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RESEARCH PAPER

Assessment of diversity among trifoliate citrus accessions based on floral traits

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Abstract : The study was planned to assess genetic diversity among citrus trifoliate rootstocks. Sixteen *Citrus trifoliate* (*Poncirus trifoliate* and its hybrids) rootstocks were evaluated for different characters as per IPGRI citrus descriptors. Genotypes were significantly different for start of flowering, full bloom and end of flowering. Swingle was earliest to flower 21st February followed by X-639 (2nd March) and flying dragon (15th March) was the last to start flowering. Significant variation for all the quantitative flower traits was recorded. The highest mean flower diameter of 51.38 mm was recorded in U-852 and the lowest in flying dragon (20.14 mm). Flower length was highest in C-32 (25.59 mm) followed by U-852 (23.39 mm) and the lowest mean flower length was recorded in chethali trifoliate (8.60 mm). C-32 exhibited highest pedicel diameter of 2.14 mm and the lowest was observed in swingle citrumelo (1.37 mm). Pedicel length was in the range of 0.00-9.81 mm. Longer mean petal length of 27.04 mm was observed in C-32. The highest stamen number of 29.50 was observed in C-32 which was statistically at par with carrizo (27.80) and the lowest number of 13.41 was observed in rubidoux. The dendrogram based on un-weighted pair group method with arithmetic mean (UPGMA) divided all the sixteen genotypes into six clusters (Group I, II, III, IV, V and VI).

Key Words : UPGMA,Citrus, Trifoliate, Rootstock, Characterization

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INTRODUCTION

In India, citrus occupies the second position with an area of 1.1 million hectares and third in term of production (12.7 million tonnes). Among citrus fruits, mandarin predominates in area and production and commercially produced in Maharashtra, Madhya Pradesh, Punjab and Northeast states followed by sweet oranges in Maharashtra, Andhra Pradesh, Tamil Nadu and Northwestern region (Anonymous, 2018). In Punjab state, citrus ranks first in the area (51649 hectares) and production (1.2 million tonnes). Among the citrus fruits, Kinnow mandarin predominates by sharing 92.83 per cent of the area and 97.36 per cent citrus production of the state almost all the kinnow mandarin.

Rootstock plays a crucial role in determining the phenology of scion in a specific area. Various rootstocks are being used for different scion cultivars in the various citrus-producing countries. Based on the performance of the scion, rootstocks are selected for a particular region

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(Syvertsen *et al.*, 1985). The performance of a rootstock varies from place to place and by abiotic and biotic stresses (Sites *et al.*, 1985). In the present scenario, molecular characterization is a vital task, but the morphological characterization is the basis of the breeding programme.

Furthermore, most of the essential horticultural traits cannot be assessed by molecular markers so that the phenotypic characterization could be an essential component (Kinley and Chinawat, 2011). Many researchers have used morphological characters to describe and characterize different mandarin varieties and their hybrids (Domingues *et al.*, 1999). Morphological characterization is the first option to recognize diverse traits. In the absence of morphological variations in a population, no formal taxonomic status would be assigned to a particular plant species (Lohwasser *et al.*, 2010). Citrus is the major crop of the world and as well as in India, but unfortunately, a few rootstocks are being used for propagation of scion genotypes. There is an immediate need to identify or develop the substitute for standard rootstocks. In this regard, characterization and evaluation of available germplasm are essential to find suitable cultivar or parents for developing new hybrids. The synchronization and duration of flowering among genotypes, flower earliness, length of style and filament, pollen viability, stigma receptivity and pollen dehiscence are the significant aspects for planning the breeding strategy under crop improvement programme (Uppal *et al.*, 2016). The present paper is focused on the morphological description of sixteen rootstocks for their floral characters.

MATERIAL AND METHODS

The present investigations were carried out in the College Orchard and Pathological Molecular Laboratory of Department of Fruit Science, Punjab Agricultural University, Ludhiana. Sixteen genotypes collected from the different parts of the country and the world were used in the study *viz.*, Benton, C-32, C-35, chethali carrizo, carrizo, chethali trifoliate, flying dragon, gonicoppal, kuharski, rich16-6, rubidoux, swingle, trifoliate orange, troyer, U-852 and X-639. The experiment was laid out in Randomized Block Design (Gomez and Gomez, 2010) with three plants per replications were considered. The morphological observations on start date of flowering, full bloom period end date of flowering, flower diameter, flower length, pedicel diameter, pedicel length, calyx diameter, petal length, petal width, stamen no., pollen viability, filament length and style length were recorded as per International Plant Genetic Resources Institute, Italy (IPGRI) descriptors of citrus (IPGRI, 1999). All the observations were recorded during the year 2017 and 2018. A critical difference at 5 per cent level of probability was computed to compare the statistical significance of different treatments. Analysis of variance was conducted for various quantitative traits using SAS (Statistical analysis system) 9.3 version software. The genetic diversity among the mandarin genotypes was computed on the basis floral quantitative characters by using computer software programme DARwin (Perrier and Jacquemoud, 2006). The data were subjected to the un-weighted pair group method with arithmetic mean (UPGMA) analysis to generate dendrogram and cluster analysis.

RESULTS AND DISCUSSION

There was significant variation in the start date of flowering among the investigated rootstock genotypes (Fig. 1). Start date of flowering varied from 21st February to 15th March. Swingle was the earliest to flower (21st February) followed by X-639 (2nd March). Flying dragon was last to bloom (15th March). Full bloom was the earliest in swingle (2 to 7 March), followed by X-639 (12-21 March). Whereas, kuharski (23 to 31 March) was the last to full bloom. Flowering was earlier to end in swingle (16th March) followed by C-35 (28th March) and C-32 (29th March). Flying dragon and rich 16-6 were the last to end flower on 1st April. Similarly Kaur (2015) reported the earliest flowering in swingle citrumelo followed by trifoliate orange and rough lemon was last

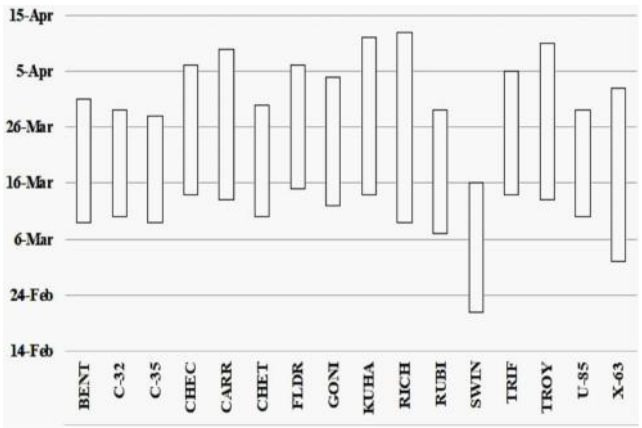

Fig. 1 : Variation for flowering among different genotypes

to full bloom.

The respective range and mean of flower diameter and flower length was observed 23.34-51.38 mm and 8.60-25.59 mm and 37.07 and 17.34, respectively. The highest mean flower diameter of 51.38 mm was recorded in U-852 followed by C-32 (45.12 mm) and rubidoux (43.77 mm). Flying dragon was noted to have the lowest mean flower diameter of 20.14 mm. The highest flower length of 25.59 mm was recorded in C-32 followed by U-852 (23.39 mm) and the lowest mean flower length was recorded in chethali trifoliate (8.60 mm). In a study on mandarin, Uppal (2016) observed variation in flower diameter from 17.63 (N-43) to 27.10 mm (N-38) and flower length was in the range of 10.47 mm (N-43) to 13.75 mm (Kinnow). Similarly Kaur (2015) observed variation in flower diameter in the range of 27.60 mm (Trifoliate orange) to 51.63 mm (Rangpur lime) and highest flower length in the range from 11.00 mm (Rangpur lime) to 24.07 mm (Rough lemon).

Pedicel diameter varied from 1.00 mm to 2.14 mm with an average of 1.47 mm among the rootstock genotypes (Table 2). The highest pedicel diameter of 2.14 mm was recorded in C-32 and lowest pedicel diameter of 1.37mm was observed in swingle citrumelo. Pedicel length was in the range of 0.00-9.81 mm with an average of 4.22 mm. The longest mean pedicel length of 9.81 mm was observed in C-32 followed by benton (8.27 mm). Flowers of chethali trifoliate, flying dragon, gonicoppal, rich 16-6 and rubidoux were sessile. In a similar study on morphological diversity among 39 mandarin accessions, Dorji and Yapwattanaphun (2011) reported that pedicle length varied from 4.27 mm in trongsa mandarin to 4.82 mm in shumar mandarin.

The data on variation for calyx diameter among the investigated rootstock genotypes are presented in Table 2. Calyx diameter ranged from 3.79-7.09 mm with an average of 5.06 mm. Flying dragon was having the highest mean calyx diameter of 7.09 mm, which was at par with U-852 (6.94 mm). The lowest mean calyx diameter of 3.79 mm was observed in C-35 which was at par with benton (3.81 mm).

The respective range of petal length and petal width was 14.93-27.04 mm and 6.27-18.38, respectively (Table 3). Significantly longer mean petal length of 27.04 mm was noted in C-32, which was statistically at par with Swingle (26.60 mm) and U-852 (26.42 mm). The lowest mean petal length of 14.93 mm was noted in chethali trifoliate. Swingle registered the highest petal width of 18.38 mm followed by U-852 (13.61 mm) and C-32 (12.51 mm). The lowest petal width of 6.27 mm in troyer and X-639. Kinley and Chinawat (2011) also reported similar variation in petal length, according to their observations, petal length of 11.30 mm was observed in samtse and zhemgang mandarin which was higher than that of tsirang and dagana mandarin (10.90 mm each). Similarly, Dorji and Yapwattanaphun (2011) reported the

Table 1: List of investigated citrus genotypes

Genotype	Latin name/Parentage/Source
Benton	*Poncirus trifoliata* X *Citrus sinensis* (unknown cultivars)
C-32	*Citrus sinensis cv.* Ruby *X Citroncirus webberii cv.* Webber-Fawcett
C-35	*C. sinensis* cv. Ruby *X Citroncirus webberii* cv. Webber-Fawcett
Carrizo citrange	*C. sinensis* cv. Washington X *Poncirus trifoliata*
Chethali Carrizo	Selection made at HRS Chethali (India)
Chethali Trifoliate	Selection made at HRS Chethali (India)
Flying Dragon	*Poncirus trifoliata* L.
Gonicoppal Trifoliate	Selection made at CES Gonicoppal (India)
Kuharski	*Citrus sinensis*L. Osbeck X *Poncirus trifoliata*L.
Rich 16-6	*Poncirus trifoliata* L.
Rubidoux	*Poncirus trifoliata* L.
Swingle citrumelo	*Citrus paradisi* X *P. trifoliata*
Trifoliate orange	*P. trifoliata* L.
Troyer citrange	*C. sinensis* L. cv. Washington X *P. trifoliata* L.
U-852	*C. reticulata* L. X *P. trifoliata*L.
X-639	*C. reshni* L. X *Poncirus trifoliata* L.

variation in petal length and width in mandarin accessions.

Stamen number differed considerably among rootstock genotypes under observation (Table 3). It ranged from 13.40 to 29.5. The highest stamen number of 29.50 was noted in C-32 which was statistically at par with Carrizo (27.80). Least stamen number of 13.41 was noted in rubidoux. The highest stamen number of 20.07 in N-43 and lowest stamen number of 13.43 was recorded in khasi, mudkhed seedless and Nagpur seedless (Uppal, 2016). In a similar study, Kinley and Chinawat (2011) reported the maximum stamen number of 14.90 in tsirang and dagana mandarin and lowest number of 14.60 in samtse mandarin. Similarly, Dorji and Yapwattanaphun (2011) also observed variation in stamen number from 13.90 (Yadi mandarin) to 16.00 (Sjkhar and mandarin).

Mean pollen viability ranged from 60.36 to 93.58 per cent with an average of 82.07 per cent in all the rootstock genotypes.The highest mean pollen viability of 93.58 per cent was noted in rich 16-6, which was at par with rubidoux (93.02%) whereas, the lowest mean pollen viability of 60.32 per cent was in recorded in trifoliate orange. Filament length was in the range of 6.38-17.46 mm with an average of 12.05 mm. The highest mean filament length of 17.46 mm was noted in U-852 followed by kuharski (15.83 mm), whereas, the lowest mean filament length of 6.38 mm was observed in chethali trifoliate, trifoliate orange which was at par with rich 16-6 (7.34 mm). Wide variation in style length was noted in the rootstock genotypes. Style length varied from 1.27 to 6.95 mm with an average length of 3.38 mm. The highest mean style length of 8.39 mm length was recorded in C-32 whereas, the lowest mean style length of 1.27 mm was recorded in kuharski.

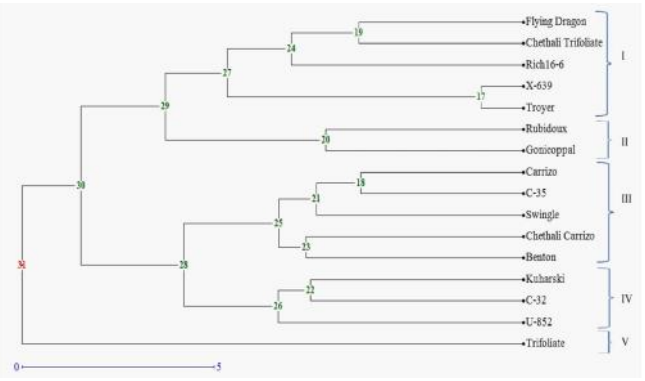

Fig. 2 : Dendrogram illustrating the phylogenetic relationship among the sixteen rootstock genotypes based on morphological traits

Table 2: Quantitative flower characters

Genotype	Flower diameter (mm)	Flower length (mm)	Pedicel diameter (mm)	Pedicel length (mm)	Calyx diameter (mm)
Benton	35.85^{g}	20.87^{c}	1.68^{cd}	8.27^{b}	3.81^{g}
C-32	45.12^{b}	25.59^{a}	2.14^{a}	9.81^{a}	4.25^{fg}
C-35	41.51^{cde}	18.33^{d}	1.53^{def}	4.67^{efg}	3.79^{g}
Carrizo citrange	39.61^{def}	20.43^{c}	1.52^{def}	5.28^{c}	6.07^{b}
Chethali Carrizo	39.48^{ef}	18.92^{d}	1.43^{ef}	4.85^{def}	6.00^{b}
Chethali Trifoliate	28.68^{hi}	8.60^{g}	0.00^{g}	0.00^{i}	4.65^{ef}
Flying Dragon	23.34^{j}	12.47^{e}	0.00^{g}	0.00^{i}	7.09^{a}
Gonicoppal Trifoliate	39.64^{def}	10.03^{fg}	0.00^{g}	0.00^{i}	5.32^{c}
Kuharski	40.59^{def}	20.93^{c}	1.74^{bc}	4.61^{fg}	4.60^{ef}
Rich 16-6	30.91^{h}	11.25^{ef}	0.00^{g}	0.00^{i}	4.24^{fg}
Rubidoux	43.77^{bc}	10.59^{f}	1.42^{ef}	2.07^{h}	5.22^{cd}
Swingle citrumelo	38.24^{fg}	22.73^{b}	1.37^{f}	8.02^{b}	5.91^{b}
Trifoliate orange	42.69^{bcd}	18.05^{d}	1.57^{cde}	4.35^{g}	4.77^{de}
Troyer citrange	26.60^{i}	17.63^{d}	1.44^{ef}	5.06^{cde}	4.18^{fg}
U-852	51.38^{a}	23.39^{b}	1.89^{b}	5.34^{c}	6.94^{a}
X-639	25.76^{ij}	17.63^{d}	1.36^{f}	5.27^{cd}	4.18^{fg}
Range	23.34-51.38	8.60-25.59	0.00-2.14	0.00-9.81	3.79-7.09
Mean	37.07	17.34	1.19	4.22	5.06
S.E.±	1.97	1.31	0.18	0.77	0.27
C.V. (%)	25%	25%	25%	25%	25%

Table 3: Quantitative flower characters

Genotype	Petal length (mm)	Petal width (mm)	Stamen no. (mm)	Pollen viability (mm)	Filament length (mm)	Style length (mm)
Benton	19.91^{ef}	8.04^{fg}	19.40^{f}	80.53^{def}	10.29^{h}	4.64^{b}
C-32	27.04^{a}	12.51^{c}	29.50^{a}	91.76^{ab}	14.66^{cd}	6.95^{a}
C-35	21.81^{cd}	10.81^{d}	23.68^{c}	72.53^{g}	13.59^{de}	1.68^{fg}
Carr-Chetalli	17.75^{gh}	6.81^{hi}	27.80^{ab}	80.74^{def}	15.10^{bc}	4.15^{c}
Carrizo	24.25^{b}	12.02^{c}	20.06^{ef}	78.58^{ef}	14.32^{cd}	3.95^{c}
Chet. Trifol	14.93^{i}	7.79^{fg}	21.36^{def}	84.59^{cd}	6.38^{j}	1.87^{ef}
Flyi. Dragon	18.57^{fg}	8.44^{f}	21.52^{de}	84.42^{cd}	9.38^{hi}	3.17^{d}
Gonicoppal	20.50^{de}	8.44^{f}	19.44^{f}	86.63^{bc}	9.57^{hi}	2.23^{e}
Kuharski	24.32^{b}	12.04^{c}	27.27^{b}	88.96^{abc}	15.83^{b}	1.27^{g}
Rich16-6	16.63^{hi}	7.29^{gh}	17.11^{g}	93.58^{a}	7.34^{j}	1.74^{f}
Rubidoux	22.47^{c}	8.52^{f}	13.41^{h}	93.02^{a}	8.75^{i}	3.11^{d}
Swingle	26.60^{a}	18.38^{a}	21.24^{def}	77.23^{fg}	14.15^{cd}	4.96^{b}
Trifoliate orange	21.30^{cde}	9.67^{e}	24.24^{c}	60.32^{h}	11.41^{g}	3.36^{d}
Troyer	16.72^{h}	6.27^{i}	23.52^{c}	78.05^{fg}	12.88^{ef}	4.84^{b}
U-852	26.42^{a}	13.61^{b}	26.73^{b}	84.12^{cde}	17.46^{a}	3.01^{d}
X-639	17.39^{gh}	6.27^{i}	22.77^{cd}	78.05^{fg}	12.48^{fg}	3.14^{d}
Range	14.93-27.04	6.27-18.38	13.41-29.50	60.32-93.58	6.38-17.46	1.27-6.95
Mean	21.04	9.81	22.44	82.07	12.10	3.38
S.E.±	0.97	0.81	1.05	2.11	0.80	0.38
C.V.	25%	25%	25%	25%	25%	25%

The dendrogram based on un-weighted pair group method with arithmetic mean (UPGMA) divided all the sixteen genotypes into six clusters (Group I, II, III, IV, V and VI) (Fig. 2). Interestingly nine hybrids of *P. trifoliata* were in the group II, IV and V. Among the selections, chethali trifoliate was in the cluster I with flying dragon and rich 16-6 and chethali carrizo was in group IV and gonicoppal was in group III with rubidoux. Among the four accessions of *P. trifoliata*, flying dragon and rich 16-6 were in cluster I, rubidoux and rubidoux in group III and trifoliate orange in cluster VI. The results revealed the existence of diverse accessions in trifoliate citrus germplasm despite accessions exhibiting similar morphological qualitative characters. The similarities were observed in floral qualitative characters among different genotypes. However, quantitative floral characters varied significantly among investigated trifoliate genotypes.

The genotypic variation in different rootstock for flowers indicated that the investigated rootstocks were comprised of phenotypically different individuals. These differences could be assigned to mutations and cross-pollination (due to male sterility and self-incompatibility). It has been reported that natural hybridization and the occurrence of spontaneous mutations are very common in citrus species. Furthermore, cross-pollination and the occurrence of zygotic twins have resulted in considerable variation in plant types (Das *et al.*, 2007). The geographical distribution of the same genotypes may also enhance variation to germplasm. The genetic constitution in combination with biotic and abiotic stresses also cause variation in the germplasm. The conclusion scould be supported by the hypothesis of Paudyal and Haq (2008) who stated that ecological factors contributed to a variation of 40 per cent in pummelo accessions from un-controlled field survey. Similarly, Dorji and Yapwattanaphun (2011a) reported that morphological variability could be assigned to mutations, cross-pollination and environment interactions. The bud sport, introduction of materials in a location different from its original habitat and lack of reproductive barriers (Self or cross-incompatibility, male sterility, synchronization of flowering) both within species and genus might have recurrently supplemented to its variation and heterogeneity (Darji and Yapwattanaphun, 2011b).

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