest in P × S -117 (12.14 mg $g^{-1}$ FW), however, it was statistically at par with P × TO-112 and P × T-125. Other resistant hybrids namely P × TO-103, P × S-119 and P × T-130 also exhibited the higher chlorophyll content (10.55-11.50 mg $g^{-1}$ FW) than other hybrids. The lowest content of total chlorophyll was recorded in P × S -121 (2.86 mg $g^{-1}$ FW). Over pummelo, the total chlorophyll content was 121.94% higher in P × S-117 hybrid. The highest concentration of $H_2O_2$ was shown by P × T- 132 (173.22 nM $mg^{-1}$ FW), which was statistically similar with P × TO- 104, P × TO-106, P × TO-108, P × TO-110, P × S-113, P × S-121, P × S-122, P × T-123, P × T- 125 and P × T- 126. The lowest concentration of $H_2O_2$ was shown by P × S -117 (100.72 nM $mg^{-1}$ FW), which had similarity statistically with P × TO-103, P × S-119, P × T- 127 and P × T-130. The study of per cent change in the content of $H_2O_2$ over pummelo indicated very high reduction in Troyer, P × TO-103, P × TO-112, P × S-117, P × S-119, P × T-127 and P × T- 130 (–21 to –34.91%), whereas P × TO-106, P × T-126 and P × T-132 exhibited the increase (11.35 to 11.94%) in the level of $H_2O_2$ over pummelo.

The inoculation of oomycete in citrus hybrid showed the highest lipid peroxidation product in the leaves of P × T-132 (25.99 μmol $g^{-1}$ FW). P × S-116 was the next hybrid to have highest lipid peroxidation content (23.79 μmol $g^{-1}$ FW) but was found statistically similar with P × T- 124 and P × T- 125. The lowest lipid peroxidation was observed in the leaves of P × S- 119 (6.44 μmol $g^{-1}$ FW) without showing any significant difference with P × T-130. The highest change in the level of lipid peroxidation

Table 3 — Biochemical response of *Phytophthora nicotianae* inoculated progenies of citrus hybrids (adjusted mean)

| Hybrid No. | Total chlo.'a+b' (mg $g^{-1}$ FW) | $H_2O_2$ (mM $mg^{-1}$ FW) | Lipid peroxidation (µmole $g^{-1}$ FW) | Glycine betaine (mg $g^{-1}$ FW) |
|---|---|---|---|---|
| Citrumelo (control) | $8.57^{f}$ | $133.99^{m}$ | $14.7^{p}$ | $0.17^{d}$ |
| Pummelo (control) | $5.47^{jk}$ | $154.75^{ghj}$ | $17.54^{lm}$ | $0.05^{o}$ |
| Troyer (control) | $10.30^{e}$ | $121.18^{n}$ | $11.29^{q}$ | $0.20^{c}$ |
| P × TO-103 | $10.88^{cd}$ | $103.86^{p}$ | $12.53^{q}$ | $0.33^{a}$ |
| P × TO-104 | $7.46^{g}$ | $165.69^{abcdef}$ | $16.91^{lmno}$ | $0.16^{def}$ |
| P × TO-105 | $6.04^{i}$ | $159.18^{defghj}$ | $15.75^{nop}$ | $0.11^{hijklm}$ |
| P × TO-106 | $4.41^{m}$ | $172.32^{ac}$ | $14.91^{p}$ | $0.16^{defg}$ |
| P × TO-107 | $6.12^{i}$ | $158.89^{defghj}$ | $18.46^{hijkl}$ | $0.16^{def}$ |
| P × TO-108 | $7.48^{g}$ | $164.30^{abcdefg}$ | $17.36^{klmn}$ | $0.14^{efghijk}$ |
| P × TO-109 | $6.33^{hi}$ | $160.50^{bdefghi}$ | $15.30^{op}$ | $0.15^{defg}$ |
| P ×TO-110 | $10.44^{de}$ | $168.28^{abcd}$ | $16.65^{mno}$ | $0.09^{lmn}$ |
| P × TO-111 | $4.96^{klm}$ | $148.24^{jkl}$ | $15.62^{op}$ | $0.15^{defghij}$ |
| P × TO-112 | $12.08^{ab}$ | $115.39^{no}$ | $9.04^{rs}$ | $0.30^{a}$ |
| P × S-113 | $5.23^{jkl}$ | $165.23^{abcde}$ | $21.99^{cde}$ | $0.11^{ijklm}$ |
| P × S-114 | $6.08^{i}$ | $140.78^{klm}$ | $19.21^{hijk}$ | $0.13^{efghijkl}$ |
| P × S-115 | $5.70^{ij}$ | $135.46^{lm}$ | $21.54^{defg}$ | $0.05^{no}$ |
| P × S-116 | $7.02^{g}$ | $153.70^{fghj}$ | $23.79^{b}$ | $0.12^{efghijklm}$ |
| P × S-117 | $12.14^{a}$ | $100.72^{p}$ | $9.02^{rs}$ | $0.33^{a}$ |
| P × S-118 | $6.87^{gh}$ | $158.15^{defghj}$ | $21.86^{cdef}$ | $0.14^{efghijkl}$ |
| P × S-119 | $11.50^{bc}$ | $110.57^{op}$ | $6.44^{t}$ | $0.24^{bc}$ |
| P × S-120 | $7.16^{g}$ | $159.54^{cdefghij}$ | $18.12^{jklm}$ | $0.08^{mno}$ |
| P × S-121 | $2.86^{n}$ | $162.02^{abcdefgh}$ | $17.99^{jklm}$ | $0.13^{efghijkl}$ |
| P × S-122 | $6.05^{i}$ | $166.15^{abcde}$ | $20.05^{ghi}$ | $0.10^{klm}$ |
| P × T-123 | $4.76^{lm}$ | $164.48^{abcdefg}$ | $18.50^{ijklm}$ | $0.15^{defghi}$ |
| P × T-124 | $7.45^{g}$ | $158.09^{defghj}$ | $22.18^{bcd}$ | $0.16^{de}$ |
| P × T-125 | $11.85^{ab}$ | $163.41^{abcdefg}$ | $23.73^{bc}$ | $0.24^{b}$ |
| P × T-126 | $10.33^{de}$ | $172.53^{ab}$ | $19.92^{fhij}$ | $0.10^{jklm}$ |
| P × T-127 | $6.23^{hi}$ | $107.27^{op}$ | $9.47^{r}$ | $0.15^{defghi}$ |
| P ×T-128 | $8.71^{f}$ | $149.43^{hjk}$ | $20.25^{efgh}$ | $0.12^{ghijklm}$ |
| P × T-129 | $7.02^{g}$ | $137.21^{lm}$ | $23.02^{bcd}$ | $0.12^{fghijklm}$ |
| P × T-130 | $10.65^{de}$ | $112.30^{nop}$ | $7.21^{st}$ | $0.33^{a}$ |
| P×T-131 | $7.28^{g}$ | $153.73^{efghjk}$ | $21.73^{deg}$ | $0.15^{defgh}$ |
| P × T-132 | $10.55^{de}$ | $173.22^{ab}$ | $25.99^{a}$ | $0.14^{efghijk}$ |
| LSD ($P \leq 0.05$) | | | | |
| Control Treatment Means | 0.33 | 6.48 | 1.003 | 0.02 |
| Test Treatment in the same Block | 0.57 | 11.23 | 1.73 | 0.04 |
| Test Treatment not in the same Block | 0.66 | 12.97 | 2.00 | 0.04 |
| Test Treatment and a Control Treatment | 0.51 | 9.90 | 1.53 | 0.03 |

[C, Citrumelo; P, Pummelo; TO, Trifoliate Orange; S, Sacaton; and T, Troyer Citrange]

in comparison to pummelo was observed in P × S-119 (–63.28%) and P × T-132 (42.88%) hybrids.

The highest content of GB was registered in P × TO-103, P × S-117 and P × T-130 (0.33 mg $g^{-1}$ FW in each), and showed the similarity statistically with P × TO-112. The content of GB was lowest in pummelo and P × S-115 (0.05 mg $g^{-1}$ FW in each). The per cent increase in the content of GB in hybrid populations due to inoculation of oomycete over pummelo was observed in all the hybrids including citrumelo and Troyer, except P × S-115, and its highest increase was observed in the leaves of P × TO-103, P × S-117 and P × T-130 (560.00% in each). The lowest increase was shown in P × S-120 hybrid (80.00%).

**Leaf nutrient status**

The concentrations of leaf N, $K^{+}$ and $Ca^{+2}$ in the tested progenies of citrus hybrids exhibited the significant difference due to inoculation of *P. nicotianae* (Table 4). Of the various hybrids, highest concentration of leaf N was recorded in P × T-125 (2.74%). P × S -119 was found next best treatment, but was similar statistically with P × T-130. Other three resistant hybrids (P × TO-103, P × TO-112 and P × S-117) also showed higher leaf N (2.56 - 2.63%) than many other hybrids. The lowest content of leaf N was noticed in P × S-113 (1.59%). There were 23 hybrids which had lower leaf N content than pummelo (control), while highest increase in N over control was observed in P × T-125 (19.13%) followed

Table 4 — Leaf nutrient status of *Phytophthora nicotianae* inoculated progenies of citrus hybrids (adjusted mean)

| Hybrid No. | Nitrogen (N) (% DW) | Phosphorus ($K^+$) (% DW) | Calcium ($Ca^{+2}$) (% DW) |
|---|---|---|---|
| Citrumelo (control) | $2.40^{e}$ | $1.25^{cdefghi}$ | $3.19^{g}$ |
| Pummelo (control) | $2.30^{f}$ | $0.97^{jk}$ | $1.91^{q}$ |
| Troyer (control) | $2.61^{c}$ | $1.34^{bcdefh}$ | $4.03^{d}$ |
| P × TO-103 | $2.63^{c}$ | $1.43^{abcdefg}$ | $3.70^{f}$ |
| P × TO-104 | $2.24^{g}$ | $0.92^{ijk}$ | $2.96^{h}$ |
| P × TO-105 | $2.13^{j}$ | $0.98^{fghijk}$ | $2.55^{j}$ |
| P × TO-106 | $2.05^{k}$ | $0.71^{jk}$ | $2.78^{i}$ |
| P × TO-107 | $2.11^{j}$ | $0.79^{jk}$ | $2.17^{lm}$ |
| P × TO-108 | $1.94^{m}$ | $0.95^{hijkl}$ | $2.02^{op}$ |
| P × TO-109 | $2.18^{hi}$ | $0.91^{ijk}$ | $2.2^{1l}$ |
| P ×TO-110 | $1.84^{no}$ | $0.80^{jk}$ | $2.56^{j}$ |
| P × TO-111 | $1.99^{l}$ | $0.72^{jk}$ | $2.39^{k}$ |
| P × TO-112 | $2.56^{d}$ | $1.60^{abcd}$ | $4.46^{c}$ |
| P × S-113 | $1.59^{q}$ | $0.64^{k}$ | 1.06u |
| P × S-114 | $1.70^{p}$ | $0.83^{jk}$ | $1.41^{t}$ |
| P × S-115 | $2.29^{f}$ | $0.81^{jk}$ | $1.79^{r}$ |
| P × S-116 | $1.93^{m}$ | $0.90^{gijkl}$ | $1.91^{s}$ |
| P × S-117 | $2.59^{cd}$ | $1.54^{abcde}$ | $4.90^{a}$ |
| P × S-118 | $1.88^{n}$ | $0.62^{k}$ | $1.78^{r}$ |
| P × S-119 | $2.70^{b}$ | $1.71^{ab}$ | $3.85^{e}$ |
| P × S-120 | $2.15^{ij}$ | $0.87^{ijk}$ | $1.97^{p}$ |
| P × S-121 | $2.21^{gh}$ | $0.74^{jk}$ | $2.06^{no}$ |
| P × S-122 | $1.81^{o}$ | $0.63^{k}$ | $2.10^{mn}$ |
| P × T-123 | $2.04^{k}$ | $1.11^{defghijk}$ | $2.09^{no}$ |
| P × T-124 | $1.99^{l}$ | $1.22^{bcdefghj}$ | $2.16^{lm}$ |
| P × T-125 | $2.74^{a}$ | $1.76^{a}$ | $4.87^{a}$ |
| P × T-126 | $2.11^{j}$ | $1.16^{defghijk}$ | $2.93^{h}$ |
| P × T-127 | $2.33^{f}$ | $1.04^{efghijk}$ | $2.61^{j}$ |
| P ×T-128 | $2.24^{g}$ | $1.08^{defghijk}$ | $2.40^{k}$ |
| P × T-129 | $1.86^{n}$ | $1.21^{bcdefghj}$ | $2.12^{mn}$ |
| P × T-130 | $2.69^{b}$ | $1.65^{abc}$ | $4.66^{b}$ |
| P×T-131 | $2.13^{j}$ | $1.05^{efghijk}$ | $2.84^{i}$ |
| P × T-132 | $2.01^{kl}$ | $1.07^{defghijk}$ | $2.58^{j}$ |
| LSD *(P ≤0.05)* | | | |
| Control treatment means | | 0.02 | 0.27 | 0.03 |
| Test treatment in same block | | 0.04 | 0.46 | 0.06 |
| Test treatment not in same block | | 0.05 | 0.54 | 0.07 |
| Test and a control treatment | | 0.03 | 0.41 | 0.05 |

[C, Citrumelo; P, Pummelo; TO, Trifoliate Orange; S, Sacaton; and T, Troyer Citrange]

by P × T-130 (16.96%), P × TO-103 (14.35%) and Troyer citrange (13.48%).

Hybrid P × T-125 exhibited the highest content of leaf $K^+$ (1.76 %), which did not show any significant difference with the $K^+$ content of P × TO- 103, P × TO- 112, P × S-117, P × S-119 and P × T-130 hybrids. P × S- 118 had the lowest $K^+$ content (0.62%) without showing any significant difference with pummel in majority of the remaining hybrids. In the study of the per cent change in leaf $K^+$ content over pummelo rootstocks, highest increase was noticed in P × T-125 (81.44%) followed by P × S-119 (76.29%), P × T-130 (70.10%), P × TO-112 (64.95%) and P × S-117 (62.89%).

The highest content of leaf $Ca^{+2}$ was found in the leaves of P × S-117 (4.90%), closely followed by P × T-125 (4.87%) without showing any significant difference, while P × S-113 hybrid had the lowest $Ca^{+2}$ content (1.06%). P × TO-103, P × TO-112, P x T-125 and P × T-130 identified as resistant rootstocks had quite higher leaf $Ca^{+2}$ (3.70-4.87%) than other hybrids. Many hybrids like P × S-113, P × S-114, P × S-115 and P × S-118 proved worse for $Ca^{+2}$ content over Pummelo. The level of the $Ca^{+2}$ in hybrids over pummelo was very much higher in Troyer, P × TO-112, P × TO-117, P × TO-119, P × TO-125 and P × TO-130 (101.57-156.54%) than rest of the hybrids tested in the present study.

**Regression analysis**

In the present study, the relationship with *P. nicotianae* caused lesion length and other physio-chemical parameters were studied using regression approach. Variables namely A, ELWL and N were found to be highly significant having inverse relationship with lesion length. The coefficient of determination with these three variables was 67%, while remaining share (33%) of coefficient of determination was contributed by rest of the seven characters (RWC, MDA, total chlorophyll, $H_2O_2$, glycine betaine, K and Ca) studied.

## Discussion

Fungal diseases in general, and *Phytophthora* species in particular are stated to be the major cause of concern for low productivity of citrus fruits, leading to huge economic losses throughout the world. The diseases caused by *Phytophthora* become more devastating particularly in trees on susceptible rootstocks. Besides known resistant rootstocks (Citrumelo and Troyer citrange), two hybrids *viz*., P × TO-112 and P × T-130 were found without any sign of *P. nicotianae* caused lesions, while four hybrids (P × TO-103, P × S -117, P × S- 119 and P × T-125) were having very small size of lesions (≤1.5 mm), hence found resistant against the oomycete. Afek, Sztejnberg & Solel[11] determined the degree of resistance of citrus plants by comparing the lesions length on the seedlings of species of unknown resistance with lesions length on seedlings of rootstock species of known resistance. The lesion

length on the seedlings of resistant species were 2.8 mm for *Citrus macrophylla* and 3.2 mm for *Poncirus trifoliata*, while on susceptible species like *C. jambhiri* (rough lemons) and *C. sinensis* (oranges), it was up to 11.0 mm. Of the 32 hybrids (*P. trifoliate* × 'Poorman orange') tested by Mohammed, Belmehdi & Zemzami[20], only 14 were found resistant against *Phytophthora* species. Troyer citrange and Sacaton citrumelo (*P. trifoliata* x *C. paradisi*) had also been reported to show a good degree of resistance similar to sour orange, whereas rough lemon and Rangpur lime proved highly susceptible. In all the cross combinations tested in the present study, *P. trifoliata* was the common part in all the male parents. However, out of thirty hybrids, the resistance against *Phytophthora nicotianae* was shown by six hybrids only. A study on *Phytophthora* caused root-rot resistance in the hybrid progeny of *C. sunki* × *P. trifoliata*[21] indicated two QTL maps for the species *P. trifoliata*, which could explain 16-24% of the genetic variation. The genetic basis for some disease resistance in citrus has been characterized by a simple and oligogenic inheritance[22] in *P. trifoliata*. Siviero, Cristofani, Furtado, Garcia, Coelho & Machado[21] established that resistance to gummosis was quantitatively inherited with at least 3 significant QTLs in linkage groups and heritability of 18.7% for resistance. *P. trifoliata* has been reported to have the tolerance against diseases[23], which has also been transferred successfully in US-802 rootstock, the progeny of *C. grandis* and *P. trifoliata*[24]. Boava, Cristofani-Yaly, Stuart & Machado[25] suggested the involvement of several proteins with higher levels, including B1, 3-endo-glucanase, chalcon synthase, lipoxygenases and peroxidases in the resistant interaction between *P. trifoliata* and *P. nicotianae*.

Of the various hybrids inoculated with *P. nicotianae* during the course of present study, P × S-117 hybrid exhibited the highest *A*, followed by P × TO-112 and P × S-119. Singh, Sharma, Dubey, Kamil, Lekshmi, Awasthi & Jha[26] also observed significant difference in the *A* of different citrus species of rootstock importance, inoculated with *P. nicotianae*. The photosynthetic capacity of leaves and carbohydrates allocation from source leaves to reproductive or vegetative sinks are strongly influenced by the rootstocks[27]. Besides, rootstock-specific hydraulic conductance has positive correlation with leaf gas exchange parameters, influencing the water relations, leaf gas exchange, nutrients and hormone status of plants[28]. The decrease in canopy gas exchange (particularly stomatal conductance) has been found to be correlated with the proportion of roots infected by *P. cinnamomi*[29]. Due to primary root infection and destruction, plants suffer severe from drought stress. Thus, minimum water potential values become more negative, and in consequence, plants close their stomata and reduce the rate of photosynthesis. The rates of photosynthesis decrease with the extent of phloem tissue destruction by the pathogen at the base of infected stem[30].

Among the various hybrid progenies of citrus root-stocks tested in the present study against *P. nicotianae*, P × S -119, Troyer citrange, P × TO- 103, P × TO-112, P × S-117, P × T-125, and P × T- 130 showed the lower ELWL than other hybrids. Moreover, the higher RWC was recorded in leaves of P × T-125, P × S-119, P × TO-103 and P × TO-112 than other hybrids. Significant variations in the RWC and ELWL due to citrus rootstocks have also been reported by Sharma, Dubey & Awasthi[31]. Low ELWL values and *E*, and higher RWC values in leaves can be considered a selection criteria to breed plants for drought tolerance[32], which can develop following root dysfunctionality due to feeder root infection by *P. nicotianae*.

The content of total chlorophyll was significantly higher in P × S -117, P × TO-112 and P × T -125 than other hybrids tested. Besides, Troyer, P × S-119, P × TO-103, P × T -126, P × T -130 and P × T -132 also showed relatively higher content of total chlorophyll than other hybrids. Manter, Kelsey & Karchesy[33] also observed the significant difference in chlorophyll aspects among *Phytophthora* infected plants. *Phytophthora* spp. pathosystem is best explained by the development of stem lesions and root necrosis, affecting water transport capacity, which lead to decline in the chlorophyll fluorescence and photosynthetic activity[34].

The exposure of plants to stress can lead to increased generation of reactive oxygen species (ROS) including $H_2O_2$, which react with proteins and lipids, damaging cellular structures and metabolism, especially photosynthesis[35], however, their regulation is rootstock specific[31]. Glycine betaine (GB), an amphoteric quaternary amine, plays an important role as a compatible solute in plants under stress, and plant species vary in their capacity to synthesize GB[36]. The high level of $H_2O_2$ was registered in P × T- 132, P × TO- 104, P × TO-106, P × TO-108, P × TO-110, P ×

S-113, P × S – 121, P × S -122, P × T-123, P × T- 125 and P × T- 126 rootstocks in the present study. The lowest generation of $H_2O_2$ was shown by P × S -117, which had similarity statistically with P × TO-103, P × S-119, P × T- 127 and P × T-130. The level of lipid peroxidation product in the leaves was highest in P × T-132. Hybrids P × S-116, P × T-124 and P × T-125 were among the progenies to have very high level of lipid peroxidation product, while it was lowest in the leaves of P x S-119 without showing any significant difference with P × T-130. The higher content of GB was registered in P × TO-103, P × S-117 and P × T-130, and P × TO-112 than other hybrids. The content of GB was lowest in pummelo and P × TO-115. Singh, Sharma, Dubey, Kamil, Lekshmi, Awasthi & Jha [26] reported lower increase in $H_2O_2$ in Troyer citrange, sour orange and RLC-5, and rated as tolerant rootstock to *P. nicotianae*. *Phytophthora* inoculation tends to increase the several ROS in the leaves of infected plants, however, stress resistant rootstocks have been reported to have less production of $H_2O_2$ in leaves as well as roots then susceptible rootstocks[37]. Resistant rootstocks maintain their structural cell integrity under stress, producing smaller increases in the levels of $H_2O_2$ and MDA[38], which increases largely in susceptible rootstocks, as has also been observed higher in many hybrids during the course of present study.

Nutrient uptake is the function of roots, and disturbance in the activity of feeder roots tend to affect the root malfunctioning and subsequently the leaf nutrient status of the plants[26]. Of the various hybrids tested in the present study, highest concentration of N was recorded in the leaves of P × T-125 followed by P × S -119 and P × T-130. Other resistant hybrids namely P × TO-103, P × TO-112 and P × S-117 also performed better to have high leaf N over other hybrids. Hybrid P × T-125 exhibited the highest content of leaf $K^+$ followed by P × TO- 103, P × TO- 112, P × S-117, P × S-119 and P × T-130 hybrids without any significant difference. The high content of leaf $Ca^{+2}$ was found in the of P × S-117 and P × T-125. The content of other resistant hybrids (P × TO-103, P × TO-112 and P × T-125) also proved to be the good $Ca^{+2}$ accumulators. *Phytophthora* inoculation tends to reduce the length and number of feeder roots, causing generalized dysfunction in water relations, reducing root hydraulic conductivity, and uptake of nutrients[39]. Singh, Sharma, Dubey, Kamil, Lekshmi, Awasthi & Jha [26] found that most of the macro and micro-nutrients decreased in leaf tissues of susceptible rootstock genotypes following the inoculation by oomycete. However, rootstock genotypes, which showed lower ROS generation (sour orange and Troyer citrange) could maintain leaf nutrient concentration even under *Phytophthora* induced stress. Sour orange and Troyer citrange are able to maintain N, P, K, Ca and Cu content even under stress caused by *Phytophthora* inoculation. *Phytophthora* root rot reduces leaf concentration of N, P, S, Zn and B to below critical values for optimum growth[40], as it attacks the unsuberized roots causing severe loss of the primary organs of water and mineral nutrient uptake. The regeneration of roots at higher rates even in the presence of damaging population of *P. nicotianae* has been reported to be a tolerant trait against the fungus by Graham[41], hence the maintenance of higher leaf nutrient in some hybrids in the present studies reflects the root functionality of the infected hybrids having *P. trifoliata* as one of the male parents.

Against *P. nicotianae*, citrumelo Swingle-4475 (*C. paradisi* × *P. trifoliata*) and citrange C-35 (*C. sinensis* × *P. trifoliata*) have been regarded as resistant and moderately tolerant rootstocks, respectively[42] (Use of Swingle citrumelo, Carrizo citrange and X-639 citrandarin as male parent have been reported to impart the resistance against the *Phytophthora* in the hybrid progenies. The rough lemon (commercially used rootstock in India) × Trifoliate orange rootstocks showed minimum lesion and sporangia in the leaf discs followed by Swingle citrumelo and X-639 citrandarin[43]. They suggested that hybrids from the crosses of rough lemon × *P. trifoliata* and rough lemon × Swingle citrumelo can be exploited to improve *Phytophthora* resistance in Citrus. In a transcriptomic analysis to provide new insight into tolerance mechanism among *P. nocotianae and* two germplasms — tolerant sour orange (SO, *Citrus aurantium*) and susceptible Madam Vinous (MV, *C. sinensis*) — in both the biotrophic and necrotrophic phases of host-pathogen interaction, the necrotrophic phase as a decisive turning point, since it included stronger modulation of a number of genes implicated in pathogen perception, signal transduction, HR-like response, transcriptional reprogramming, hormone signaling, and cell wall modifications. In particular, the pathogen perception category reflected the ability of SO to perceive the pathogen even after its switch to

necrotrophy, and thus to cope successfully with the infection, while MV failed. The concomitant changes in genes involved in the remaining functional categories seemed to prevent pathogen spread. This investigation provided further understanding of the successful defense mechanisms of *C. aurantium* against *P. nicotianae*, which might be exploited in post-genomic strategies to develop resistant *Citrus* genotypes[44]. *P. trifoliata* has shown to transmit the resistance to *Phytophthora* caused gummosis governed by at least two genes[45].

Recently[46], the transcriptome analysis of a commonly used citrus rootstock Carrizo citrange in response to *P. parasitica* infection using the RNA-seq technology was performed, wherein 6692 differentially expressed transcripts (DETs) among *P. parasitica*-inoculated and mock-treated roots were identified. Of these, 3960 genes were differentially expressed at 24 h post inoculation and 5521 genes were differentially expressed at 48 h post inoculation. Gene ontology analysis of DETs suggested substantial transcriptional reprogramming of diverse cellular processes particularly the biotic stress response pathways in Carrizo citrange roots. Many R genes, transcription factors, and several other genes putatively involved in plant immunity were differentially modulated in citrus roots in response to *P. parasitica* infection. Quantitative real-time PCR analysis suggested that the resistance in *C. reticulata* var. Guanggan to *P. nicotianae* may be associated with high basal and induced expression of some defense-related genes, particularly the PR3 gene[47].

## Conclusion

*P. nicotianae* is one of the most widespread *Phytophthora* spp., which is known to cause root rot and foot rot/ gummosis in citrus. Given the growing awareness of fungicidal problems, genetic improvement of citrus rootstocks, as attempted in the present study proved to be a good alternative. Overall, the inherited resistance of *P. trifoliata* against *P. nicotianae* was expressed by six hybrids *viz.*, P × TO-110, P × TO-112, P × S-117, P × S-119, P × T-125 and P × T-130 in terms of lesion development. The resistance in these hybrids was also evidenced by relatively lower ELWL and ROS generation and higher retention of *A*, RWC and leaf nutrient status then rest of the hybrids studied.

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## Conflict of interest

The authors declare no conflict of interest.

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