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Morphological and genetic diversity in citrus genotypes to substantiate rootstock breeding for root rot resistance

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ABSTRACT

Investigations were conducted on six citrus rootstocks, *viz*., rough lemon (RL), trifoliate orange (TO), Swingle citrumelo (SC), X 639 (X), Rangpur lime (RA) and Gou Tou (GT), to assess the morphological and genetic variability; and reaction against *Phytophthora*. Smooth trunk surface was recorded in all the genotypes. Three genotypes had erect growth habit, while it was spreading in Gou Tou (GT), Rangpur lime (RA) and rough lemon (RL). The leaves of GT, RA and RL were of unifoliate, while trifoliate orange (TO) had trifoliate leaf and Swingle citrumelo (SC) and X 639 (X) had multifoliate leaf division. No variation was recorded with respect to flowering season in all citrus rootstocks, *i.e.* mid February to last week of March. Fifty-five SSR markers were used for evaluation of genetic diversity amongst the six rootstocks. Twenty markers exhibited high polymorphism and showed wide allelic diversity. Capacity of each SSR to show polymorphic loci, varied from 0.29 (F98) to 0.83 (CCSME46 and CCSMEc4) with an average PIC value of 0.61. The resolving power (Rp) was highest for the primer CCSME43 (8.33) and was lowest for F90 (1.33). Significant differences were observed in the value of MI and were found to be the highest for primer F40 (9.25), while minimum MI was recorded for F98 (0.38). The PIC values of a marker vary with the crop and the set of the genotypes used. The reduction in the number of sporangia and lesion size in trifoliate orange and Swingle citrumelo indicated their tolerance against *Phytophthora nicotianae* var. *parasitica*. The number of sporangia counted after 48 h of incubation showed that all leaf baits of each rootstock were attacked by large number of sporangia. The number of sporangia on each leaf disc of rootstocks decreased after 48 h as the sporangia germinated into mycelium on the edges of leaf discs.

Key words: Citrus, *Phytophthora*, SSR markers, variability.

INTRODUCTION

Citrus occupies an important place in the horticultural wealth of India by covering around 0.95 million ha area with an annual production of 11.66 million tonnes (Anon, 2). The average productivity of citrus in India is 10.44 MT/ha, which is far behind the highest productivity in world. This is mainly attributed to *Phythophthora* root rot, citrus decline, fruit drop, poor nutrition and non-availability of quality planting material. Most of these factors are influenced by rootstock, which is the major contributor to tree performance and longevity as it determines tolerance to various biotic and abiotic stresses. In order to understand the genetic background and the breeding value of the available germplasm, systematic study related to characterization and evaluation of germplasm is of great importance for current and future breeding and genetic improvement of the citrus. A large number of citrus species/ progenitors of commercial citrus fruits are believed to have originated in India.

Molecular markers based on DNA sequence proved to be an ideal means for the identification and estimation of relatedness among genotypes of different origin. Due to the limited number of morphological and biochemical markers, molecular markers have been reported to be powerful tools for elucidating genetic diversity, determining parentage and revealing phylogenetic relationships among various *Citrus* species. Several molecular markers have been used for practical application in citrus (Aleza *et al*., 1).

The rough lemon rootstock has superiority for tree vigour, high yield, resistance against tristeza virus and suitability to high pH soils, thus, it occupied most prominent place among citrus rootstocks in India. However, it is vigorous in nature, which limits its use in high density planting and imparts poor quality to fruits of scion varieties. Hence, there is an urgent need to develop *Phytophthora* tolerant rootstock of citrus suitable for Kinnow mandarin under Punjab conditions. Sour orange, several hybrids of trifoliate orange, *viz*. X 639, citranges and Swingle citrumelo and Rangpur lime are also tolerant to *Phytophthora,* which are widely being used as rootstocks in the citrus growing regions (Castle, 4). These rootstocks can be used to develop

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new rootstocks, which are suitable under Indian conditions. A more precise system for identification of genotypes and for assessing the genetic variation in the existing genetic resources is a fundamental requirement for establishing breeding programmes and the registration of new cultivars in citrus. Hence, the present study was conducted to assess the morphological and genetic variability; and reaction against *Phytophthora* of six citrus rootstocks.

MATERIALS AND METHODS

Six citrus rootstocks, *viz.*, rough lemon (RL), trifoliate orange (TO), Swingle citrumelo (SC), X 639 (X), Rangpur lime (RA) and Gou Tou (GT) planted in the College Orchard, Department of Fruit Science, PAU, Ludhiana, is located at 29.3° N latitude and 76.5° E longitude, 270 m amsl were evaluated during the years 2015-2016. Twenty quantitative and 27 qualitative characters based on Descriptors for Citrus (Anon, 3) were studied for each genotype. The rootstocks were screened against *Phytophthora nicotianae* var. *parasitica* by using leaf bait method as described by Dhakad *et al*. (6) for quick detection of resistance or susceptibility. Pathogen was isolated from root zone soil of infected plant on selective PARPH media (Naqvi, 12) by using soil plating method. Multiplication of pathogen was done on sorghum seeds as described by Kaur *et al*. (9). Spore suspension was made as described by Naqvi (13).

DNA was isolated from young leaf using the modified CTAB with some modifications (Cheng *et al*., 5). The purified DNA, approximately 50 ng, was used for amplification with SSR primers in polymerase chain reaction (PCR). Amplification was carried out in a 10 µl reaction mixture (2.5 mM *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primer and 1.0 U of *Taq* DNA polymerase) in a gradient thermo-cycler (Veriti 96-Well Thermal Cycler, Thermo Fisher Scientific). PCR cycling conditions for amplification of SSR fragments consisted of an initial denaturation at 94°C for 4 min. followed by 35 SSR cycles consisting of 1 min. denaturation at 94°C, 1 min. annealing at 52-56°C (primer-specific) and 1½ min. elongation at 72°C, and finally single extension cycle of 7 min. at 72°C. The amplified product was resolved on 6.0% polyacrylamide gel electrophoresis (PAGE) at 300 V for 1½ h. The gels stained with 100 µl of ethidium bromide (Promega, USA) were visualized under UV gel documentation (Syngene, G: Box, USA).

Fifty five SSR markers were used for evaluation of genetic diversity of the six citrus rootstocks. The SSR amplicons were recorded as described by Kulhari *et al.* (11). Marker index (MI) for SSR markers was calculated according to Powell *et al*. (15). Diversity index/ genetic diversity are the expected heterozygosity and were calculated according to Weir (21). Polymorphism information content (PIC) values and resolving power (Rp) of the primers were calculated as per the formulae of Roldan-Ruiz *et al*. (17) and Prevost and Wilkinson (16), respectively. The morphological data was analyzed as per randomized block design. For *Phytophthora* screening test the observations were recorded for three leaf baits per replication and with three replications per treatment. Data were analysed by using completely randomized block design, or *t*-test statistics using SAS (9.4 version) computer software.

RESULTS AND DISCUSSION

Smooth trunk surface, high density of branches and glabrous shoot tip surface was recorded in all the citrus genotypes (Table 1). Three rootstocks had ellipsoid tree shape, while GT and RA trees have obloid shape but RL has spheroid. Variability was studied for growth habit (erect and spreading) among different genotypes. The three genotypes had erect growth habit, while it was spreading in GT, RA and RL. The variation was recorded in branch angle of all rootstocks. The spine shape did not vary among different genotypes and was straight in all rootstocks except TO, which had curved shape. However, variability was observed in the colour of shoot tip of various rootstocks. The shoot tip colour was green in X and GT and yellow green in TO. Study on tree behaviour of these genotypes was made to describe their vegetative life cycle under Punjab conditions due to their differential ability to tolerate the low temperature and high temperature, which prevailed in the region during winter and summer season, respectively. Genotypes TO, SC and X were deciduous; whereas RA, GT and RL were evergreen. Similarly, differences in growth habit and tree shape were also observed by Singh (19) among different strains and species of citrus. In a similar study Kaur *et al.* (10) recorded glabrous shoot tip surface in rough lemon and trifoliate orange, while it was uneven among different strains of Rangpur lime.

The leaves of GT, RA and RL were of unifoliate type but, TO had trifoliate leaf and SC and X had multifoliate leaf division (Table 2). Leaves were unifoliate in Box orange, Cleopatra mandarin, *pectinifera*, Rangpur lime, rough lemon and trifoliate in case of Carrizo and Troyer citrange. Earlier, Singh (19) also found unifoliate leaves in Schaub rough lemon and Brazilian Rangpur lime; and trifoliate leaves in Flying Dragon. The variation in intensity of green colour of leaf blade was observed in citrus rootstocks varied from light green to dark green colour. Three rootstocks had dark green colour

Table 1. Qualitative tree traits in different citrus rootstocks used in hybridization.

Trait \ Genotype	PT	SC	X	RA	GT	RL
Trunk surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Tree shape	Ellipsoid	Ellipsoid	Ellipsoid	Obloid	Obloid	Spheroid
Tree growth habit	Erect	Erect	Erect	Spreading	Spreading	Spreading
Density of branches	Dense	Dense	Dense	Dense	Dense	Dense
Branch angle	Narrow	Narrow	Narrow	Wide	Wide	Medium
Spine density	High	High	High	Low	Low	High
Spine shape	Curved	Straight	Straight	Straight	Straight	Straight
Shoot tip colour	Yellow green	Purple	Green	Purple	Green	Purple
Shoot tip surface	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Vegetative life-cycle	Deciduous	Deciduous	Deciduous	Evergreen	Evergreen	Evergreen

Table 2. Qualitative leaf characteristics of different citrus rootstocks used in hybridization.

Trait \ Genotype	TO	SC	X	RA	GT	RL
Leaf division	Trifoliate	Trifoliate	Multifoliate	Simple	Simple	Simple
Intensity of leaf blade green colour	Medium green	Dark green	Dark green	Light	Dark green	Medium
Leaf colour variegation	Absent	Absent	Absent	Absent	Absent	Absent
Leaf lamina attachment	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate
Leaf lamina shape	Ovate	Lanceolate	Ovate	Elliptic	Elliptic	Elliptic
Leaf lamina margin	Dentate	Dentate	Dentate	Dentate	Entire	Entire
Leaf apex	Obtuse	Obtuse	Obtuse	Acute	Acute	Acute
Presence/ absence of petiole wings	Present	Present	Present	Absent	Absent	Absent
Petiole wing width (mm)	Narrow	Narrow	Narrow	Absent	Absent	Absent
Petiole wing shape	Linear	Obdetate	Obovate	Absent	Absent	Absent
Junction between petiole and lamina	Articulate	Articulate	Articulate	Fused	Fused	Fused
Leaf emergence	22nd February	10th February	4th February	25th February	12th February	25th February

intensity, while it was light green colour in RA, but RL had the medium green colour intensity. The leaf colour variegation was absent among all the genotypes. Singh (19) reported medium and dark leaf colour intensity in trifoliate orange and red fleshed pummelo, respectively. All the rootstocks had brevipetiolate leaf lamina attachment. The variation was observed in leaf lamina shape of all four genotypes, which was observed lanceolate in SC, ovate in TO and X and elliptic in GT, RA and RL. Singh (19) also reported elliptic leaf lamina shape for various rough lemon strains. In all the citrus rootstocks, the variation was recorded in leaf lamina margin. Rootstocks RL and GT showed entire leaf margin, whereas in TO, SC, X and RA rootstocks dentate leaf margins were observed. Acute leaf apex was observed in RA, RL and GT, whereas, it was obtuse in TO, SC and X. Earlier, Singh (19) also recorded similar findings for leaf lamina margin in Rangpur lime, Swingle citrumelo and trifoliate orange. Presence or absence of petiolar wing was also studied in citrus rootstocks, which was found present in TO and its hybrids, but absent in RA, GT and RL. TO and its hybrids had narrow petiole wings as compared to absent in RA, GT and RL. Some scientists reported that the width of petiole wing is a morphological marker for screening of hybrids in citrus. Absence of petiole wing in RL and RA was also confirmed by Singh (19). The petiolar wing was

obdurate in SC and obovate in X and absent in RA, GT and RL. Variability was recorded for junction between petiole and lamina; it was articulate in TO and its hybrids. Fused junction was studied in RA, GT and RL. The leaf emergence started from 4th February in X and it was observed in RL on 25th February. The leaf emergence in all the rootstocks was recorded between 4th February (X 639) to 25th February (rough lemon). Leaf lamina length, width and their ratio varied significantly among parentage used in hybridization during the year of study (Table 3). Maximum leaf lamina length (72.75 mm) and width (43.83 mm) were recorded in RL, which was significantly higher than all the other rootstocks. The maximum leaf lamina length: width ratio (1.99 mm) was observed in SC, which was significantly higher than that of all other rootstock genotypes. Similar results were recorded by Kaur *et al*. (10) on citrus rootstocks. The leaf lamina length: width ratio was found highest (2.78) in SC. The maximum petiole wing length (15.52 mm) was recorded in GT. The maximum petiole wing width (2.28 mm) was recorded in SC, whereas, petiole was not reported in rough lemon. Leaf thickness was found non-significant for all the parents.

Data regarding qualitative flower characters, *viz.* flowering season, flowering month, length of anthers relative to stigma, flower type, colour of open flower, colour of anthers, petal colour, number of sepals and petals per flower were studied (Table 4). No variation was recorded with respect to flowering season in all citrus rootstocks. Mid of February to last week of March was observed as main flowering season in all the rootstocks. All the genotypes of citrus under study were found to bloom from mid of February to second week of April. Among all rootstocks X was earliest to flower (February 13), while RL was last (March 1). X was observed to be earliest with respect to mean full bloom with date (February 20-28) and the RL was last too attain full bloom (March 22-28). All the citrus rootstocks also differed with respect to end of flowering. The end bloom was studied late in RL (April 20). Similar variation with respect to start of flowering, full blooming period and end of flowering was obtained by Singh (19) in different citrus

Table 3. Morphological traits of different citrus rootstocks used in hybridization.

Trait / Genotype	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf lamina length: width ratio	Petiole wing length (mm)	Petiole wing thickness (mm)	Leaf thickness (mm)
TO	48.33d^{e}	27.67^{c}	1.75^{bc}	15.23^{ab}	0.58^{c}	0.41^{a}
SC	62.60b^{c}	31.47b^{c}	1.99^{a}	15.48^{ab}	0.62^{bc}	0.23^{d}
X	44.90^{e}	28.98^{c}	1.55^{c}	11.73^{c}	0.65^{c}	0.23^{d}
RA	55.23^{cd}	30.76^{bc}	1.80^{ab}	14.2^{bc}	0.55^{bc}	0.36^{b}
GT	65.49^{ab}	35.03^{b}	1.87^{ab}	15.52^{ab}	0.84^{a}	0.26^{d}
RL	72.75^{a}	43.83^{a}	1.66^{bc}	17.18^{a}	0.74^{ab}	0.31^{c}

Table 4. Qualitative inflorescence traits of different citrus rootstocks used in hybridization.

Genotype / Trait	TO	SC	X	RA	GT	RL
First bloom	4th March	20th February	13th February	17 March	17th February	1st March
Date of full bloom	8-12th March	1th-8th March	20th-28th February	22-26 March	25thFeb.-3rd March	22-28 March
Date of end bloom	17th March	20th March	12th March	20 April	17th March	20th April
Length of anthers relative to stigma	Shorter	Shorter	Shorter	Longer	Longer	Shorter
Flower type	Hermaphrodite and male	Hermaphrodite and male	Hermaphrodite and male	Staminate	Staminate	Hermaphrodite and male
Colour of open flower	White	White	White	Violet	Violet	Violet cream
Colour of anthers	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow
Petal olour	White	White	White	White	White	White
No. of sepals per flower	5/6	5	5	5	5	5
No. of petals per flower	5	5	5	5	5	5

rootstocks. Little variation was observed for flower type as both perfect and staminate flowers were observed on a single tree in all the citrus rootstocks. The variation was also observed in the length of anther in all the rootstocks. Length of anthers was longer relative to stigma in GT, when compared to all other rootstocks showed shorter length of anthers relative to stigma. Similarly, Singh (19) reported only hermaphrodite flowers were observed in rough lemon strains. The flower colour was white in TO and its hybrids but, violet in RA, RL and GT. Little variation was observed in anther colour. The colour of petal was white in all the citrus genotypes. The variability with respect to petal colour, flower type and anther colour in different genotypes might be due to their inherent varietal characteristics. The results of this investigation were similar with those of Singh (19) and Kaur *et al.* (10) regarding petal colour and anther colour and flower types, while white coloured flowers were noted in trifoliate, Rangpur lime and rough lemon. No variation was recorded in number of sepals and petals among different genotypes. The maximum mean flower diameter (45.12 mm) was recorded in TO (Table 5). Maximum flower length (26.40 mm) was recorded in TO, which was significantly higher than all other rootstocks. Kaur *et al*. (10) investigated the maximum flower length in rough lemon. Maximum pedicel diameter (2.89 mm), pedicel length (11.23 mm) was recorded in TO and calyx diameter (9.15 mm) was recorded in RL. Maximum petal length (25.45 mm) was observed in GT, which was significantly higher than all other rootstocks. The maximum petal width of 16.60 mm was observed in RL, which was significantly higher than all other rootstocks. The present findings are in accordance with the findings of Singh (19), who reported the higher petal length (21.2 mm) in TO as compared to RL and RA. Width is also observed higher in TO and minimum was recorded in RL, whereas, RA showed results in between. The maximum number of stamens (28.30) was recorded in RL, which were significantly higher than all the other rootstock genotypes under study. Similarly, Singh (19) and Kaur *et al*. (10) also recorded variability for number of stamens among different rootstocks. Style length was recorded maximum (7.94 mm) for GT. While minimum length (6.31 mm) was found in SC, which was significantly less than all other rootstock. This variability may be attributed to the differences in genetic make-up of different genotypes. However, Singh (19) observed minimum filament and style length in trifoliate orange and maximum in rough lemon and Rangpur lime.

Analysis of variance revealed significant variation in lesion size and number of sporangia among the genotypes. Tolerance was judged by the lesser lesion size and minimum number of sporangia.

Table 5. Quantitative floral traits of different citrus rootstocks used in hybridization.

Trait / Genotype	Flower dia. (mm)	Flower length (mm)	Pedicel dia. (mm)	Pedicel length (mm)	Calyx dia. (mm)	Petal length (mm)	Petal width (mm)	No. of stamens	Filament length (mm)
TO	45.12^{a}	26.4^{a}	2.89^{a}	11.23^{a}	8.34^{b}	25.45^{a}	14.27^{b}	27.56^{a}	16.34^{a}
SC	41.11^{ab}	19.18^{cd}	2.17^{b}	6.47^{d}	6.02^{c}	20.58^{cd}	12.24^{cd}	23.71b^{c}	14.56^{b}
X	28.20^{d}	18.31^{d}	1.77^{d}	5.9^{d}	6.18^{c}	18.64^{d}	10.45^{d}	21.67^{d}	11.46^{c}
RA	43.67^{a}	23.12^{abc}	1.99^{c}	9.67^{ab}	7.56^{b}	21.34^{cd}	13.12^{bc}	22.45^{cd}	13.78^{b}
GT	35.31^{bc}	21.89^{bcd}	2.28^{b}	7.5^{cd}	8.15^{b}	24.35^{ab}	13.38^{bc}	24.08^{b}	13.7^{b}
RL	32.66^{d}	23.93^{ab}	2.30^{b}	9.15^{bc}	9.60^{a}	22.23^{bc}	16.60^{a}	28.30^{a}	15.97^{a}

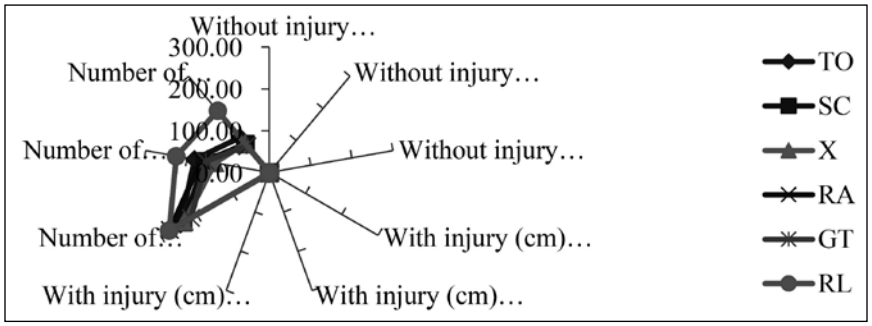

Fig. 1. Screening of citrus rootstocks by leaf bait technique against *Phytophthora* root rot.

After 48 h of incubation, smallest lesion size (0.41 cm) was observed in TO (Fig. 1), while, maximum lesion size was observed for RL (1.00 cm). After 96 and 120 h of incubation, TO show the minimum lesion size (1.12 and 1.67 cm, respectively) and RL showed the maximum lesion size (above 2.0 cm). Rough lemon leaf discs developed the largest lesions on the wounded leaf (2.42 cm) after 120 h of *P. parasitica* incubation. In the leaf discs with injury after 48 h of incubation, the minimum lesion size was recorded with TO (0.61 cm) and largest on RL (1.25 cm). The lesion size exceeded up to 1.34 cm after 72 h incubation on RL. The rootstock, TO showed consistently smallest lesions after 96 (1.22 cm) and 120 h (1.77 cm) of incubation. The number of sporangia counted after 48 h of incubation showed that all leaf baits of each rootstock were attacked by large number of sporangia. The minimum number of sporangia after 48 h of inoculation were observed on TO (216.33) and maximum on RL (276.67). The number of sporangia on each leaf disc of rootstocks decreased after 48 h as the sporangia germinated into mycelium on the edges of leaf discs. Sharma (18) used leaf baiting for screening against *Phytophthora* in potato. While, Harada and Kondo (8) utilized detached leaf method for evaluation of resistance for *Phytophthora* in beans who observed water soaked large spreading lesions as susceptible reaction. Dhakad *et al*. (6) also used leaf bait screening against *Phytophthora* in Kinnow mandarin. The reduction in the number of sporangia and lesion size in TO and SC indicates their tolerance against *Phytophthora nicotianae* var. *parasitica.*

A total of 655 alleles were amplified by 55 markers and the number of alleles ranged from 2 (CCSMEc8) to 11 (F40) with an average of 5.69 alleles per locus

Table 6. Primer sequence and other details of information generated by 24 SSR markers used in characterization of six citrus rootstocks.

Primer code	G + C content (%)	Anneal. temp. (°C)	PIC	Genetic diversity	Resolving power (Rp)	Marker index (MI)
CCSMEc3-F	50.00	58.00	0.69	0.88	5.00	7.03
CCSMEc4-F	60.00	60.00	0.83	0.97	2.00	5.83
CCSMEc10-F	50.00	58.00	0.63	0.83	3.67	4.14
CCSMEc12-F	50.00	58.00	0.63	0.83	3.00	3.31
CCSME5-F	50.00	58.00	0.75	0.92	2.67	5.53
CCSME23-F	50.00	58.00	0.67	0.88	2.67	3.50
CCSME27-F	45.00	56.00	0.67	0.84	6.00	7.56
CCSME33-F	55.00	58.00	0.60	0.79	6.33	6.31
CCSME42-F	45.00	58.00	0.63	0.81	3.67	4.03
CCSME46-F	55.00	60.00	0.83	0.97	2.00	5.83
CCSME49-F	47.62	58.00	0.63	0.84	3.00	3.36
TAA15-F	52.38	58.00	0.72	0.91	3.33	5.44
Ci03C08-F	52.94	53.00	0.71	0.90	4.00	6.33
F02-F	45.83	60.00	0.67	0.87	2.00	2.61
F13-F	41.67	60.00	0.63	0.78	3.00	3.14
F29-F	41.67	60.00	0.73	0.89	2.67	4.44
F40-F	43.48	60.00	0.65	0.84	7.67	9.25
F43-F	37.50	58.00	0.67	0.83	2.00	2.50
F50-F	45.83	60.00	0.76	0.94	3.33	6.56
F53-F	50.00	60.00	0.67	0.87	2.00	2.61
F77-F	45.83	58.00	0.71	0.87	2.33	3.47
F90-F	55.00	60.00	0.67	0.89	1.33	1.78
TAA1-F	50.00	60.00	0.62	0.83	7.67	8.25
CAC33-F	55.00	60.00	0.72	0.91	3.33	5.44

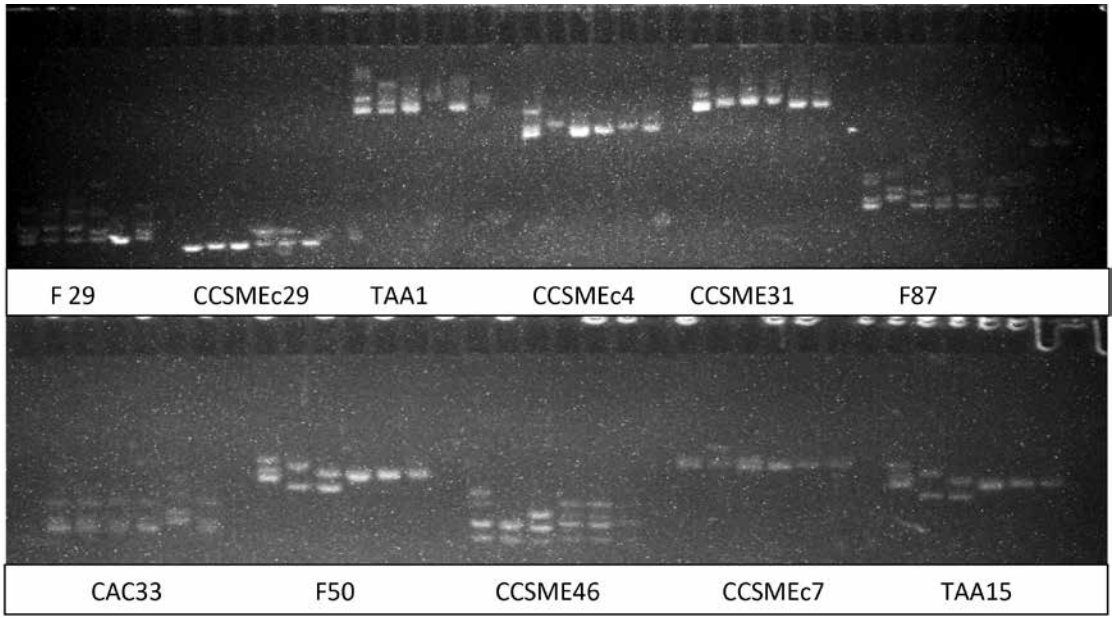

Fig. 2. PCR amplification of DNAs from six citrus genotypes. RL = rough lemon, SC = Swingle citrumelo, X = X639, TO = trifoliate orange; GT = Gou Tou, and RA= Rangpur lime.

(Table 6). All primers showed polymorphism among all genotypes, while six primers (F29, CCSMEc29, TAA1, CCSMEc4, CCSME31 and F87) showed polymorphism between all the genotypes RL, TO, RA, X, GT and SC (Fig. 2). The PIC value provides an estimate (Table 1) of the discrimination power of a marker by taking into account not only number of alleles at locus but also the relative frequencies of those alleles in the rootstocks and analyzed to characterized the capacity of each SSR to show polymorphic loci, varied from 0.29 (F98) to 0.83 (CCSME46 and CCSMEc4) with an average PIC value of 0.61. The Rp (resolving power) was the highest for the primer CCSME43 (8.33) and was lowest for F90 (1.33). Significant differences were observed in the value of MI (Marker Index) and were found to be the highest for primer F40 (9.25), while minimum MI was recorded for F98 (0.38). The PIC values of a marker vary with the crop and the set of the genotypes used. Froelicher *et al*. (7) reported that PIC value from 0.05 to 0.70 over the four loci in 77 genotypes. Whereas, in Rangpur lime it ranged from 0.32 (CCME8, F16, CCSMEc3, CCSME49) to 0.828 (CCSME29), with an average of 0.51 across all strains, Similarly, PIC values of 0.68, 0.63, 0.61, 0.64 and 0.41 were recorded in lemon, mandarin, grapefruit, natural hybrids and sweet orange, respectively (Novelli *et al*., 14). The data on number of alleles amplified and PIC values showed that higher the number of alleles amplified, the higher is the PIC value but, the trend was not followed consistently. The markers, which have two alleles, had a PIC value ranging from 0.37 to 0.5. However, the markers, which amplified four alleles had PIC value 0.40 to 0.72. The markers with 5 alleles had PIC value of 0.78 and 6 alleles of 0.78 to 0.81. Hence, the data depicted that the highest PIC value did not confirm with the higher number of alleles. The PIC value across all loci ranged between 0.09 to 0.71 with an average of 0.37 (Soriano *et al*., 20). The PIC values vary with markers, species and set of the genotypes used. The lower PIC value may be due to closely related genotype and higher PIC value may be due to the diverse genotypes. Marker loci with an average number of alleles running at equal frequencies will have the highest PIC value. The higher PIC values in the present studies could be due to the use of polyacrylamide gels in comparison to agrose gels.

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