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Selecting donor parents for future breeding aiming at tolerance to downy mildew and powdery mildew diseases under foot hills of Himalayas

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Abstract
Assessment on genetic diversity and principal component analysis among the genotypes is important for planning an effective hybrid breeding program as the genetically diverged genotypes are known to produce high heterotic effects. The present study was carried out at the HRS and KVK, Kandaghat, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) India during pre-kharif seasons of 2017-18. All the characters under study showed considerable divergence and the genotypes were grouped into five clusters. Among the five clusters, cluster V was the largest, comprising of 10 genotypes. The inter-cluster distance was larger than the intra-cluster distance suggesting wider genetic diversity among the genotypes of different groups. The maximum and minimum intra- cluster distances were found in cluster I and cluster IV, respectively. The inter-cluster D^2 values was maximum between the cluster II and V indicating wide range of genetic diversity between these two clusters. The desirable cluster mean value was observed in cluster V for all the traits except seed cavity size and TSS. The cluster analysis and PCA revealed that considerable variation existed among genotypes that could be implicated in selection of cucumber for the development or improvement of germplasm and cultivars.

Keywords: Diversity, clustering pattern, principal component analysis, cucumber

Introduction
Cucumber (*Cucumis sativus* L. 2n=2x=14) belongs to the family cucurbitaceae is an important vegetable crop grown throughout the world under tropical and subtropical climates (Tatlioglu, 1993) [23]. It is thought to have been originated in India (Harlan, 1975) [6] because of the fact that *Cucumis sativus* L. var. *hardwickii* (Royle) Alef., primogenitor of cultivated cucumber is found in the Himalayan foot hills of India. Tender fruits are consumed either as salad, cooked as a vegetable and as pickling cucumber in its immature stage (Shah *et al*., 2016) [21]. Cucumber is a low energy and high water content vegetable, which is a rich source of vitamin B and C, carbohydrates, calcium and phosphorus (Yawalkar, 1985) [25]. According to FAOSTAT (2017) [5], the total area lined by cucumber in the world is 3.50 million hectare with an annual production of 148.62 million tonnes and productivity of 42.46 t/ha. In India, area covered by cucumber cultivation is 82.04 thousand hectares with annual production of 1259.94 thousand tonnes and 15.35 t/ha productivity (Anonymous, 2018) [1].
Inbred lines are the prerequisite for hybrid variety development in crop plants. For developing high yielding hybrids in cucumber, inbred lines need to be evaluated for their diverged gene pool. Genetic diversity makes it possible to establish genetic distances within and between species, identify species and strains by unique and specific genetic profiles with the help of advanced biometrical methods such as multivariate analysis (Rao, 1952) [19] based on Mahalanobis' (1936) [14] D^2 statistics. Such studies are also useful in selection of parents for hybridization to recover superior progenies which can further be released as an improved open pollinated variety. Principal component analysis simplifies the complex data by transforming number of correlated variables into a smaller number of variables called principal components. The first principal component accounts for maximum variability in the data as compared to each succeeding component (Leilah and Al-Khateeb, 2005) [13].

Materials and Methods
A field experiment was conducted at Horticultural Research Station and Krishi Vigyan Kendra, Kandaghat under Dr YS Parmar University of Horticulture and Forestry, Nauni-

Solan, (HP) India wherever located at an altitude of 1425 meters above mean sea level, having the latitude of 30.59ºN and longitude of 77.07ºE. Experiment was laid in Randomised Complete Block design (RCBD) with three replications. Row to row and plant to plant spacing of 100 × 75 cm was kept in a plot having size 2.0 × 4.5 m, that accommodated 12 plants per plot. All the recommended package of practices was followed to raise a healthy crop. Data were taken from five randomly selected plants for days to first female flower appearance, node number bearing first female flower, days to first fruit harvest, number of primary branches per plant, length of vine (m), fruit length at edible stage (cm), fruit breadth at edible stage (cm), seed cavity size (cm), 100 seed weight (g), number of fruits per plant, average weight of edible fruit (g), total soluble solids of the pulp (°Brix.), total sugars (%), ascorbic acid content of the pulp (mg per 100 g), cucurbitacin content of the fruit [CC (µg/100g)], PDI of downy mildew disease at 120 DAS, PDI of powdery mildew disease at 120 DAS and marketable fruit yield per plant (kg). The genetic diversity existing between the genotypes with respect to the set of characters was estimated using Mahalanobis' D^2 statistic (Mahalanobis, 1936) [14]. Treating D^2 as a generalized statistical distance, the criterion used by Tocher (Rao, 1952) [19] was applied for determining the group constellation. Average intra and inter-cluster distances were determined following the method of Singh and Chaudhary (1985) [22]. Principal component analysis (PCA), to identify the factor dimension of the data, was used to summarize varietal information in a reduced number of factors for selection of the best performing genotype(s).

Results and Discussion

Grouping of genotypes into various clusters

Twenty eight genotypes were grouped into five different non over lapping clusters as per Tocher's method as described by Rao (1952) [19]. Cluster V had highest number of genotypes (10) followed by cluster I had 6 genotypes, cluster IV had 5 genotypes, cluster II had 4 genotypes, while, cluster III had 3 genotypes (Table 1). Similar results were also obtained by Rao *et al*. (2003) [20], Khan (2006) [10], Kabir *et al*. (2009) [8], Kumar *et al*. (2013) [11], Tyagi *et al*. (2017) [24] and Das *et al*. (2019) [2].

The grouping pattern of genotypes was observed to be random, indicating that geographical diversity and genetic divergence were unrelated. Such observation has been reported by Lalramhlimi *et al.* (2018) [12]. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection are responsible for genetic diversity. Therefore, selection of genotypes for hybridization should be based on genetic divergence rather than geographic divergence (Mehta *et al.*, 2004 and Tyagi *et al*., 2017) [15, 24].

Table 1: Distribution of 28 cucumber genotypes into different clusters.

Clusters	Number of genotypes	Genotype
I	6	LC-9, UHF-16, RK-40, LC-18, Super Star, PCUC-8
II	4	KTC-7, Panipat Local, Tania, Pant Khira
III	3	LC-19, Poinsett, Punjab Naveen
IV	5	2870 G, Pusa Barkha, Priya, Kalyanpur Green, Swarna Sheetal
V	10	K-90, LC-8, LC-10, LC-11, 1983 G, KTG 40, LC-17, LC-20, Pusa Uday, Kohinoor Local

Average intra- and inter cluster distances

The estimates of intra- and inter cluster values represented by D^2 values (Table 2). The intra- cluster distance ranged from 8.82 (Cluster IV) to 15.04 (cluster I). Among the five clusters, the intra- cluster distance was maximum in cluster I (15.04) followed by cluster II (12.37) and cluster III (12.23), while the minimum intra- cluster distance was observed in cluster IV (8.82) followed by cluster V (10.58). Similar kind of results in cucumber was also reported by several researchers (Kumar *et al*., 2013 and Das *et al*., 2019) [11, 2]. The intra - cluster values are lesser than the inter- cluster values which indicates the homogenous and heterogenous nature of the genotypes within and between the clusters, respectively. The maximum inter-cluster distance was observed between cluster II and clusters V (71.19) followed by cluster II and IV (45.31), cluster I and clusters II (43.26), whereas the minimum inter-cluster distance was observed between Cluster III and IV (18.15) indicating close relationship among the genotypes belonging to these clusters. These results are in agreement with the findings of Kabir *et al*. (2009) [8], Kumar *et al*. (2013) [11], Tyagi *et al*. (2017) [24] and Das *et al*. (2019) [2]. Hence, inter mating between the genotypes included in these clusters could be expected to give transgressive segregates in the advanced generation as suggested by Kalloo *et al.* (1980) [9].

Table 2: Intra-and inter-cluster distances of 28 cucumber genotypes.

Clusters	I	II	III	IV	V
I	15.04	43.26	25.66	23.09	42.70
II		12.37	29.36	45.31	71.19
III			12.23	18.15	43.13
IV				8.82	27.11
V					10.58

*Bold diagonal values indicate intra cluster distance, rest of the values show the inter cluster distances.

Mean value of the clusters

The diversity among the genotypes was also substantiated by the considerable amount of variation among cluster means for different characters which might be the reason for large inter cluster distances. The cluster means of 28 genotypes showed that the mean values of the clusters varied in magnitude for all the 18 characters (Table 3). Cluster V (4.75) was the highest marketable fruit yield per plant (kg) followed by cluster IV

(3.72). Regarding earliness traits cluster V found best as minimum days for days to first female flower appearance (40.11), node number bearing first female flower (2.37) and days to first fruit harvest (48.00). Highest number of number of primary branches per plant, length of vine (m), fruit length at edible stage (cm), fruit breadth at edible stage (cm) was produced in the genotypes cluster V (5.48, 4.00, 21.01 and 5.11, respectively). Maximum seed cavity size (cm) was observed in genotypes under cluster I (3.76) followed by cluster IV (3.51) whereas, 100 seed weight (g) was highest in cluster V (3.67). Maximum number of fruits per plant was exhibited in cluster V (18.38) whereas, average weight of edible fruit (g) was highest in cluster V (258.73). Total soluble solids of the pulp (°Brix) was observed in cluster IV (3.82) however, highest total sugars (%) and ascorbic acid content of the pulp (mg per 100 g) was observed in cluster V (2.51 and 4.21, respectively) even as, low cucurbitacin content of the fruit [CC (µg/100g)] found in cluster V (100.58) followed by cluster III (105.56). Least PDI of downy mildew disease at 120 DAS was shown in the genotypes cluster V (14.63) while, cluster V (17.11) was also found best as minimum PDI of powdery mildew disease at 120 DAS is desirable.

Thus, the maximum cluster mean was observed in cluster V for all the characters except seed cavity size and total soluble solids of the pulp, while Cluster I for seed cavity size; cluster IV for total soluble solids of the pulp. These clusters could be regarded as useful sources of gene for important yield component traits. Hence, it can be suggested from the present study that a high yielding, early flowering type, number of fruits per plant with least infestation of downy mildew and powdery mildew diseases could be bred by utilizing the genotypes from cluster V as parents in the future breeding programme. Earlier workers like Ram (2001) [18], Khan (2006) [10], Kumar *et al*. (2013) [11], Tyagi *et al*. (2017) [24] and Das *et al*. (2019) [2] have also indicated the significance of genetic divergence.

Genetically distant parents usually able to produce higher heterosis (Falconar, 1960, Moll *et al*., 1962 and Mian and Bhal, 1989) [4, 17, 16]. Endang *et al*. (1971) [3] stated that the clustering pattern could be utilized in choosing parents for cross combinations which likely to generate the highest possible variability for effective selection of various economic traits. Keeping this in view, the findings from the present study indicated that the cluster II and V, II and IV showed higher distance between them. Parental material selection from these clusters would give high manifestation of heterosis as well as wide spectrum of variation when they are hybridized.

Table 3: Cluster means for 18 characters in cucumber genotypes

Clusters	Days to first female flower appearance	Node number bearing first female flower	Days to first fruit harvest	Number of primary branches per plant	Length of vine (m)	Fruit length at edible stage (cm)	Fruit breadth at edible stage (cm)	Seed cavity size (cm)	100 seed weight (g)	Number of fruits per plant	Average weight of edible fruit (g)	Total soluble solids of the pulp (°Brix.)	Total sugars (%)	Ascorbic acid content of the pulp (mg per 100 g)	Cucurbitacin content of the fruit [CC (µg/100 g)]	PDI of Downy mildew disease at 120 DAS	PDI of powdery mildew disease at 120 DAS	Marketable fruit yield per plant (kg)
I	51.16	4.52	59.70	3.50	2.98	17.34	4.90	3.76	2.95	13.55	230.51	3.53	2.39	3.26	111.69	36.18	28.47	3.13
II	47.01	4.20	54.70	4.20	3.15	17.04	4.10	3.35	2.62	13.20	190.06	2.97	2.09	3.31	108.75	26.00	19.64	2.53
III	47.71	4.19	55.70	4.11	3.05	17.28	4.38	3.44	3.03	13.16	218.08	3.60	2.40	3.38	105.56	18.26	17.70	2.88
IV	44.09	3.48	51.19	4.28	3.67	18.99	4.58	3.51	3.33	15.84	234.32	3.82	2.39	3.85	109.03	18.44	20.51	3.72
V	40.11	2.37	48.00	5.48	4.00	21.01	5.11	2.88	3.67	18.38	258.73	3.73	2.51	4.21	100.58	14.63	17.11	4.75

Principal component analysis

The analysis had grouped the estimated cucumber variables into five main components. The PCA was performed to obtain a simplified view of the relationship between the characters days to first female flower appearance, node number bearing first female flower, PDI of downy mildew disease at 120 DAS, PDI of powdery mildew disease at 120 DAS and marketable fruit yield per plant that explained 100% contribution toward divergence, and variable loadings for components PC_1 (days to first female flower appearance), PC_2 (node number bearing first female flower), PC_3 (PDI of downy mildew disease at 120 DAS), PC_4 (PDI of powdery mildew disease at 120 DAS), and PC_5 (marketable fruit yield per plant) were determined (Table 4). These components were chosen because their eigenvalues exceeded 1.0 and explained 79.80% of total variance. The first component (PC_1) explained 44.20% of total accounted for variance in which an increase in days to first female flower appearance leads to increase early node number bearing first female flower, PDI of downy mildew disease at 120 DAS and marketable fruit yield per plant whereas, decrease in PDI of powdery mildew disease at 120 DAS (Table 5). The second component (PC_2) explained an additional 11.40% of the variance in which a increase in days to first female flower appearance leads to increase early node number bearing first female flower, PDI of downy mildew disease at 120 DAS, PDI of powdery mildew disease at 120 DAS and marketable fruit yield. Increased yield potential is a stated goal for plant breeders. The PCA was also used to determine relationships among cucumber genotypes of Indian origin (Das *et al*., 2019) [2]. There are no clear guidelines to determine the importance of a trait coefficient for each principal component. Johnson and Wichern (1988) [7] regard a coefficient greater than half of the coefficient, divided by the square root of the standard deviation of the eigenvalue of the respective principal component, as significant. Accessions in close proximity are perceived as being similar in PCA; accessions that are further apart are more diverse.

Table 4: Principal component analysis (PCA) for quantitative characters contributing to divergence.

Principal component (PC)	Eigenvalues (%)	Variance (%)	Cumulative variance (%)
Eigenvalues and variance accounted for (%) by PCA based on correlation matrix			
PC_1	7.95	44.20	44.20
PC_2	2.06	11.40	55.60
PC_3	1.94	10.80	66.40
PC_4	1.29	7.20	73.60
PC_5	1.12	6.20	79.80

Table 5: Contribution of diverse traits in the principal components of cucumber.

Variable	PC_1	PC_2	PC_3	PC_4	PC_5
Factor loadings due to PCs with eigenvalues >1					
Days to first female flower appearance	0.305	0.094	0.198	0.102	0.236
Node number bearing first female flower	0.283	0.009	0.163	-0.052	0.029
PDI of downy mildew disease at 120 DAS	0.210	0.334	0.005	-0.467	0.038
PDI of powdery mildew disease at 120 DAS	-0.047	0.381	-0.065	-0.107	0.634
Marketable fruit yield per plant (kg)	0.323	0.182	-0.102	-0.035	0.109

Conclusion

Grouping of genotypes by multivariate and principal component analysis in the study is of practical value for the cucumber breeders. Representative genotypes may be chosen from the particular groups for hybridization programs with other approved cultivars. This will aid in identification, selection and combining genotypes to obtain important traits in one line with a broad genetic base.

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