Genetic divergence evaluation of apple germplasm by D^2 multivariate analysis

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Abstract: Genetic divergence was worked out in the new germplasm of apple (*Malus × domestica* Borkh.) including 42 genotypes. Mahalanobis's D^2 statistics was applied for identifying the potential parents to be involved in the hybridization programme for trait specific improvement or selecting better segregants. On the basis of performance for various traits, genotypes were grouped into three clusters and maximum numbers of genotypes i.e. 28 were accommodated in cluster I , while 10 and 4 genotypes were arranged in cluster II and III respectively. The average intra cluster distance was maximum in cluster II (2.214) and minimum in cluster III (1.212). Inter cluster distance was maximum between cluster II and III (5.077) indicating that hybridization between genotypes from cluster II and III can be utilized for getting the superior recombinants in segregating generations. On the basis of cluster means for various characters , cluster II was found superior for shoot thickness (0.34cm), inter - nodal length (2.38cm), number of lenticels (62.10) and leaf blade length (8.79cm) whereas cluster III was found superior for leaf blade width (5.10cm), petiole length (2.64cm) and leaf blade ratio (3.62). Hence, hybridization between parents from cluster II and cluster III for these characters can produce better recombinants in segregating generations.

Keywords: Apple*,* Cluster analysis*,* D^2 statistic*,* Genetic divergence

INTRODUCTION

Apple (*Malus × domestica* Borkh.) is an important temperate fruit crop of India with respect to acreage, production, demand and economic value. It is a member of rosaceae family and pomoideae sub family. In 1930, Vavilov suggested that Turkistan was the area where *M. sieversii* and *M. domestica* could have originated (Robinson *et al.,* 2001). Zhou (1999) referred China as its origin since about 80 per cent of all species of the genus are found in that country.

Consumers have a special preference for apple grown in high hills of Himachal Pradesh due to its characteristic color, flavor, crispiness, compactness and sweetness. There is considerable scope for its cultivation in Himachal Pradesh but the productivity of apple is very low i.e. 2.7 MT/ha (Anonymous, 2013) due to monoculture of Delicious varieties, low proportion of self fruitful varieties in orchards, inappropriate sites, irregular bearing to adverse climatic conditions and improper management practices, poor soil conditions, lack of suitable adaptable cultivars and lack of diversification in pollinizers and their inadequate proportion. Hence, there is a need to develop and utilize the new germplasm of apple for meeting the desired objectives by following suitable breeding strategy, so as to have suitable varieties to overcome constraints listed above

in enhancing productivity and sustaining production. Variation in the existing germplasm is pre-requisite for initiating any breeding programme. Reduced number of cultivars used in breeding programmes of apple can be explained by the lack of information of the germplasm banks, which reduces their possible use (Noiton and Alspach, 1996). The main problem when using a reduced number of cultivars is the inbreeding among future generations, in comparison with other fruit trees as peach, raspberry or chestnut. Nowadays, new approaches can be afforded to increase genetic variability in commercial releases such as collecting seedling from the supposed original species *M. sieversii* or using old cultivars (Forsline and Aldwinckle, 2004). But inbreeding problems are not yet visible in seeding populations due to the elevated heterozygocity of the genus *Malus*. The first cultivar obtained by crossing was attributed to Thomas Andrew Knight (1759–1838). Another method to obtain new genotypes/ cultivars is through selection of natural mutants and chimeras (Janick *et al.,* 1996).

 For improvement of those characters with nonadditive type of gene action and didn't respond to selection, there is a need for partitioning of nonadditive component of genetic variance further by hybridization which is achieved through genetic divergence studies. D^2 multivariate analysis is one of the

Table 1. Clustering pattern of 42 genotypes of apple on the basis of genetic divergence.

Cluster number	Number of genotypes	Name of genotype	Accession Number	Sources of availability
I	28	Spartan I	EC-492553	NBPGR, Phagli
		Red Gold	EC-328842	NBPGR, Phagli
		Tydeman's Early Worchester	EC-044005	NBPGR, Phagli
		Royal Deliciuous	EC-552628	NBPGR, Phagli
		Tropical Beauty	EC-552616	NBPGR, Phagli
		Black Ben Davis	EC-036439	NBPGR, Phagli
		Red Baron	EC-115820	NBPGR, Phagli
		Anna	EC-100218	NBPGR, Phagli
		Early Red One	EC-513657	NBPGR, Phagli
		Real Mecoy	EC-558098	NBPGR, Phagli
		York-a-Red	EC-558022	NBPGR, Phagli
		Red Delicious	EC-451348	NBPGR, Phagli
		Cox's Orange Pippin	IC-013509	NBPGR, Phagli
		Ambroyal	-	RHRS, Mashobra
		Co-Fuji	-	RHRS, Mashobra
		Early Red Bird	-	RHRS, Mashobra
		Gloster	-	RHRS, Mashobra
		Hardeman	-	RHRS, Mashobra
		Lady Sudeley	-	RHRS, Mashobra
		Lody Early Golden	IC-558091	RHRS, Mashobra
		Lobo	-	RHRS, Mashobra
		Lord Lambourne	-	RHRS, Mashobra
		Mclntosh Double Red	IC-558023	NBPGR, Phagli
		Red Gravenstein	-	RHRS, Mashobra
		Red Fuji	EC-513662	NBPGR, Phagli
		Starkrimson Delicious	-	RHRS, Mashobra
		Tydeman's Late Orange	-	RHRS, Mashobra
		Spartan II	-	RHRS, Mashobra
II	10	Granny Smith	EC-114117	NBPGR, Phagli
		Gold Spur	EC-160161	NBPGR, Phagli
		Gala	EC-144039	NBPGR, Phagli
		Mutsu	EC-100445	NBPGR, Phagli
		Gibson Golden	-	RHRS, Mashobra
		Gale Gala	-	RHRS, Mashobra
		Golden Delicious	-	RHRS, Mashobra
		Yellow Transparent	EC-044217	NBPGR, Phagli
		Yellow Newton	-	RHRS, Mashobra
		Scarlet Gala	IC-558100	RHRS, Mashobra
III	4	Skyline Supreme	EC-027801	NBPGR, Phagli
		Red Chief	EC-200817	NBPGR, Phagli
		Lalla Delicious	EC-110765	NBPGR, Phagli
		Baldwin	IC-558017	NBPGR, Phagli

valuable tools for obtaining quantitative estimates of genetic divergence between biological populations. Further, grouping of genotypes based on Tocher's method will be more useful in choosing reliable parents for obtaining superior segregants. The crossing of two parents is now, as it always has been, the main method in apple breeding. Therefore, an attempt has been made in the present study to estimate genetic divergence among forty two genotypes of apple.

MATERIALS AND METHODS

Studies were carried out at Regional Horticultural Research & Training Station , Dr Y S Parmar University of Horticulture & Forestry, Mashobra, Shimla, Himachal Pradesh, India (lat 31° 7′ 48″ N, long 77° 13′ 48″ E, alt 2146 m) on forty two genotypes of

apple. The genotypes along with their accession number and sources of availability have been presented in Table 1. Trees were planted at a spacing of (3m × 1.5m) in the year 2011-12. The cultural practices were followed as per package and practices of fruit crops recommended by Dr Y S Parmar University of Horticulture and Forestry, Nauni-Solan, HP, India. The observations were recorded on one year old plants on shoot thickness (cm), shoot - inter-nodal length (cm), no. of lenticels, leaf blade length (cm), leaf blade width (cm), leaf blade ratio (l/w) and petiole length (cm). The statistical analysis was carried out for each observed character under the study using MS-Excel, SPSS 16.0 and SPAR 2.0 packages. The mean values of data were subjected to analysis of variance (ANOVA) as per Gomez and Gomez (1983) for Ran-

Table 2. Average intra and inter cluster distance (D^2).

Cluster	I	II	III
I	2.128		
II	2.635	2.214	
III	3.589	5.077	1.212

Table 3. Cluster means for different characters among 42 genotypes of apple.

Characters	Clusters		
	I	II	III
One year old shoot thickness (cm)	0.32	0.34	0.27
One year old shoot - Inter-nodal length (cm)	2.22	2.38	1.63
One year old - No. of lenticels	28.29	62.10	36.75
Leaf blade length (cm)	7.51	8.79	5.28
Leaf blade Width (cm)	3.70	5.03	5.10
Leaf blade ratio (l/w)	2.03	1.75	3.62
Petiole length (cm)	2.36	2.54	2.64

domized Block Design. The data were subjected to Mahalanobis's D^2 statistics as per Mahalonobis (1936). Treating D^2 as the generalized statistical distance between a pair of populations (genotypes), all populations were grouped into different clusters according to method described by Rao (1952).

RESULTS AND DISCUSSION

The analysis of variance indicated highly significant differences among the genotypes for all the traits studied. On the basis of mean performance of various traits, the clustering pattern of 42 genotypes of apple from different geographical locations has been presented in the Table 1. All the genotypes were grouped into 3 clusters. Maximum numbers of genotypes were arranged in cluster-I (28) while second and third cluster comprised of 10 and 4 varieties/cultivars respectively. Pattern of grouping of genotypes into different clusters indicated that genetic diversity is independent of geographical diversity. The studies on genetic divergence in apple corroborated with the findings of Srivastva *et al.* (2013), Sharma and Sharma (2006) and Sharma *et al.* (2013). Sharma *et al.,* (2013) grouped 16 cultivars of apple into 4 clusters on the basis of net variability and reported that pattern of grouping of genotypes into different clusters was independent of their place of collection. Maximum number of genotypes (7) were accommodated in cluster IV while minimum genotypes in cluster II (2). On the other hand Sharma and Sharma (2006) worked out genetic divergence among 30 scab resistant genotypes of apple and grouped them into 6 clusters. Maximum number of genotypes (14) entered into cluster III followed by cluster VI (9), cluster II (3), cluster I (2), cluster IV (1) and cluster V (1). Since genotypes collected from a single place were grouped into different clusters thus indicated that genetic diversity is independent of geographical diversity. Pereira *et al.* (2003) clustered apple genotypes into different groups on the basis of

traits like internodal length, spur frequency, spur coefficient, number of long shoot. Saran *et al.* (2007) grouped 35 ber genotypes into 7 clusters using D^2 statistics.

The average inter and intra cluster divergence (D^2) values are presented in the Table 2. The diagonal figures in the table represent the intra cluster distances. The intra cluster distance was highest in cluster II (2.214) and lowest in cluster III (1.212). Whereas, highest (5.077) inter cluster distance was recorded between cluster II and III and lowest (2.635) was observed between cluster I and II. It is well known that crosses between divergent parents usually produce greater heterotic effect than closely related ones. Since, crossing of genotypes belonging to same cluster will not expect to yield superior hybrids or segregants therefore, inter cluster distances were also worked-out. Inter cluster distance was maximum between cluster II and III (5.077). Study revealed that cluster II was most diverse among three clusters as it accommodated maximum number of genotypes (28) while cluster III was least diverse and accommodated only 4 genotypes. On the other hand maximum inter cluster distance was reported between cluster II and III indicated that these are most distantly related. Since crossing between most distantly related individuals results into superior hybrids or superior transgressive segregants in segregating generations, thus crossing between the genotypes of cluster II and III which are most distantly related may result into superior hybrids or better recombinants in segregating generations of apple. Sharma *et al.*, 2013 reported maximum inter cluster distance between clusters I and II (30.331) and revealed that maximum variability will be achieved when hybridization between the cultivars accommodating these clusters is attempted.

Furthermore, on the basis of cluster means for various characters studied, the cluster means for various horticultural traits are presented in the Table 3. The average shoot thickness was maximum in cluster II (0.34cm) followed by cluster I (0.32cm), and cluster III (0.27cm). Similarly, the maximum inter – nodal length was recorded in cluster II (2.38cm) followed by cluster I (2.22cm). Cluster II further recorded the maximum number of lenticels (62.10) followed by cluster III (36.75) and cluster I (28.29). Length of leaf blade was found to be maximum cluster II (8.79cm) followed by cluster I (7.51cm) while width of leaf blade was maximum in cluster I (5.10cm) followed by cluster II (5.03cm). Maximum Petiole length was obtained in cluster III (2.64cm) followed by cluster II (2.54cm) and cluster I (2.36cm). Leaf blade ratio was maximum in cluster III (3.62) and minimum in cluster II (1.75).Cluster II was found superior for shoot thickness, inter nodal length, number of lenticels and leaf blade length whereas cluster III was found superior for leaf blade width and petiole length. Sharma *et al.,* (2013) studied the average cluster means and recorded

highest values for the characters like fruit weight (8.81 g), fruit set after 50 days (64.66) fruit retention (49.88%), TSS (11.79°B) in Cluster I. The cluster II had better mean performance for the traits like fruit yield per plant (39.08 kg), duration of flowering (19.25 days), flesh firmness (12.34 kg/cm^2), fruit diameter (6.59 cm), fruit length (5.74 cm) and plant spread (4.92 m). In similar way, cluster III revealed superior mean performance for the characters like trunk girth (46.76 cm), followed by spur frequency (12.66%), total sugars (7.62%), reducing sugars (6.78%). The characters like shoot length (9.67 cm), plant height (4.95 m), plant spread (3.89 m) had higher values in Cluster IV. The character fruit set after 50 days (64.66) in cluster I, yield per plant (39.78 Kg) in cluster II, trunk girth (46.76 cm) in cluster III and shoot length (19.47 cm) in cluster IV showed the highest values.

On the basis of cluster means in the current study it is depicted that hybridization between genotypes of cluster- II and cluster- III can be effectively utilized for getting superior recombinants through further partitioning of genetic variance of the above mentioned characters.

Conclusion

The study on apple germplasm by D^2 multivariate analysis concluded that the genetic diversity was independent of geographic diversity. Highest inter cluster distance was recorded between cluster II and III indicating that hybridization between genotypes from cluster II and III can be utilized for getting the superior recombinants/transgressive seggregants in segregating generations in this crop. Furthermore, cluster II was found superior for shoot thickness, inter – nodal length, number of lenticels and leaf blade length whereas cluster III was found superior for leaf blade width and petiole length depicted that genotypes of cluster- II and cluster- III can be effectively utilized for improvement of these traits through further partitioning of genetic variance by hybridization. Hence it is concluded that genotypes of most distantly related clusters accompanied with useful characteristics could be effectively utilized in inter cluster crosses with the hope that this would lead to improvement over the existing cultivars in one or more horticultural traits of apple.

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