GENETIC VARIABILITY AND HERITABILITY AMONG MANDARIN (*CITRUS RETICULATA* BLANCO) GENOTYPES UNDER INDIAN SUB-TROPICAL CONDITIONS

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Abstract. An effort has been made to study the genotypic variance, heritability, and genetic advance among nineteen mandarin genotypes grown under Indian sub-tropical conditions. The cluster analysis differentiated diverse genotypes which were grouped independently of their geographical origin. Wide variability in morphological characteristics indicated the genetic variability among mandarin genotypes. Rootstock diameter, scion diameter, fruit weight and seed weight showed a higher genotypic coefficient of variance, genetic advance and heritability, and genetic advance percentage of means. However, the phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all the traits under study. The study indicated the existence of diversity in analyzed mandarin accessions. Furthermore, it concluded that the occurrence of additive gene action with low environmental influence is responsible for the determination of rootstock diameter, scion diameter, fruit weight, and seed weight as compared to other traits under study. Fruit yield was strongly correlated with the leaf lamina thickness and fruit weight followed by seed weight, indicating that fruit weight and seed weight can be used as selection criteria in an early year of bearing under crop improvement programme. Principal component analysis (PC1) distinguished positive correlations between fruit weight, fruit diameter, and fruit yield.
Keywords: *citrus, genetic diversity, genetic advance, genotypic variance, phenotypic variance*

Introduction

Citrus is grown extensively in tropical and sub-tropical provinces worldwide. The profitable cultivation of mandarins is concentrated between 40° North and South of the equator (Patil et al., 2012). Citrus is reported to have originated in Southeast Asia, extended up to North Burma, East India, and Southwest of China (Gmitter and Hu, 1990; Soost and Roose, 1996). The maximum citrus germplasm diversity identified in Northeast India including 23 species, one subspecies, and 68 varieties (Sharma et al., 2004). This variation in germplasm might be due to the occurrence of bud sports, natural hybridization, cross-pollination, and the presence of a high percentage of zygotic seedlings (Das et al., 2007; Golein et al., 2011; Singh et al., 2021).

Taxonomic interactions within citrus groups are highly complicated. This complexity in relations might be due to the recurrent occurrence of spontaneous mutants and interspecific hybrids in nature. The crop improvement programs in citrus are mainly based on the depiction of morphological, cytological, and genetic characters of various cultivars (Fatima et al., 2015; Ahmad et al., 2018). The morphological flexibility is considered a major hindrance in the validation of phenotypic diversity. However, the inheritance of agronomic traits of citrus was reported to be governed by multiple genes and evaluated only by their morphological characterization (Liu and Deng, 2007). Morphological

descriptions are the basic passport data of the genotype under the given environmental conditions and that is why they are being used widely (Domingues et al., 1999; Monteverde, 2000; Koehler et al., 2003; Campos et al., 2005; Josan and Kaur, 2006; Lin et al., 2007; Khan et al., 2008, 2014; Kinley and Chinawat, 2011; Dorji and Yapwattanaphun, 2014; Sharma et al., 2015; Baswal et al., 2017; Sunaina et al., 2018; Kaur et al., 2022). The identification of superior genotypes within the collected germplasm is valuable for genetic up-gradation and crop conservation (Clark and Hoy, 2006; Lin et al., 2007; Gaikward et al., 2018; Singh et al., 2022).

The morphological and genetic diversity has been considered as independent from one another (Koehler et al., 2003; Campos et al., 2005; Singh et al., 2017; Rattanpal et al., 2018; Singh et al., 2020). Genetic variability is the basis of any crop improvement program. However, little effort has been made to study the genotypic variance, heritability, and genetic advance among collected accessions. Such a study reveals the nature of inheritance of fruit quantitative and qualitative parameters to design citrus breeding strategies. Moreover, there is a necessity to diversify scion cultivars of citrus for North Indian regions to reduce the new insect-pest load scattering over a narrow genetic pool. Secondly, mandarins represent maximum phenotypic heterozygosity and variation in characters in comparison to other citrus species (Moore, 2001; Faralli et al., 2019).

Thus, the hypothesis for the study is that genetic diversity may be present in mandarin germplasm grown under sub-tropical conditions. Therefore, the present study was carried out to assess the nature and genetic variability in mandarin germplasm.

Materials and methods

Experimental site and climate

The experiment was conducted at the citrus breeding block of Punjab Agricultural University, Ludhiana during 2014 and 2015. This site location is marked at the latitude and longitude 30° 54' N and 75° 48' E, respectively. The climatic zone is subtropical and is situated 247 m above the mean sea level. The mean maximum and minimum monthly temperature for both the years (2014 and 2015) was 40.1 °C and 7.0 °C, respectively. However, the mean maximum monthly relative humidity was 78.2% and the mean minimum monthly relative humidity was 40.75%. The average annual rainfall was 705.8 mm (*Fig. 1*).

Plant material and methodology

A total of 19 mandarin genotypes introduced from the United States of America and different states of India were used in the study (*Table 1*). Fourteen genotypes (CRS-4, Clone-11, Coorg, Darjeeling, Khasi, Mudhkhed Seedless, N-4, N-28, N-34, N-38, N-43, N-51 belonging to *Citrus reticulata* Blanco were introduced from different states of the country while five hybrids (Daisy, Kinnow, Fremont, Nova, and W. Murcott) were introduced from the USA. Rough lemon (*C. jambhiri* Lush.) was used as rootstock for all the genotypes. The age of the studied plants was six years and all the recommended package of practices were followed as per Punjab Agricultural University, Ludhiana, Punjab (India) for citrus cultivation. The morphological descriptors of citrus framed by the International Plant Genetic Resources Institute, Italy (IPGRI, 1999) were used throughout the research period. The number of replication in this experiment was three and each replication included one plant. The total number of plant under study was 57 i.e.

three plants per treatment. The diameter of rough lemon rootstock was measured at 10 cm under graft union; however, scion diameter was noted at 10 cm overhead union. Twenty copiously developed leaves and ten randomly selected fruits were taken for each replication. Leaf quantitative parameter data, viz. leaf lamina length (LLL), leaf lamina width (LLW) and leaf lamina thickness (LLT) and petiolar wing length (PWL) and petiolar wing width (PWW) were measured by using the Vernier's calipers (Mitutoyo Inc., Japan). Fruit parameters data viz. fruit weight, fruit yield, fruit length, fruit diameter, fruit number per tree, seed number per fruit and seed weight were also recorded. The data on total soluble content, as degree Brix, were recorded by using Digital Hand Refrectrometer. The acidity was estimated as the percentage of citric acid by titrating a well-known volume of juice content against 0.1 N sodium hydroxide (NaOH).
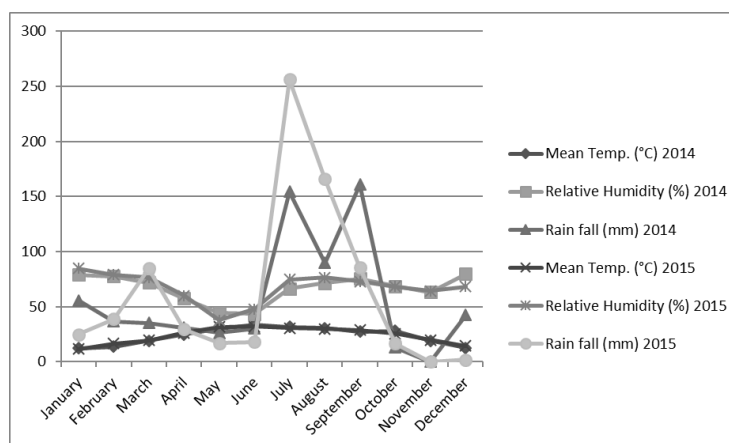
Figure 1. Climatic conditions of the experimental site during the study years 2014 and 2015

Data analysis

The experiment was laid out in Randomized Block Design at the time of planting as described by Gomez and Gomez (2010). Significant differences among genotypes were calculated using one-way ANOVA followed by post hocTukey's honestly significant difference (HSD) test at 5% level (p<0.05) for pair wise comparison of genotypes for each of parameters using software version 9.3 (SAS Institute Inc., Cary, NC, USA). The genetic variance (GV) (*Eq.1*), phenotypic variance (PV) (*Eq.2*), genotypic coefficient of variance (GCV) (*Eq.3*) and phenotypic coefficient of variance (PCV) (*Eq.4*), were computed (Mulualem and Mohammed, 2012).

$$Genotypic\ variance = \frac{MSg - MSe}{nr} \qquad (Eq.1)$$

$$Phenotypic\ variance = \frac{MSg}{nr} \qquad (Eq.2)$$

where MSg is genotypic mean square, MSe is error mean square and nr is number of replication.

Table 1. *List of mandarin genotypes used in the study*

S. No.	Common Name	Latin name	Parentage	Source
1	CRS-4	*Citrus reticulata* Blanco	Selection	Assam Agricultural University, Tinsukia, Assam
2	Clone-11	*Citrus reticulata* Blanco	Selection	Central Horticultural Experiment Station, Chettali, Karnataka, India
3	Coorg	*Citrus reticulata* Blanco	Selection	Central Citrus Research Institute, Nagpur, Maharashtra, India
4	Daisy	*Citrus reticulata* Blanco	Fortune x Fremont	United States of America
5	Darjeeling	*Citrus reticulate* Blanco	Selection	Central Citrus Research Institute
6	Fremont	*Citrus reticulata* Blanco	Clementin mandarin x Ponkan Tangelo	United States of America
7	Khasi	*Citrus reticulata* Blanco	Selection	Central Citrus Research Institute
8	Kinnow	*Citrus nobilis* Lourx *Citrus deliciosa* Tenore	Willow leaf x King Mandarin	United States of America
9	Mudhkhed Seedless	*Citrus reticulata* Blanco	Selection	Central Citrus Research Institute
10	Nagpur -4	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
11	Nagpur -28	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
12	Nagpur -34	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
13	Nagpur- 38	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
14	Nagpur -43	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
15	Nagpur-51	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
16	Nagpur Seedless	*Citrus reticulata* Blanco	Nagpur Selection	Central Citrus Research Institute
17	Nagpur	*Citrus reticulata* Blanco	Selection	Central Citrus Research Institute
18	Nova	*Citrus reticulata* Blanco	Clementin mandarin x Orlando Tangelo	United States of America
19	W. Murcott	*Citrus reticulata* Blanco x *Citrus sinensis*	Murcott and Unknown	United States of America

$$GCV = \frac{\sqrt{VG}}{Grand\ Mean} \times 100 \qquad (Eq.3)$$

$$PCV = \frac{\sqrt{VP}}{Grand\ Mean} \times 100 \qquad (Eq.4)$$

Broad sense heritability (h2) (*Eq.5*) in percentage was estimated in each character using variance components as described (DeLacy et al., 1996). Genetic advance (GA) and genetic advance percent mean) (GAM) were computed as per *Eq.6* and *Eq.7*, respectively.

$$Heritability\ (h^2) = \frac{Genotypic\ Variance}{Phenotypic\ Variance} \qquad (Eq.5)$$

$$Genetic\ advance = \sqrt{VP} \times h^2 \times k \qquad \text{(Eq.6)}$$

where k is the differential selection constant and value for k is 2.06.

$$GAM = \frac{Genetic\ Advance}{Grand\ Mean} \times 100 \qquad \text{(Eq.7)}$$

The genetic diversity among the mandarin genotypes was computed based on quantitative data by using the Computer Software DARwin (Perrier and Jacquemoud-Collet, 2006). The data generated dendrogram on subjection to an un-weighted pair group method with arithmetic mean (UPGMA) analysis. The Pearson's Correlation Coefficient method was used to calculate genotypic and phenotypic correlation among important quantitative traits of different mandarin accessions as per the correlation method described by Al-Jibouri et al., 1958. Principle component (PCA) analysis was performed with XLSTAT 14.0 to identify the principal components that contributed the maximum to variability and to identify the association of traits with yield in mandarin germplasm.

Results

Tree traits

All the mandarin genotypes differ significantly in consideration of rootstock diameter (*Table 2*). The range of rootstock diameter among mandarin genotypes was 36.91 to 124.82. Rootstock diameter (124.82 mm) was the maximum in Darjeeling mandarin and was at par with Nova, Nagpur, Mudhkhed Seedless, Khasi, and Coorg. The maximum scion diameter was observed in Khasi mandarin (97.44 mm) and did not show any significant difference with Nagpur Seedless, Nagpur, Nova, Mudhkhed Seedless, Darjeeling, and Coorg. However, the minimum rootstock diameter (36.91 mm) and scion diameter (27.75 mm) were recorded in the CRS-4 genotype. Scion to rootstock diameter ratio was found to be the maximum in Kinnow (0.90) and was statistically at par with W. Murcott, Nagpur, Nagpur Seedless, Mudhkhed Seedless, Khasi, Fremont, Coorg, and Clone-11. Minimum scion to rootstock diameter ratio (0.69) was observed in N-43 and N-4.
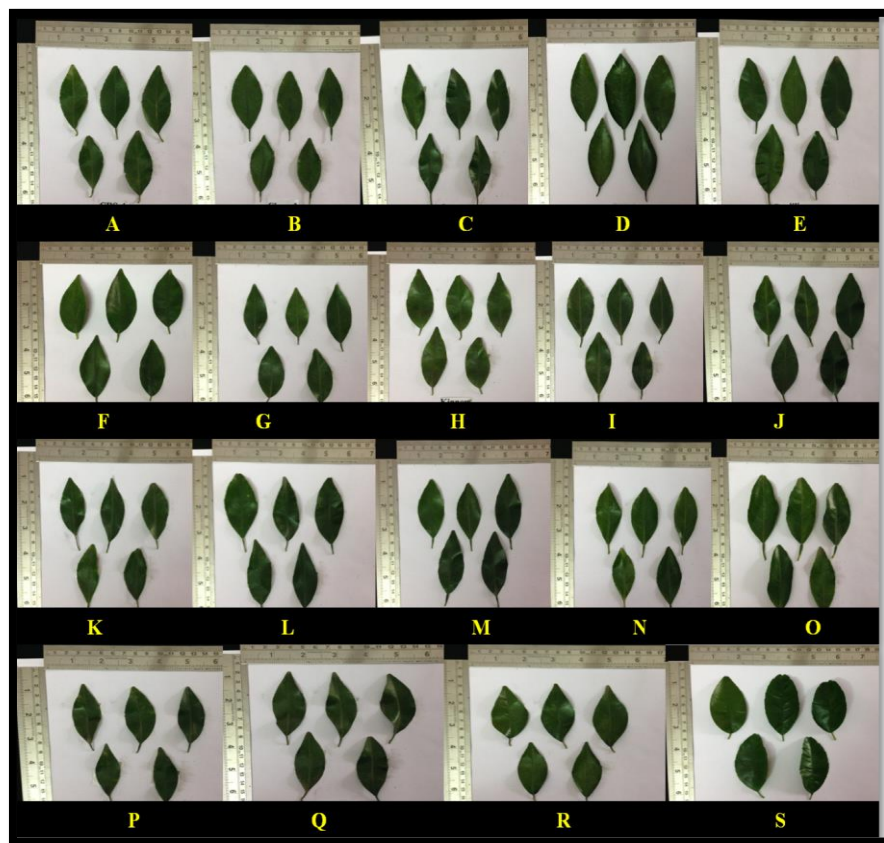
Leaf traits

The data (*Table 2, Fig. 2*) illustrated significant deviation in quantitative leaf traits amid mandarin genotypes. The maximum mean LLL to width ratio was recorded in Nagpur (2.23) and it was at par with Mudhkhed Seedless, Nagpur Seedless, N-34, N-28, N-4, Khasi, Fremont, Darjeeling, CRS-4, and Coorg. The least average length to width ratio (1.97) of leaf lamina was registered in Kinnow. The maximum mean LLT (0.33 mm) was recorded in Darjeeling and N-43 and both were at par with W. Murcott, N-51, N-28, Kinnow, Daisy, and Coorg. However, the LLT (0.25 mm) was observed to be minimum in Nova genotype. The outcome of the studies depicted that the highest PWL was recorded in N-38 (16.36 mm) which was at par with Nagpur Seedless, Nagpur, N-51, N-43, N-28, and N-4. The least LLW (2.47 mm) was noticed in N-51 that was found statistically at par with N-43, N-38, N-4, Darjeeling, Clone-11, and CRS-4. However, the minimum mean PWL (10.35 mm) and PWW (1.46 mm) were recorded in W. Murcott.

Table 2. *Vegetative characters of different mandarin genotypes grown under Punjab conditions (Pooled data 2014 and 2015)*

Genotypes	Rootstock Diameter (mm)	Scion diameter (mm)	Scion/Diameter ratio	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf length/width	Leaf lamina thickness (mm)	Petiole wing length (mm)	Petiole wing width (mm)
CRS-4	36.91^i	27.75^i	0.75^{de}	68.60^{bc}	31.38^{efg}	2.19^{ab}	0.30^{bcde}	13.96^{bcd}	2.25^{abc}
Clone-11	57.22^{gh}	47.69^{fgh}	0.83^{abcd}	67.71^{bc}	32.63^{cdefg}	2.08^{bcdef}	0.28^{efgh}	12.72^{def}	2.29^{abc}
Coorg	109.04^{ab}	92.34^{ab}	0.85^{abc}	63.81^c	30.10^g	2.12^{abcde}	0.31^{abcd}	12.55^{defg}	1.80^{fg}
Daisy	87.53^{cde}	71.35^{cd}	0.82^{bcd}	74.85^a	37.05^a	2.02^{def}	0.31^{abcd}	10.81^{gh}	1.83^{ef}
Darjeeling	124.82^a	94.59^{ab}	0.76^{de}	71.78^{ab}	33.28^{bcdefg}	2.16^{abcd}	0.33^a	13.76^{cd}	2.24^{abc}
Fremont	74.89^{efg}	64.09^{de}	0.85^{bc}	68.50^{bc}	31.58^{defg}	2.19^{ab}	0.28^{efg}	10.96^{fgh}	1.47^h
Khasi	114.27^{ab}	97.44^a	0.85^{abc}	67.45^{bc}	30.85^{fg}	2.19^{ab}	0.29^{def}	12.45^{defg}	2.16^{bc}
Kinnow	87.18^{cde}	78.94^{bcd}	0.90^a	68.58^{bc}	34.75^{abcde}	1.97^f	0.31^{abcd}	11.69^{efgh}	2.13^{cd}
Mudhkhed Seedless	106.01^{abc}	87.81^{ab}	0.83^{abcd}	67.93^{bc}	32.65^{cdefg}	2.11^{abcdef}	0.30^{cdef}	13.13^{de}	2.09^{cde}
N-4	66.11^{fg}	45.66^{gh}	0.69^e	71.13^{ab}	34.06^{abcdef}	2.12^{abcdef}	0.27^{fgh}	15.99^a	2.31^{abc}
N-28	80.48^{def}	63.00^{def}	0.79^{cd}	72.91^{ab}	35.00^{abcde}	2.08^{abcdef}	0.31^{abcd}	15.74^{ab}	2.13^{cd}
N-34	81.18^{def}	63.82^{de}	0.78^{cd}	68.25^{bc}	32.75^{bcdefg}	2.09^{abcdef}	0.30^{bcde}	13.94^{bcd}	1.84^{ef}
N-38	60.01^{gh}	49.04^{efg}	0.82^{bcd}	69.26^{bc}	34.40^{abcdef}	2.01^{def}	0.26^{gh}	16.36a	2.41^{ab}
N-43	44.31^{hi}	32.72^{hi}	0.69^e	69.62^{ab}	33.70^{abcdefg}	2.07^{bcdef}	0.33^{ab}	15.61^{abc}	2.23^{abc}
N-51	59.93^{gh}	44.44^{gh}	0.75^{de}	71.85^{ab}	35.42^{abc}	2.04^{cdef}	0.31^{abcd}	16.22^a	2.47^a
Nagpur Seedless	97.15^{bcd}	86.78^{abc}	0.89^{ab}	69.03^{bc}	31.60^{defg}	2.19^{abc}	0.28^{efgh}	15.76^{ab}	2.16^{bc}
Nagpur	110.61^{ab}	94.67^{ab}	0.85^{abc}	72.63^{ab}	32.77^{bcdefg}	2.23^a	0.29^{cdef}	15.56^{abc}	1.87^{def}
Nova	107.00^{ab}	86.81^{abc}	0.81^{cd}	72.10^{ab}	35.12^{abcd}	2.05^{bcdef}	0.25^h	11.68^{efgh}	1.55^{gh}
W. Murcott	83.03^{def}	70.98^{cd}	0.86^{abc}	71.73^{ab}	36.38^{ab}	1.98^{ef}	0.32^{abc}	10.35^h	1.46^h
Mean	**83.56**	**68.42**	**0.81**	**69.88**	**33.45**	**2.1**	**0.3**	**13.64**	**2.04**
LSD (p≤0.05)	**19.13**	**15.99**	**0.08**	**5.5**	**3.68**	**0.14**	**0.03**	**1.89**	**0.26**
CV	**19.97**	**20.4**	**8.74**	**6.88**	**9.61**	**6.23**	**7.86**	**12.12**	**11.43**

Different alphabets show significant difference among genotypes

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Figure 2. *Variation in leaf size of different mandarin genotypes A) CRS-4, B) Clone-11, C) Coorg, D) Daisy, E) Darjeeling, F) Fremont, G) Khasi, H) Kinnow, I) Mudhkhed Seedless, J) N-4, K) N-28, L) N-34, M) N-38, N) N-43, O) N-51, P) Nagpur Seedless Q) Nagpur, R) Nova and S) W. Murcott*

Fruit and seed traits

The findings of the study illustrated that significant variation in fruit size and weight existed in mandarin genotypes (*Table 3, Fig. 3*). Daisy (228.50 g) recorded the maximum mean fruit weight which was at par with W. Murcott, N-34, and Kinnow. However, the minimum fruit weight was measured in N-38 (110.17 g) which was significantly lower than all genotypes except Fremont (112.83 g). Fruit diameter (75.12 mm) and fruit length (65.55 mm) were found to be the highest in Daisy as compared to all other genotypes (*Table 2*). Mean fruit diameter (56.82 mm) and fruit length (53.37) were recorded minimum in N-38 and Fremont, respectively. The highest fruit number per tree was recorded in Kinnow (403) followed by Mudhkhed Seedless (402) and W. Murcott (365) which was significantly higher than all other genotypes. However, the lowest number of fruits per tree was recorded in Nova (210) mandarin. Daisy recorded the highest fruit yield (74.26 kg/tree) followed by Kinnow (71.06 kg/tree) and W Murcott (62.84 kg/tree) which was significantly higher than all other genotypes. Nova mandarin recorded the lowest fruit yield (28.52 kg/tree). The total soluble solids (9.76 Brix) were recorded maximum in Kinnow that was statistically at par with Daisy, Nova, Fremont, and Darjeeling. The acid content ranged from 0.56 to 0.96 percent among all the genotypes.

Table 3. *Fruit characters of different mandarin genotypes grown under Punjab conditions (Pooled data 2014 and 2015)*

Genotypes	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit number per tree	Fruit yield per tree (Kg)	TSS (^{0}Brix)	Acidity (%)	Av. Seed Number/fruit	Av. Seed weight of 20 seeds
CRS-4	133.00^{gh}	63.13^e	57.72^{ghij}	275^{fghi}	36.58^{fghij}	8.25^{def}	0.81^{bc}	15.03^{bcd}	1.07^i
Clone-11	135.00^{fgh}	66.73^{cd}	59.57^{defgh}	298^{defgh}	40.23^{efghij}	9.07^{bc}	0.66^g	12.80^{def}	0.97^i
Coorg	140.50^{efg}	69.70^{bc}	64.95^a	292^{defghi}	41.03^{efghij}	7.35^i	0.63^g	13.33^{def}	1.81^d
Daisy	228.50^a	75.12^a	65.55^a	325^{bcde}	74.26^a	9.70^a	0.56^h	18.60^a	2.03^c
Darjeeling	150.33^{de}	69.88^{bc}	63.70^{ab}	286^{defghi}	42.99^{efghi}	9.23^{ab}	0.80^{bc}	14.02^{de}	2.14^{bc}
Fremont	112.83^i	61.80^e	53.37^k	301^{defg}	33.96^{fghi}	9.45^{ab}	0.82^b	18.33^{ab}	1.49^{fg}
Khasi	141.50^{efg}	68.23^c	61.23^{bcde}	352^{bc}	49.81^{cde}	7.65^{hi}	0.71^f	11.55^{def}	1.73^{de}
Kinnow	176.33^b	74.03^a	59.27^{defgh}	403^a	71.06^{ab}	9.76^a	0.73^{def}	19.77^a	2.30^b
Mudhkhed Seedless	146.33^{ef}	67.33^{cd}	61.53^{bcd}	402^a	58.82^{bcd}	8.17^{defgh}	0.92^a	12.23^{def}	2.11^c
N-4	138.50^{efgh}	64.22^{de}	63.05^{abc}	292^{defghi}	40.44^{efghij}	7.71^{ghi}	0.96^a	10.78^{ef}	1.47^{fg}
N-28	144.33^{efg}	72.45^{ab}	60.22^{defg}	312^{cdef}	45.03^{ef}	7.76^{fghi}	0.75^{de}	14.58^{cd}	1.56^{efg}
N-34	161.83^{cd}	62.18^e	55.55^{jk}	252^{ghi}	40.78^{efghij}	8.21^{defg}	0.72^{ef}	17.65^{abc}	1.72^{de}
N-38	110.17^i	56.82^f	55.30^{jk}	262^{ghi}	28.86^j	7.97^{efgh}	0.81^{bc}	14.25^{cde}	2.08^c
N-43	126.67^h	68.93^c	58.23^{fghi}	289^{defghij}	36.61^{fgij}	8.08^{efgh}	0.77^{cd}	14.10^{de}	1.62^{ef}
N-51	137.67^{efgh}	66.70^{cd}	57.00^{hij}	248^{ij}	34.14^{fghij}	7.79^{fghi}	0.80^{bc}	14.07^{de}	1.72^{de}
Nagpur Seedless	138.00^{efgh}	63.07^e	60.65^{cdef}	312^{cdef}	43.06^{efgh}	8.63^{cd}	0.76^{def}	10.28^f	1.25^h
Nagpur	135.67^{fgh}	68.65^c	58.23^{fghi}	329^{bcd}	44.64^{efg}	8.40^{de}	0.67^g	13.77^{def}	1.39^{gh}
Nova	135.83^{fgh}	74.40^a	55.67^{ijk}	210^j	28.52^j	9.59^{ab}	0.73^{def}	20.62^a	2.20^{bc}
W. Murcott	172.17^{bc}	73.33^a	58.70^{efgh}	365^{ab}	62.84^{abc}	9.11^{bc}	0.83^b	14.60^{cd}	3.60^a
Mean	**145.54**	**67.72**	**59.45**	**305.53**	**44.93**	**8.52**	**0.76**	**14.76**	**1.8**
LSD (p≤0.05)	**12.81**	**3.36**	**2.63**	**49.86**	**13.05**	**0.54**	**0.04**	**3.54**	**0.17**

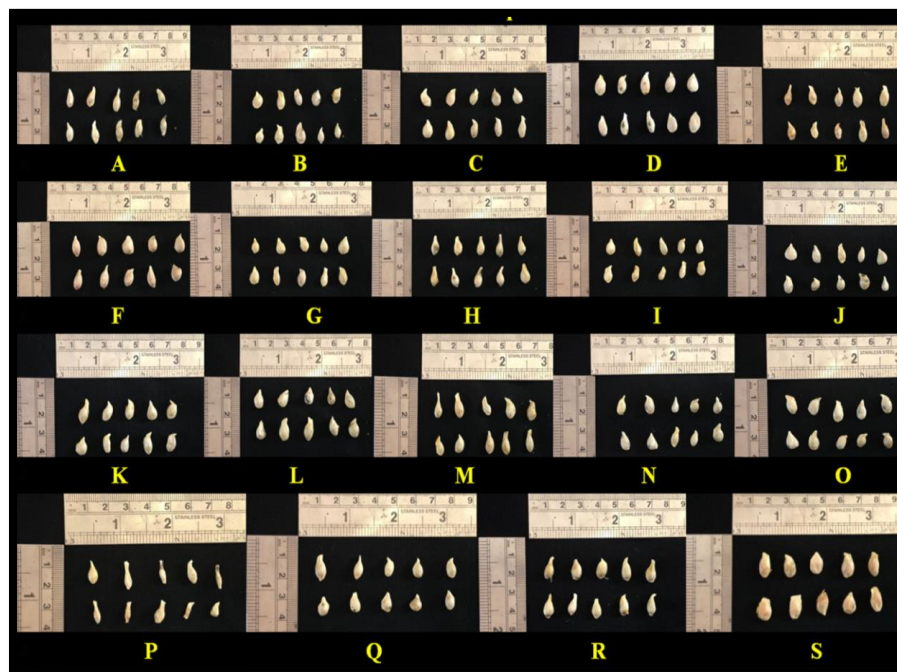Different alphabets show significant difference among genotypes
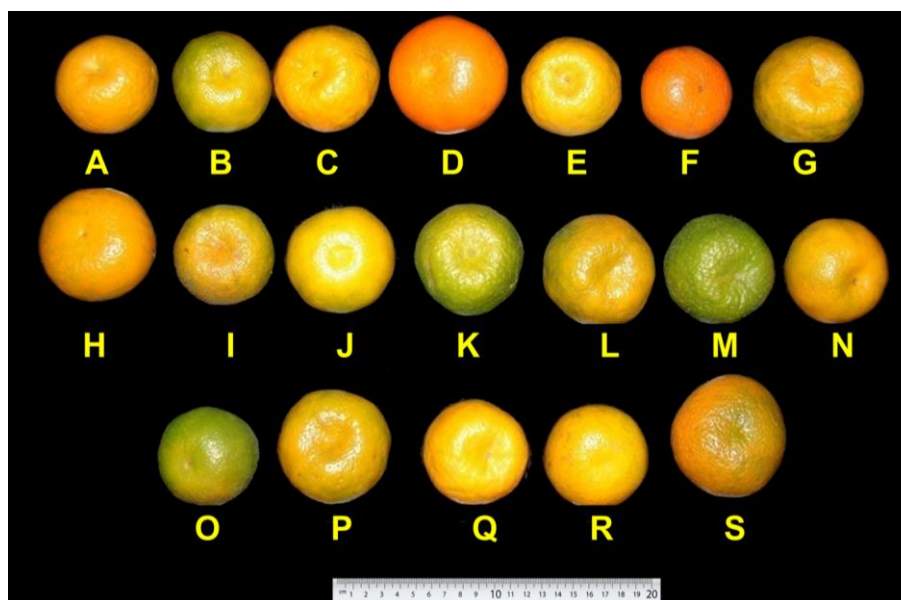
Figure 3. *Diversity in fruit size of different mandarin genotypes A) CRS-4, B) Clone-11, C) Coorg D) Daisy, E) Darjeeling, F) Fremont, G) Khasi, H) Kinnow, I) Mudhkhed Seedless, J) N-4, K) N-28, L) N-34, M) N-38, N) N-43, O) N-51, P) Nagpur Seedless Q) Nagpur, R) Nova and S) W. Murcott*

The highest (0.96%) and the lowest (0.56%) acid content were recorded in N-4 and Daisy, respectively. The mean seed number per fruit had a significant deviation among all the mandarin genotypes. The number of seeds per fruit varied from 10.28 to 20.62. The maximum number of seeds per fruit was counted (20.62) in Nova while the minimum seed number per fruit was recorded in Nagpur Seedless (10.28). The data illustrated that the maximum number of seeds per fruit was at par with Kinnow, Daisy, Fremont, and N-34. It was evident from the data that the maximum seed weight (3.60 g per 20 seeds) was observed in W. Murcott followed by Kinnow, Nova, Darjeeling, and Mudhkhed Seedless (*Fig. 4*). However, the minimum mean seed weight was observed in Clone-11 (0.97 g per 20 seeds) which was significantly lower than all genotypes except CRS-4 (1.07 g per 20 seeds).

Genetic variance and heritability

Phenotypic variance for all the important traits was calculated through genotypic variance and genotypic environment variance (*Table 4*). A wide range of variability was observed for all the entire traits except for LLT and fruit acidity. Phenotypic coefficient of variance which represents total variability in germplasm, was higher for scion diameter (45.9), followed by average seed weight of 20 seeds (45.4), rootstock diameter (42.2), an average number of seeds per fruit (28.3), and fruit weight (25.3) whereas, the genotypic coefficient of variance which represents heritable variability was higher for average seed weight of 20 seeds (45.1), followed by scion diameter (44.3), rootstock diameter (40.6) and fruit weight (24.9). The genetic advance was higher for fruit number per tree (82.21) followed by fruit weight (73.4), rootstock diameter (67.3), and scion diameter (60.4). However, the genetic advance percentage of means was found to be higher for the average seed weight of 20 seeds (92.4) followed by scion diameter (88.3), rootstock diameter (80.5), and fruit weight (50.5).

Figure 4. *Variation in seed size of different mandarin genotypes A) CRS-4, B) Clone-11, C) Coorg, D) Daisy, E) Darjeeling, F) Fremont, G) Khasi, H) Kinnow, I) Mudhkhed Seedless, J) N-4, K) N-28, L) N-34, M) N-38, N) N-43, O) N-51, P) Nagpur Seedless Q) Nagpur, R) Nova and S) W. Murcott*

Table 4. *Variability, heritability and genetic advance in mandarin genotypes for 16 traits*

Traits	Grand mean	Ranges	GV	PV	PCV	GCV	h_2	GA	GAM
Rootstock diameter (mm)	83.6	36.91-124.82	1152.9	1245.8	42.2	40.6	0.9	67.3	80.5
Scion diameter (mm)	68.4	27.75-97.44	919.8	984.7	45.9	44.3	0.9	60.4	88.3
Leaf lamina length (mm)	69.9	63.81-74.85	5.5	13.2	5.2	3.4	0.4	3.1	4.5
Leaf lamina width (mm)	33.4	30.10-37.05	3.8	7.2	8.0	5.8	0.5	2.9	8.7
Leaf lamina thickness (mm)	0.3	0.25-0.33	0.0	0.0	8.9	8.9	1.0	0.1	18.3
Petiole wing length (mm)	13.6	10.35-16.22	7.3	8.2	21.0	19.8	0.9	5.2	38.4
Petiole wing width (mm)	2.0	1.46-2.47	0.2	0.2	21.3	20.3	0.9	0.8	39.9
Fruit weight (g)	145.5	110.17-228.50	1311.4	1353.1	25.3	24.9	1.0	73.4	50.5
Fruit diameter (mm)	67.2	56.82-75.12	46.7	49.6	10.5	10.2	0.9	13.7	20.3
Fruit length (mm)	59.4	53.37-65.95	20.9	22.7	8.0	7.7	0.9	9.1	15.2
Fruit number per tree	5810.3	210-403	3281.1	3317.45	7.56	7.51	0.9	82.21	1.41
Fruit yield (kg/tree)	853.6	28.52-74.26	209.54	227.10	5.16	4.95	0.9	21.20	2.48
Average no. of seeds per fruit	14.8	10.28-20.62	14.2	17.4	28.3	25.5	0.8	7.0	47.5
TSS (ºBrix)	8.5	7.35-9.76	1.1	1.2	12.9	12.5	0.9	2.1	24.8
Acidity (%)	0.8	0.56-0.96	0.0	0.0	17.9	17.7	1.0	0.3	36.0
Average seed weight of 20 seeds (g)	1.8	0.97-3.60	0.7	0.7	45.4	45.1	1.0	1.7	92.4

Legends: GV = genotypic variance, PV= phenotypic variance, PCV = phenotypic coefficient of variance, GCV = genotypic coefficient of variance, h2= heritability in broad sense, GA= genetic advance, Genetic advance as percent of the Mean (GAM)

Cluster analysis

Nineteen mandarin genotypes were divided into three major clusters in dendrogram drawn based on UPGMA (*Fig. 5*). Cluster-I consisted of four genotypes namely N-34, W. Murcott, Daisy, and Kinnow. Likewise, cluster -II included seven genotypes, viz. Nagpur Seedless, Nova, Mudhkhed Seedless, Coorg, Nagpur, Darjeeling, and Khasi. Under cluster –III eight genotypes were grouped (N-28, Fremont, N-4, N-51, Clone-11, N-38, CRS-4, and N-43).
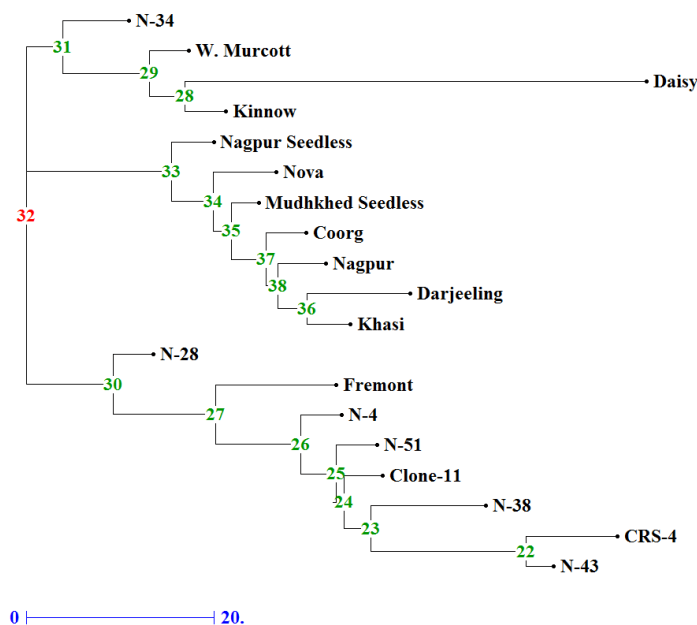
Figure 5. Dendrogram illustrating genetic relationship among 19 mandarin genotypes generated by UPGMA tree analysis based on tree and fruit traits. Cluster -I included N-34, W. Murcott, Daisy, Kinnow, Cluster -II included Nagpur Seedless, Nova, Mudhkhed Seedless, Coorg, Nagpur, Darjeeling, Khasi and Cluster –III included N-28, Fremont, N-4, N-51, Clone-11, N-38, CRS-4, N-43

Correlation studies

Pearson correlation between all the traits was calculated and unrelated traits depicting the highest correlation are represented in *Figure 6*. Fruit yield was strongly correlated with the fruit weight followed by seed weight, indicating that fruit weight and seed weight can be used as selection criteria during early years of fruit bearing in breeding programs for high yield. Similarly, LLT can give an idea about productivity in the early stages of growth, when trees are not in bearing. Most of the verities are concentrated in 95% confidence interval; some of the values lying outside depict high variability for the combination of traits. Linear fit of traits is shown by a straight line representing the direction of variability.
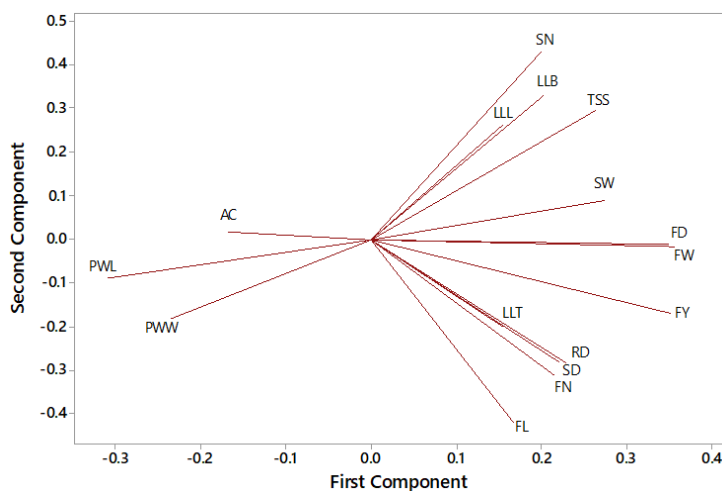
Principal Component Analysis

PCA simplified 18 parameters into five PC with eigen value > 1 (1.2672 to 5.5204) and cumulative per cent of the variance of 82.6 (*Table 5*). PC1 had effective parameters like fruit weight (0.356), fruit yield (0.351), and fruit diameter (0.351). PC2 had a high

contribution factor load from the average number of seeds per fruit (0.430), fruit TSS (0.296) and LLL (0.262). PC3 explained the difference with the highest positive factor loads from scion diameter (0.349) and rootstock diameter (0.309) and negative load from leaf lamina width (-0.384) and LLL (-0.333). PC4 explained the highest positive factor load of fruit length (0.236) and LLL (0.186) and negative factor load of fruit acidity (-0.642) and average seed weight of 20 seeds (-0.436). PC5 had highest contribution from rootstock diameter (0.446) and LLL (0.440). PCA allows the reduction of data dimensions (*Fig. 7*). The two main components of the analysis (herein referred to as PC1 and PC2) explained 34.5% and 17.2% of the total variance of the data, respectively.
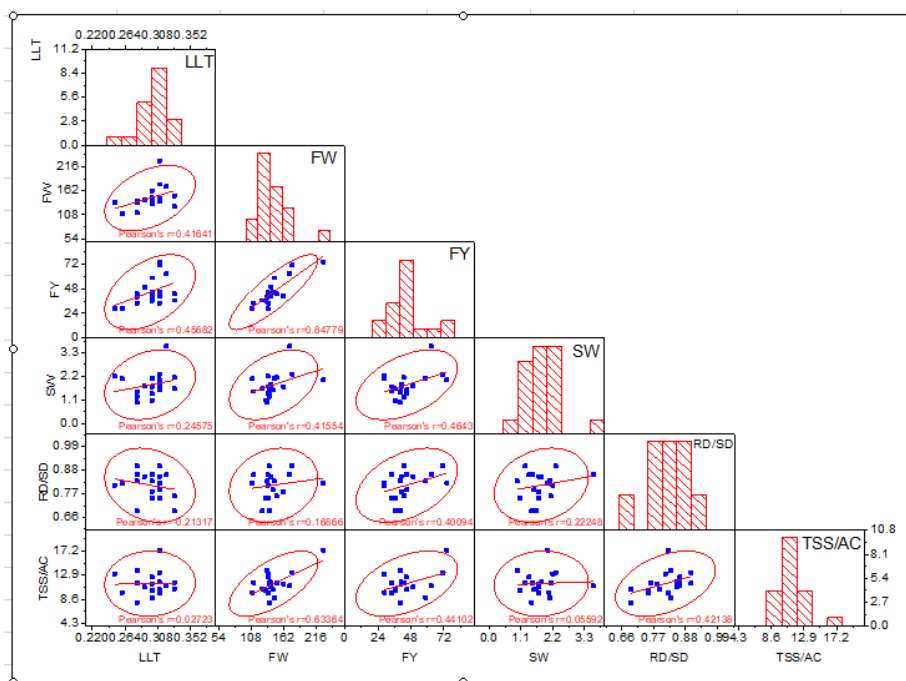
Figure 6. The scatter-matrix, hostogram, 95% confidence limit eclipse and Pearson's correlation matrix for leaf lamina thickness (LLT), fruit weight (FW), fruit yield (FY), seed weight (SW), rootstock diameter/scion diameter (RD/SD), and TSS/acidity (TSS/AC)

Figure 7. Principal component analysis (PCA) plot of the two principal axes

Table 5. *Eigen values, factor and contribution of first five principal components axes in mandarin genotypes for 14 traits*

Variables	Eigen values				
	PC1	PC2	PC3	PC4	PC5
Eigenvalue	5.5204	2.7584	2.302	1.3687	1.2672
Proportion	0.345	0.172	0.144	0.086	0.079
Cumulative	0.345	0.517	0.661	0.747	0.826
Rootstock diameter (mm)	0.22	-0.28	0.309	0.096	0.446
Scion diameter (mm)	0.23	-0.283	0.349	0.064	0.365
Leaf lamina length (mm)	0.155	0.262	-0.333	0.186	0.44
Leaf lamina breadth (mm)	0.203	0.33	-0.384	-0.021	0.245
Leaf lamina thickness (mm)	0.154	-0.201	-0.327	-0.025	-0.203
Petiole wing length (mm)	-0.309	-0.089	-0.285	0.162	0.304
Petiole wing width (mm)	-0.234	-0.182	-0.377	0.122	-0.017
Fruit weight (g)	0.356	-0.016	-0.212	0.161	-0.152
Fruit diameter (mm)	0.35	-0.012	-0.093	0.174	0.069
Fruit length (mm)	0.168	-0.419	-0.194	0.236	0.018
Fruit number per tree	0.216	-0.312	-0.08	-0.409	-0.192
Fruit yield per tree	0.351	-0.169	-0.184	-0.144	-0.209
Fruit TSS	0.264	0.296	0.123	-0.011	-0.102
Fruit acidity	-0.167	0.016	-0.145	-0.642	0.27
Av. no of seeds per fruit	0.201	0.43	0.157	0.091	-0.165
Av. seed weight of 20 seeds (g)	0.274	0.09	-0.037	-0.436	0.249

Discussion

The diameter of the rootstock and scion is crucial in determining the degree of compatibility of the stionic relationship between scion and rootstock. A significantly higher mean rootstock diameter was recorded in Darjeeling (124.82 mm) and the lowest was recorded in the CRS-4 (36.91 mm). However, the mean scion diameter was recorded in Khasi (97.44 mm) and the lowest scion diameter was found in genotype CRS-4 (27.75 mm). The maximum scion to rootstock ratio was observed in Kinnow which clearly shows its adaptive suitability under tropical conditions and compatibility with Rough lemon rootstock. The results further showed that Darjeeling, Khasi, Nagpur, Coorg, Nova, and genotype had higher scion to rootstock diameter ratio which indicated better compatibility of these genotypes with Rough lemon rootstock among all genotypes under Punjab conditions whereas, the CRS-4 genotype is not best suited for Rough lemon rootstock under these conditions due to its poor scion to rootstock diameter ratio. The variation in the scion to rootstock diameter ratio might be due to the interaction of genotypes with rootstock and environment. Furthermore, it had been hypothesized that the variation in rootstock diameter of different mandarin genotypes might be due to limited plant growth by bud union resistance to water transport and xylem anatomical characteristics, particularly the number and diameter of vessels and carbohydrate distribution (Martínez-Alcántara et al., 2013; Forner-Giner et al., 2014). Similarly, significant variation in rootstock diameter and scion to rootstock diameter ratio among citrus varieties had already been reported (Sayad et al., 2016; Baswal et al., 2017; Rattanpal et al., 2018; Sunaina et al., 2018; Singh et al., 2022).

The LLL to width ratio showed significant variation in diverse mandarin genotypes. This might be due to the close interaction among genotypes and the environment. It has been observed that variation in leaf characters was due to genotype and environmental factors (Hovenden and Schimanski, 2000; Hovenden, 2001; Gomez et al., 2003). Significant variation has been reported for leaf morphological characters in citrus accessions (Khan et al., 2008; Singh et al., 2010, 2021, 2022; Dorji and Yapwattanaphun, 2011; Sunaina et al., 2018).

The variation in fruit weight among mandarin genotypes ranged from 228.50 g (Daisy) to 110.17 g (N-38). The variation in fruit weight among mandarin genotypes might be due to differential translocation of photosynthates from fully developed leaves to developing fruits. Furthermore, it had been reported that increased pulp tissue caused an increase in fruit weight and fruit size. This can be attributed to cell division during the early stages of fruit development, caused mainly by the thickness of the peel tissue (Dalal et al., 2013). The fruit weight in mandarin genotypes ranged from 59.46 g in Cleopatra to 266.33 g in King, in the studies undertaken by Campos et al. (2005). The highest fruit number per tree was recorded in Kinnow which was significantly higher than all other genotypes except Mudhkhed seedless and W. Murcott. However, Daisy recorded the highest fruit yield (74.26 kg/tree) followed by Kinnow (71.06 kg/tree) and W Murcott (62.84 kg/tree) which was significantly higher than all other genotypes except Kinnow and W Murcott. Nova mandarin recorded the lowest fruit number and fruit yield among all genotypes under study. Significant variation in seed number per fruit among mandarin genotypes was observed during the investigation. Similarly, the variation in the number of bold seeds and the abortive seeds were also observed previously (Altaf et al., 2008; Fatima et al., 2010). Similarly, Kinley and Chinawat (2011) and Dorji and Yapwattanaphun (2011) observed significant variation in fruit number, fruit yield, and seed number in mandarin germplasm.

The maximum TSS was recorded in Kinnow fruits followed by Daisy and Nova. However, the minimum fruit acidity was observed in mandarin genotype Daisy. The variation in fruit TSS and acidity among mandarin genotypes might be due to the seasonal dynamics of carbohydrates and acids in the fruits due to starch hydrolysis during the maturation period. Similarly, in different studies, significant variation in fruit weight, diameter, length, TSS, and acidity had been reported in mandarin genotypes (Khan et al., 2008; Kinley and Chinawat, 2011; Dorji and Yapwattanaphun, 2011; Sunaina et al., 2018; Singh et al., 2022).

A significant variation in morphological characters among mandarin genotypes was observed in this study. The findings are in agreement with the observations made by some other authors (Dorji and Yapwattanaphun, 2011) who reported the highest significant dissimilarity in quantitative traits between 39 mandarin accessions in Bhutan. It has been reported that the mandarin genotypes represented variations of single clones (Dorji and Yapwattanaphun, 2015). Consequently, resemblance among genotypes in our findings also supported the statement that mandarins in the USA and India might occur from a variation of a single clone. The present findings also revealed the fact that the presence of assorted accessions in mandarin genotypes despite accessions exhibited the same morphological qualitative traits. This might be due to the diverse action of evolutionary forces and environmental attributes. In an open field survey, Paudyal and Haq (2008) attributed 40% of variation to environmental factors in pummelo accessions. Similarly, vegetative differences in individual accessions were also reported by Dorji and Yapwattanaphun (2011), they attributed this phenotypic variation to cross-pollination,

natural or induced mutations, and environment interactions. During the current investigation, the high level of significant variation in quantitative vegetative attributes specified the existence of diversity in mandarin germplasm and hence the hypothesis of this study has been accepted. The cluster studies revealed that the mandarin germplasm though collected from the different agro-ecological zones formed sub-groups without clear demarcation by region.

Furthermore, in this study, rootstock diameter, scion diameter, fruit weight, the weight of 20 seeds showed a higher genotypic coefficient of variance, genetic advance, heritability, and genetic advance percentage of means. The phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all the traits under study. High heritability, high genetic advance along higher genotypic coefficient of variance gives good information in terms of selection advance than any parameter alone (Paudyal and Haq, 2008). This suggests the occurrence of additive gene action with low environmental influence for the determination of these traits, compared to other traits, and could be valuable in the phenotypic selection of mandarin genotypes through devising appropriate correlated inheritance breeding strategies (Wera et al., 2014). However, low genetic advance with low heritability observed for leaf characters, fruit juice TSS and acidity indicated the presence of intra and inter allelic interactions. In terms of character scores, PC1 distinguishes positive correlations between fruit weight, fruit diameter, and fruit yield, and negative relationships between petiole wing length and petiole wing width. Similarly, PC2 highlights the positive correlation between seed number per fruit, LLB, LLW, and TSS besides, it contrasts with the highly negatives value of fruit length.

No doubt, quantifying the seed parameters is more complicated than simply measuring the fruit yield directly. However, the positive correlation between yield and other traits can be exploited in early stages of fruit tree breeding. In this study, fruit yield was positively correlated with fruit weight, seed weight, and leaf lamina thickness (LLT). Though high seed weight is undesirable, yet this trait along with fruit weight and LLT will help in selection of genotype during early years of its bearing. It is pertinent to note that hybrid mandarin seedlings come into bearing after 6-7 years. At this stage variety are selected for yield and quality. Though fruit quality potential of seedlings can be assessed in first year of bearing, but for evaluating yield potential of seedlings additional 4 to 6 years are required. These correlations will help in reducing this breeding period.

Conclusion

This study showed that the mandarin genotypes used in the current investigation differ phenotypically. The study concluded that the occurrence of additive gene action with low environmental influence is responsible for the determination of rootstock diameter, scion diameter, fruit weight, and seed weight compared to other traits under study. Furthermore, a strong correlation of fruit yield with LLT, fruit weight and seed weight had been observed in our study, indicating that LLT, fruit weight and seed weight can be used as selection criteria for the genotypes at early years of fruit bearing to shorten the crop improvement programme in mandarin.

Conflict of interests. The authors declare that they have no conflict of interests in publishing this manuscript.

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