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Assessment of Genetic Variability of Wild Apple (*Malus spps*) Genotypes in Kashmir Valley

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Authors' contributions

This work was carried out in collaboration between all authors. Author SH carried out the study, wrote the protocol and wrote the first draft of the manuscript. Authors SH and HUR collectively managed the literature searches and author HUR carried out statistical analysis using windowstat software and managed the experimental process. Author KMB gave research topic and designed the study and acted as main mentor in research. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out in order to survey, document and characterize available germplasm of wild apple to obtain information on the variability and genetic divergence with respect to various traits. 33 genotypes of wild apple were collected. A significant difference in various fruit and leaf traits was observed although maximum range was observed for fruit weight i.e. 1.06 g in Selection 3 and 81.34 g in selection 20 followed by TSS (6.70°Brix in Selection 10 and 16.30 °Brix in Selection 4). Estimates of divergence among 33 wild apple genotypes revealed that significant divergence existed among them. The genotypes under study were grouped into 8 clusters as per Mahalanobis D^2 analysis with maximum number of genotypes in cluster I (17 genotypes) followed by cluster III (8 genotypes), cluster II (3 genotypes) and rest were monogenotypic. The maximum intra cluster distance was observed in cluster III (139.24) followed by cluster I (67.77) where as the inter cluster distance was maximum between cluster II and V (5213.52) followed by cluster II and IV (4895.20). Cluster means also showed significant differences in terms of various observed traits with maximum range in fruit weight (1.34 g in cluster IV to 79.19 g in cluster II) followed by TSS

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(6.70 ℃Brix in cluster IV to 16.30 ℃Brix in cluster V) . The crosses between the genotypes of cluster II with V and II with IV and cluster I with II are likely to exhibit high heterosis and produce recombinants with desired traits in segregating generations. Besides this, principle component analysis (PCA) was performed to study correlation and to interpret relationship among genotypes. Results revealed that PC1 variation observed was 88.19% while from PC2 variation was observed as 8.84%.

Keywords: Selection; range; cluster; heterosis; variation.

1. INTRODUCTION

Apple is economically the most important fruit tree crop due to its abundance in most of the temperate regions, fruit quality and propagation ease [1]. Apples have been cultivated since 4000 BC. The primary centre of *Malus* is within the region from Asia Minor to the Western China. The Old Silk Road from Central Asia to the Danube played an important role in the dispersal of the cultivated apple.

The apple belongs to the *Rosaceae* and has been classified into the subfamily *Pomoideae* and genus *Malus*. *Malus* wild species are widely distributed throughout North America, Europe, Asia Minor, and Asia and serve as potential genetic resources for the development of new apple cultivars and/or rootstocks adapted to diverse environmental conditions [2].

The genus *Malus* consists of about 27 wild species. Some of wild species are *Malus sieversii* (Lodeb), *Malus orientalis*, *Malus sylvestris* (L.) Mill, *Malus floribunda*, *Malus baccata* (L.) Borkh, *M. sikkimensis* (Wenzig) Koehne, *M. Komarovii* (Sarg.) Rehd. Among wild *Malus* species, *Malus sieversii* Lebed., native to Central Asia, has been recognized as a major progenitor of the domesticated apple, *Malus* × *domestica* Borkh [3]. The Himalayas are abundant in wild fruit species that are distinct from the tropical types found elsewhere in India. Himalayas provide suitable ecological niches for the prevalence of large number of temperate fruit germplasm [4].

The Indigenous crab apples distributed throughout Himalayan region constitute different *Malus* species. Seven types of *Malus baccata* and two types of *Malus sikkimensis* from different agro climatic regions have been collected at the IARI Regional Station for Horticulture at Shimla, India [5]. The genus *Malus* is characterized by a large diversity resulted from the accumulation of somatic mutations and fostered by the human activities during the long history of the cultivation, artificial crossing and transportation to distant habitats [6]. The novel diversity can be utilized in apple breeding programme to develop new varieties that will meet future market needs and opportunities, increase productivity, offer increased health benefits, reduce growing or handling costs, and reduce the risk of inbreeding depression [7]. Genetic diversity in crop species can be determined using morphological and agronomic characteristics, as well as biochemical and DNA marker analysis [8]. However, morphological and agronomic characteristics are greatly influenced by growth and environmental conditions.

Apple germplasm and maintenance of genetic diversity are important to future breeding programmes as this diversity gives species the ability to adapt to changing environments [9] and provide the raw material to breed new cultivars *via* hybridization [10] or selection [11].

This research was initiated to work toward preserving the genetic diversity of *Malus* germplasm in Kashmir region. The purpose of this work was to quantify, characterize and describe the variability of 33 local wild apple genotypes using phenotypic characters, to promote conservation and management of genetic resources, plant breeding strategies in all directions and to give opportunities to the local farmers. Also, the objective was to evaluate the fruit parameters in the wild apple gene pool and identify and utilize significant correlations between some pomological characters of interest in order to improve the choice of production objectives by using a limited number of characters. In addition, multivariate analysis was carried out to detect associations among genotypes and to identify the most useful variables for discrimination among genotypes.

2. MATERIALS AND METHODS

The present investigation was carried out in two districts of Kashmir valley viz Srinagar and Pulwama, located at an altitude of 1630 and 1585 meters above mean sea level respectively. The survey was conducted during 2014-2016

and observations were recorded as per the standard methods.

Thirty three diverse genotypes of wild apple were selected during survey on the basis of variability for various phenological traits. For the purpose of obtaining the data, trees of wild apple were located with the help of local habitants and earmarked for recording of observations. On each tree four branches from four sides were marked. 20 fruits were selected randomly out of minimum 100 fruits collected from the four marked branches. Fruit characteristics were measured on fruit harvested at full maturity stage. Data were collected for two consecutive years (2015-16). The following traits were characterized for each tree.

Standard methods were used for estimation of total 10 fruit physical and vegetative characters i.e. fruit length, fruit diameter, length of fruit stalk, fruit weight, number of seeds per fruit, leaf blade length, leaf blade width, petiole length, TSS and acidity [12]. Fruit length (cm) and its diameter (cm), length of stalk (cm), petiole length (cm), leaf blade length (cm) and its width (cm) were estimated using vernier calliper. Fruit weight (g) was measured with electronic balance. Number of seeds per fruit was estimated by cutting fruit and thereafter seeds were counted. Total soluble solids (°Brix) was recorded with the help of refractometer. Acidity was determined by taking a known volume of juice and titrating it against 0.1N NaOH solution using phenolphthalein as indicator. Acidity was expressed as percent maleic acid and calculated using the formula given below.

Titratable Acidity (TA) =

$$\frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Volume made up} \times 67}{\text{Weight of sample} \times \text{Aliquot taken} \times 1000} \times 100$$

3. RESULTS AND DISCUSSION

For the success of any breeding programme and for the selection to be effective genetic variability must be present in the breeding material. It is therefore necessary to assess the relative magnitude of variability in order to use such information, together with other selection parameters for the improvement of yield and quality of any fruit crop through adoption of effective breeding methods [13]. The extent of genetic variability indicates the potential of exercising selection of a particular genotype whereas heritability (h^2) along with genetic advance (per cent of mean) is most useful in predicting the resultant effect of selection of best genotype. Knowledge of extent of genetic variation and diversity for fruit phenology, quality, maturity and yield component traits in locally available wild apple accessions and subsequent identification of adapted superior genotypes as potential donors for yield and quality improvement is therefore essential. The accessions observed in this study could serve as an outstanding basis and source of germplasm for apple breeding aimed at developing new cultivars with desired characters. The apple being highly cross pollinated crop, each seedling raised plant is therefore a distinct genotype due to its heterozygous nature. Thus tremendous genetic variability is created which on the outer play of environmental conditions produces some excellent genotypes (possessing many desirable traits in a single plant).

3.1 Fruit and Leaf Characters

The measured fruit and leaf parameters are shown in the Table 1. The wild apple genotypes studied showed significant variation in fruit length (1.14 cm in Sel-3 to 5.21 cm in Sel-20). Other selections had intermediate values for fruit length. The maximum fruit diameter of 6.42 cm was recorded in Sel-20 followed by Sel-28 (5.83 cm). The minimum fruit diameter (1.17 cm) was recorded in Sel-3. The length of fruit stalk ranged from 0.60-5.20cm with maximum (5.20 cm) recorded in Sel-15 and minimum stalk length of 0.60 cm was recorded in Sel-17. Fruit weight showed variation among the 33 wild apple genotypes with maximum fruit weight in Sel-20 (81.34 g) followed by Sel-28 (80.68 g) and minimum fruit weight was recorded in Sel-3 (1.06 g). The maximum (10) number of seeds was recorded in Sel-7 whereas minimum (2.12) number of seeds was recorded in Sel-3. The TSS of the wild genotypes studied ranged from 6.70-16.30 °Brix. The maximum TSS (16.30 °Brix) was recorded in Sel-4 followed by Sel-2 (15.60 °Brix). The minimum TSS of 6.70 °Brix was recorded in Sel-10. The acidity of 33 wild genotypes studied ranged from 0.06-0.79%. The maximum (0.79%) was recorded in Sel-4 whereas minimum (0.06 %) in Sel-20.

Leaf blade length of the selected wild apple genotypes ranged from 6.26-12.06 cm. The maximum leaf blade length of 12.06 cm was recorded in Sel-29 whereas minimum leaf blade length of 6.26 cm was recorded in Sel-9. Leaf blade width of genotypes under study ranged from 2.73-7.23 cm with maximum (7.23 cm)

recorded in Sel-29 and minimum (2.73 cm) recorded in Sel-25. Petiole length ranged from 1.53-4.30 cm with maximum (4.30 cm) recorded in Sel-21 while minimum (1.53 cm) recorded in Sel-25.

Fruit size is an important marketing parameter determining economical value in horticultural crops especially for pear and apple [14]. Fruit size is also important parameter for selection of superior genotypes through breeding programmes [15]. All the genotypes in this study showed variation as for as the fruit size is concerned. The fruit length and diameter showed variation and ranged from 1.14 to 5.21 cm and 1.17 to 6.42 cm, respectively. The results were close to results found by Reim et al. [16] who recorded the fruit size between 1.8-5.1 cm and Reim et al. [16] also reported that the majority of the trees had fruit size less than 3.5 cm thus indicating a true type *Malus sylvestris*.

Another character observed was fruit weight which showed a huge variation and ranged from 1.06 to 81.34 g. Variation in fruit size and weight might be under control of genetic factors involving their phylogenic behaviour [17,18]. It is a well known fact that the genetics, environment and cultural practices all interact to determine fruit weight. Producing bigger fruits might be the inherent ability of genotype to utilize resources efficiently to achieve a certain fruit size [19]. The results of this study were close to results of Mratinic and Aksic [12] who reported fruit weight of 70 to 193.33 g in some Turkish *Malus* species and Gordana [20] who reported that fruit weight in some wild apples was 3.828 and 3.668 g.

Chemical aspects of fruits such as total soluble solid and acidity provide important information to the consumers in terms of recognizing a more nutritious fruit [21]. TSS is influenced by environmental factors such as temperature, light (duration and intensity), rainfall/supply of water and locations [22]. The variability in fruit characteristics especially in fruit composition is not only genetic factor but also influenced by climatic factors [23]. In the present study, total soluble solids (TSS) showed a range of 6.70-16.30 (^{o}Brix) and the titratable acidity showed a range of 0.06-0.79%. These results are in concordance with Mratinic and Aksic [24] who in their studies on phenotypic diversity of apple germplasm in South Serbia reported that the soluble solid in ^{o}Brix content varied from 12.55 to 19.24 and titratable acidity varied between 0.10 and 0.82%. Similarly, Khan et al. [25] also reported that the fruits of wild *Malus* species were small in size with high acidity and low sugar content. Kaya et al. [26] in their studies on fruit quality characters and genetic variability of apple germplasm in Turkey reported that the range for soluble solids content was 9.0%–14.4% and 0.15%–1.75% for titratable acidity.

The variability in various leaf characters might be due to genetic makeup and interaction with environment. The results were in close confirmation with Wohner et al. [27] who reported that the leaves in *Malus* species could reach a length of 8-11 cm.

3.2 Cluster Analysis

Based upon the performance of genotypes, 33 wild genotypes were grouped in to eight clusters (Table 2) as per Mahalanobis D^2 analysis employing Tocher's method [28]. The cluster anlysis (Fig. 1) indicated that maximum number of genotypes fall in cluster I (17) followed by cluster III (8), cluster II (3), cluster IV (1), cluster V (1), cluster VI (1) cluster VII (1) and cluster VIII (1). Cluster I consisted of Sel-14, Sel-27, Sel-32, Sel-1, Sel-21, Sel-5, Sel-33, Sel-3, Sel-13, Sel-6, Sel-8, Sel-18, Sel-16, Sel-15, Sel-2, Sel-19 and Sel-11, cluster II consisted of Sel-20, Sel-28 and Sel-24, Cluster III consisted of Sel-9, Sel-22, Sel-25, Sel-26, Sel-17, Sel-29, Sel-7and Sel-30 whereas genotypes Sel-10, Sel-4, Sel-12, Sel-31 and Sel-23 fall into cluster IV, V, VI, VII and VIII respectively.

In addition to grouping of selections into different clusters, non hierarchical analysis was also performed to identify the diverse and desirable selections in terms of inter cluster and mean performance of clusters for various characters, respectively. The average intra and inter cluster distance (D^2) values (Table 3 and Fig. 2) revealed that the cluster III had the highest intra cluster distance value of 139.24 followed by cluster I (67.77) and cluster II (27.72). The inter cluster distance was highest between cluster II and cluster V (5213.52) followed by inter cluster distance between cluster II and cluster IV (4895.20), cluster I and cluster II (4057.50), cluster II and cluster III (2324.69), cluster IV and cluster VIII (2239.19) and cluster V and cluster VIII (2239.19). The clusters IV and V were least divergent with inter cluster distance (79.16).

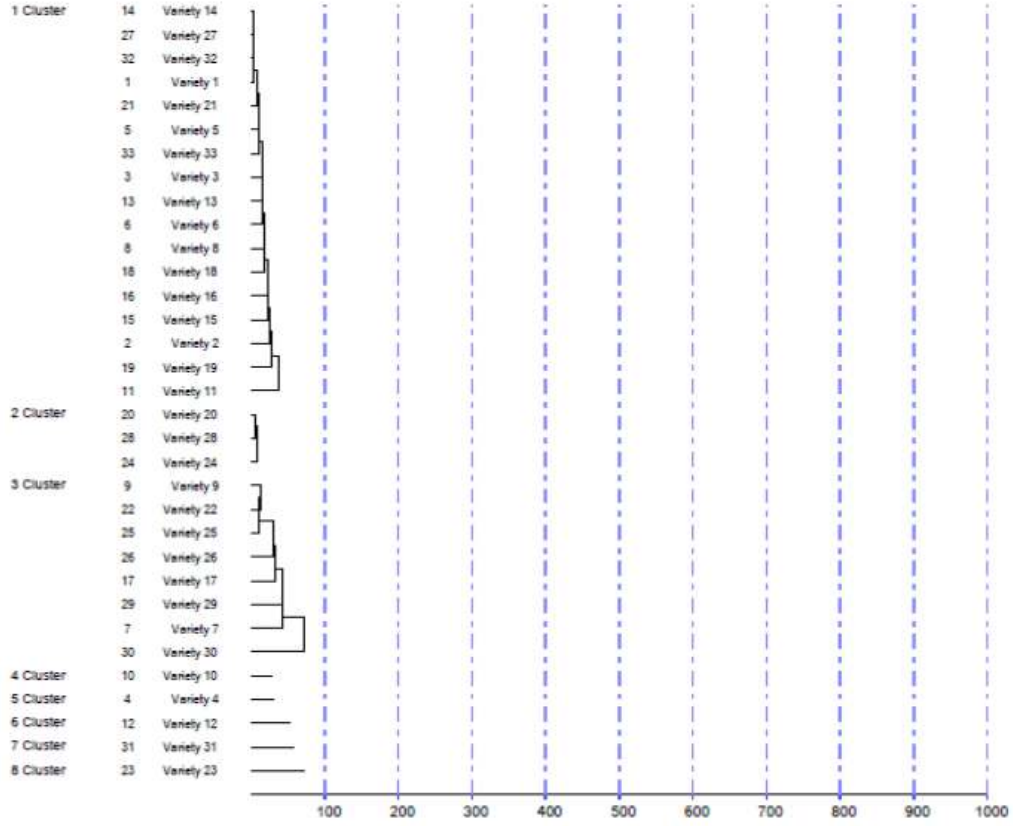

Fig. 1. Clustering of collected wild apple genotypes by Toucher method

The cluster means for different characters (Table 4) revealed that cluster I had maximum mean values for TSS (12.30 ºBrix), leaf blade length (8.64 cm) and fruit weight (5.52 g), cluster II had maximum mean values for fruit weight (79.19 g), TSS (13.20 ºBrix) and leaf blade length (7.50 cm). Cluster III had maximum mean value for fruit weight (22.89 g), TSS (12.25 ºBrix) and leaf blade length (8.19 cm). Cluster IV had maximum mean value for leaf blade length (8.75 cm) and TSS (6.70 ºBrix). Cluster V had maximum mean value for TSS (16.30 ºBrix), leaf blade length (10.60 cm), number of seeds (8.00). Cluster VI had maximum mean value for fruit weight (46.85 g), TSS (11.00 ºBrix), leaf blade length (8.90 cm). Cluster VII had maximum mean value for fruit weight (43.75 g), leaf blade length (7.60 cm) while minimum mean was observed for acidity (0.67%) where as cluster VIII had maximum mean value for fruit weight (47.43 g) followed by TSS (13.27 ºBrix).

Multivariate technique using D^2 statistics [29] is a very powerful and multivariate statistical tool in quantifying the degree of divergence among the genotypes [30]. In order to identify genetically diverse parents for hybridization, Mahalanobis D^2 statistics has been used in almost all crop species. Use of Mahalanobis D^2 statistics to estimate or evaluate the net/total divergence in breeding for crop improvement has been indicated by number of workers in different fruit crops [31,32]. The use of genetically divergent parents in hybridization under transgressive breeding programme depends on the categorization of breeding material on the basis of appropriate criteria [32,33,34]. Apart from providing requisite assistance or help in selection of divergent parents in hybridization, D^2 statistics also adequately assists in the measurement of diversification and the contribution of the relative proportion of each component trait towards the total genetic divergence or variation. This estimation of genetic divergence helps in reducing the large data of genotypes to manageable proportions. It is assumed that the parents showing wide genetic divergence are best suited for being used in the hybridization programmes. The utility of multivariate analysis in quantifying the degree of divergence between

populations so as to understand the trend of their evolutionary pattern and assess the relative contribution of different components to the total divergence together with the nature of forces operating at intra and inter cluster levels had greatly been emphasized [35].

In the present investigation 33 wild apple genotypes were evaluated to estimate the diversity as per Mahalanobis D^2 statistics and the analysis for divergence revealed that value of V statistics measuring Wilk's criteria were high and significant, indicating the presence of substantial genetic diversity in the material. This genetic variation can be effectively employed in intra-specific crosses with hope that would lead to transmission of higher genetic gain for desirable traits. The results were supported by Mir [36] who also studied D^2 statistics for wild apple germplasm.

The clustering of genotypes from different eco-geographic locations into one cluster could be attributed to exchange of breeding materials from one place to another and may be due to unidirectional selection practiced for a particular character at several places produced similar phenotypes which were aggregated in one cluster irrespective of their distant geographic origin [37]. Another case is where many genotypes originating from one place were scattered over different clusters and such diversity among genotypes of common geographic origin could be attributed to factors like heterogeneity, genetic architecture of the populations, past history of selection, developmental traits and degree of general combining ability [30].

The average intra and inter cluster distance (D^2) values were also measured and results revealed that the cluster III had the highest intra cluster distance value of 139.24 followed by cluster I (67.77) where as the inter cluster distance was highest between cluster II and cluster V (5213.52) followed by inter cluster distance between cluster II and cluster IV (4895.20), cluster I and cluster II (4057.50), cluster II and cluster III (2324.69). The cluster IV and V were least divergent with inter cluster distance (79.16).The results clearly indicate that tremendous potential exists for introgressing the allelic resource present in these genotypes through a systematic breeding and selection approach so as to recover the best quality recombinants. Cluster means for various morpho-taxonomic and quality related characters revealed that substantial variability existed for all the traits and identify the traits to be chosen for hybridization. The results were in conformity with Saran et al. [31], Sharma et al. [34], Mir [36], Pereira et al. [38] and Bhat and Dhillon [39].

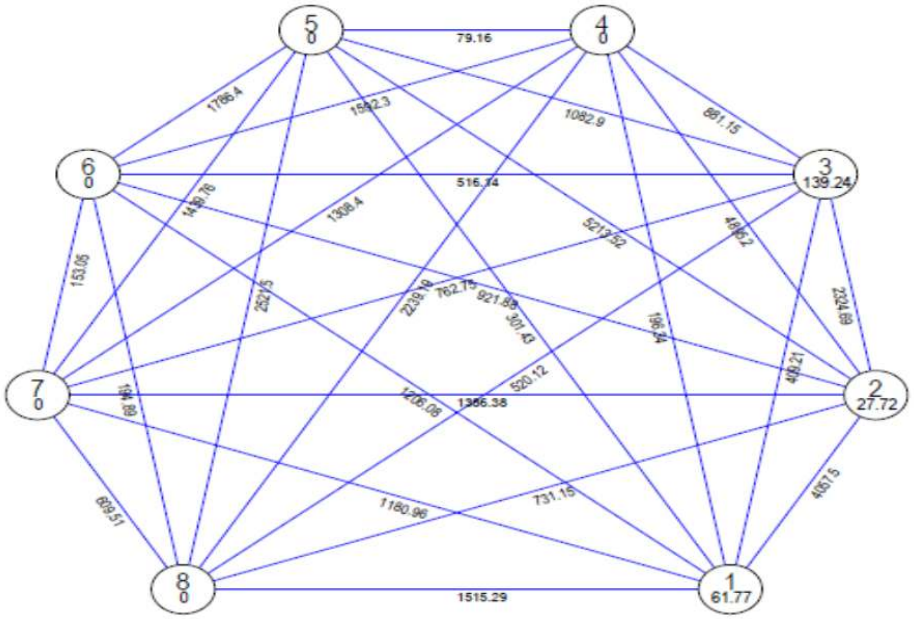

Fig. 2. Malalonobis Euclidean distance of various clusters by Toucher method

Table 1. Mean values of different traits of wild apple genotypes

S. no.	Selection	Fruit length (cm)	Fruit diameter (cm)	Length of stalk (cm)	Fruit weight (g)	Number of seeds/fruit	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (cm)	TSS (^{o} Brix)	Acidity (%)
1	Sel-1	1.43	1.37	3.60	1.37	2.32	8.24	4.20	4.06	10.20	0.58
2	Sel-2	2.76	2.66	1.63	6.77	2.51	9.67	7.16	2.90	15.60	0.54
3	Sel-3	1.14	1.17	3.60	1.06	2.12	8.44	4.23	1.90	15.50	0.43
4	Sel-4	1.30	1.34	3.96	2.22	8.44	10.60	5.66	2.73	16.30	0.79
5	Sel-5	2.54	2.49	3.53	7.70	3.25	9.53	4.30	3.40	12.00	0.46
6	Sel-6	1.21	1.36	3.50	1.19	3.56	9.90	5.00	1.86	15.80	0.48
7	Sel-7	3.38	3.38	2.43	20.48	10.00	9.50	5.60	2.60	12.60	0.26
8	Sel-8	2.23	2.29	0.90	6.20	6.89	10.70	5.46	3.73	9.70	0.39
9	Sel-9	2.11	2.60	1.80	18.76	4.91	6.26	3.20	3.86	14.70	0.20
10	Sel-10	1.18	1.32	3.40	1.34	5.34	8.56	5.33	3.23	6.70	0.72
11	Sel-11	2.26	2.73	2.73	9.17	6.72	7.00	3.50	3.46	13.00	0.25
12	Sel-12	3.16	3.79	1.06	46.85	7.33	8.900	4.90	2.30	11.00	0.37
13	Sel-13	1.78	1.95	2.60	3.68	3.21	9.73	5.93	2.50	11.00	0.40
14	Sel-14	1.82	2.00	2.70	3.75	6.53	7.70	3.26	3.20	11.10	0.44
15	Sel-15	2.16	2.66	5.20	8.66	4.23	9.06	4.30	3.13	11.60	0.38
16	Sel-16	2.18	2.56	4.33	10.34	5.29	9.06	4.46	2.66	10.50	0.42
17	Sel-17	2.92	4.05	0.60	27.08	3.26	8.83	4.26	2.73	7.40	0.10
18	Sel-18	1.72	2.09	1.46	5.16	2.31	7.36	4.76	1.90	14.90	0.40
19	Sel-19	2.49	2.87	4.33	11.08	7.56	7.20	4.50	2.53	11.10	0.50
20	Sel-20	5.21	6.42	0.93	81.34	7.00	7.30	5.13	2.00	11.90	0.06
21	Sel-21	1.94	2.33	3.73	6.06	5.26	7.50	4.30	4.30	14.30	0.48
22	Sel-22	2.48	2.94	2.03	14.60	3.33	8.06	4.26	2.56	16.00	0.18
23	Sel-23	4.29	4.67	1.10	47.43	2.51	7.93	4.33	1.66	13.27	0.10
24	Sel-24	4.19	4.48	1.76	75.55	6.00	7.90	4.26	2.23	13.70	0.11
25	Sel-25	2.54	3.40	0.80	16.41	2.51	6.56	2.73	1.53	12.60	0.08
26	Sel-26	3.52	3.92	2.23	26.92	3.22	7.21	4.00	4.00	13.00	0.21
27	Sel-27	1.97	2.14	2.86	3.91	3.53	9.16	3.70	3.86	11.30	0.53
28	Sel-28	4.79	5.83	0.90	80.68	4.21	7.30	4.86	2.10	14.00	0.17
29	Sel-29	3.23	3.85	3.30	26.20	3.71	12.06	7.23	4.13	11.60	0.24
30	Sel-30	4.23	4.37	1.50	32.69	5.00	7.00	5.00	2.50	10.10	0.36
31	Sel-31	4.98	4.78	1.90	68.75	6.33	7.60	4.40	1.76	12.50	0.67
32	Sel-32	1.23	1.45	3.14	1.45	2.27	7.43	3.82	2.90	10.10	0.51
33	Sel-33	2.86	2.78	2.33	6.34	2.52	9.23	5.21	2.63	11.40	0.57
Mean		2.67	3.04	2.48	19.88	4.64	8.44	4.64	2.81	12.31	0.37
CV %		17.23	16.03	15.41	3.66	23.46	6.63	8.13	15.52	7.34	5.29
CD@5%		0.75	0.79	0.62	1.18	1.63	0.91	0.61	0.71	1.47	0.03

Table 2. Distribution of wild apple genotypes into clusters based on D^2 statistics

S. no.	Cluster	Number of genotypes	Name of genotypes
1.	I	17	Sel-14, Sel-27, Sel-32, Sel-1, Sel-21, Sel-5, Sel-33, Sel-3, Sel-13, Sel-6, Sel-8, Sel-18, Sel-16, Sel-15, Sel-2, Sel-19, Sel-11
2.	II	3	Sel-20, Sel-28, Sel-24
3.	III	8	Sel-9, Sel-22, Sel-25, Sel-26, Sel-17, Sel-29, Sel-7, Sel-30
4.	IV	1	Sel-10
5.	V	1	Sel-4
6.	VI	1	Sel-12
7.	VII	1	Sel-31
8.	VIII	1	Sel-23

Table 3. Average intra cluster (Diagonal) and inter cluster (Above Diagonal) distance values in wild apple

S. no.	Cluster	I	II	III	IV	V	VI	VII	VIII
1.	I	**61.77**	4057.50	409.21	196.34	301.43	1206.08	1180.96	1515.29
2.	II		**27.72**	2324.69	4895.20	5213.52	921.88	1386.38	731.15
3.	III			**139.24**	881.15	1082.90	516.14	762.75	520.12
4.	IV				**0.00**	79.16	1592.30	1308.40	2239.19
5.	V					**0.00**	1786.40	1439.76	2521.50
6.	VI						**0.00**	153.05	194.89
7.	VII							**0.00**	609.51
8.	VIII								**0.00**

Table 4. Cluster means for various characters in different clusters of wild apple genotypes

S. no.	Cluster	Fruit length (cm)	Fruit diameter (cm)	Length stalk (cm)	Fruit weight (g)	Number of seeds/fruit	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (cm)	TSS (^{o}Brix)	Acidity (%)
1.	I	1.99	2.17	3.07	5.52	3.69	8.64	4.60	3.00	12.30	0.46
2.	II	4.99	5.91	1.20	79.19	5.67	7.50	4.76	2.11	13.20	0.11
3.	III	3.05	3.57	1.84	22.89	4.13	8.19	4.54	2.99	12.25	0.20
4.	IV	1.18	1.32	3.40	1.34	5.00	8.57	5.33	3.23	6.70	0.72
5.	V	1.31	1.35	3.97	2.22	8.00	10.60	5.67	2.73	16.30	0.79
6.	VI	3.16	3.80	1.07	46.85	7.00	8.90	4.90	2.30	11.00	0.37
7.	VII	5.21	6.02	1.90	43.75	6.00	7.60	4.40	1.77	12.50	0.67
8.	VIII	4.29	4.67	1.10	47.43	2.00	7.93	4.33	1.67	13.27	0.10

Table 5. Correlation between original variables and the first two principal components (PC) and contributions to the total variation (%) in apple germplasm

S. no.	Variable	Component loadings	
		PC1	PC2
1	Fruit length	0.066	0.020
2	Fruit diameter	0.045	-0.006
3	Fruit: length of stalk	-0.028	0.092
4	Fruit weight	0.921	0.254
5	Number of seeds	-0.222	0.562
6	Leaf blade length	-0.201	-0.058
7	Leaf blade width	0.061	-0.092
8	Petiole length	0.124	-0.256
9	TSS	-0.136	-0.073
10	Acidity	-0.126	0.725
Eigen value		13012.64	1304.72
% Var.		88.169	8.84

3.3 Principal Component Analysis

Principal component analysis (PCA) was performed to evaluate the wild apple genotypes and to study the correlation among different traits of these 33 wild apple genotypes (Table 5). Two components of PCA viz., PC1 and PC2 were studied to explain the trait variation. The first two principal components were able to explain the total variation among traits. PC1 accounted for 88.16% variation whereas the PC2 accounted for 8.84% variation. In particular the PC1 was positively and strongly associated with fruit weight (0.921) and negatively associated with number of seeds, TSS and acidity. Highest PC2 scores were for number of seeds (0.562) and acidity (0.725). The most desirable trait which showed variation was fruit weight (0.921).

Principal component analysis (PCA) one of the multivariate statistical procedures, has been used to study correlations among fruit traits to establish genetic relationship among cultivars within sets of apple cultivars. Associations between traits obtained from PCA may correspond to genetic linkage between loci controlling traits or a pleiotropic effect [40]. Principal component analysis has been studied previously to evaluate apple germplasm [41,42]. In the present study PCA was used to explain the variation among different genotypes of wild apple and the results revealed that PC1 accounted for 88.16% variation where as the PC2 accounted for 8.84% variation. In particular the PC1 was positively and strongly associated with fruit weight (0.921) and negatively associated with number of seeds, TSS and acidity. The most desirable trait which showed variation was fruit weight. The results were supported by Mratinic and Aksic [12] who reported that PC1, PC2 and PC3 accounted for 30.825%, 22.562% and 13.709% respectively of total variation.

4. CONCLUSION

It could be concluded that Jammu and Kashmir has rich germplasm of wild apple in terms of variability and genetic divergence with respect to various traits. High heritability estimates coupled with high genetic advance were observed for all the characters were indicative of additive gene action and selection based on these characters would be more reliable. The characters with high heritability coupled with high genetic advance further indicated the possibility of making selections in earlier generations. Also, this information can be efficiently utilized in future apple improvement programmes for evolving new and superior varieties. Besides these, this research could effectively be referred before initiating a work toward preserving the genetic diversity of *Malus* germplasm in Jammu and Kashmir.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pereira LS, Ramos CAM, Fischer. Breeding apple (*Malus* × *domestica* Borkh). In: Breeding plantation tree crops: Temperate species (Jain S.M., Priyadarshan P.M., eds). Springer New York, NY. 2009;33-82.
2. Hokanson S, Lamboy W, Szewc-McFadden A, McFerson J. Microsatellite (SSR) variation in a collection of Malus (apple)species and hybrids. Euphytica. 2001;118:281–294.
3. Forsline PL, Aldwinckle HS, Dickson EE, Luby JJ, Hokanson HC. Collection, maintenance, characterization, and utilization of wild apples of Central Asia. [Ed., Jules Janick]. Horticultural Reviews 2003;29:1-62.
4. Sharma YP, Pramanick KK. Utilization of plant genetic resources for the improvement of temperate fruit crops. Indian Journal of Genetics and Plant Breeding 2012;72(2):130-135.
5. Kishore DK, Pramanick KK, Sharma SK. Significance of crab apples in the Improvement of Apples. Acta Horticulturae. 2005;696:39-41.
6. Muzher BM, Younis RAA, EL-Halabi O, Ismail OM. Genetic identification of some Syrian local apple (*Malus* sp.) cultivars using molecular markers. Research Journal of Agriculture and Biological Sciences. 2007;3(6):704-713.
7. Kumar S, Volz RK, Alspach PA, Bus VGM. Development of a recurrent apple breeding programme in New Zealand: A synthesis of results and a proposed revised breeding strategy. Euphytica. 2010;173(2): 207-222.
8. Liu CJ. Geographical distribution of genetic variation in *Stylosanthes scabra* revealed by RAPD analysis. Euphytica. 1997;98: 21–27.
9. Bull JJ, Wichmann HA. Applied evolution. Annual Review of Ecology and Systematics. 2001;32:183-217.
10. Doebley JF, Gaut BS, Smith BD. The molecular genetics of crop domestication. Cell. 2006;7:1309–1321.
11. Dzhangaliev AD, Salova TN, Ture-khanova PM. The wild fruit and nut plants of Kazakhistan. Hoticulture Review. 2003;29:305-371.
12. Mratinic E, Aksic MF. Evaluation of phenotypic diversity of apple (*Malus* sp.) germplasm through the principle component analysis. Genetika. 2011; 43(2):331-340.
13. Williams EB, Shay JR. The relationship of genes for pathogenicity and certain other characters in *Venturia inaequalis* (Cke.) Wint. Genetics. 1957;42:704–711.
14. Gillaspy G, David H, Grussem W. Fruits: A developmental perspective. The Plant Cell. 1993;5:1439-1451.
15. Westwood MN, Blaney LT. Non-climatic factors affecting the shape of apple fruits. Nature. 1963;200:802-803.
16. Reim S, Proft A, Heinz S, Hofer M. Diversity of the European indigenous wild apple *Malus sylvestris* (L.) Mill. in the East Ore Mountains (Osterzgebirge), Germany: I. Morphological Characterization. Genetic Resources and Crop Evolution. 2012;59: 1101–1114.
17. Cowman AK, Cripps RF, Richings EW, Taylor NJ. Fruit size: Towards an understanding of the metabolic control of fruit growth using avocado as a model system. Physiologia Plantarum. 2001;111: 127-136.
18. Harada T, Kurahashi W, Yanai M, Wakasa Y, Satoh T. Involvement of cell proliferation and cell enlargement in increasing the fruit size of *Malus* species. Scientia Horticulturae. 2005;105: 447–456.
19. Stanley CJ, Tustin DS, Lupton GB, McArthney S, Cashmore WM, De Silva HN. Towards understanding the role of temperature in apple fruit growth responses in three geographical regions within New Zealand. Journal of Horticultural Science and Biotechnology. 2000;5:413-422.
20. Gordana S. Morphological characteristics of fruits of selected types of wild apple (*Malus sylvestris* L.) in the area of Bijelo

Polje. Agriculture & Forestry. 2013;59(2): 167-171.

21. Drogoudi PD, Michailidis Z, Pantelidis G. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Scientia Hortculturae. 2008;115:149-153.
22. Ahmed M. Biodiversity in pears (*Pyrus spp.*): Characterization and conservation of germplasm from Azad Jammu and Kashmir. Ph.D. Thesis submitted to Department of Horticulture University College of Agriculture Bahauddin Zakariya University Multan; 2008.
23. Wang CY. Pear fruit maturity, harvesting, storage and ripening. In: The Pear (Eds. Zwet, T. and Childers, N.F.). Horticultural Publisher, Gainesville. 1982;431-443.
24. Mratinic E, Aksic MF. Phenotypic Diversity of Apple (*Malus* sp.) Germplasm in South Serbia. Brazilian Archives of Biology and Technology 2012;55(3):349-358.
25. Khan M A, Olsen KM, Sovero V, Kushad MM, Korban SS. Fruit quality traits have played critical roles in domestication of the apple. Crop Science. 2014;1-43.
26. Kaya T, Balta F, Sensoy S. Fruit quality parameters and molecular analysis of apple germplasm resources from Van Lake Basin, Turkey. Turkish Journal of Agriculture and Forestry. 2015;39:1-12.
27. Wohner T, Ali ME, Peil A. Wildspecies Apple: *Malus* × *robusta* In: Julius Kühn-Institut Federal Research Centre for Cultivated Plants, JKI data sheets- Fruit Varieties. 2014;1:1-20.
28. Rao CR. Advanced statistical methods in Biometrical Research. John Willey and Sons, Inc. New York. 1952;381.
29. Mahalanobis PC. A statistical study of Chinese head measurement. Man in India 1928;8:32-64.
30. Murty BR, Arunachalam V. The nature of divergence in relation to breeding system in some crop plants. The Indian Journal of Genetics and Plant Breeding. 1966;30:47-58.
31. Saran PL, Godara AK, Dalal RP. Biodiversity among Indian jujube (*Ziziphus mauratiana* Lamh.) genotypes to powdery mildew and other traits. Notulae Botanicae Horti Agrobotanici Cluj. 2007;35(2):15-22.
32. Sharma G, Bashir R, Sharma N, Jamwal M. Multivariate analysis and choice of parent for hybridization in apple (*Malus* x *domestica* Borkh.). Asian Journal of Bioscience. 2013;8(1):39-42.
33. Santos CAF, Correa LC, Costa SR. Genetic divergence among *Psidium* accessions based on biochemical and agronomic variables. Crop Breeding and Applied Biotechnology. 2011;11:149-156.
34. Sharma G, Lata S, Yadav A, Sharma OC, Sharma N. Assessment of variation in pear genotypes through genetic divergence and cluster analysis using D^2 statistics. African Journal of Agricultural Research. 2015; 10(10):1063-1066.
35. Mishra SB, Singh UK, Sahu PK. Studies on genetic divergence in five land rand races of rice in northern Bihar. Annals of Agricultural Research. 1994;15(2):217-221.
36. Mir SA. Genetic studies on wild apple in South Kashmir. M.Sc. Thesis submitted to the Division of Fruit Science, SKUAST-Kashmir, Shalimar, Srinagar, J&K; 2015.
37. Singh RK, Bains SS. Genetic divergence for ginning out turn and its components in upland cotton. Indian Journal of Genetics and Plant Breeding. 1968;28(2):151-157.
38. Pereira LS, Ramos CAM, Ascasibar EJ, Pineiro AJ. Analysis of apple germplasm in north Spain. Journal of American Society for Horticulture Science. 2003;120(1):67-84.
39. Bhat ZA, Dhillon WS. Genetic studies on variability, heritability, genetic advance and cluster analysis in pear (*Pyrus* spp.). Indian Journal of Horticulture. 2015;77(2): 167-172.
40. Oraguzie NC, Hofstee ME, Brewer LR, Howard C. Estimation of genetic parameters in a recurrent selection programme in apple. Euphytica. 2001;118: 29-37.
41. Currie AJ, Ganeshanandam S, Noiton DAM, Garrick DJ, Shelbourne CJA, Oraguzie NC. Quantitative evaluation of apple (*Malus* x *domestica* Borkh.) fruit

shape by principal component analysis of Fourier descriptors. Euphytica. 2000;111: 221-227.

42. Echeverria G, Graell J, Lopez ML, Brezmes J, Correig X. Volatile production in Fuji apples stored under different atmospheres measured by headspace/ gas chromatography and electronic nose. Acta Horticulturae. 2005;682(2): 1465-1470.

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