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Genetic divergence in trifoliate citrus rootsctocks under sub-tropical conditions of Punjab

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Abstract
The present investigation was aimed to assess genetic diversity in citrus trifoliate rootstocks. The research work was carried out on five citrus trifoliate (*Poncirus trifoliate* and its hybrids) rootstocks. Their evaluation was done for characters as per IPGRI citrus descriptor. Most of the quantitative trees, leaves, flowers and fruit characters were found statistically significant. Leaf lamina length was recorded maximum in strain Flying Dragon (54.4 mm) which was at par with Rich 16-6 and X-639 and minimum in Gonicoppal Trifoliate (32.4 mm). The leaf lamina width was maximum for Trifoliate (22.1 mm) and was found minimum for Gonicoppal Trifoliate (15.7 mm). The average leaf chlorophyll content ranged from 50.7 in Flying Dragon strain of trifoliate to 65.5 in Trifoliate. The strains were differed with respect to full bloom.The total soluble solids was recorded maximum in strain Flying Dragon (12.1 ^{o}Brix), which was at par with Rich 16-6, and the lowest was recorded in strain Trifoliate (8.8 ^{o}Brix). Highest acidity was recorded to be 3.5 per cent in X-639, Rich 16-6 and Trifoliate while the lowest was recorded for Flying Dragon (1.3 %). The mean number of seeds per fruit was highest in Rich 16-6 (26.0) and the lowest was found in X-639 (7.0), which was at par with Flying Dragon. The strains also differed significantly for seed length and breadth.The study concluded a significant genetic variance in strains under investigation.

Keywords: Divergence, trifoliate, rootstock and sub-tropical

1. Introduction
Citrus is an important fruit crop of tropical and sub-tropical region of the world. It is reported to be grown in every tropical country and in those regions of sub-tropical countries, where winter temperature does not fall below 0 ˚C [15]. As per the citrus classification, important centres of origin of citrus are South-east Asia, Australia, central Africa and islands between Asia and Australia [23, 25]. In Indian context, north-east India adjoining north Mayanmar are claimed to be the primitive centres with regard to origin of citrus species. The presence of large number of species of citrus in northeastern India described this area as treasure house of citrus germplasm [16]. However, detailed description of this major germplasm is lacking in India. Morphological characterization is an important task for the breeder which is highly desirable for planning crop improvement strategies.

It is a well known fact that agronomic characters are much influenced by environmental and genotypic interaction relationships. The knowledge of genetic relationships and their variation among accessions have important consideration for making the diverse crosses for creating diversity among the germplasm. Furthermore, it has been reported that important agronomic traits of citrus are controlled by multiple genes which can only be assessed through morphological characterization [13]. It has been reported that morphological diversity is independent from its genetic diversity [5, 11]. Secondly, the important characters are of low heritability and they are controlled by multiple genes [5, 13].

Rootsctock plays a major role in defining the phenology of the tree grown in a specific area. A large number of different citrus rootstocks are used in the various citrus-producing areas of the world. On the baisis of the performance of the scion productivity and quality paramerters, rootstocks are selected as best adapted to a specific region [24]. The performace of each rootstock may vary in accordance with abiotic and biotic stresses [22].

Now days, molecular characterization is an important task for the breeder but phenotypic characterization is the foundation of the breeding programme. Moreover, most of the agronomic characters cannot be evaluated through molecular markers, the morphological characterization could be an essential component [10]. Citrus contribute a significant prosperity to the farmers but unfortunate part is that, a few rootstocks have been recommended for the propagation of scin cultivars. There is an urgent need to search atlternatives to rough lemon rootstock and in this regard evaluation/characterization of the available germplasm is must to select the better types as such or suitable parents for breeding programmes. Furthermore, the testing of advanced selection and of new cultivars is an important aspect of fruit breeding [9]. Hence this paper is focused on the physical and morphological description of five rootstocks for their tree, fruit and seed characteristics. Therefor the present study is having due importance both for scientists and students.

Materials and Methods

Plant material: The research work was carried out on five citrus rootstocks viz., Flying Dragon, Gonicoppal Trifoliate, Rich 16-6, Trifoliate and X-639.

Methodology

The investigation was carried out at Punjab Agricultural University, Ludhiana. The university is situated in Punjab (India) at latitude 30° 54’ N and longitude 75° 48’ E with subtropical climatic conditions. The height above mean sea level is 247 m. The experiment was laid out in RBD as described by Singh *et al* (1998) [21] with three replications for each strain and one plant per replication. Morphological characterization of tree, leaves, fruits and seeds was done using descriptors developed for Citrus by International Plant Genetic Resources Institute (IPGRI), Rome, Italy [1]. Chlorophyll content was estimated in ten intact leaves per plant using a portable chlorophyll meter (SPAD-502). Quantitative data was statistically analysed and mean, range and coefficient of variation (CV) was calculated.

Results and Discussion

Variability was observed for tree shape. Two shapes viz., spheroid and ellipsoid were observed, in trifoliate strains.However, no variability was observed for trunk surface among different strains of trifoliate. Tree growth habit was spreading in X-639 and Trifoliate while the strains Gonicoppal, Rich 16-6 and Flying Dragon had erect tree growth habit. Likewise, density of branches ranged from medium in Gonicoppal Trifoliate and Trifoliate to highly dense in Rich 16-6, X-639 and Flying Dragon. Similarly, branch angle in different strains of trifoliate varied from wide to narrow. The mean spine length ranged from 13.5 mm in Flying Dragon to 25.9 mm in Rich 16-6 (Table 1). Spine length of Trifoliate was found to be statistically at par with Flying Dragon. The results obtained for spine length are supported by Singh (2006) [17] who reported variation in qualitative traits among lime strains.

Leaf characters

Not much variation was recorded in qualitative leaf parameters. Leaf division among trifoliate strains also did not show any variability, all the strains had trifoliate leaf shape. Singh (2006) [17] also found unifoliate leaves for Schaub rough lemon and Brazilian Rangpur lime, and trifoliate leaf shape for Flying Dragon.

Variability for leaf lamina margin was also observed in trifoliate group. Strains viz., Rich 16-6, X-639, Trifoliate and Flying Dragon had dentate leaf lamina margin while the Gonicoppal Trifoliate had crenate leaf margin. The result for leaf margin corresponds to those obtained by Singh (2006) [17]. Leaf lamina shape was elliptic in Rich 16-6, X-639 and Trifoliate while, it was ovate in Gonicoppal Trifoliate and obovate in Flying Dragon strain of trifoliate. Leaf lamina attachment was found to be Brevipetiolate.It was found that petiole wings were present in all the strains of Trifoliate. Presence of petiolar wing in Ale mow is also supported by Singh (2006) [17].

A significant variation was recorded in quantitative morphological parameters (Table 1). The leaf lamina length was recorded maximum in strain Flying Dragon (54.4 mm) which was at par with Rich 16-6 and X-639 and minimum in Gonicoppal Trifoliate (32.4 mm). The leaf lamina width was maximum for Trifoliate (22.1 mm) and was found minimum for Gonicoppal Trifoliate (15.7 mm). The result obtained for petiole wing was significant for the strains of trifoliate. The mean petiole wing width ranged from 1.8 mm in strain X-639 to 5.2 mm in Trifoliate. The strain Flying Dragon of trifoliate was found at par with Rich 16-6. The average leaf chlorophyll content ranged from 50.7 in Flying Dragon strain of trifoliate to 65.5 in Trifoliate (Table 1). The findings are supported by the results of Baswal *et al* (2017b) [4] who reported high diversity among pummelo germplasm under sub tropics.

Flower characters

The strain Rich 16-6 showed early flowering (March 10) while the strain Flying Dragon was last to put forth flowering on March 18 (Table 2). Similarly, the strains of trifoliate also differed with respect to full bloom, the strain Rich 16-6 was earliest to full bloom (March 18-24) while the strain Flying Dragon was last to reached full bloom (March 28 to April 5). The flowering season in trifoliate group terminated first in strains Rich 16-6 and X-639 on April 6 for both strains. While in strain Trifoliate and Flying Dragon flowering ended at the last i.e. April 10 (Table 2). Gonicoppal Trifoliate, Rich 16-6, Trifoliate and Flying Dragon had hermaphrodite flowers. Strain X-639 had both male as well as hermaphrodite flower on a same tree. The flowers from different strains did not differ with respect to flower colour on anthesis (Plate 1). The colour of open flower was found to be white. Singh (2006) also obtained white coloured flowers in Chethali and Katazamir strains of rough lemon. Likewise, most of the strains viz., Gonicoppal Trifoliate, Rich 16-6, Trifoliate and Flying Dragon had pale yellow anther while strain X-639 had yellow coloured anther.

The results pertaining to pedicel length for various trifoliate strains were also significant (Table 3). Great variability was observed among trifoliate strains for calyx diameter. Strains of trifoliate also differed significantly for both filament and style length. The length of filament was recorded maximum in Rich 16-6 (11.2 mm), which was at par with Trifoliate and X-639, and minimum in Flying Dragon (4.5 mm). Similarly style length was found to be maximum in strain Trifoliate (4.3 mm), which was at par with X-639, and minimum in Flying Dragon (0.5 mm) (Table 3). Maximum mean petal length was observed in X-639 (21.8 mm), which was at par with Trifoliate, Rich 16-6 and Gonicoppal Trifoliate, while minimum was recorded to be 15.1 mm in Flying Dragon. Similarly, the variability for petal width ranged from 7.34 mm in X-639, which was at par with Flying Dragon, to 9.9 mm in Gonicoppal Trifoliate. Likewise, the strains showed significant variability, the mean number of stamens was recorded the highest in strain X-639 (24.3) and lowest was recorded in Rich 16-6 (18.0). The highest pollen viability was recorded to be 80.4 per cent in X-639 and the lowest was recorded in Gonicoppal Trifoliate, being 39.4 per cent (Table 4). The results are similar to the findings of Baswal *et al* (2017b) [4] who reported high diversity among grapefruit germplasm with respect to floral biology under sub tropics.

Fruit characters

The fruit characters Gonicoppal Trifoliate had not been studied because of pre-mature fruit drop in this genotype. The Flying Dragon and Rich16-6 were scored as mid-season (December-January) while X-639 was found to be early maturing (October-November). Fruit maturity took place during February-March in Trifoliate and thus scored as late maturing. Fruits from strain Rich 16-6, X-639 and Flying Dragon had spheroid shape while fruit shape was pyriform in Trifoliate. Similarly, the shape of fruit base for different trifoliate strains was highly variable. The shape of fruit base was convex in Rich 16-6 and Flying Dragon strains and concave-collared in Trifoliate while it was truncate in X-639. The shape of fruit apex was rounded for all strains. Fruit skin colour was dark yellow in strain Rich 16-6 and Trifoliate while it was observed as orange in X-639 and yellow green in Flying Dragon. The strains of trifoliate were also found to differ with respect to fruit surface texture. Fruit surface texture was pitted in Trifoliate while in Rich 16-6, X-639 and Flying Dragon the fruit surface was found to be rough. Similarly, nature of oil glands were found to be conspicuous in X-639 and Trifoliate while oil glands were scored as inconspicuous in Rich 16-6 and Flying Dragon strains. Oil gland density was found to be intermediate in Rich 16-6 and Trifoliate strain while in X-639 it was found to be high and was scored as low in Flying Dragon. Fruit axis was found to be solid in all the strains.

Maximum fruit weight was recorded for both X-639 and Trifoliate (53.3 g each) while Flying Dragon had the lowest weight (5.0 g) (Table 4). Strains under this group had great variability in terms of fruit weight. Likewise, trifoliate strains also had significant variability for fruit diameter. The maximum fruit diameter was recorded to be 67.4 mm in Trifoliate and minimum was recorded in Flying Dragon, being 20.9 mm. The maximum fruit length (77.8 mm) was recorded in strain Trifoliate while minimum was observed for Flying Dragon (17.6 mm). Total soluble solids was recorded to be the highest in strain Flying Dragon (12.1 ºBrix), which was at par with Rich 16-6, and the lowest was recorded in strain Trifoliate, being 8.8 ºBrix, which was at par with X-639. Among trifoliate strains the highest acidity was recorded to be 3.5 per cent in X-639, Rich 16-6 and Trifoliate while the lowest was recorded for Flying Dragon, being 1.3 per cent. The results concerning acidity closely resembles to findings of Singh (2006) [17]. The maximum TSS: Acid ratio was recorded in Flying Dragon, being 8.8, while minimum was recorded to be 2.5 for Trifoliate which was at par with Rich 16-6 and X-639.

Highest fruit rind thickness was recorded to be 5.3 mm in Trifoliate and the lowest was recorded in strain Rich 16-6 (0.7 mm), which was at par with Flying Dragon (Table 5). The diameter of fruit axis was recorded to be maximum in X-639 (3.6 mm) while minimum was recorded in Trifoliate, being 1.1 mm, which was at par with Flying Dragon. The strains of trifoliate group also differed significantly for oil gland size on fruit surface. The oil gland size on fruit surface was recorded to be maximum in Flying Dragon (0.9 mm), which was at par with Trifoliate, and minimum in Rich 16-6 (0.1 mm), which was at par with X-639. Strains of trifoliate differed significantly for width of epicarp at equatorial region.The width of epicarp at equatorial region was found to be maximum in Trifoliate (6.2 mm) while minimum was observed in Rich 16-6 (1.5 mm), which was at par with Flying Dragon. Trifoliate strains also did not show significant variability for number of segment. The maximum numbers of segments were observed in fruits of Trifoliate, being 9.0 while number of segments was equal in rest of the strains being 8.0. The results are similar to the findings of Singh and Singh (2004) [18] and Singh (2016) [19] who reported variation among fruit morphological and physiological characters among mandarin cultivars under sub-tropical conditions of Punjab.

Seed characters

Seed shape was uneven for different strains of trifoliate. However, clavate seed shape was most frequent and was observed in Rich 16-6, X-639 and Flying Dragon while in strain Trifoliate ovoid shaped seeds were observed.Seed colour among the trifoliate strains was found to be highly variable. Seed colour was observed as white in Rich 16-6 while it was Creamish brown in X-639. Similarly, cream coloured seeds were found in Trifoliate while seeds were white in Flying Dragon. The mean number of seeds per fruit was highest in Rich 16-6 (being, 26.0) and the lowest was found in X-639 (7.0), which was at par with Flying Dragon (Table 5). The strains of trifoliate also differed significantly for seed length. The seed length was recorded to be maximum in strain Trifoliate (9.4 mm), which was at par with Rich 16-6 and X-639, and minimum in Flying Dragon (5.6 mm). Similarly, for trifoliate group, seed width was recorded as maximum in Rich 16-6 (6.8 mm) while minimum seed width was recorded in Trifoliate (4.8 mm), which was at par with Flying Dragon and X-639.

Morphological characterization of citrus genotypes is of great importance because inheritance of agronomic traits of citrus is controlled by multiple genes which can be assessed only through morphological assessment [13]. The genotypic variation in different rootstock strains with respect to tree, leaves, flowers and fruits indicated that the rootsctocks under study were consisted of phenotypically different individuals. These differences could be attributed to mutation and cross pollination. The similarity justify the reports that stated that almost all the citrus scion and rootscock stains are emerged as chance seedlings friom single clone. It has been reported that natural hybridization and the occurrence of spontaneous mutations are very common in citrus species. Secondly, cross-pollination and the reported high percentage of zygotic twins have not only resulted in greater variation in the plant types but also a lack of uniformity in fruit quality [6]. The geographical distribution of the same genotypes may also add variation to germplasm. The genetic makeup in combination with biotic and abiotic stresses contributes to variation in the germplasm. The findings are supported by the hypothesis given Paudyal and Haq (2008) [14] who reported that environmental factors contributes variation to the tune of 40% in pummelo accessions from un-controlled field survey. The findings are also supported by Dorji and Yapwattanaphun (2011a) [7] who reported that phenotypic variation could be attributed to mutations, cross pollination and environment interactions. The bud sport mutations, introduction of materials in location different from its original habitat and lack of reproductive barriers both within species and genus might have continually added to its variation and heterogeneity (Dorji and Yapwattanaphun 2011b) [8].

Table 1: Quantitative leaf and spine characters of trifoliate strains

Strains	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf length:width ratio	Petiole wing width (mm)	Spine length (mm)	Chlorophyll
Flying Dragon	54.4	16.5	3.2	2.6	13.5	50.7
Gonicoppal Trifoliate	32.4	15.7	2.0	3.4	19.5	61.2
Rich 16-6	34.4	16.0	2.1	2.3	25.9	57.3
Trifoliate	41.7	22.1	1.9	5.2	14.5	65.5
X-639	36.3	17.0	2.1	1.8	22.7	63.1
Mean	39.8	17.4	2.3	3.1	19.2	59.5
Range	32.4-54.4	15.7-22.1	1.9-3.2	1.8-5.2	13.5-25.9	50.7-65.5
LSD (5%)	4.9	NS	0.3	0.4	1.8	NS

Table 2: Quantitative flower characters of trifoliate strains

Strains	Pedicel length (mm)	Calyx diameter (mm)	Filament length (mm)	Style length (mm)	Petal length (mm)	Petal width (mm)	Stamens per flower	Pollen viability (%)	Number of petals per flower
Flying Dragon	0.4	3.2	4.5	0.5	15.1	7.4	20.3	77.7	5.0
Gonicoppal Trifoliate	0.5	2.4	5.5	3.5	19.9	9.9	21.3	39.4	5.0
Rich 16-6	1.9	8.8	11.2	3.5	20.2	8.7	18.0	73.2	5.0
Trifoliate	3.2	9.4	10.9	4.3	21.2	8.6	20.0	63.4	5.0
X-639	2.2	6.1	10.5	3.8	21.8	7.3	24.3	80.4	5.0
Mean	1.6	6.0	8.5	3.1	19.6	8.4	20.7	66.8	5.0
Range	0.4-3.2	2.4-9.4	4.5-11.2	0.5-4.3	15.1-21.8	7.3-9.9	18.0-24.3	39.4-80.4	5.0-5.0
LSD (5%)	0.8	0.5	0.8	0.6	2.0	1.0	1.1	2.3	NS

Table 3: Quantitative fruit characters of trifoliate strains

Strains	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	TSS (ºBrix)	Acidity (%)	TSS : Acid ratio
Flying Dragon	5.0	20.9	17.6	12.1	1.3	8.8
Rich 16-6	16.6	33.9	31.9	11.6	3.5	3.2
Gonicoppal Trifoliate	53.3	67.4	77.8	8.8	3.5	2.5
X-639	53.3	45.4	41.6	9.6	3.5	2.7
Mean	32.0	41.9	42.2	10.5	3.0	4.3
Range	5.0-53.3	20.9-67.4	17.6-77.8	8.8-12.1	1.3-3.5	2.5-8.8
LSD (5%)	6.9	9.7	6.5	1.5	0.4	0.9

Table 4: Quantitative fruit characters of trifoliate strains

Strains	Fruit rind thickness (mm)	Fruit axis diameter (mm)	Oil gland size (mm)	Width at equatorial region (mm)	No. of segments per fruit	Seed length (mm)	Seed width (mm)	No. of seeds per fruit
Flying Dragon	1.1	1.2	0.9	2.0	8.0	5.6	4.9	8.0
Rich 16-6	0.7	2.3	0.1	1.5	8.0	9.3	6.8	26.0
Gonicoppal Trifoliate	5.3	1.1	0.7	6.2	9.0	9.4	4.8	18.7
X-639	2.5	3.6	0.2	2.9	8.0	8.7	5.2	7.0
Mean	2.4	2.0	0.5	3.1	8.2	8.3	5.4	14.9
Range	0.7-5.3	1.1-3.6	0.1-0.9	1.5-6.2	8.0-9.0	5.6-9.4	4.8-6.8	7.0-26.0
LSD (5%)	0.7	0.7	0.2	0.8	NS	1.2	0.6	1.9

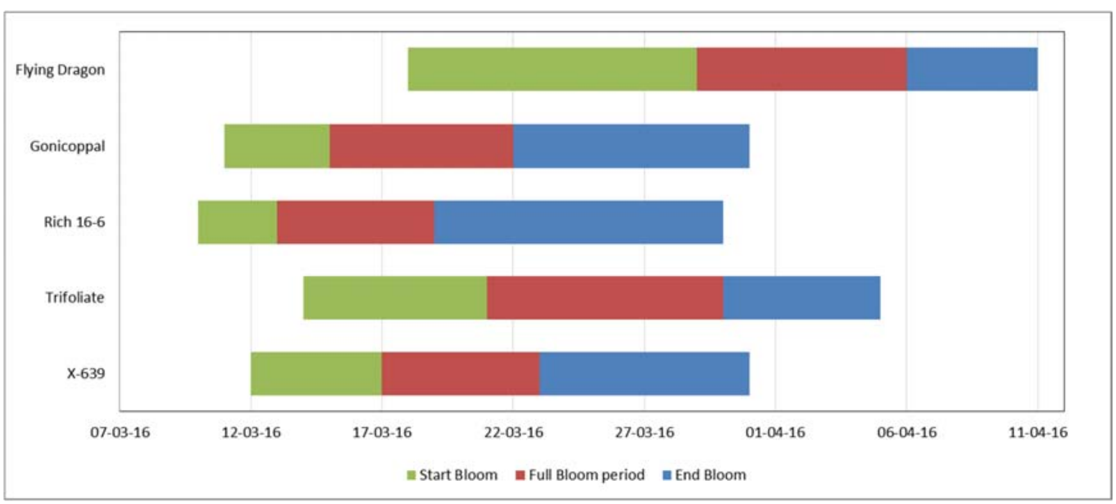

Fig 1: Flowering period in different strains of trifoliate orange and its hybrid

Plate 1: Flowers of different strains of trifoliate; (a) Gonicoppal, (b) Rich 16-6 and (c) Trifoliate

Conclusions

The study concluded that accessions under investigation consisted of phenotypically different individuals. The results revealed that diverse accessions existed in the germplasm in spite of similar morphological qualitative characters. The present study will help the breeders to select the diverse accessions so as to use them in citrus rootsctock breeding programme.

References

1. Anonymous. Descriptors for Citrus. International Plant Genetic Resources Institute, Rome, Italy, 1999.
2. Barrett HC, Rhodes AM. A numerical study of affinity relationships in cultivated citrus and its close relatives. *Syst Bot.* 1976; 1:105-36.
3. Baswal AK, Rattanpal HS, Sidhu GS, Uppal GS. Studies on pollen viability and floral biology in grapefruit (*Citrus paradisi* Mac Fadyen) cultivars under subtropical conditions of Punjab Eco. Env. & Cons. 2017a **23**(1): 381-84
4. Baswal AK, Rattanpal HS, Sidhu GS, Uppal GS. Varietal evaluation and genetic variability analysis in pummel (*Citrus grandis* M.) genotypes under subtropics of Punjab. Green Farming. 2017b; 8(2):342-45
5. Campos ET, Espinosa MAG, Warburton ML, Varela AM, Monter AV. Characterization of mandarin (*Citrus* spp.) using morphological and AFLP markers. For Docum Elect (ISO). 2005; 30:687-93.
6. Das A, Mondal B, Sarkar J, Chaudhuri S. Occurrence of zygotic twin seedlings in mandarin orange plants of the northeastern Himalayan region. Curr Sci. 2007; 92:1488-89
7. Dorji K, Yapwattanaphun C. Morphological Identification of Mandarin (*Citrus reticulata* Blanco) in Bhutan Kasetsart. J Nat Sci. 2011a; 45:793-802.
8. Dorji K, Yapwattanaphun C. Assessment of morphological diversity for local mandarin (*Citrus reticulata* Blanco) accessions in Bhutan. J Agri Tech. 2011b; 7:485-95.
9. Harding PH. Testing and cultivar evaluation. In: Method in fruit breeding. (Eds.): J N Moore and J Janick Purdue University Press, West Lafayette. 1983, 371-81.
10. Kinley D, Chinawat Y. Morphological Identification of Mandarin (*Citrus reticulata* Blanco.) in Bhutan. *Kasetsart J* (Nat Sci). 2011; 45:793-02.
11. Koehler SP, Dornelles ALC, Freitas LB. Characterization of mandarin citrus germplasm from southern Brazil by morphological and molecular analyses. Pesq Agropec Bras. 2003; 38:797-06.
12. Kumar K, Singh B, Arora PK, Kumar A, Gill JS. Characteriztion of citrus rootstocks for morphological traits. New Frontiers and Future Challenges inb Horticulture Crops, 2011, 2012, 8.
13. Liu YZ, Deng XX. Citrus breeding and genetics in China. Asian Australas J Plant Sci Biotech. 2007; 1:23-28.
14. Paudyal K, Haq N. Variation of pomelo [*Citrus grandis* (L.) Osbeck] in Nepal and participatory selection of strains for further improvement. Agroforestry Systems. 2008; 72:195-04.
15. Reitz HJ. The World Citrus Crop. Outlook on Agriculture. 1984; 13(3):140-46.
16. Sharma BD, Hore DK, Gupta SG. Genetic resources of Citrus of north-eastern India and their potential use. *Genet Resour Crop Evol.* 2004; 51:411-18.
17. Singh S. Citrus Monograph. NRCC, Nagpur, 2006, 1-96.
18. Singh IP, Singh S. Evaluation of seedless cultivar of mandarin (*Citrus* species) for export. Ind J Agric Sci. 2004; 74:376-78.
19. Singh G, Aulakh PS, Rattanpal HS. Genetic divergence of indigenous and exotic mandarin (*Citrus reticulata* Blanco) accessions based on fruit morphological and physiological traits. Res on Crops. 2016; 17(3):538-44
20. Singh H, Rattanpal HS, Sidhu GS, Chahal TS. Study on physio-morphological characteristics among six rangpur lime (*Citrus limonia* Osbeck.) strains. J Tree Sci. 2010; 29:48-56.
21. Singh S, Bansal ML, Singh TP, Kumar R. Statistical Method for Research Workers. Kalyani Publishers, New Delhi, India, 1998, 310-17.
22. Sites JW, Reitz HJ. The variation in individual Valencia oranges from different locations of the tree as a guide to sampling and spot-picking for quality. Part II. Titratable acid and soluble solids/titratable acid ratio of the juice. Pro Amer Soc Hort Sci. 1950; 55:73-80.
23. Swingle WT, Reece PC. The botany of citrus and its wild relatives. In: W. Reuther H J, Webber and L D Batchelor (Eds.), The Citrus Industry, (rev). University of California Press, Berkley, California. 1967; 1:190-430.
24. Syvertsen JP, Graham JH. Hydraulic conductivity of roots, mineral nutrition, and leaf gas exchange of citrus rootstocks. J Amer Soc Hort Sci. 1985; 110:865-69.
25. Tanaka T. The origin and dispersal of citrus fruits having their centre of origin in India. Indian J Hort. 1958; 15:101-15.