Environment and Ecology 40 (3) : 1046—1052, July—September 2022
ISSN 0970-0420

Varietal Assessment and Genetic Variability Studies of Pummelo (*Citrus grandis* (L.) Osbeck) Accessions under Mid Hill Conditions

Thejangulie Angami, H. Kalita, Lungmuana, Letngam Touthang, Badapmain Makdoh, Gerik Bagra, Raghuveer Singh, K. Suraj Singh

Received 6 February 2022, Accepted 19 April 2022, Published on 15 July 2022

ABSTRACT

Preliminary work was conducted for varietal assessment and genetic variability studies of pummelo found in mid hill conditions of Arunachal Pradesh. Wide variability was observed among the accessions which provides opportunities for genetic gain through selection or hybridization viz. fruit weight (567.52 – 1581.48 g), number of segments per fruit (13.00 - 114.33), peel thickness (1.22 - 3.26 cm), number of segments per fruit (13.17 - 18.00), juice percent (14.40 - 20.54), TSS (7.50 - 10.75 ºB), titratable acidity (0.39 - 1.71 %), ascorbic acid (27.57 - 48.28 mg/100 ml), total sugar (5.53 - 9.85 %) and total phenols (2.01 - 3.31 mg/100 ml). The PCV and GCV was higher for the characters like number of seeds per fruit (63.94 and 64.09) followed by titratable acidity (42.61 and 63.29), fruit weight (33.14 and 33.20) and peel thickness of (31.49). The higher genetic advance was recorded by number of seeds per fruit (131.40) followed by fruit weight (68.17), peel thickness (64.79), titratable acidity (56.96) respectively making these characters as an important tool for selection process. The Principal Component Analysis (PCA) verified the first four components accounted for 84.9 % of the total variance into variables (PC-1: 36 %, PC-2: 19.6 %, PC-3: 16.5 % and PC-4: 12.9 %) respectively.

Keywords Pummelo, Variability, PCV, GCV, Heritability.

INTRODUCTION

Pummelo (*Citrus grandis* (L.) Osbeck.), one of the monoembryonic species of citrus fruit is considered an underutilized citrus fruit in the North-eastern region of India, mostly found as one of the backyard crop in homestead garden without commercial cultivation. Pummelo is an important component to human diet providing a variety of constituents important to human nutrition including vitamin C (Bharali *et al*. 2017). It is a fat, sodium and cholesterol free, making it a very good source for dieters. The North-eastern region of India with its diverse soil physiographic and climatic variability is considered an abode for many *citrus* species (Hazarika *et al*. 2016). Till now,

Thejangulie Angami[1]*, H. Kalita[1], Lungmuana[2], Letngam Touthang[1], Badapmain Makdoh[1], Gerik Bagra[1], Raghuveer Singh[1], K. Suraj Singh[3]

[1]ICAR (RC) for NEH Region, Arunachal Pradesh Center, Basar 791101
[2]ICAR (RC) for NEH Region, Mizoram Center, Kolasib 976081
[3]ICAR – KVK, West Siang 791101, Arunachal Pradesh, India

Email: thejaangami@yahoo.com
*Corresponding author

no standard variety of pummelo has been released. However, the diverse eco-geographical, occurrences of spontaneous mutation and natural hybridization have given rise to a wide variability in pummelo in the region but have minimally been used for improvement programs due to lack of characterization. The variability study is an essential component for the assessment of genetic diversity and selection. Clonal selection is considered an effective method for genetic improvement and selection of new genotypes in fruit crops. Even today, it has been deployed as an initial step for cultivar identification and diversity assessment. For any crop improvement program, assessment of genetic variability of collected germplasm is an important step. Being a complex character, yield is influenced by a number of yields and yield attributing characters, by environment, and by polygenes. Thus, the variability in the collections for these characters is the sum total of heredity effects of concerned genes and the influence of the environment. Hence, it is very essential to partition the observed variability into heritable and non heritable components measured as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), narrow sense heritability (h^2), broad sense heritability (H^2), genetic advance (GA), and genetic advance expressed as percent mean (GAM %). Surveys of genetic variability with the help of suitable parameters such as GCV, heritability estimates and GA are absolutely necessary to start an efficient breeding program as reported by Atta *et al*. (2008). Heritability value alone may not provide clear predictability of the breeding value. However, heritability in combination with genetic advance over mean (GAM) is more effective in predicting the resultant effect of selection (Patil *et al*. 1996, Ramanjinappa *et al*. 2011). Genetic advance is also of considerable importance as it indicates the magnitude of the expected genetic gain from one cycle of selection (Hamdi *et al*. 2003). In spite of tremendous potentiality and huge genetic diversity, very little work has been done and due importance has been given highlighting the distinguishable morphological features. Keeping in view the availability of wide range of pummelo strains, preliminary study was carried out with the aim to screen the superior accessions based on physico-biochemical attributes under mid hill condition considered most suitable with respect to horticultural aspects.

MATERIALS AND METHODS

Considering the vast spread of pummelo trees, assessment of genetic diversity in their natural habitat and identification of superior accessions from different villages in West Siang district of Arunachal Pradesh, were carried out during 2016 and 2017. The details and photos of different accessions of pummelos along with their sources of collection with their GPS coordinates are given in Table 1 and Fig.1. The experiment was laid out in Completely Randomized Block Design with three replications. Plants of uniform age (10-15 years) of seedling origin were selected during the course of investigation. Three fully mature, healthy and disease free fruits from each replication were collected randomly from different direction for

Table 1. Sources of collection with GPS coordinates.

Accessions	Color of fruit flesh	Collection site(village)	Latitude(N)	Longitude(E)	Elevation(m)
P-1	White fleshed	Pagi	27º57' 38" N	94º43'05" E	838
P-2	White fleshed	Nyigam	27º58'22" N	94º41'02" E	624
P-3	Light reddish fleshed	Lipu Namchi	28º00'12" N	94º44'42" E	850
P-4	Light reddish fleshed	Nyodu	27º58'58" N	94º39'52" E	653
P-5	Light reddish fleshed	Eshi Chiku	28º00'08" N	94º45'23" E	927
P-6	Reddish fleshed	Gori - III	27º59'24" N	94º42'04" E	664
P-7	Reddish fleshed	Bam	28º01'46" N	94º40'41" E	710
P-8	White fleshed	Bam	28º01'14" N	94º40'47" E	695
P-9	Light reddish fleshed	Bam	28º00'57" N	94º40'52" E	648
P-10	Reddish flesh	Daring	27º51'57" N	94º48'04" E	349
P-11	Reddish flesh	Soi	27º58'55" N	94º42'10"E	731
P-12	Light yellow flesh	Bagra	28º04'10" N	94º45'38" E	459

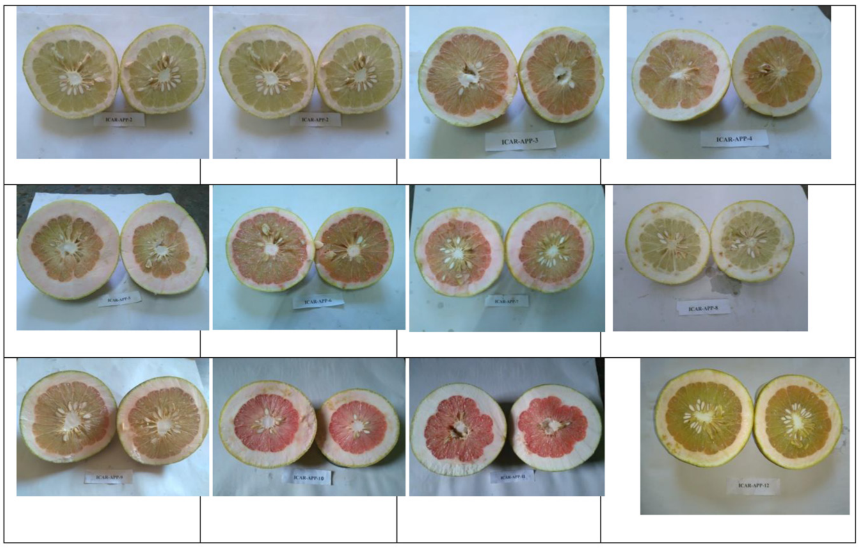

Fig. 1. Different accessions of pummelos.

recording different observations. Physico-chemical characterization of these pummelo accessions was done using citrus descriptor cited by IPGRI, Rome, Italy). Fruit characteristics were observed for fruit weight, number of seeds per fruit, peel thickness, number of segments per fruit, juice percentage, TSS, titratable acidity, ascorbic acid, total sugar, total phenols. Total soluble solids content of fully mature fruits was recorded using Digital Hand Refractometer. Titratable acidity was estimated by titrating a known volume of pulp juice extracted against 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator (AOAC 2000). Ascorbic acid content was determined by titrating sample filtrate in 4% oxalic acid using 2, 6-Dichlorophenol indophenol dye to a pink point and expressed as mg/100 g (AOAC 2005). Total sugar was estimated by Anthrone method as described by (Sadasivam and Manickam 2005). Total phenol was determined using the Folin-Ciocalteau method (Singleton and Rossi 1965). The phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) were computed as per the method suggested by Singh and Chaudhury (1985), heritability % (h^2) were estimated according to Falconer (1989), genetic advance (GA) and genetic advance percentage of mean were calculated as per the procedure recommended by Singh and Chaudhury (1985) and Allard (1960). Statistical analysis were performed using Statistical Analysis System 9.3 computer software (SAS Institute Inc. 13). DMRT procedure was used at p = 0.05 level to determine if there were significant differences among the means. A principle component analysis was conducted with XLSTAT to assess the patterns of variation considering all the characters. A frequency analysis was performed with the SAS package.

RESULTS AND DISCUSSION

The analysis of variance for different characteristics studied were found significant ($p < 0.05$) among the 12 pummelo accessions. The physico-chemical traits studied given in Tables 2 - 3 revealed a wide variability and thus can be considered as traits of interest in breeding programs of pummelo viz., fruit weight (567.52 - 1581.48 g per fruit), number of seeds per fruit (13.00 - 114.33), peel thickness (1.22 - 3.26 cm), number of segments per fruit (13.17 - 18.00), juice percent (14.40 - 20.54), TSS (7.50 - 10.75 °B), titratable acidity (0.39 - 1.71 %), ascorbic acid (27.57 - 48.28 mg/100 ml), total sugar (5.53 - 9.85 %) and total phenols (2.01 - 3.31 mg/100 ml). The highest mean fruit weight (1581.48 g) was recorded in the accession P-5 while the lowest (567.52 g) was recorded in the accession P-4. Least number of

Table 2. Physical characteristics of different pummelo accessions.

Accessions	Fruit weight(g)	Number of seeds per fruit	Peel thickness (cm)	Number of segments per fruit	Juice%
P-1	1043.72[d]	44.67[d]	2.07[f]	16.33[abc]	16.61[bc]
P-2	942.74[e]	104.67[b]	1.24[k]	16.50[abc]	15.91[bc]
P-3	606.93[g]	13.00[g]	1.57[j]	15.33[bc]	18.08[ab]
P-4	567.52[h]	29.67[e]	1.70[i]	15.67[bc]	14.40[c]
P-5	1581.48[a]	30.00[e]	3.26[a]	15.50[bc]	15.61[bc]
P-6	772.31[f]	20.67[f]	1.22[k]	16.83[ab]	20.54[a]
P-7	1089.00[c]	102.67[b]	2.32[e]	14.33[cd]	15.92[bc]
P-8	753.72[f]	70.67[c]	2.86[c]	15.33[bc]	16.50[bc]
P-9	1120.50[c]	67.33[c]	1.94[g]	17.33[ab]	17.47[b]
P-10	784.01[f]	42.33[d]	2.54[d]	13.17[d]	17.94[ab]
P-11	1272.41[b]	27.00[e]	2.96[b]	15.83[abc]	18.14[ab]
P-12	596.22[g]	114.33[a]	1.85[h]	18.00[a]	17.15[bc]
Mean	927.55	55.58	2.13	15.85	17.02
Range	567.52 - 1581.48	13.00 - 114.33	1.22 - 3.26	13.17 - 18.00	14.40 - 20.54
CV	33.16	63.99	31.50	8.21	9.29
SE	88.79	10.27	0.19	0.38	0.46
LSD (0.05)	33.05	5.37	0.05	1.96	2.49

seeds per fruit (13.00) was observed in the accession P-3 meanwhile accession P-12 recorded the highest number of seeds per fruit (114.33). Minimum peel thickness (1.22 cm) was recorded in the accession P-6 while the accession P-11 exhibited the maximum peel thickness (3.26 cm). Highest number of segments per fruit (18.00) and juice percent (20.54) was observed in the accessions P-12 and P-6 respectively. Bio-chemical traits like total soluble solids (TSS) was reported maximum (10.75 °Brix) in the accession P-6 which also exhibited highest total sugar content (9.85 %) and lowest titratable acidity (0.39 %). Meanwhile the accession P-4 recorded the highest phenol content with 3.31 mg/100 ml whereas the accession P-12 exhibited the lowest (2.01 mg/100 ml) phenol content. Consequently, the present study illustrated the existence of wide ranges of variations for most of the characters among the pummelo accessions, which provides opportunities for genetic gain through selection or hybridization. These variations in

Table 3. Bio-chemical characteristics of different pummelo accessions.

Accessions	TSS(°Brix)	Titratable acidity (%)	Ascorbic acid (mg/100 ml)	Total sugar(%)	Total phenols (mg/100 ml)
P-1	10.17[abcd]	0.64[bc]	43.35[ab]	9.12[a]	3.02[ab]
P-2	9.17[ef]	1.24[ab]	48.28[a]	8.15[a]	2.91[bc]
P-3	9.33[cde]	0.68[bc]	40.08[abc]	8.74[a]	3.13[ab]
P-4	10.67[a]	0.42[c]	39.11[abc]	9.35[a]	3.12[ab]
P-5	8.33[fg]	1.24[ab]	36.07[abc]	7.64[ab]	3.31[a]
P-6	10.92[a]	0.39[c]	34.57[bc]	9.85[a]	2.87[bc]
P-7	9.67[bcde]	0.68[bc]	43.00[ab]	9.16[a]	2.49[d]
P-8	10.25[abc]	0.64[bc]	40.08[abc]	9.80[a]	3.01[ab]
P-9	10.75[a]	0.43[c]	42.49[ab]	9.83[a]	2.65[cd]
P-10	7.50[g]	1.71[a]	34.47[bc]	5.53[b]	2.18[e]
P-11	10.33[ab]	0.64[bc]	30.56[bc]	9.52[a]	2.65[cd]
P-12	9.25[de]	0.75[bc]	27.57[c]	8.87[a]	2.01[e]
Mean	9.69	0.79	38.30	8.80	2.78
Range	7.50 - 10.92	0.39 - 1.71	27.57 - 48.28	5.53 - 9.85	2.01 - 3.31
CV	10.67	50.77	15.35	14.00	14.26
SE	0.30	0.12	1.70	0.36	0.11
LSD (0.05)	0.85	0.62	11.51	2.14	0.32

Table 4. Estimates of variability among fruit characteristic traits in different pummelo accessions. (GCV: Genotypic coefficient of variance, PCV: Phenotypic coefficient of variance).

Characters	Genotypic variance	GCV	Phenotypic variance	PCV	Heritability in broad sense	Genetic advance	Genetic advance (% mean)
Fruit weight (g)	94509.07	33.14	94805.86	33.20	99.69	632.29	68.17
No. of seeds per fruit	1262.83	63.94	1268.94	64.09	99.52	73.03	131.40
Peel thickness (cm)	0.45	31.49	0.45	31.49	100.00	1.38	64.79
Number of segments per fruit	1.35	7.33	2.38	9.74	56.57	1.80	11.36
Juice (%)	1.70	7.67	4.09	11.89	41.57	1.73	10.16
TSS (°Brix)	0.98	10.22	1.25	11.54	78.40	1.82	18.78
Acidity (%)	0.11	42.61	0.25	63.29	44.00	0.45	56.96
Ascorbic acid (mg/100 ml)	20.28	11.76	63.19	20.76	32.09	5.25	13.71
Total sugar (%)	1.02	11.48	2.51	18.00	40.64	1.33	15.11
Total phenols (mg/100 ml)	0.15	13.78	0.18	21.74	83.33	0.74	26.62

the traits may have been influenced by many factors such as variety, climate, soil and thus bound to vary (Heshi *et al*. 2001).

In the variability studies, genotypic coefficient of variance, phenotypic coefficient of variance, broad sense heritability and genetic advance (per cent mean) for ten recorded traits are shown in Table 4. The co-efficient of variance (both genotypic and phenotypic) was higher for the characters like number of seeds per fruit (63.94 and 64.09 respectively) followed by titratable acidity (42.61 and 63.29 respectively), fruit weight (33.14 and 33.20 respectively) and peel thickness of (31.49). PCV was found higher than GCV for all the characters studied, which signifies the presence of environmental influence to some degree in the phenotypic expression of characters (Mishra *et al*. 2015). A high PCV and GCV for the characters studied indicated that environment influences on the expression of these traits were minor as reported by Ahsan *et al*. (2015). PCV and GCV with higher value signified that the accessions showed evidence of much variation among them with respect to morphological and biochemical characters meanwhile, lowest values of PCV and GCV indicated that the accessions do not show much variation among themselves reported by Singh *et al*. (2008) and Punetha *et al*. (2011). GCV associated with high heritability indicated that selection would be effective for the improvement of these characters but for a character with low heritability, selection may be comparatively impractical due to masking effect of the environment on the genotypic effects (Burton 1952). This indicated that selection for number of seeds per fruit, titratable acidity, fruit weight and peel thickness would be effective. The genetic advance as percentage of means for ten traits ranged from 10.16 to 131.40. The higher genetic advance as percentage of mean was recorded by number of seeds per fruit (131.40) followed by fruit weight (68.17), peel thickness (64.79), titratable acidity (56.96) respectively. The characters exhibiting high heritability and genetic advance as percentage of mean could be used as powerful tool in selection process such characters are controlled by the additive genes and less influenced by the environment (Panse and Sukhatme 1995). Thus, in the present investigation high genetic advance (percentage of mean) coupled with high heritability was observed for number of seeds per fruit, fruit weight and peel thickness. These characters also showed the highest GCV and PCV, so selection would be highly effective for these traits.

Principal component analysis, a data reduction tool plays an important role in identifying important traits responsible for differentiation between genotypes which at the same time, revealed some aspects of interrelation that were not interpretable by the cluster analysis. Hair *et al*. (1998) suggested that eigen values greater than one are significant and should only be considered so that fewer components are dealt with. Furthermore, according to Guei *et al*. (2005), first three principal components are often the most important in reflecting the variation patterns among the different genotypes and the characters associated with these are most important in differ-

Table 5. Principal component loadings of different studied parameters.

Characters	PC 1	PC 2	PC 3	PC 4
Fruit weight	-0.27	0.57	-0.21	0.56
No. of seeds	-0.04	-0.36	0.77	0.49
Peel thick	-0.52	0.38	-0.39	0.56
No. of segment	0.69	-0.26	0.10	0.32
Juice	0.18	-0.51	-0.61	-0.22
TSS	0.94	0.13	-0.09	0.08
Acidity	-0.94	-0.07	0.14	-0.10
Ascorbic acid	0.13	0.54	0.66	-0.30
Total sugar	0.93	0.14	-0.05	0.25
Phenol	0.26	0.84	-0.09	-0.33
E value	3.60	1.96	1.65	1.29
Prop	36	19.6	16.5	1.29
Cumm	36	55.6	72.1	84.9

entiating various genotypes. Hence, the first four components were extracted to explain the variability existed among the 12 accessions shown in Table 5 and biplot diagram in Fig. 1.

The PCA verified the first four components accounted for 84.9 % of the total variance into variables (PC-1: 36 %; PC-2: 19.6 %; PC-3: 16.5 and PC-4: 12.9%) respectively. PC-1 showed high positive loadings on parameters such as number of seeds per fruit, TSS and total sugar suggesting that these parameters are have high relationship between each other and increased with the decrease peel thickness and titratable acidity. PC-2 remarks the relation of fruit weight and parameters such as ascorbic acid and total phenols and the increase in these parameters may reduce the juice percentage. PC-3 again showed the potential of juice reduction due to number of seeds per fruit and ascorbic content while PC-4 remarks the relationship between fruit weight, number of seeds per fruit and peel thickness. The relationship between the studied parameters and local accessions were done by plotting PC-1 and PC-2 (Fig. 2). P-1, P-3, P-4, P-6, P-8, P-9, P-11 and P-12 were on the positive side of PC-1 and these accessions were highly differentiated by parameters like TSS, TS and number of seeds per fruit while ascorbic acid and total phenols influence P-1, P-2, P-3, P-4, P-5, P-7, P-8 and P-11.

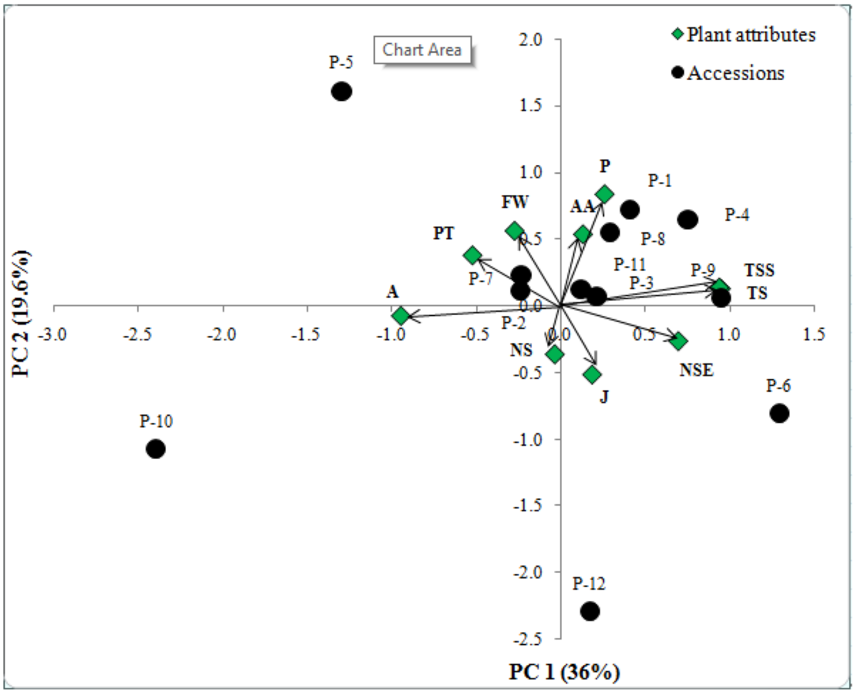

Fig. 2. Scoring biplot of PCA (PC1 Vs PC2) for pummelo accessions based on physico-chemical characters.

ACKNOWLEDGEMENT

To the Director, ICAR (Research Complex) for NEH Region, Umiam, Meghalaya. Name of project: Evaluation of Tropical and Sub-tropical Fruit Trees (IXX08906)

REFERENCES

Ahsan MZ, Majidano MS, Bhutto H, Soomro AW, Panhwar FH, Channa AR, Sial KB (2015) Genetic variability, coefficient of variance, heritability and genetic advance of some *Gossypium hirsutum* L. accessions. *J Agric Sci* 72 (2): 147-151.

Allard RW (1960) Principles of Plant Breeding. John Wiley and Sons. New York, USA.

Association of Official Analytical Chemists (2000) Official methods of analysis of the AOAC international. 17th (ed). Washington, DC.

Association of Official Analytical Chemists (2005) Official methods of analysis of the AOAC. 18th (ed). Washington, DC.

Atta BM, Haq MA, Shah TM (2008) Variation and interrelationships of quantitative traits in chickpea (*Cicer arietinum* L.). *Pak J Bot* 40 : 637-647.

Bharali R, Bhattacharyya RK, Das P (2017) Comparison of fruit quality parameters among white, pink and red pulp pummelos of Assam. *Bull Env Pharmacol Life Sci* 6 Special issue (1) : 26-28.

Burton GW (1952) Quantitative inheritance in grasses. Proc. VI International Grassland Congress, pp 277-283.

Guei RG, Sanni KA, Fawole AFJ (2005) Genetic diversity of rice (*Oryza sativa* L.). *Agronomie Africaine* 5: 17-28.

Hair JF, Anderson RE, Tatham RL, Black WC (1998) Multivariate data analysis. Prentice Hall, New Jersey.

Hamdi A, El-Ghareib AA, Shafey SA, Ibrahim MAM (2003) Genetic variability, heritability and expected genetic advance for earliness and seed yield from selection in lentil. *Egypt J Agric Res* 81: 125-137.

Hazarika TK, Lalthlamuani, Lalchhanmawia J, Lalrinfeli, Nautiyal BP (2016) Variability in physico-chemical characteristics and selection of superior types among local pummelo (*Citrus grandis* (L.) Osbeck) germplasms from Mizoram, North East India. *Curr Sci* 111(3): 1355-1361.

Heshi AB, Garande VK, Wagh AN, Katore HS (2001) Effect of pre-harvest sprays of chemicals on the quality of pomegranate fruit (*Punica granatum* L.) cv G-137. *Agric Sci Digest* 21: 25-27.

Mishra PK, Ram RB, Kumar N (2015) Genetic variability, heritability and genetic advance in strawberry (*Fragaria* × *ananassa* Duch.). *Turkish J Agric For* 39: 451-458.

Panse VG, Sukhatme PV (1995) Statistical methods for agricultural workers. 3rd (ed). ICAR, New Delhi pp 58.

Patil YB, Madalageri BB, Biradar BD, Hoshmani RM (1996) Variability studies in okra (*Abelmoschus esculentus* L. Moench.). *Karnataka J Agric Sci* 9: 289-293.

Punetha P, Rao VK, Sharma SK (2011) Evaluation of different chrysanthemum (*Chrysanthemum morifolium*) genotypes under mid hill conditions of Garhwal Himalaya. *Ind J Agric Sci* 81: 830-833.

Ramanjinappa V, Arunkumar KH, Hugar A, Shashibhaskar MS (2011) Genetic variability in okra (*Abelmoschus esculentus* L. Moench.). *Pl Arch* 11: 435-437.

Sadasivam S, Manickam A (2005) Biochemical methods, Revised 2nd (ed). New Age International Publication, pp 8-9.

Singh RK, Chaudhury BD (1985) Biometrical Methods in Quantitative Analysis. Kalayani Publishers, New Delhi, India.

Singh N, Raju KP, Prasad DVS, Bharadwaj KV (2008) Studies on genetic variability, heritability and genetic advance in French marigold (*Tagetes patula*) genotypes. *J Ornam Hortic* 12: 30-34.

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Vitic Enol* 16:144-158.