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Genetic divergence studies in tulip (*Tulipa gesneriana* L.)

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ABSTRACT

Twenty one tulip genotypes were assessed for their genetic divergence based on 16 agro-morphological traits following Mahalanobis D^2-statistic. On the basis of D^2 values, the 21 genotypes were grouped in to five clusters, of which cluster II accommodated 6 genotypes, while cluster IV had single genotype. The high magnitude of D^2 cluster means indicated that there is a considerable diversity in the population studied. The D^2 and inter-cluster coverage divergence were utilized for the choice of parents to decide the cross combination to produce heterotic effect. The highest inter cluster D^2 value was recorded between clusters III and V (11005.75) indicating that crosses may be attempted between the genotypes of cluster III (Character, Christian Dream, Hamilton and Horizon) and cluster V (Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb) to obtain new desirable recombinants in tulip. The study of cluster mean value of 5 clusters indicated high range of variation for days to sprout, days to flower, wrapper leaf area, percent sprouting & flowering, plant height and scape length among the different clusters. The cluster V includes genotypes with earliness and exhibited longest duration of flowering, highest wrapper leaf area and bulb weight. Genotypes of this cluster also possessed desirable floral traits (scape length, floral size and scape thickness) and bulb traits (number of bulbs per plant and bulb weight). Hence, genotypes from this cluster could serve as valuable parents to develop superior cultivars. Out of 16 principal components first six accounted 91.88% of total variability. The first principal component accounted for 57.25% of variability, while, the second and third accounted for 12.37 and 7.54% of total variability, respectively. Hierarchical cluster analysis (HCA) was performed for getting more clear idea among the genotypes. Based on HCA one dendrogram was constructed with two major clusters. These clusters could be divided into 8 minor sub-clusters.

Key words: Tulip, D^2 analysis, genetic diversity.

INTRODUCTION

Tulip (*Tulipa gesneriana* L.) is one of the most important ornamental crops in the world. It is ranked third among the top ten flowers sold worldwide (Podwyszynska and Sochacki, 12), being extremely popular for landscaping, and also as garden plant and cut flower. Tulips are highly valued for their attractive, coloured, upright flowers, mainly produced in springs. In, India tulip has been recently introduced in the Kashmir valley. It is gaining great popularity there over the last few years. There is tremendous scope for its commercial cultivation in Himachal Pradesh, Jammu & Kashmir, Uttarakhand and similar other hilly terrains of India (Jhon *et al*., 4). However, this crop has never been opted as commercial crop in India due to lack of adaptive genotypes, agro-techniques and planting material (Bhatia *et al*., 2).

In tulip, most of the varieties have been imported from Holland, and the performance of these varieties depends upon climatic conditions of the region under which they are grown. As a result, cultivars which perform well in one region may not perform same in other regions of varying climatic conditions (Kamble *et al*., 5). It is also important to study the performance of existing cultivars for their superior desirable characters (Archana *et al*., 1). The extent of genetic variability is of paramount importance for the improvement of a crop as greater is the genetic variability in the existing germplasm better would be the chances of selecting superior genotypes. Improvement through selection depends upon the variability existing in the available cultivars, which may be due to the difference either in genetic constitution of cultivars or in the environments in which they grow (Sestra *et al*., 14). A breeding strategy becomes purposeful and effective when it is based on genetic diversity present in particular species (Patil *et al*., 11). Genetic diversity is being used as source of genes in crop improvement for production of high yielding varieties, hybrids and to effect ecologically sustainable economic and social development (Kameshwari *et al*., 6). The present study was an attempt to investigate the extent of divergence among various genotypes of tulip using D^2 analysis.

MATERIALS AND METHODS

The present study was carried out at the research farm of Indian Agricultural Research Institute, Regional Station, Katrain, Kullu, Himachal Pradesh

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(32°12N'; 77°13'E; 1,560 m asl) during 2012-14. The Field received 950-1,000 mm annual rainfall and 1,000-1,100 mm snowfall annually. The experimental material comprised 21 tulip genotypes introduced from Holland and collection from various parts of Jammu & Kashmir. The morphological characteristics of these tulip genotypes have been given in Table 1. Healthy and uniform sized bulbs weighing about 10-15 g were planted 8-10 cm deep at a distance of 20 cm × 15 cm. Eighteen bulbs were planted per treatment per replication in randomized block design with three replications. After planting of bulbs the crop was mulched with grass in order to maintain the soil temperature and moisture. The recommended package of practices along with plant protection measures was followed to raise the successful crop. The data were recorded on five randomly selected plants per replication. The observations were recorded for 16 quantitative traits, namely days to sprouting, percentage sprouting, number of leaves/plant, wrapper leaf area, days to flower, plant height, scape length, scape thickness, flower diameter, percentage flowering, duration of flowering, number of bulbs per plant, bulb weight (g), bulb size (cm), number of bulblets per plant and bulblets weight. The D^2 statistic was used for assessing the genetic divergence among the populations as suggested by Mahalanobis (9). Based on the D^2 values thus obtained, the entire germplasm was classified into distinct clusters, grouping together the less divergent

Table 1. Morphological characterization of 21 tulip genotypes.

Genotype	Cultivar group	Flowering time	Morphological description
Ali bi	Triumph Tulip	Mid-late spring	Medium cup shaped, lavender pink flowers
American Dream	Darwin Hybrid	Mid spring	flower is pale yellow, edged in glowing red with a golden apricot-sheen
Apeldoorn	Darwin Hybrid	Mid spring	Bowl shaped, cherry red flowers with signal red margins. Inside signal red with contrasting black center encircled with yellow border
Apeldoorn Elite	Darwin Hybrid	Mid spring	Crimson flowers with a contrasting yellow edge, black anthers and black basal marks inside
Blushing Apeldoorn	Darwin Hybrid	Mid spring	Cup shaped, sunny yellow with delicate red outline and tangerine blush,
Cassini	Triumph Tulip	Mid- late spring	Dark red, goblet shape, fragrant
Character	Darwin Hybrid	Mid Spring	Bowl shaped, golden yellow flowers with black anthers.
Christmas Dream	Single Early Tulip	Early spring	Pink to rose colored, cup shaped blooms
Ganders Rhapsody	Triumph Tulip	Late spring	Cup shaped, deep pink flowers with white stripes
Golden Apeldoorn	Darwin Hybrid	Mid Spring	Cup shaped golden yellow flowers with black anthers, contrasting black center with bronze green border
Golden Melody	Triumph Tulip	Late spring	
Golden Oxford	Darwin Hybrid	Mid Spring	Bowl shaped, golden yellow flowers with black anthers.
Hamilton	Fringed Tulip	Late Spring	Buttercup yellow flowers with dark yellow fringes
Horizon	Double Late Tulip	Late spring	Peony flowered
Leen Vander Mark	Triumph Tulip	Mid spring	Vibrant red with white blooms
Lle De France	Triumph Tulip	Mid-late spring	Cardinal red flowers with dark bronze-green basal marks and yellowish brown margins
Monte Carlo	Double Early tulip	Mid spring	Double, sulphur yellow flowers with small red feathers
Oxford Wonder	Darwin hybrid		Large, golden yellow with an orange-red flame.
Pretty Women	Lily flowered Tulip	Mid-late	Cardinal red flowers with pointed and slightly reflexed petals
Strong Gold	Triumph Tulip	Late spring	Cup shaped, primrose yellow exterior with faint orange flames, canary yellow interior
Tulip Hb	Darwin Hybrid	Mid spring	Blood red flowers with signal red flames and buttercup yellow bases with greenish yellow margins

genotypes (Rao, 13). Principal Component Analysis was conducted by SPSS 16. Mean values registered for each variable were used for statistical analysis. The Principal Components (PC) for the dataset, eigen values (variance) for the PCs loadings (correlation of each variables with PCs) and PC score for each genotypes under the concerned PCs were used for interpretation of the analysis. PCs showing eigen values lesser than one was considered non-significant. PC loadings greater than selection criterion (SC) were considered significant. The SC value was calculated as 0.5/ √PC eigen value (Ovalles and Collins, 10). A dendrogram was constructed based on hierarchical cluster analysis using SPSS 16.0 statistical package.

RESULTS AND DISCUSSION

There was highly significant genotypic differences for all the traits studied revealing the existence of substantial amount of variation among the genotypes. Based on D^2 analysis the 21 genotypes were classified into five clusters (Table 2). The cluster II had the maximum (6) genotypes and cluster IV had only one genotype. The cluster I and cluster V had 5 genotypes in them. The cluster III had four genotypes. The pattern of distribution of genotypes from different eco-geographical regions into different clusters with different divergence values was at random, supporting that geographical diversity is not related to genetic diversity. The main forces other than geographical origin responsible for this genetic diversity may be natural/ artificial selection, exchange of breeding material, genetic drift and environmental variation. Similar conclusions were drawn by Kavitha and Anburani (7) in African marigold and Kumar *et al*. (8) in Snapdragon.

There was considerable amount of genetic divergence in the present collection as evident from inter- and intra-cluster distances among five clusters (Table 3). Intra-cluster distance was highest (2067.0) in cluster III with four genotypes and lowest (0.0) in cluster IV as represented by only one line (Ganders Rhapsody). This indicated that the genotypes in cluster III were highly diverse. Highest inter-cluster distance was between cluster III and V (11005.8) followed by I and III (7395.9); III and IV (6882.3), and II and III (5542.4). The lowest inter-cluster distance was between cluster I and V (1641.1). From D_2 analysis it was evident that crosses may be attempted between the genotypes of cluster III (Character, Christian Dream, Hamilton, Horizon) and cluster V (Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb) to obtain new desirable recombinants in tulip. Since all kinds of gene actions and interactions are possible in the expression of quantitative traits it is advisable to make crosses between genotypes selected from the clusters with high mean performance to get desirable transgressive segregants. According to Patil *et al*. (11), the highly divergent genotypes would produce a broad spectrum of variability enabling further selection and improvement. The hybrids developed from these genotypes within the limit of compatibility of these clusters may produce high magnitude of heterosis or desirable transgressive segregants, which would be rewarding for successful breeding programme.

Table 3. Inter/ intra distance matrix among 5 clusters based on D^2 analysis.

Cluster	1	2	3	4	5
1	1226.1	2004.3	7395.9	3324.4	1641.1
2		719.1	5542.4	3902.1	2383.3
3			2067.0	6882.3	11005.8
4				0.0	4335.1
5					772.5

The study of cluster mean value of 5 clusters indicated considerable differences for the traits studied (Table 4). After leaving out the solitary cluster IV, range of variation for number of leaves, scape thickness, flower size, number of bulbs & bulblets were low among the multi-member cluster. The characters, *viz.*, duration of flowering, bulb size and bulb weight exhibited moderate variations. The high range of variation was observed for days to sprout, days to flower, wrapper leaf area, percent sprouting & flowering, plant height and scape length among

Table 2. Cluster classification of 21 tulip genotypes based on D^2 analysis.

Cluster No.	Genotype(s)
I	Ali Bi, American Dream, Golden Melody, Golden Oxford, Oxford Wonder
II	Apeldoorn Elite, Cassini, Lean Vander Mark, Lle de France, Monte Carlo, Pretty Women
III	Character, Christian Dream, Hamilton, Horizon
IV	Ganders Rhapsody
V	Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb

Table 4. Cluster means of 21 genotypes for 16 traits based on D^2 analysis.

Cluster	Days to sprout	Percent sprouting	No. of leaves/ plant	Wrapper leaf area (cm^2)	Days to flower	Plant height (cm)	Spike length (cm)	Scape thickness (mm)	Flower size (cm)	Percent flowering	Flower duration (days)	No. of bulbs/ plant	Bulb wt. (g)	Bulb size (cm)	No. of bulblets/ plant	Bulblet wt. (g)
1	52.0	85.3	4.1	114.9	126.9	29.15	23.3	5.4	6.1	88.0	17.5	2.2	15.1	10.8	2.6	3.1
2	39.8	96.0	3.6	85.3	119.8	27.97	22.3	5.2	6.6	83.9	19.2	2.3	14.8	10.8	2.5	2.6
3	61.5	55.0	3.5	59.4	122	19.7	15.7	5.2	5.3	45.4	16.2	1.4	9.2	8.7	1.5	1.3
4	40.3	44.4	5.1	98.4	114	44.3	38.4	6.8	5.4	97.6	21.3	3.2	16.4	13.6	1.5	2.0
5	37.1	100.0	3.8	123.3	114.2	34.0	25.5	6.2	6.3	96.8	22.0	2.8	21.5	12.4	1.8	3.3

the different clusters. The variation observed in cluster means also point out the degree of variability. The cluster V includes genotypes with earliness and exhibited longest duration of flowering, highest wrapper leaf area and bulb weight. Genotypes of this cluster also possessed desirable floral traits (scape length, floral size and scape thickness) and bulb traits (number of bulbs per plant and bulb weight). Hence, genotypes from this cluster could serve as valuable parents to develop superior cultivars. The cluster IV that contained only one genotype (Ganders Rhapsody) was early with taller plant height and scape length. This cluster had thicker scape and high bulb multiplication potential. The cluster III genotypes were late and produced small sized flowers on thin and short scapes. The genotypes of this cluster were dwarf and produced least number of bulbs and bulblets with minimum bulb size and bulb weight. The genotypes of cluster II had larger flowers and were early in sprouting and flowering. The genotypes of cluster II possessed medium plant height, scape length and had moderate bulb multiplication potential. The genotypes of cluster I was late with medium plant height, scape length and produced relatively larger flowers. The cluster I genotypes showed moderate bulb multiplication potential and produced comparatively larger bulbs than cluster III. The intercrossing genotypes of cluster IV and V with other genotypes of cluster I, II and III may create wider variability, which is expected to produce high yielding transgressive segregants in tulip improvement programme.

In the present investigation, the first six principal components with eigen values more than 0.5 contributed to 91.88 per cent of cumulative variability among the 21 tulip genotypes evaluated for 16 morphological characters (Table 5). The first principal component accounted for 57.25 per cent of variability while, the second and third accounted for 12.37 and 7.54 per cent of total variability respectively. The percent of variability from fourth to sixth principal component accounted for 7.54, 6.67, 4.63 and 3.42 in decreasing order, respectively. The PCs from 7 to 16 which recorded the eigen values less than 0.5 were ignored as they were unlikely to have any practical significance. It was therefore inferred that essential features of dataset had been represented in the first 6 PCs. The significance of the variables in each PC was determined by comparing the loading with corresponding SC. Days to sprouting (0.90) and percent sprouting (0.43) explained the maximum variance in PC1. The PC2 which accounted for 12.37% of total variance showed higher variance for number of leaves (0.65), scape thickness (-0.43) and scape length (-0.42) signifying their importance

Table 5. Component loading of 16 traits, eigen values, proportion of the total variability represented by first 6 principal components (PC), cumulative percent variability and Selection Criterion (SC) in 21 tulip genotypes.

Trait	Principal component					
	1	2	3	4	5	6
Days to sprout	0.90	0.00	-0.39	0.00	0.00	0.00
Percent sprouting	0.43	0.03	0.70	0.00	0.00	0.01
No. of leaves/ plant	-0.01	0.65	-0.06	0.00	0.01	0.30
Wrapper leaf area (cm^2)	-0.08	-0.03	-0.59	0.00	0.00	-0.01
Days to flower	0.00	-0.13	0.00	0.97	0.11	0.16
Plant height (cm)	0.00	-0.34	0.00	-0.22	0.76	0.38
Spike length (cm)	0.00	-0.42	0.00	-0.10	-0.64	0.51
Scape thickness (mm)	0.00	-0.43	0.00	0.01	-0.01	-0.31
Flower size (cm)	0.00	-0.21	0.00	0.01	-0.01	-0.43
Percent flowering	0.00	0.16	0.00	0.09	-0.06	-0.19
Flower duration (days)	0.00	0.08	0.00	0.00	0.00	-0.32
No. of bulbs/ plant	0.00	-0.02	0.00	0.00	0.00	0.10
Bulb wt. (g)	0.00	0.02	0.00	0.00	0.00	0.21
Bulb size (cm)	0.00	0.00	0.00	0.00	0.00	0.00
No. of bulblets/ plant	0.00	0.00	0.00	0.00	0.00	0.00
Bulblet wt. (g)	0.00	0.00	0.00	0.00	0.00	0.00
Eigen values (variance)	9.16	1.98	1.21	1.07	0.74	0.55
Percent variability	57.25	12.36	7.54	6.67	4.63	3.42
Cumulative percent Variability	57.25	69.62	77.16	83.83	88.46	91.88
SC	0.16	0.36	0.45	0.45	0.58	0.68

in quality improvement in tulip. The PC3 reflected significant loadings for traits like percent sprouting (0.70) and wrapper leaf area (-0.59). The PC4 showed significant variance for days to flower (097). The PC5 reflected the significant loading for plant height (0.76) and scape length (-0.64).

Principal component analysis (PCA) reduces the large dataset to a small numbers of unrelated groups of variables of their components. Variables strongly associated with same group may share some underlying biological relationship. These associations are often useful for generating hypothesis or for understanding behavior of complex traits (Dey *et al.*, 3). The components like days to sprout and percent sprouting, number of leaves, scape length and scape thickness explained considerably higher amount of variations in PC1 and PC2 that accounts for 69.62% of cumulative variability. Scape length is one of the economically important traits that determine the quality of cut tulips. Significant positive correlation of scape length with plant height, number of leaves per plant, wrapper leaf area, flower size, number of bulbs per plant, bulb size and bulb weight was earlier reported by Bhatia *et al.* (2). Hence, there is a large scope for improvement of these traits through selection based on scape length.

Based on hierarchical cluster analysis, 21 genotypes were divided into two major clusters at a distance co-efficient of 25 (Fig. 1). The first cluster had four genotypes, namely, Character, Hamilton, Horizon and Christmas Dream. Among them first three genotypes were very close to each other. The second major cluster had rest of the 17 genotypes. The second cluster had two sub-cluster with five genotypes in first sub-cluster had five genotypes (Cassini, Oxford Wonder, Leen Vender Mark, Monte Carlo and Lle De France) and rest of the 12 genotypes were represented in second sun-cluster. In the second sub-cluster the cultivars, Apeldoorn, Golden Apeldoorn and American Dream were very closely related to each other. Similarly, Strong Gold and Tulip Hb were also closely related with each other. Clustering pattern based on the dendrogram make it possible to visualize the distance among the cultivars very clearly. Selection of cultivars becomes easier with dendrogram. Similar results were reported by Kameswari *et al.* (6) in chrysanthemum.

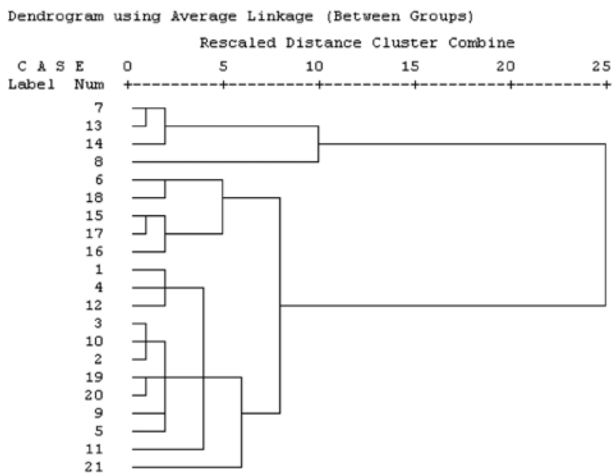

Fig. 1. Clustering pattern of tulip genotypes based on morphological traits.

In total, 8 distinct minor clusters were formed with largest distance between the genotypes, Character and Tulip Hb. The clustering pattern was similar to the D^2 analysis. However, they were separated through dendrogram with 8 minor clusters.

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