

Full Length Research Paper

Genetic divergence and cluster analysis studies of different apple genotypes using D^2 statistics

Girish Sharma¹, Nirmal Sharma^{2*}, Rubiqua Bashir¹, Amit Khokhar² and Mahital Jamwal²

¹Department of Fruit Breeding and Genetic Resources, Dr. YSP UHF, Solan, HP, India.

²Division of Fruit Science, SKUAST-J, Jammu and Kashmir, India.

Accepted 8 August, 2013

Medium quantum of genetic divergence was observed among sixteen apple genotypes under the present study. All the genotypes, on the basis of total variability were grouped into four distinct clusters. Maximum number of cultivars were accommodated in Cluster IV (Fuji, Gala, Jonadel, Jonagold, Red Fuji, Royal Gala and Spigon) followed by Cluster I (Arlet, Ruspippin, Sinta and Summerred), Cluster III (Crimson Gold, Elstar and Neomi) and Cluster II ('Spartan' and 'Quinte'). Cluster IV had highest intra cluster value so was most divergent and Cluster I having least intra cluster value was least divergent. Highest value for inter cluster distance was recorded between Cluster I and II while it was lowest between Cluster III and IV. Cluster means were maximum in Cluster II followed by Clusters I, III and IV. Neomi is best cultivars for fruit yield/plant, fruit length, fruit diameter, fruit weight, total sugars and non-reducing sugars. However, Jonagold is best for TSS. Cultivars Spartan, Elstar, Royal Gala, Jonagold and Summerred would prove best for different vegetative characters.

Key words: Apple, cluster analysis, D^2 statistics, genetic divergence.

INTRODUCTION

The cultivated apple (*Malus × domestica* Borkh.) is a member of family rosaceae and sub family pomeoideae, have originated in south western Asia, Asia Minor, the Caucasus mountains of Russia, central Asia and the Himalayan region of India and Pakistan (Juniper et al., 1999). It is an important temperate fruit crop of India with respect to acreage, production, economic value and above all popularity among the consumers. In India it is a prime commercial fruit crop of Himachal Pradesh, Jammu and Kashmir and Uttranchal and some parts of north eastern states including Arunachal Pradesh, Sikkim, Nagaland, Meghalaya and Nilgiri hills of Tamil Nadu (Awasthi and Chauhan, 2001). Apple productivity has

gradually declined since 1975 till date and on the basis of low production and productivity, India is now ranked 10th in the world apple cultivation scenario (Sardana, 2012). The important factors which are responsible for low productivity are age old varieties, inappropriate sites, irregular bearing, poor soil conditions, lack of suitable adaptable cultivars and poor selection of pollinizers and their inadequate proportion. Therefore urgent need is felt for development/introduction of new improved varieties which could help in elevating the apple productivity in India. For the success of any breeding programme the basic requirement is the variability found within the members of the population. It is this variation which if

*Corresponding author. E-mail: nirmalsharma.77@rediffmail.com.

heritable could be used for cultivar improvement, as improved cultivars are the backbone of any orchard system. Therefore, prior to initiation of any breeding programme they should be tested and extent of variability present must be adequately assessed so that the breeding programme could yield the desired results. To use or exploit the available variability present in the genetic material in the form of some specific groups or classes, the divergence studies based upon some desirable/suitable parameters is of very essential and of utmost significance. Keeping in view the above the genetic divergence and cluster analysis using D^2 statistics was undertaken with the objectives to assess the variability present among the sixteen apple genotypes and potential use of this variability for hybridization programmes. 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 (Saran et al., 2007). The use of genetically divergent parents in hybridization under transgressive breeding programme is dependent upon categorization of breeding material on the basis of appropriate criteria (Santos et al., 2011). 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.

MATERIALS AND METHODS

Studies were carried out in the Department of Fruit Breeding and Genetic Resources, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India on sixteen apple genotypes viz. Arlet, Crimson Gold, Elstar, Fuji, Gala, Jonadel, Jonagold, Neomi, Quinte, Red Fuji, Royal Gala, Ruspippin, Sinta, Spartan, Spijon and Summered. Plant height and spread (North-South and East-West) were measured with the help of measuring pole and were expressed in meters. The trunk girth was measured at a height of 9 cm above graft union with the help of measuring tape and was expressed in centimeters. Shoot length was measured by selecting twenty uniform shoots on periphery of each tree and recording the length of shoots with the help of measuring tape and expressed in centimeters. Internodal length was worked out by dividing the shoot length with number of nodes. Spur frequency was recorded by selecting ten uniform branches in each tree of 1 m length more than one year old and number of spurs were counted and mean was worked out dividing number of spurs with length of branch taken multiplied by 100. Number of days from the date of opening of first flower to the date of opening of last flower was taken as the duration of flowering. Fruits retained in all the genotypes were recorded one week before harvesting of fruits and expressed in percentage by dividing number of fruit retained with total number of flowers multiplied by 100. The dates on which fruits were harvested was recorded as date of harvesting. The crop load of apples harvested from each plant was recorded and the results were expressed in yield per plant in kilograms. The length and diameters of ten fruits was measured with the help of digital vernier calliper and mean was worked out and expressed in centimeters. The fruit weight was worked by weighing ten fruits

selected randomly from each tree and weighed on a single pan kitchen balance and mean was expressed in grams (g). Flesh firmness of fruit was measured after removing the skin (0.8 cm) and using effigy penetrometer (model FT 327) with plunger of 11 mm dia. The results were expressed in kg/cm². Specific gravity was measured by dividing the weight of the fruits by their volume. In counting number of seeds per fruit chaffy and shrivelled seeds were discarded. Total soluble solid contents of five uniformly ripened fruits of each tree were determined with an Erma hand refractometer (0 to 32°Brix) by placing few drops of juice on the prism and reading was taken. Titratable acidity, total sugars, reducing sugars and non-reducing sugars were determined as per the method suggested by AOAC (1990). Mahalanobis D^2 statistic was used for assessing the genotypic divergence between populations (Mahalanobis, 1936). The generalized distance between any two populations is given by formula:

$$D^2 = \sum \sum \lambda_{ij} \sigma_{ai} \sigma_{aj}$$

Where, D^2 = Square of generalized distance; λ_{ij} = Reciprocal of the common dispersal matrix; $\sigma_{ai} = (\mu_{i1} - \mu_{i2})$; $\sigma_{aj} = (\mu_{j1} - \mu_{j2})$; μ = General mean.

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (gs) values of any two uncorrelated genotype of D^2 value. All $n(n-1)/2$ D^2 value were clustered using Toucher's method described by Rao (1952). The intra cluster distances were calculated by the formula given by Singh and Choudhary (1997):

$$\text{Square of the intra cluster distance} = \sum D_i^2 / n$$

Where, $\sum D_i^2$ is the sum of distance between all possible combinations of the entries included in a cluster and n is number of all possible combinations.

The inter cluster distances were calculated by the formula described by Singh and Choudhary (1997):

$$\text{Square of the intra cluster distance} = \sum D_i^2 / n_i n_j$$

Where, $\sum D_i^2$ is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the clusters under study. n_i is number of entries in Cluster I and n_j is number of entries in cluster j. The criterion used in clustering by this method was that any two genotypes belonging to the same cluster, at least on an average, show a small D^2 value than those belonging to two different clusters.

RESULTS AND DISCUSSION

The clustering pattern of sixteen cultivars of apple on tree, shoot, flowering and fruit characters are presented in Table 1. The genetic divergence in the present study observed among the sixteen cultivars is of medium quantum. The sixteen cultivars on the basis of net variability were grouped into four distinct clusters. Maximum number of cultivars (7) were accommodated into Cluster IV (Fuji, Gala, Jonadel, Jonagold, Red Fuji, Royal Gala and Spijon) while the minimum number (2) were in Cluster II which included Spartan and Quinte.

Table 1. Clustering pattern of sixteen cultivars of apple on the basis of genetic divergence.

Cluster number	Number of cultivars	Cultivars included
I	4	Arlet, Ruspippin, Sinta and Summered
II	2	Spartan and Quinte
III	3	Crimson Gold, Elstar and Neomi
IV	7	Fuji, Gala, Jonadel, Jonagold, Red Fuji, Royal Gala and Spijon

Table 2. Intra and inter cluster distance (D^2).

Clusters	I	II	III	IV
I	8.201	30.331	16.428	14.598
II		8.410	24.595	21.102
III			9.156	9.994
IV				9.321

Inter and intra cluster divergence values (D^2) between and within four clusters are presented in the Table 2. The intra cluster distance was maximum (9.32) for Cluster IV and minimum (8.20) for Cluster I. Highest value (30.331) for inter cluster distance was recorded between Cluster I and II while it was lowest (9.994) between Cluster III and IV. On the basis of results it is inferred that subsequent hybridization between the genotypes having broad genetic base should result in maximum heterotic performance and eventually the desirable transgressive recombinants, as broad genetic base is a fundamental requirement for any crop improvement programme. The Cluster IV accommodating cultivars Fuji, Gala, Jonadel, Jonagold, Red Fuji, Royal Gala and Spijon were more divergent, followed by Cluster III having three cultivars namely Crimson Gold, Elstar and Neomi. Wide diversity in the progeny is expected when hybridization is attempted within cultivars which are more divergent. Since inter cluster distance is maximum (30.331) between Clusters I and II so maximum variability will be achieved when hybridization between the cultivars accommodating these clusters is attempted. The cluster means of the various tree, shoot, flowering and fruiting characters are presented in Table 3. The average cluster means revealed highest values for the characters like fruit weight (8.81 gm), 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/cm²), 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 (39.78Kg) in Cluster II, trunk girth (46.76 cm) in Cluster III and shoot length (19.47 cm) in Cluster IV showed the highest values. 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. In the present study the above said characters revealed variation but in a narrow sense, probably the cultivars in the present study were not much divergent in respect of these traits. Saran et al. (2007) grouped 35 ber varieties into 7 clusters using D^2 statistics. Linoaiah et al. (1998) after canonical variate analysis in apple found that plant height, stem girth, flowering shoot per square meter and percentage flowering per square meter contributed much towards the genetic diversity. These characters though not very significant in our study still they could be effectively exploited in future crop improvement. While studying the clonal variability in mango Manchekar et al. (2011) reported substantial variation after applying D^2 statistics. Hence it is concluded that genotypes of cultivars with wide genetic variation accompanied with useful characteristics could be effectively employed in intra specific crosses with the hope that this would lead to the transmission of higher genetic gain for different putative traits major being yield from practical utility point of view. On the basis of the performance of different cultivars and the cluster analysis, the sixteen apple cultivars have been identified for different characters (Table 4), which are potential parents for hybridization programmes. Neomi is best cultivars for fruit yield/plant, fruit length, fruit diameter, fruit weight, total sugars and non-reducing sugars. However, Jonagold is best for TSS. Cultivars Spartan, Elstar, Royal Gala, Jonagold and Summerred would prove best for different vegetative characters.

Table 3. Cluster means of sixteen apple cultivars.

Character	Clusters			
	I	II	III	IV
Plant height (m)	4.06	4.76	4.61	4.95
Plant spread (NS)	2.96	3.76	3.76	3.89
Plant spread (EW)	4.06	4.92	4.26	4.67
Trunk girth (m)	31.46	41.96	46.76	42.33
Shoot length (cm)	17.71	16.22	17.08	19.47
Internodal length (cm)	2.88	3.06	2.82	2.94
Spur frequency (%)	12.04	12.25	12.66	11.66
Duration of flowering (days)	17.54	19.25	18.56	17.83
Fruit set after 50 days (%)	64.46	44.50	60.00	59.33
Fruit retention (%)	49.88	32.25	46.94	46.52
Fruit yield per plant (Kg)	29.47	39.08	28.13	36.59
Fruit length (cm)	5.73	5.74	5.38	5.68
Fruit diameter (cm)	6.36	6.59	6.17	6.28
Fruit weight (g)	8.81	8.46	8.48	8.68
Flesh firmness (kg/cm ²)	11.56	12.34	11.16	11.61
TSS (°B)	11.79	11.34	11.40	11.57
Acidity (%)	0.69	0.72	0.60	0.64
Total sugar (%)	7.20	7.59	7.62	7.48
Reducing sugar (%)	6.40	6.50	6.78	6.43
Number of seeds per fruit	6.96	7.00	5.55	5.83

Table 4. Promising cultivars of apple for different characters.

Characters	Highest cultivars	Lowest cultivars	Promising cultivar at par with highest
Plant height (m)	Spartan	Fuji	Royal Gala, Jonadel, Neomi
Trunk girth (cm)	Elstar	Summered	Fuji, Royal Gala
Plant spread NS (m)	Royal Gala	Neomi	Spartan
Plant spread EW (m)	Jonadel	Summered	Royal Gala, Elstar
Shoot length (cm)	Jonagold	Quinte	Arlet, Gala
Internodal length (cm)	Summered	Elstar	Crimson Gold
Spur frequency (%)	Fuji	Jonagold	-
Duration of flowering	Neomi, Ruspippin	Crimson Gold	-
Fruit retention (O.P.)	Ruspippin	Spartan	Gala and Summered
Date of harvest			
Fruit yield/plant (Kg)			
Fruit length (cm)	Neomi	Crimson Gold	-
Fruit diameter (cm)	Neomi	Crimson Gold	-
Fruit weight (g)	Neomi	Crimson Gold	-
Flesh firmness (kg/cm ²)	Red Fuji	Fuji	Arlet, Elstar
Number of seeds/fruit	Ruspippin	Sinte	Fuji, Spartan, Spijon and Summered
Total soluble solid (°B)	Jonadel	Quinte	
Fruit acidity (%)	Spijon	Neomi	Crimson Gold
Total sugar (%)	Neomi	Crimson Gold	
Reducing sugar (%)	Neomi	Spijon	

REFERENCES

- AOAC (1990). Official Methods of Analysis. 15th edition. Association of official Agricultural chemists, Washington, D.C.
- Awasthi RP, Chauhan PS (2001). Apple. In: *Handbook of Horticulture*. K. L. Chadha, (ed.). Indian Council of Agricultural Research, New Delhi, pp. 119-131.
- Juniper BE, Watkin R, Harries SA (1999). The origin of apple. *Acta Hort.*

484:27-30.

Linoiaiah HB, Reddy NS, Kulkarni RS, Thomas KK (1998). Genetic divergence in cashew (*Anacardium occidentale* L.) genotypes. In: Development in plantation crop research.

Mahalanobis PC (1936). The generalized distance in statistics. Proc. Nat. Acad. Sci. (India). 2:79-85.

Manchekar MD, Mokashi AN, Hegde RV, Venugopal CK, Byadgi AS (2011). Clonal variability studies in alphonso mango (*Mangifera indica* L.) by genetic divergence (D^2) analysis. Karnataka J. Agric. Sci. 24(4):490-492.

Pereira LS, Ramos CAM, Ascasisbar EJ and Pineiro AJ (2003). Analysis of apple germplasm in North Western Spain. J. Amer. Soc. for Hort. Sci. 120(1):67-84.

Rao CR (1952). Advanced statistical methods in biometrical research. John Wiley and Sons, Inc. New York. pp. 357-363.

Santos CAF, Corrêa LC, Costa SR (2011). Genetic divergence among *Psidium* accessions based on biochemical and agronomic variables. Crop Breed. App. Biotech. 11:149-156.

Saran PL, Godara AK, Dalal RP (2007). Biodiversity among indian jujube (*Ziziphus mauritiana* Lamk.) Genotypes for powdery mildew and other traits. Not. Bot. Hort. Agrobot. Cluj. 35(2):15-22.

Sardana MMK (2012). Towards increasing productivity and improving post harvest management in apple cultivation in Himachal. <http://isidev.nic.in/pdf/DN1003>.

Singh PK, Choudhary RD (1997). Biometrical Methods. In. Quantitative Genetic Analysis, Kalayani Publishers, New Delhi, pp. 178-185.