Indian Journal of Agricultural Sciences **83** (11): 1217–20, November 2013/Article

Genetic divergence in respect to qualitative traits and their possible use in precision breeding programme of apple (*Malus* × *domestica*)

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Received: 12 November 2011; Revised accepted: 1 May 2013

ABSTRACT

The domestic apple (*Malus* × *domestica* Borkh) has been originated in western Asia, eastern Europe, and south western Siberia and presences of wild relatives revealed that Indian subcontinent have ample diversity of apple. The genetic variability and divergence were studied during 2008-10 to evaluate 88 apple accessions for 10 fruit quality traits of apple used in DUS testing as per UPOV guidelines grown at Experimental Farm of CITH, Srinagar. Analysis of variance revealed the significant difference among accessions for 10 fruit quality traits. Maximum numbers of accessions (29) grouped in cluster II average fruit diameter was recorded highest (83.34 mm) in cluster II, and lowest average fruit weight was noted in cluster I. Intra cluster distance was recorded highest 1.69 in cluster II and minimum in cluster I, whereas highest inter cluster distance was noted between cluster I (6.49) and cluster II.

Key words: Coefficient of variation, D^2 analysis, Genetic divergence, Mahalanobis, *Malus domestica* Qualitative traits

The apple (*Malus* × *domestica* Borkh) is most ubiquitous of temperate fruits and has been in the cultivation in Europe and Asia from antiquity. The genus *Malus* consists of 15 primary species, two from Europe, four from North America and the rest from Asia. Most of our domestic cultivars derived from *M. pumila* Mill, the common apple of Europe, large fruited apple derived from human intervention. Asia is witness of 9 primary species. Large numbers of *M. baccata* strains found in Kashmir which evident that the north-western mountains are centre of diversity which are locally known as Trel apple. Survey conducted on apple in Kashmir resulted identification of more than 10 *M.baccata* races (Anonymous 2010). The genetic variability found in the apple has allowed adapted types to be selected for different environments and still selection continuous for new types to extend apple culture into both colder and warmer regions. Apples are grown in Siberia and northern China where temperature falls below 40°C and high elevations of Columbia and Indonesia where two crops can be produced in single year (Janick 1974). More than 10 000 apple cultivars are in record but only few dozen are grown on commercial scale worldwide (Way *et al.* 1990) .In apple developing new varieties required two diverse accessions for and the registration and protection of new varieties relies mostly on fruit quality traits on the basis of distinctiveness, uniformity and stability. Hence understanding of the genetics of DUS traits (based on UPOV guideline) helps the breeder to develop distinct variety. Choosing the genetically diverse parents will enable the expansion of genetic base and evolution of superior types. The choice of genetically divergent plants for hybridization under transgressive breeding programme is dependent on cauterization of breeding materials on the basis of suitable criteria. In this regard Mahalanobis D^2 distance was used to measure the genetic divergence in breeding programme. Intercrossing between more divergent parents shall be helpful to develop a broad spectrum of variability and further selection can be executed in the segregating generations. With the passage of time lot of clonal variations have taken place, further Kashmir Division possess wide range of Ambri and *Malus baccata* strains which required to be evaluated on the basis of qualitative traits with an objective to have systematic and rational genetic improvement programme of apple genotypes.

MATERIALS AND METHODS

The experiment was carried out for three consecutive years, i e 2008-10 at the Central Institute of Temperate Horticulture, Srinagar. The experimental site is located in

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dry area of Kashmir at latitude of 34° 45' N and longitude of 74° 50' E, and elevation is 1649 masl, area experienced average minimum and maximum temperature 6.52° – 19.63°C, respectively and rainfall 650-1000 mm. A total of 88 accessions, i.e. *M. floribunda* (Kashmir)*, M.baccata-01* (Kashmir), *M. baccata-02* (Kashmir), *M. baccata-03* (Kashmir), *M.baccata-04* (Kashmir), *M.baccata-05* (Kashmir), *M.baccata-06* (Kashmir), *baccata-07* (Kashmir), *M.baccata-08* (Kashmir), *M.baccata-09* (Kashmir), AAS/GSP/BSP/13(Kashmir), AAS/GSP/BSP09 (Kashmir), American Apirouge (NBPGR), Benoni (NBPGR), Summer Red (NBPGR), Michal (NBPGR), June Eating (NBPGR), Cooper IV (NBPGR), Starkrimson Gold (NBPGR), EC-539446 (NBPGR), Lemon Guard (NBPGR), Black Ben Davis (NBPGR), Kaseri (Kashmir), EC-38785 (NBPGR), Anna (NBPGR), Wilson Red June (NBPGR), Starkrimson (NBPGR), EC-239451 (NBPGR), EC-539447 (NBPGR), EC-539448 (NBPGR), Gold Spur (NBPGR), Parkin's Beauty (NBPGR), Silver Spur (NBPGR), Red Fuji (NBPGR), Golden Delicious (UK), EC- 539451,Chanpora Selection (Kashmir), Ionica (NBPGR),EC-539450 (NBPGR), Star Summer Gold (NBPGR), Wealthy Apple (NBPGR), EC-539453 (NBPGR), Vista Bella (NBPGR), Pink Lady (NBPGR), Lal Ambri (Kashmir), Ambri (Kashmir),Skyline Supreme, Green Sleeves, Top Red, Granny Smith (Italy), Vista Bella, Pink Lady, Firdous (SKUASTK), Spartan, Oregon Spur (Canada), Well Spur (USA), Tallisare (USSR), Gala Mast (Himachal Pradesh), Gala (Switzerland), Hardi Spur (USA), Starking Delicious, Red Baron, King Hascias, AAS/GSP/BSP/04, Early MC-Intosh, EC-539452, Red Gold (USA), Red Delicious (HP), Tydeman's Early Worcestor(USA), EC-83683,EC-539449, Indo, MC Spur, Florina, Mai Gold H-15, Antinovka (NBPGR), Jonathan (NBPGR). Laxtone Fortune(USA), Rich-a-Red (NBPGR), Shireen (SKUAT), Winter Commercial (NBPGR), Maayan (Israel), Prima (NBPGR), Jonathan (NBPGR), Coe Red Fuji (Israel), Gold Spur (USA), Oregon Spur(Canada), Royal Delicious (USA), Hardiman (HP), Jonica (USA), Vance Delicious (NBPGR), Red Chief (Italy) and Scarlet Gala(HP). During survey programme , all the varieties were obtained from respective countries through premier plant introduction agency for India (NBPGR, New Delhi). *Malus baccata* strains were obtained through survey and selection. The characteristics chosen for this study were selected among a larger set defined by International Union for the Protection of New Varieties of Plants (UPOV 1974) and International Board for Plant Genetic Resources (IBGRI 1982) from the descriptor for apple fruit diameter, fruit polar length, length of fruit stalk, fruit stalk thickness, depth of stalk cavity, depth of eye basin, width of eye basin, were recorded with scientific digital vernier caliper (Mitutoyo, Japan), and fruit weight, was observed with digital scientific balance, fruit firmness with penetrometer were recorded on 10 randomly collected fruits from each variety. In order to record the data of aforesaid traits 20 fruits from each accession was collected randomly. The observations were collected for the three consecutive years. Three years data were subjected to stastical analysis general variability was calculated by using the procedure suggested by Gomez and Gomez (1984) and clustering was done by multivariate analysis (Mahalanobis 1949). All the genotypes were grouped on the basis of generalized distance using the Tocher's method as suggested by Rao (1952).

RESULTS AND DISCUSSION

Highest SD and CV 79.95% and 48.98% respectively were recorded in fruit weight. Whereas SD was recorded highest in fruit diameter. So for as coefficient of variations is concerned, highest recorded in depth of eye basin (45%), followed by length of stalk (41.73%), SD is best measure of variation in a population with respect to recorded traits. SD gives more weightage to extreme values and less to those which are near to the mean (Singh and Naranayam 2009). They further reports that coefficient of variation gives the absolute idea of variation. A trait in which CV is higher would have greater variation than the one in which it is lower. When the CV is high the traits are more variable, when CV is low accessions are less variable. Fruit diameter registered (70.23 mm), and mean fruit weight 163.22 g recorded in 88 apple varieties. It is evident from the Table 1 that coefficient of variation 48.98 % noted in fruit weight followed by 45% in depth of eye basin, minimum CV (23.23%) noted in width of stalk cavity . Based on the relative value of the magnitude of divergence of the 88 genotypes were grouped into five clusters (Table 2). The maximum varieties (29) were grouped in cluster II followed by cluster III (21), cluster V (17), cluster I, have nine genotypes. All the *Malus* series were grouped in the cluster I except *M. baccata* - 06 (Cluster V) (Table 2). Grouping pattern showed that of Indian apple accessions were find place with International origin varieties. These grouping patterns of accessions are according to the findings of the Srivastava *et al.* (2010) in hazelnut, Barua and Sharma (2003) recorded fruit weight 113.85 g/fruit in apple at Himachal

Table 1 Mean, SD and coefficient of variation for various fruit characters

Characters	Mean	SD	CV%
Fruit diameter (mm)	70.23	18.38	26.12
Fruit length (mm)	59.85	15.68	26.20
Fruit weight (g)	163.22	79.95	48.98
Length of stalk (cm)	1.39	0.58	41.73
Fruit stalk thickness (mm)	0.36	0.12	33.33
Depth of stalk cavity (cm)	1.40	0.58	41.43
Width of stalk cavity (cm)	3.04	1.01	23.23
Depth of eye basin (cm)	1.20	0.54	45.00
width of eye basin (cm)	2.66	0.91	34.21
Fruit firmness (lb/cm^2)	15.25	5.89	38.62

Table 2 Distribution of various genotypes into various clusters.

Clusters	No. of genotypes	Genotypes
I	9	*M.floribunda* (Kashmir), *M.baccata-01* (Kashmir), *M. baccata-02* (Kashmir), *M. baccata-03* (Kashmir), *M.baccata-04* (Kashmir), *M.baccata-05* (Kashmir), *M.baccata-06* (Kashmir), *M.baccata-07* (Kashmir), *M.baccata-08* (Kashmir), *M.baccata-09* (Kashmir)
II	29	Coe Red Fuji, Granny Smith, Starkrimson, Cooper IV, Gold Spur, Red Chief, Shireen, Royal Delicious, Red Gold, Red delicious, Top Red, Hardiman,Rich-a-Red, Lal Ambri, Ambri, Skyline Supreme, Green Sleeves, Well Spur, EC- 539451,Chanpora Selection, Ionica,EC-539450, Tallisare, Starking Delicious, EC-539452, Vance Delicious, EC-239451, EC-539447, EC-539448
III	21	Spartan, Gala Mast, Mollies Delicious, Silver Spur, Golden Delicious,Firdous,Red Fuji, Oregon Spur, Tydemans Early Worester, Prima, Winter Commercial, Rome Beauty, Parkin's Beauty, EC-83683,EC-539449, Indo, MC Spur, Florina, Mai Gold H-15, Antinovka, Jonathan
IV	12	Vista Bella, Pink Lady, Laxtone Fortune, Scarlet Gala, Red Baron, King Hascias, AAS/GSP/BSP/04, Early MC-Intosh, Maayan, Star Summer Gold, Wealthy Apple, EC-539453
V	17	American Apirouge, Benoni, Summer Red, Michal, June Eating, AAS/GSP/BSP/13(Kashmir), AAS/GSP/BSP09(Kashmir), EC-539457, Starkrimson Gold, EC- 539446, Lemon Guard, Black Ben Davis, Kaseri, EC-38785, Anna, Wilson Red June, *M.baccata*-06

Pradesh. They further reported that most important traits contributing maximum genetic divergence was yield per plant and fruit length these findings are in consonance of the findings by Gasi *et al.* (2010) who observed less differentiation between traditional and international accessions of apple from Bosnia accessions were studies.

The cluster means indicated substantial variation among 5 clusters (Table 4). Mean fruit diameter recorded highest (83.34 mm) in cluster II, whereas very small fruit diameter (23.86 mm) noted in cluster I, since cluster I contain majority of *M. baccata* genotypes which fruits are very small. Fruit diameter is one of the most important traits to be considered while initiating apple hybridization programme, size is one of the important criteria for fruit grouping in various grades, fruit diameter of 80-85 mm graded in super fancy grade. Most of the accessions whose fruit diameter are high have been grouped in cluster II, selection of parents from cluster I and cluster II which had high inter cluster distance (indicate wide genetic distance between these clusters) for hybridization programme would help to evolve novel hybrids. The number of genotypes falling in these clusters was 9 and 29. The parents for hybridization could be selected on the basis of their large inter cluster distance for isolating useful recombinants in the segregating generations. Length of stalk, stalk thickness, depth of stalk cavity, width of stalk cavity, depth of eye basin have similar trend. However, fruit firmness recorded maximum (27.01 lb/cm^2) in cluster IV, whereas least firmness (12.19 lb/cm^2) in cluster (V) (Table 4). It is obvious from the grouping pattern that geographical distribution and genetic divergence did not follow the same pattern. Maximum intra cluster distance 1.68 for cluster II, followed by cluster IV (1.61), whereas least intra cluster distance (1.33) was noted in cluster I. Clustering pattern depicts that accession of different geographical origin have been placed in one cluster had high intra cluster distance (Cluster II). Cluster I had least intra cluster distance might be due to similar origin of accessions and having common ancestry. Cluster II have genotypes of diverse origin whereas cluster I had least intra cluster distance, as such this cluster comprise *Malus baccata* series of similar genetic makeup. Accessions within the cluster with high degree of divergence

Table 3 Intra-cluster (in bold) and inter cluster distance for 10 characters in apple.

Cluster	I	II	III	IV	V
I	1.333				
II	8.382	1.687			
III	6.492	2.186	1.581		
IV	6.205	3.566	2.350	1.607	
V	4.557	3.953	2.047	2.846	1.547

Table 4 Mean of various fruit characters falling into various clusters of apple

Character	Cluster				
	I	II	III	IV	V
Fruit Diameter (mm)	23.86	83.34	76.7	71.17	63.77
Fruit length (mm)	22.37	71.65	63.79	59.30	55.10
Fruit weight (g)	8.52	235.84	176.36	144.52	118.2
Length of stalk (cm)	0.40	1.96	1.28	1.31	1.15
Fruit stalk thickness (mm)	0.24	0.44	0.34	0.32	0.32
Depth of stark cavity (cm)	0.40	1.96	1.35	1.37	1.04
Width of stalk cavity (cm)	0.76	3.87	3.34	3.02	2.46
Depth of eye basin (cm)	0.24	1.63	1.35	1.16	0.8
width of eye basin (cm)	0.63	3.35	2.94	2.69	2.19
Fruit firmness (lb/cm^2)	13.66	13.58	13.99	27.01	12.19

would produce more desirable breeding material for achieving maximum genetic advance, however in this case intra- cluster distance is low, hence it can't be used within cluster crossing programme. The maximum inter cluster distance (6.49) was observed between cluster I and cluster II (2.18) followed by cluster II and cluster III (2.35) least inter cluster distance was noted between cluster II and Cluster IV showing their close proximity, inter cluster distance lowest between clusters indicting close relationship and similarity for most traits in the genotypes hence selection of parents from these clusters be avoided. However, in all cases the inter cluster distance were greater than similar studies conducted on apple and other fruit crops by Barua and Sharma (2003) in apple ,Singh *et al*. (2003), in pomegranate, Rai and Misra (2005) in bael, Rajan *et al*. (2009) in mango, and Nagar and Fageria (2006) in lehusua.Hence, suggested selection of distant parents based on D^2 analysis the intra cluster distance indicating the presence of genetic diversity among the varieties between different groups. The grouping pattern of the varieties of different geographical locations into one cluster may be due to presence of some common genes controlling the most important characters through modifying effect of micro-climate affecting genetic expressions. Another virtue that varieties from same locations were placed in separate clusters indicating wide diversity among varieties from common locations. This means that the geographical diversity may not necessarily be related to genetic diversity. These statements are in consonance with findings of Barua and Sharma (2003) in apple genotypes at Barapani, Meghalaya, Dwivedi and Mitra (1996), Srivastava *et al.* (2007) in apricot and Kumar *et al.* (2010) in litchi.

Standard Deviation is the best measure of variation in the population, high SD in fruit weight and diameter, length of fruit stalk and fruit length are indicative of high variability. Genetic divergences in order to utilize such variability in precision breeding programme were studied among 88 apple varieties grown at CITH. Highest number of varieties were grouped into cluster II, which indicates that these genotypes are having similar genetic constituents, accordingly crossing among the genotypes belonging to cluster II shall not produce desirable recombinants. Highest inter cluster D^2 values were recorded between cluster I and cluster II . Crossing between the clusters I and II shall result good recombinants and hybrid vigour.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Protection of Plant Varieties and Farmer's Right Authority, GOI, NASC Complex, New Delhi for providing financial assistance to undertake this study.

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