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Multivariate and genetic diversity assessment in summer squash (*Cucurbita pepo* L.) genotypes

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Abstract
Summer squash (*Cucurbita pepo* L), is a significant vegetable crop with significant nutritional, therapeutic and financial importance. Assessing the degree of genetic diversity and population structure among the available genotypes is crucial for successful breeding. Using forty different genotypes arranged in a randomized full block design, the current study was carried out at SKUAST-Kashmir during Kharif 2024. To evaluate genetic diversity and cluster the genotypes, data on morphological, yield and quality variables were examined using Ward's technique and Mahalanobis D2 statistics. Analysis of variance indicated the existence of extensive variability by showing substantial differences across all genotypes. Four clusters were formed from the genotypes using cluster analysis; Cluster II had the most genotypes (17) and the highest fruit yields per plant (4.52 kg) and per hectare (226.64 q). Early flowering and fruiting, a desired feature for creating hybrids that mature early, was displayed by Cluster I. With the highest levels of vitamin C (13.42 mg/100 g), total soluble solids (10.03 °Brix), and crude fiber (2.13%), Cluster III was notable for its quality qualities despite its smaller size. The greatest inter-cluster distance was found between Clusters I and II, indicating that crossings between these genotypes will result in more variety and high heterotic combinations. Breeding efficiency will be improved by choosing parents from genetically distant clusters, particularly those that combine high yield and superior quality features, according to the study. The findings offer a solid basis for creating summer squash cultivars that are early maturing, high yielding and nutritionally enhanced and suited to temperate climates.

Keywords: Mahalanobis D2, cluster analysis, genetic divergence, population structure and breeding potential

Introduction

Summer squash (*Cucurbita pepo* L.), a crucial annual vegetable crop in the Cucurbitaceae family, is distinguished by its compact plant architecture and strong development. Its petioles are covered with prickly hairs and carry orbicular, shallow- to deep-lobed leaves, while its stems have closely spaced, shorter internodes. It is a cross-pollinated, monoecious plant with short tendrils that usually grows in a compact, bushy habit. It is ideal for cultivation in temperate and subtropical regions with mild summers (Bisognin, 2002) [5]. The summer squash, which includes horticultural varieties including zucchini, crookneck, scallop, straight neck, acorn, cocozelle and vegetable marrow, is regarded as one of the most varied species in the genus Cucurbita. The crop thrives on a variety of soil types, including marginal and less fertile areas, it is quick to grow, early to harvest, and versatile. A temperature range of 16-27 ºC is ideal for growth. Its production greatly enhances the socioeconomic growth of farming communities in addition to promoting nutritional security. It is harvested before seed development, at the glossy rind stage, the crop is prized worldwide for its soft, immature fruits. Humans can benefit from zucchini's many health benefits and possiable medical uses (Mohammad *et al*., 2003) [11], Summer squash is a popular ingredient in cuisines all over the world because of its mild flavor and delicate texture, which make these fruits frequently devoured. In addition to its culinary applications, summer squash is known for its great nutritional and medicinal value. According to (Balluz *et al*., 2005) [4], it is a good source of dietary fiber and vitamins A, C, niacin and folate, which help prevent inflammatory conditions like arthritis and asthma as well as chronic diseases like cancer and cardiovascular conditions.

A common summer squash, zucchini is especially well-known for its high vitamin C content, which boosts immunity and lowers homocysteine levels to improve cardiovascular health. With 95% water, the fruit is extremely hydrating while yet being a low-calorie food choice, with only 25 calories per medium-sized fruit. Squash cultivation has become more and more popular in recent years due to rising consumer health consciousness.

According to Al Brifcany (2015) [1], almost half of the world's total land used for squash and pumpkin cultivation is in Asia. In 2023-2024, their aggregate yearly production in India was 2,703.61 thousand metric tonnes (NHB, 2024). About 3,200 hectares of summer squash are farmed in the Kashmir region alone, yielding about 64,800 tonnes of produce (Anonymous, 2024) [3]. Summer squash is now a highly valued vegetable crop in many different countries due to its short growing season, high productivity, nutritional value and robust market demand.

To initiate the development of high-yielding and superior-quality summer squash varieties suitable for cultivation in the temperate conditions of the Kashmir Valley, it is essential to evaluate a wide range of existing genotypes. These genotypes should be selected based on their performance from diverse sources. Superior genotypes can then be identified and utilized as parents in hybridization programs. However, selecting superior parents from a large pool of genotypes is a complex task. Genetic divergence analysis assists in identifying genetically diverse parents, which are more likely to produce significant heterotic effects in crosses and generate a broad spectrum of variability during gene segregation and recombination in heterozygous polygenic blocks. Multivariate techniques, such as D^2 statistics (Mahalanobis, 1928) [9], are powerful tools for quantifying the genetic divergence between genotypes. This helps in identifying potential parents for breeding programs aimed at achieving high heterotic responses and transgressive segregants. Estimating genetic divergence reduces large data sets of genotypes to more manageable proportions. It is generally assumed that parents showing wide genetic divergence are most suitable for hybridization; however, this is not always guaranteed, as seen in wild species where crosses with locally adapted genotypes may result in low heterosis. In the process of improving summer squash through hybridization and creating variability to enhance yield and other desirable traits, understanding the nature and degree of genetic divergence within the available germplasm is critical. The utility of multivariate analysis in quantifying divergence between populations, understanding evolutionary trends and assessing the relative contribution of different components to total divergence has been extensively discussed (Murty and Qadri, 1966; Anand and Murty, 1968; Mishra *et al.,* 1994) [12, 2, 10].

Materials and Methods

The study was conducted at the Vegetable Experimental Farm, Division of Vegetable Science, Faculty of Horticulture, SKUAST-Kashmir, Shalimar Srinagar, Jammu and Kashmir, during the Kharif season of 2024. The experimental location is 15 kilometers from Srinagar city in Shalimar, amid the foothills of Zabarvan. At 340.14" North latitude and 740.86" East longitude, the site is 1685 meters above mean sea level. The soil is alluvial and the pH of the water is neutral. One of the characteristics of the temperate climate is the mild summers. In August, the average temperature is 31.40°C, while in October, it is 5.260°C. Rainfall is greatest in April. Forty genotypes of summer squash with phenotypically distinct qualities that were collected from multiple sources were examined for yield and yield-attributing traits: Days to appearance of 1st male flower, Days to appearance of 1st female flower, Days to 1st Fruit Harvest, Days to Last Fruit Harvest, Number of Fruits/Plant, Average Fruit Weight (kg), Fruit Length (cm), Fruit Diameter (cm), Flesh Thickness (cm), Rind Thickness(mm), Flesh Weight (g), Fruit Yield/Plant (kg), Fruit Yield/ha (q), Total Sugars (%), Dry Matter content (%), Vitamin C content (mg/100g), TSS (0Brix), Crude Fibre content (%) during Kharif-2024. The seeds were sown on April 23, 2024. The single factor experiment was set up with three replications using a Randomized Complete Block Design (RCBD). Each replication contained five plants of each genotype, with rows and plants separated by 2 x 1 m. During the growth and development stage, standard cultural procedures were followed to ensure a healthy crop. In the process of improving summer squash through hybridization and creating variability to enhance yield and other desirable traits, understanding the nature and degree of genetic divergence within the available germplasm is critical. The utility of multivariate analysis in quantifying divergence between populations, understanding evolutionary trends and assessing the relative contribution of different components to total divergence has been extensively discussed (Murty and Qadri, 1966; Anand and Murty, 1968; Mishra *et al.,* 1994) [12, 2, 10].

Results and Discussion

The analysis of variance for dispersion revealed significant variability among the traits under study. Genetic divergence was estimated for 40 genotypes of summer squash using Mahalanobis D^2 analysis and Ward's technique (Ward, 1963), grouping them into four clusters (Table 1). The dendrogram (Fig. 1) showed the highest number of genotypes in Cluster II (17), followed by Cluster I (10) and Cluster III (3) and Cluster IV (10). Cluster I included SKUA-SQ-7, SKUA-SQ-3, SKUA-SQ-11, SKUA-SQ-10, SKUA-SQ-19, SKUA-SQ-21, SKUA-SQ-25, SKUA-SQ-12, SKUA-SQ-1, SKUA-SQ-39. Cluster II comprised SKUA-SQ-4, SKUA-SQ-6, SKUA-SQ-28, SKUA-SQ-13, SKUA-SQ-18, SKUA-SQ-9, SKUA-SQ-40, SKUA-SQ-5, SKUA-SQ-33, SKUA-SQ-27, SKUA-SQ-15, SKUA-SQ-22, SKUA-SQ-17, SKUA-SQ-24, SKUA-SQ-34, SKUA-SQ-2, SKUA-SQ-14. Cluster III included SKUA-SQ-16, SKUA-SQ-8 and SKUA-SQ-20. Cluster IV contained SKUA-SQ-29, SKUA-SQ-30, SKUA-SQ-31, SKUA-SQ-35, SKUA-SQ-32, SKUA-SQ-36, SKUA-SQ-37, SKUA-SQ-38, SKUA-SQ-23, SKUA-SQ-26. Despite originating from the same geographic location, the genotypes were scattered across different clusters. This diversity among genotypes with a common geographic origin could be due to factors such as heterogeneity, genetic architecture, selection history and developmental traits, as well as the extent of general combining ability (Murty and Arunachalam, 1966) [12]. The clustering pattern indicated that geographical diversity alone is not a determining factor in grouping genotypes into specific clusters. Therefore, the selection of varieties for hybridization should prioritize genetic diversity over geographic diversity. Statistical distance (D^2) provides a useful genetic diversity index for these four clusters. The observed diversity among genotypes of common geographic origin may be ascribed to factors such as heterogeneity, genetic architecture of populations, historical selection pressures, developmental traits and the extent of general combining ability (Murty and Arunachalam, 1966) [12].

The highest inter-cluster distance between Cluster I and Cluster II reflects a significant genetic difference between the genotypes in these clusters shown in table 2. In contrast, the lowest inter-cluster distance between Cluster II and Cluster IV suggests a closer genetic relationship between the genotypes. Within Cluster I, the maximum intra-cluster distance indicated a high level of genetic heterogeneity, while Cluster IV, with the minimum intra-cluster distance, showed greater genetic homogeneity among its genotypes. Selecting parents from genetically divergent clusters is expected to result in higher heterosis in hybridization programs. Genotypes from different clusters separated by maximum genetic distance may be used in hybridization programme to obtain a wide spectrum of variation among the segregants (Doddabhimappa *et al.,* 2010) [7]. Similar results were reported by Swain *et al*. (2022) [19], Sajid *et al* (2022) [16] in Summer Squash, Kumar *et al*. (2019) [8] in sponge gourd, Verma *et al*. (2020) [20] in pumpkin.

The genetic makeup of different clusters aids in identifying suitable clusters for hybridization. Cluster means and coefficients of variation offer insights into the nature of diversity within each group (Sardana *et al.,* 1997) [17]. While the mean values for genotypes within clusters are not statistically comparable, their comparison provides a general understanding of the genetic diversity within and across clusters. The range of means for each character helps identify the traits driving divergence within the clusters. An examination of the data presented in Table 3 (a-b) indicates notable differences in cluster means for various traits among the clusters. In Cluster I, the average fruit length of 32.36 cm and fruit diameter diameter of 7.78 cm while flesh weight with an average of 725.06 g. The average of flesh thickness is 1.85 cm, while rind thickness with an average of 0.31 mm. The number of fruits per plant with an average of 4.56, and average fruit weight with 0.88 kg average. Fruit yield per plant is 4.00 kg, resulting in a fruit yield per hectare of 201.64 quintals. The node number at which the Ist male flower appears at an average of 1.66 while the node number at which the Ist female flower appears is 3.88. The days to the appearance of the Ist female and male flowers are 62.17 and 59.54 respectively. The days to the Ist fruit harvest is 78.35 and the last fruit harvest occurs at 109.62 days. Number of seeds per fruit is 311.22, with a 100-seed weight of 24.31 g. Cluster I has an average total sugars content of 2.33% and the dry matter content is 6.97%. The vitamin C content is recorded at 11.16 mg per 100 g and total soluble solids are measured at 7.90 Brix, while the crude fiber content stands at 1.75.Cluster II exhibits a mean fruit length of 36.82 cm and for fruit diameter is 7.67 cm, while flesh weight is with an average of 837.04 g. The flesh thickness is recorded at 1.99 cm, while the rind thickness measures 0.38 mm of average. The of fruits per plant is with an average of 4.44 while average fruit weight is with 1.00 Kg average. The fruit yield per plant is 4.52 kg and the fruit yield per hectare reaches 226.64 q. The node number at which the Ist male flower appears is 1.53 and for the node number at which the Ist female flower appears is 4.1. Days to the appearance of the Ist female and male flowers are 62.60 and 60.20, respectively. On average, the days to the Ist fruit harvest are 78.26, while the days to the last fruit harvest extend to 109.60. Number of seeds per fruit is 159.30 and the 100 seed weight averages 24.82 g. Cluster II has total sugars at 2.36%, dry matter content at 6.75%, vitamin C content at 11.87 mg/100g and total soluble solids (TSS) at 8.09° Brix, while the crude fiber content is 1.82%. Cluster III, the average fruit length of 24.07 cm and fruit diameter diameter of 6.6 cm while flesh weight with an average of 498.77 g. The average of flesh thickness is 1.66 cm, while rind thickness with an average of 0.3 mm. The number of fruits per plant with an average of 4.51, and average fruit weight with 0.63 Kg average. Fruit yield per plant is 2.86 kg, resulting in a fruit yield per hectare of 143.38 quintals. The node number at which the Ist male flower appears at an average of 1.14 while the node number at which the Ist female flower appears is 4.06. The days to the appearance of the Ist female and male flowers are 63.33 and 61.06 respectively. The days to the Ist fruit harvest is 80.18 and the last fruit harvest occurs at 111.48 days. Number of seeds per fruit is 205.71, with a 100-seed weight of 29.4 g. Cluster III has an average total sugars content of 2.34% and the dry matter content is 6.47%. The vitamin C content is recorded at 13.42 mg per 100 g and total soluble solids are measured at 10.03 Brix, while the crude fiber content stands at 2.13. Cluster IV exhibits a mean fruit length of 32.32 cm and for fruit diameter is 7.36 cm, while flesh weight is with an average of 688.58 g. The flesh thickness is recorded at 1.83 cm, while the rind thickness measures 0.39 mm of average. The of fruits per plant is with an average of 4.00 while average fruit weight is with 0.85 Kg average. The fruit yield per plant is 3.43 kg and the fruit yield per hectare reaches 172.00 q. The node number at which the Ist male flower appears is 1.68 and for the node number at which the Ist female flower appears is 4.66. Days to the appearance of the Ist female and male flowers are 63.33 and 60.99, respectively. On average, the days to the Ist fruit harvest are 77.52, while the days to the last fruit harvest extend to 108.86. Number of seeds per fruit is 108.84 and the 100 seed weight averages 24.81 g. Cluster IV has total sugars at 2.23%, dry matter content at 6.96%, vitamin C content at 12.26 mg/100g and total soluble solids (TSS) at 8.32° Brix, while the crude fiber content is 1.92%.

Cluster II showed the highest flesh weight (837.04), fruit yield per plant (4.52 kg) and per hectare (226.64 q/ha), making it the most promising for yield improvement. Cluster I showed earliness with the lowest days to first female flowering (62.17) and the earliest node for female flower appearence (3.83) which is valuable for early-maturing hybrids. Cluster 3 stood out for its nutritional value, recording the highest ascorbic acid (13.42 mg/100g), TSS (10.03 °Brix), and crude fiber content (2.13%). Such data guides parent selection in hybridization programs.Therefore, traits that contribute significantly to genetic divergence can be enhanced by selecting genotypes from clusters with either high or low means for the respective traits, depending on the objectives of the breeding program. A similar comparative analysis of clusters based on mean values for each character has been conducted by Damor *et al*. (2017) [6], Quamruzzaman (2020) [14] and Rashid *et al*. (2020c) [15]. There is significant potential to harness the elite allelic resources found in these summer squash genotypes through a systematic breeding and selection strategy aimed at producing high-yielding recombinants with desirable quality traits. Parents for hybridization should be selected from multiple clusters that exhibit considerable inter-cluster distance. Additionally, the chosen parents should demonstrate strong individual performance for the traits that contribute most to genetic divergence (Singh *et al.,* 1996) [18]. Clusters comprising only a single genotype with specific traits can be effectively utilized in hybridization programs to exploit heterosis, primarily serving as testers for maximizing heterotic expression.

Table 1: Distribution of summer squash (*Cucurbita pepo* L.) genotypes into various different clusters based on D^2 Statistics

Cluster number	Genotypes	Number of genotypes in each cluster
I	SKUA-SQ-7, SKUA-SQ-3, SKUA-SQ-11, SKUA-SQ-10, SKUA-SQ-19, SKUA-SQ-21, SKUA-SQ-25, SKUA-SQ-12, SKUA-SQ-1, SKUA-SQ-39	10
II	SKUA-SQ-4, SKUA-SQ-6, SKUA-SQ-28, SKUA-SQ-13, SKUA-SQ-18, SKUA-SQ-9, SKUA-SQ-40, SKUA-SQ-5, SKUA-SQ-33, SKUA-SQ-27, SKUA-SQ-15, SKUA-SQ-22, SKUA-SQ-17, SKUA-SQ-24, SKUA-SQ-34, SKUA-SQ-2, SKUA-SQ-14	17
III	SKUA-SQ-16, SKUA-SQ-8, SKUA-SQ-20	3
IV	SKUA-SQ-29, SKUA-SQ-30, SKUA-SQ-31, SKUA-SQ-35, SKUA-SQ-32, SKUA-SQ-36, SKUA-SQ-37, SKUA-SQ-38, SKUA-SQ-23, SKUA-SQ-26.	10

Table 2: Average intra cluster (Diagonal) and inter cluster (Above Diagonal) distance values in summer squash (*Cucurbita pepo* L.)

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	146.94			
Cluster 2	365.82	128.49		
Cluster 3	271.43	214.95	107.95	
Cluster 4	242.91	186.16	229.53	94.22

Table 3(a): Cluster means for various agronomic characters in different clusters of summer squash (*Cucurbita pepo* L.)

	FL	FD	FW	FT	RT	NOFPP	AFW	FYPP	FYPH	NNAWFMFA	NNAWFFFA	DTFFF
Cluster 1	32.36	7.78	725.06	1.85	0.31	4.56	0.88	4.00	201.64	1.66	3.88	62.17
Cluster 2	36.82	7.67	837.04	1.99	0.38	4.44	1.00	4.52	226.64	1.53	4.1	62.60
Cluster 3	24.07	6.6	498.77	1.66	0.3	4.51	0.63	2.86	143.38	1.14	4.06	63.09
Cluster 4	32.32	7.36	688.58	1.83	0.39	4.00	0.85	3.43	172.00	1.68	4.66	63.33

FL: Fruit Length (cm), FD: Fruit Diameter (cm),FW: Flesh Weight (g), FT: Flesh Thickness (cm), RT: Rind Thickness (mm), NOFPP: Number of Fruits/Plant, AFW: Average Fruit Weight (kg),FYPP: Fruit Yield Per Plant (Kg), FYPH: Fruit Yield Per Hectare(q), NNAWFMFA: Node number at which 1st male flower appears, NNAWFFFA: Node number at which 1st female flower appears, DAPFF: Days to appearance of 1st female flower

Table 3(b): Cluster means for various agronomic characters in different clusters of Summer Squash (*Cucurbita pepo* L.)

	DTFMF	DTFFH	DTLFH	NOSPF	100SW	TS	DM	AA	TSS	CF
Cluster 1	59.54	78.35	109.62	311.22	24.31	2.33	6.97	11.16	7.90	1.75
Cluster 2	60.20	78.26	109.60	159.30	24.82	2.36	6.75	11.87	8.09	1.82
Cluster 3	61.06	80.18	111.48	205.71	29.4	2.34	6.47	13.42	10.03	2.13
Cluster 4	60.99	77.52	108.86	108.84	24.81	2.23	6.96	12.26	8.32	1.92

DTFMF: Days to appearance of 1st male flower, DTFFH: Days to Ist Fruit Harvest, DTLFH: Days to Last Fruit Harvest, NOSPF: No. of Seeds/Fruit, 100SW: 100 Seed Weight (g), TS: Total Sugars (%), DM: Dry Matter content (%), VITC: Vitamin C content (mg/100g), TSS: Total Soluble Solids (°Brix), CFC: Crude Fiber content (%).

Conclusion

There was substantial variation among the 40 summer squash genotypes, according to the genetic divergence analysis. Four clusters were created from the genotypes using D^2 statistics. With 17 genotypes, Cluster II had the most, followed by Cluster I and Cluster IV with 10 genotypes in each. Cluster III with 3 genotypes. Cluster I (D^2 = 148.94) had the largest intra-cluster distance, followed by Cluster II (D^2 = 128.49) and Cluster III (D^2 = 107.95). Cluster I and Cluster II had the largest inter-cluster distance (365.52), followed by Cluster III and I (271.43), Cluster I and IV (242.91), Cluster III and IV (299.53) and Cluster II and III (214.95). Significant variety was observed in the cluster means for different attributes. For hybridisation programs, choosing parents from Clusters I and IV, then II and IV, may make it easier to create unique recombinants.The findings demonstrate how crucial it is to use genotypes that are both agronomically excellent and genetically varied in order to create better summer squash cultivars. Breeders can successfully utilize heterosis and transgressive segregation by blending parents from different clusters, producing high-yielding, early-maturing and nutritionally enhanced cultivars. All things considered, the results offer a solid foundation for developing methodical breeding plans to improve summer squash yield and quality in mild climates.

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