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Inter and Intra Genetic Diversity of Sweet Cherry (*Prunus avium* L.) in Jammu and Kashmir Ecological Zone

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KMB, HUR and SH carried out study collectively whereas, authors HUR, AHP and MAM wrote the protocol and prepared the first draft of the manuscript collectively. Authors ZAD, UI and HUR collectively managed the literature searches. Authors HUR and RAL carried out statistical analysis using windowstat software and managed the experimental process. Author KMB gave research topic and designed the study and acted as main mentor in research. All authors read and approved the final manuscript.

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ABSTRACT

Nine sweet cherry accessions were used for the analysis of morphological variability and genetic diversity by means of multivariate statistical analysis. Days from full bloom to harvest was significantly lowest (66.33 days) in accession-6. Significantly highest fruit set (37.10%) was found in accession-2. However, fruit length was highest in selection-1 (24.82 mm) which was statistically at par with accession-7 (24.77 mm). Fruit weight was maximum in selection-2 (8.13 g) which was found to statistically at par with accession-1 (8.08g) and accession-7 (8.08 g). Fruit firmness was statistically maximum in selection-7 (396 gmm^{-1}). Maximum stone length (11.40mm) was found in accession-8 while as minimum was observed in accession-2 (10.10 mm). Stone weight was

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observed to be maximum in accession-7 (0.48 g) while as minimum in accession-6 (0.35 g). Statistically highest and lowest TSS was observed in accession-7 and accession-6, respectively. Most acidic fruit were of accession-6 and accession-9 while as least acidic fruits were of accession-3. Significantly maximum yield was observed in accession-2 (8.31 kg). The phenotypic variance was higher than genotypic variance for each observed trait. The phenotypic coefficient of variation and genotypic coefficient of variation was highest for TSS: acid ratio (16.21 & 15.94%) followed by fruit set (15.08 & 15.07%) and lowest for stone length (4.32 & 4.29%). The estimates of heritability (broad sense) in combination with genetic advance (as percent of mean) were high for fruit set (0.99 & 31.05%), TSS: Acid ratio (0.96 & 32.29%), fruit yield (0.97 & 28.56%), firmness (0.99 & 27.51) and fruit weight (0.99 & 21.02%). The accessions under study were grouped into 4 clusters as per Mahalanobis D^2 analysis with maximum number of accessions in cluster I (5) followed by cluster IV (2) and rest two 2 clusters were monogenotypic. The maximum intra cluster distance was observed in cluster IV (2995.47) followed by cluster I (1900.65) where as the inter cluster distance was maximum between cluster II and IV (12307.92) followed by cluster I and IV (10387.72).

Keywords: Sweet cherry; accession; variability; diversity; range; cluster; D^2.

1. INTRODUCTION

Sweet cherry (*Prunus avium* (L.) belongs to the family of Rosaceae, subfamily *Prunoideae*, to the genus *Prunus*, subgenus *Cerasus* [1]. Sweet cherries are non-climacteric stone fruits, mainly grown in temperate climate countries. In India, the state of Jammu and Kashmir, Himachal Pradesh and Uttarakhand are the main contributors of cherry production in which Jammu and Kashmir alone produces more than 95 per cent of the total production of commercial cherries. An area of 2816 ha is under cherry cultivation in J&K with an annual production of 10244 metric tonnes [2]. Sweet cherry tree is a species with a great economic importance, due to the nutritional, technological and commercial value of its fruits [3].

Genetic diversity in breeding is of paramount importance. Therefore the recognition and measurement of such diversity and its nature and magnitude are beneficial or even crucial to a breeding programme. Genetic variability among traits is important for breeding and in selecting desirable traits. The availability and informative value of plant germplasm are becoming more and more important for the future preservation and sustainable use of genetic resources [4]. Evaluation and characterization as well as estimation of diversity have been performed for various sweet cherry collections [5,6,7,8].

Heritability of a trait is important in determining its response to selection. The broad sense heritability is the relative magnitude of genotype and phenotypic variance for the traits and it gives an idea of the total variance accounted to genotypic effect [9].

Multivariate statistical techniques were suggested for analysis of genetic diversity in crop plants as a useful tool [10,11]. Mahalanobis D^2 statistic provided measure of the generalized distance in case of multiple measurements. It helps in the identification of genetically divergent genotypes that facilitated grouping and characterization by using both quantitative and qualitative characteristics [12]. The most widely used multidimensional analysis methods in PGR characterization are Principal Component Analysis (PCA) and cluster analysis [13]. Cluster analysis allows to analyze simultaneously both quantitative and qualitative traits, and each entry is treated as an individual entity of equal weight. The most appropriate approach for classification purposes is the group average clustering method [14].

Sweet cherry, as compared to other fruit species, exhibits high genetic variability. Therefore, the present study was undertaken to estimate the extent of genotypic and phenotypic variability, heritability and genetic advance among the selected characters and direct and indirect effects of component characters on yield of sweet cherry.

2. MATERIALS AND METHODS

The present investigation was carried out during 2013 and 2014 at Zangam Nursery Pattan, Baramulla, J&K. The experimental site is situated at latitude of 34°45'N and longitude of 74°5' E and an elevation of 1,649 metres above mean

sea level. Five plants from each selection were selected for recording the observations. Twenty ripened fruits were randomly collected from each direction of the tree for recording observations on yield plant^{-1}, fruit weight, fruit length, fruit, fruit firmness, stone length, stone weight, TSS and acidity. Besides, these observations, other traits like fruit set, days from full bloom to harvest, fruit stone ratio and TSS acidity ratio were also calculated.

Genotypic and phenotypic coefficients of variation were computed according to Burton [15] based on the estimate of genotypic and phenotypic variance. Heritability in broad sense was estimated as per the formula given by Lush [16]. Genetic advance as per cent mean was worked out for each fruit character adopting the formula given by Johnson et al. [17].

The data were subjected to pooled analysis. All the genotypes were grouped using Tocher's method as suggested by Rao [18]. The two varieties having smallest distance from each other were considered first to which a third variety having smallest average D^2 value from the first two varieties was added. Next come the nearest fourth variety and the process continued till the average D^2 value increased. The remaining varieties were then considered for the next cluster and the process was continued till all varieties were included in various clusters. The spatial distances between clusters were arrived at by taking square root of average intra and inter cluster D^2 values. Windowstat (9.1) package was used for statistical analysis.

3. RESULTS AND DISCUSSION

The analysis of variance pertaining to quantitative traits of cherry are summarized in Table 1. The data presented in Table 2 revealed days from full bloom to harvest was significantly lowest (66.33 days) in accession-6 and highest in accession-7 (80 days). Significantly highest fruit set (37.10%) was found in accession-2 followed by accession-3 (33.90%). Least fruit set percentage (21.95) was obtained in case of accession-4. However, fruit length was highest in accession-1 (24.82mm) which was statistically at par with accession-7 (24.77mm). Whereas, fruits of minimum length (19.59mm) were found of accession-6. Similarly, fruit weight was maximum in selection-2 (8.13g) which was found to statistically at par with accession-1 (8.08g) and accession-7 (8.08g) while as minimum average fruit weight was obtained in accession-9 (6.00g). Fruit firmness was statistically maximum in selection-7 (396 gmm^{-1}) followed by accession-2 (367), while as minimum firmness was recorded in accession-9 (271). Maximum stone length was found in accession-8 (11.40 mm) followed by accession-9 (11.32 mm), whereas, minimum was observed in accession-2 (10.10 mm). Stone weight was observed maximum in accession-7 (0.48 g) while as minimum in accession-6 (0.35 g). Statistically highest (22.50 ºBrix) and lowest TSS (17.98 ºBrix) was observed in accession-7 and accession-6, respectively. Most acidic fruit were of accession-6 (0.70%) and accession-9 (0.70%) while as least acidic fruits were of accession-3 (0.53%). Statistically highest TSS acidity ratio was found in accession-7 (40.42) followed by accession-3 (37.15), accession-2 (36.48) whereas, lowest TSS acidity ratio was in accession-6 (25.56). Similarly, fruit stone ratio came to be maximum in accession-6 (19.83) however; this value was statistically at par with accession-1 (18.96), accession-5 (18.89) and accession-2 (18.77). Significantly maximum yield was observed in accession-2 (8.31 kg) followed by accession-3 (7.81 kg).

Chemical aspects of fruits such as total soluble solid and acidity provide important information to the consumers in terms of recognizing a more nutritious fruit [19,20]. TSS and flowering is influenced by environmental factors such as temperature, light (duration and intensity), rainfall/supply of water and locations [21,22]. The variability in fruit characteristics especially in fruit composition is not only genetic factor but also influenced by climatic factors [20,23]. Variations of pomological and morphological characters in sweet cherry have earlier been reported by Srivastava et al. [8], Moghaddama et al. [20], Crisosto et al. [24]. In the present study, statistically significant differences were observed in ripening time, fruit weight, stone weight, soluble solid content, total acid content, fruit firmness, stone characteristics, fruit set and yield. This variation has been attributed to genotype, cultivar, rootstock, optimum agro-climatic regime, orchard planning regarding pollination, crop load etc [20,25,26,27].

Phenotypic coefficient of variance (PCV) was greater than genotypic coefficient of variance (GCV) for all the quantitative traits studied (Table 3) and was high for TSS acidity ratio (16.21 & 15.94%), fruit set (15.08 & 15.07%) and firmness (13.42 & 13.39%) and lowest for stone length (4.32 & 4.29%).

These results were conformity of the findings of Bandale et al. [28], Chattopadhyay et al. [29] and Yadav et al. [30]. The results were supported by Bhat and Dhillon [31], who reported high phenotypic and genotypic coefficient of variation for fruit yield, fruit length, fruit weight and TSS in pear and by Srivastava et al. [8] and Moghaddama et al. [20] in sweet cherry.

The estimates of heritability (broad sense) in combination with genetic advance (as percent of mean) were high for fruit set (0.99 & 31.05%), TSS: Acid ratio (0.96 & 32.29%), fruit yield/tree (0.97 & 28.56%), firmness (0.99 & 27.51), fruit weight (0.99 & 21.02%), fruit stone ratio (0.89 & 20.88%), fruit length (0.99 & 19.97) and low for stone length (0.98 & 8.78), days from full bloom to harvest (0.87 & 11.98) [Table 3].

High heritability coupled with high genetic advance was observed for all characters studied indicating that these characters are governed by additive gene action and phenotypic selection may be more fruitful. Hence, direct selection may be followed for the improvement of cherry for these characters. High values of heritability for the traits clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable. The results were supported by Srivastava et al. [8], Moghaddama et al. [20], Sharma et al. [32] and Sharma and Sharma [33], who reported high heritability with high genetic gain for different parameters in sweet cherry and apple.

The range of variation was high for fruit firmness (271.00-396.00) followed by fruit set (21.95-37.10), TSS acidity ratio (25.56 - 40.41), days from full bloom to harvest (66.33 – 80.00) and fruit stone ratio (13.36 – 19.83). A better idea can be gained by comparing the relative magnitude of phenotypic and genotypic co-efficient of variations for the actual strength of variability. The estimates of phenotypic coefficient were observed to be higher in magnitude than their corresponding estimates of genotypic coefficient of variations for all the traits, which indicates the expression of these traits (Table 3). Srivastava et al. [8] and Fotiric et al. [34] also found that these characters were more useful to estimate heritability value together with genetic advance in predicting the expected progress to be achieved through selection. Hence, high heritability coupled with high genetic advance over mean was observed for various fruit characters and hence simple selection will be effective for their improvement.

The genetic diversity studied for nine cherry accessions for twelve quantitative traits by employing non-hierarchical Euclidean cluster analysis and, was grouped into 4 different nonoverlapping clusters (Fig. 1). Fig. 1 clearly shows that the maximum five accessions numbering 3,5,6,8, and 9 appeared in the cluster I while as accessions numbering 1 and 2 appeared in cluster IV. However, cluster II and cluster III included accessions numbering 4 and 7 respectively and hence were monogenotypic. The discrimination of germplasm lines into many discrete clusters, suggested the presence of high degree of genetic diversity. Earlier some workers have been reported an existence of substantial high degree of genetic diversity in the cherry germplasm using cluster analysis [6,7,8].

On the basis of D^2 analysis, the intra and inter-cluster distance for four clusters are presented in Table 5 and Fig. 2. The maximum intracluster distance were found in cluster IV (2995.47) followed by cluster I (1900.65). However, maximum inter cluster distance was observed between cluster II and IV (12307.92) followed by between cluster I and cluster IV (10387.72). Since, these clusters have more inter-cluster distances within themselves; hence, the selection of parents for hybridization from such clusters would helps to evolve new hybrids.

The cluster means for different characters (Table 4) revealed that cluster I had maximum mean values for days from full bloom to harvest (77.66), fruit set (34.70), fruit length (24.51), fruit weight (8.10), acidity (0.588) and fruit yield per tree (7.82). Cluster II had maximum mean values for fruit firmness (368.83), stone length (11.19), stone weight (0.47), TSS (21.30) and TSS acidity ratio (36.78). Cluster III had maximum mean value for fruit stone ration (19.36).

Multivariate technique using D^2 statistics [35] is a very powerful and multivariate statistical tool in quantifying the degree of divergence among the genotypes [36]. In order to identify genetically diverse parents for hybridization, Mahalanobis D^2 statistics has been used in almost all crop species. 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 [37]. The use of genetically divergent

parents in hybridization under transgressive breeding programme depends on the categorization of breeding material on the basis of appropriate criteria [38]. 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. This estimation of genetic divergence helps in reducing the large data of genotypes to manageable proportions. It is assumed that the parents showing wide genetic divergence are best suited for being used in the hybridization programmes. The utility of multivariate analysis in quantifying the degree of divergence between populations so as to understand the trend of their evolutionary pattern and assess the relative contribution of different components to the total divergence together with the nature of forces operating at intra and inter cluster levels had greatly been emphasized [39]. Classification of genotypes into different groups through D^2 statistics has also been reported in cherry by Lacis et al. [7], Srivastava et al. [8], Fotiric et al. [34] and Koci and Bilgener [40].

The clustering of genotypes from different eco-geographic locations into one cluster could be attributed to exchange of breeding materials from one place to another and may be due to unidirectional selection practiced for a particular character at several places produced similar phenotypes which were aggregated in one cluster irrespective of their distant geographic origin [41]. Another case is where many genotypes originating from one place were scattered over different clusters and such diversity among genotypes of common geographic origin could be attributed to factors like heterogeneity, genetic architecture of the populations, past history of selection, developmental traits and degree of general combining ability [36].

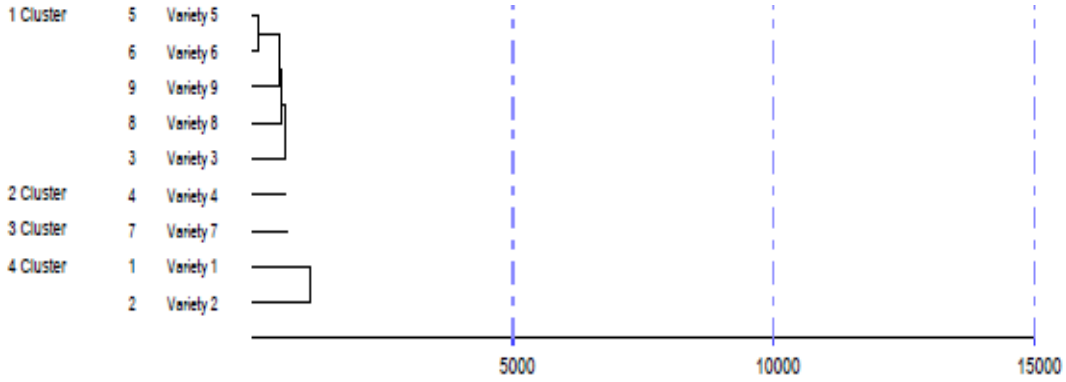

Fig. 1. Clustering of collected cherry accessions by Toucher method

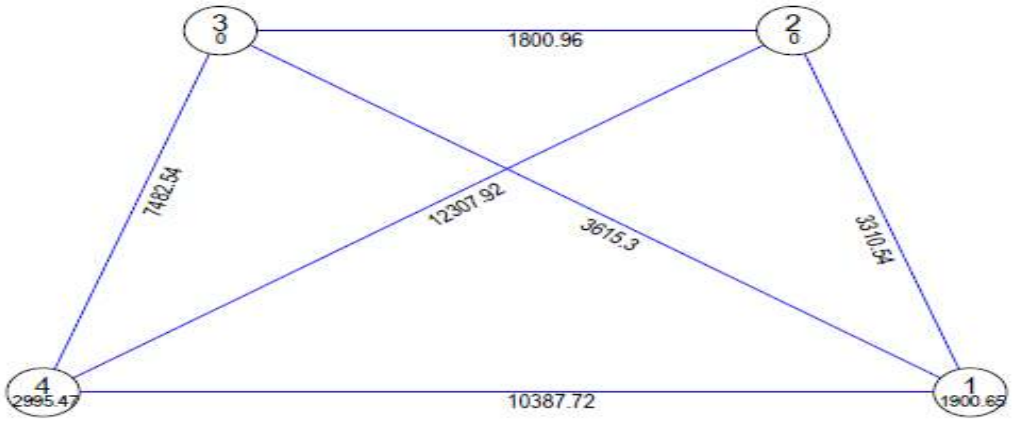

Fig. 2. Malalanobis Euclidean distance of various clusters by Toucher method

Table 1. Analysis of variance (ANOVA) for various traits in cherry accessions

Source of variation	d.f	DFBH (day)	Fruit set (%)	Fruit length (mm)	Fruit weight (g)	Firmness (gmm^{-1})	Stone length (mm)	Stone weight (g)	TSS (%)	Acidity (%)	TSS : Acidity	Fruit: Stone	Yield $tree^{-1}$ (kg)
Genotypes	8	64.59	60.27	13.96	1.713	5441.39	0.65	0.005	6.17	0.01	80.83	11.01	2.94
Error	16	2.81	0.01	0.01	0.004	9.66	0.003	0.0003	0.01	0.0004	0.91	0.41	0.02

Table 2. Mean values of different traits of sweet cherry accessions

Accession	DFBH (Days)	Fruit set (%)	Fruit Length (mm)	Fruit weight (g)	Firmness (gmm^{-1})	Stone length (mm)	Stone weight (g)	TSS (%)	Acidity (%)	TSS/ Acidity	Fruit/ stone	Yield$tree^{-1}$ (kg)
1	78.33	32.31	24.82	8.08	320	10.52	0.43	19.17	0.60	32.18	18.96	7.34
2	77.00	37.10	24.21	8.13	367	10.10	0.43	21.15	0.58	36.48	18.77	8.31
3	70.33	33.90	20.38	7.30	301	10.48	0.42	19.08	0.53	37.15	17.27	7.81
4	75.00	21.95	23.87	7.96	342	11.14	0.47	20.10	0.61	33.15	16.93	4.82
5	70.00	26.50	21.19	7.04	296	10.18	0.37	18.90	0.69	27.40	18.89	6.63
6	66.33	28.11	19.59	7.00	279	10.34	0.35	17.98	0.70	25.56	19.83	6.90
7	80.00	27.00	24.77	8.08	396	11.25	0.48	22.50	0.56	40.42	16.85	6.63
8	72.00	30.91	20.93	6.65	288	11.40	0.39	19.40	0.59	32.89	16.92	7.59
9	69.33	29.71	20.16	6.00	271	11.32	0.45	18.15	0.70	26.11	13.36	7.02
Mean	**73.15**	**29.72**	**22.21**	**7.36**	**318**	**10.75**	**0.42**	**19.60**	**0.62**	**32.37**	**17.53**	**7.01**
C.D (5%)	2.88	0.22	0.06	0.12	5.33	0.09	0.03	0.17	0.04	1.64	1.11	0.29

Table 3. Estimates of parameters of variability for different traits in sweet cherry

Observation	Mean	Range	Genotypic variance	Phenotypic Variance	Genotypic coefficient of variation	Phenotypic coefficient of variation	heritability	Genetic advance (% of mean)
DFBH	73.14	66-33-80.00	20.59	23.40	6.20	6.61	0.87	11.98
Fruit set (%)	29.72	21.95-37.10	20.08	20.10	15.07	15.08	0.99	31.05
Fruit length (mm)	22.21	19.58- 24.82	4.65	4.66	9.70	9.72	0.99	19.97
Fruit weight (g)	7.36	6.00-8.13	0.56	0.57	10.25	10.29	0.99	21.02
Firmness	**317.74**	271.00-396.00	1810.57	1820.24	13.39	13.42	0.99	27.51
Stone length (mm)	10. 84	10.18-11.40	0.21	0.22	4.29	4.32	0.98	8.78
Stone weight (g)	0.422	0.353-0.480	0.001	0.002	9.76	10.67	0.83	18.39
TSS (%)	19.68	17.98-22.50	2.05	2.06	7.28	7.30	0.99	14.96
Acidity (%)	0.61	0.53-0.70	0.003	0.004	10.11	10.69	0.89	19.71
TSS : Acid Ratio	32.37	25.56-40.41	26.63	27.55	15.94	16.21	0.96	32.29
Fruit Stone ratio	17.53	13.36-19.83	3.53	3.95	10.72	11.33	0.89	20.88
Fruit Yield /tree (kg)	7.00	4.82-8.31	0.97	1.00	14.07	14.28	0.97	28.56

Table 4. Cluster means for various traits in cherry accessions

Cluster	DFBH	Fruit set (%)	Fruit Length (mm)	Fruit weight (g)	Firmness	Stone length (mm)	Stone weight (g)	TSS (%)	Acidity (%)	TSS : Acidity	Fruit: Stone	Fruit Yield /tree (kg)
I	77.66	34.70	24.51	8.10	343.66	10.76	0.43	20.15	0.588	34.33	18.86	7.82
II	77.50	24.47	24.32	8.01	368.83	11.19	0.47	21.30	0.582	36.78	16.88	5.72
III	68.16	27.30	20.38	7.02	287.33	10.25	0.36	18.44	0.697	26.48	19.36	6.76
IV	70.55	31.50	20.49	6.65	286.66	11.06	0.42	19.11	0.607	32.05	15.85	7.47

Table 5. Average intra cluster (diagonal) and inter cluster (above diagonal) distance i.e Malalanobis Euclidean Distance in cherry accessions

S. no	Cluster	I	II	III	IV
1	I	1900.65	3310.54	3615.3	10387.72
2	II		0.00	1800.96	12307.92
3	III			0.00	7482.54
4	IV				2995.47

The results clearly indicate that tremendous potential exists for introgressing the allelic resource present in these genotypes through a systematic breeding and selection approach so as to recover the best quality recombinants. Cluster means for various morpho-taxonomic and quality related characters revealed that substantial variability existed for all the traits and identify the traits to be chosen for hybridization as reported by Lacis et al. [7], Srivastava et al. [8], Bhat and Dhillon [31] and Pereira et al. [42].

4. CONCLUSION

It could be concluded that the accessions under study exhibited morphological variability and genetic diversity in terms of fruit characteristics. This variability and diversity holds key in future cherry breeding programmes. High heritability estimates coupled with high genetic advance were observed for all the characters were indicative of additive gene action and selection based on these characters would be more reliable. The characters with high heritability coupled with high genetic advance further indicated the possibility of making selections in earlier generations. Accessions under present study belonging to different clusters can be successfully utilized for cherry improvement under Kashmir conditions which can improve overall profitability of this fruit crop.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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