



Assessment of genetic divergence in pumpkin (*Cucurbita moschata* Duchesne) through morphological and microsatellite markers for enhanced fruit yield and related traits

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Abstract

Understanding the genetic diversity of crop species is critical for developing effective breeding strategies to improve yield and related traits. This study presents an integrative assessment of genetic diversity among 72 pumpkin (*Cucurbita moschata* Duchesne) genotypes including standard check varieties, using both morphological traits and molecular markers. The experiment was carried out at ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh in augmented block design during the summer season of 2023 with data recorded on 17 quantitative traits. The analysis of variance revealed that the mean sum of squares attributable to genotype was significant to highly significant across all 17 quantitative traits, indicating that the evaluated genotypes exhibited significant genetic diversity for test traits under study. High heritability coupled with high genetic advance as per cent of mean was observed for characters such as peduncle diameter (73.34 % and 169.72 %, respectively) and seed width (99.85 % and 157.59 %, respectively), making them ideal targets for genetic improvement through selection. Principal component analysis identified seven significant principal components, with PC1 accounting for the largest proportion of variability (13.99 %), followed by PC2 (12.90 %) and PC3 (11.58 %) and highlighting fruit yield per plant, fruit weight and seed width were major contributors to the phenotypic divergence. Molecular profiling using 19 SSR markers revealed high polymorphism with polymorphic information content (PIC) values ranging from 0.36–0.97 and unweighted pair group method with arithmetic mean (UPGMA) clustering delineated seventy-two genotypes into three distinct clusters. The marker CMTm34 had the highest PIC value (0.97), suggesting its exceptional discriminatory power for detecting genetic variability in pumpkin genotypes. Allele frequencies across all markers varied widely, ranging from 8.75 % to 80.00 %, demonstrating the genetic richness and diversity of the genotypes under study. The diverse genotypes identified in this study may be utilised as potential parents in hybrid breeding programmes to develop superior pumpkin lines.

Keywords: *Cucurbita moschata*; germplasm characterisation; morphological traits; molecular marker; principal component analysis

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1. Introduction

Pumpkin (*Cucurbita moschata*, 2n = 40), an important seed-propagated climbing vegetable crop cultivated worldwide for its nutritional, medicinal and economic value. Despite its commercial and nutritional value, especially as a source of beta-carotene, pumpkin remains underutilised in breeding programs compared to other cucurbits (1). Varieties with yellow to orange flesh are especially rich in carotenoids, contributing to their medicinal and dietary appeal (2). Pumpkin is consumed in various forms, including fruits, stems, tendrils, seeds and flowers, all of which contain bioactive compounds

such as amino acids, flavonoids, phenolics and carotenoids (3, 4). The seeds are also known for oil extraction, which has therapeutic properties. Among cucurbitaceous vegetables, *C. moschata* ranks among the top ten cultivated species worldwide, with China and India as the leading producers. In India alone, pumpkin is cultivated over 121300 ha, with production of 2.7036 million metric tonnes and productivity 22.30 Mt ha⁻¹ (5). Pumpkin production in Uttar Pradesh was reported at 420.409 t in 2024 which was increased from the previous number of 416.740 t in 2023 (6). This crop was chosen in the present study because it is an economically important

cucurbit with substantial phenotypic and genetic variability, making it an excellent model for assessing molecular diversity. Furthermore, limited molecular characterisation of indigenous pumpkin germplasm highlights for effective germplasm conservation, parent selection and the development of improved cultivars through breeding (7).

This crop exhibits extensive morphological diversity, with genotypes differing in fruit shape, size, flesh thickness, colour, seed characteristics and plant architecture (8, 9). These traits are important resources for improving yield and quality through breeding. Selection based on key yield-contributing traits such as number of fruits per plant, fruit weight and flesh thickness can significantly enhance productivity (10). Furthermore, pumpkin displays considerable diversity in fruit shape, ranging from elliptical and cylindrical to oblong and oval allowing ample scope for market-specific varietal development (11, 12).

Despite its wide genetic diversity and nutritional importance, pumpkin remains comparatively underutilised in systematic breeding programmes because of limited molecular characterisation and inadequate exploitation of available germplasm resources. Consequently, many valuable alleles remain underexploited for cultivar development. While morphological traits remain vital for phenotypic characterisation, molecular markers have transformed genetic diversity analysis by providing precision and speed in breeding. Simple sequence repeats (SSR) are widely used to assess genotypic diversity and are considered as the marker of choice. These markers are highly polymorphic, reproducible (13, 14) abundant, evenly distributed across the genome

(15) and codominant in nature (16), all which aid in identification of promising parental lines, marker-assisted selection and conservation of unique germplasm. Integrating molecular data with field-based evaluations enables breeders to effectively exploit both qualitative and quantitative diversity, thereby closing the hybrid development gap and advancing pumpkin improvement programs (11, 12). Keeping the preceding points in mind, the present investigation was carried out with the objective to assess the genetic diversity in pumpkin through morphological and molecular markers and its implication for utilisation in plant breeding.

2. Materials and Methods

Experimental material

In the present investigation, a total of 72 genotypes including three standard check varieties, Pusa Vikash, Kashi Basant and Kashi Harit were evaluated at ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh (Table 1). All these test genotypes were evaluated under field conditions using an augmented block design (ABD) during the summer season of 2023 maintaining a total of 8 blocks. In each block nine test genotypes and three checks were allocated. The experimental layout was maintained with a row-to-row spacing of 4.00 m and plant-to-plant spacing of 0.70 m, ensuring optimal growth conditions and minimising competition among plants. Standard agronomic practices, as recommended, were followed throughout the cropping period to ensure the establishment of a healthy, uniform crop stand.

Table 1. List of pumpkin genotypes under study collected from ICAR-Indian Institute of Vegetable Research

Sl. No.	Name of genotypes	Sl. No.	Name of genotypes	Sl. No.	Name of genotypes
1.	VRPK-230	25	VRPK-05-01	49	VRPK -3026
2.	VRPK-18-01	26	VRPK 3024	50	VRPK-3025
3.	KASHI HARIT	27	HARP-10	51	PVN/VS/22/-26
4.	PUMPKIN V-4	28	VRPK-9-01	52	PVN/VS/22/-16
5.	VRPK-3002	29	VRPK-22-2-2-1	53	PVN/VS/22/-27
6.	AZAD PUMPKIN-1	30	VRPK-3002	54	PVN/VS/22/-28
7.	ARKA -CHANDAN	31	VRPK-5(8)	55	PVN/VS/22/-25
8.	VR-KOHRA	32	VRPK-3003	56	MAYA-75
9.	VRPK-3024	33	VRPK-3004	57	HANNAH
10.	ANAND PUMPKIN	34	VRPK-3005	58	VRPK-17-01
11.	KASHI BASANT	35	VRPK-3006	59	PUMPKIN-41
12.	NARENDRA AGRIM	36	VRPK-3007	60	VRPK-3019
13.	NARENDRA AMRIT	37	VRPK-3008	61	VRPK-3020
14.	CM-350	38	VRPK-3009	62	VRPK-9-01
15.	SWARNA AMRIT	39	VRPK-3010	63	VRPK-3021
16.	VRPK-11-6-5	40	VRPK-3011	64	VRPK-3022
17.	VRPK-3001	41	VRPK-3012	65	KKG/VKS/SKT-243
18.	VRPK-62	42	VRPK-3013	66	KKG/VKS/SKT-303
19.	VRPK-10-10	43	VRPK-3014	67	VRPK-3023
20.	PUSA VIKASH	44	VRPK-3015	68	LSV/21-80

21.	KPS-1	45	VRPK-3016	69	PUMPKIN-75
22.	PUMPKIN-CO-2	46	VRPK-3017	70	VRPK-20-02
23.	VRPK-310	47	VRPK-3018	71	KKG/VKS/SKT-172
24.	PUMPKIN-73	48	VRPK-3027	72	KKG/VKS/SKT-290

Observations recorded

The 72 genotypes of pumpkin were characterised by 17 agro-morphological traits. Five healthy and representative plants out of 10 were randomly selected from each genotype for recording morphological observations. Random sampling was adopted to minimise sampling bias and to obtain reliable estimates of the phenotypic performance of each genotype, following the standard descriptor of pumpkin proposed by previous researchers which remains widely used for pumpkin evaluation (17). The phenotypes under study include cotyledon length (CL, cm), cotyledon width (CW, cm), plant length of main vine (PLMV, cm), leaf blade length (LBL, cm), leaf blade width (LBW, cm), petiole length (PTL, cm), peduncle length (PEDL, cm), peduncle diameter (PEDD, cm), fruit length (FL, cm), fruit diameter (FD, cm), fruit flesh thickness (FFT, cm), fruit scar diameter (FDS, cm), seed length (SL, cm), seed width (SW, cm), number of fruits per plant (NFP), fruit weight at marketable stage (FW, g) and fruit yield per plant (FYP, kg). Observation on all these morphological traits were recorded at appropriate crop growth stages following standard descriptor guidelines. Vegetative traits were measured during active vegetative growth, whereas fruit and seed traits were recorded at marketable fruit maturity and physiological maturity, respectively. The mean data for the morphological traits were subjected for statistical analysis. The analysis of variance (ANOVA), estimation of genetic parameters and principal component analysis (PCA) were performed using R-Studio version 4.4.1. The PCA was performed using the seventeen quantitative morphological traits, whereas molecular clustering was conducted separately using SSR marker data through DARwin 6.0 software employing the Unweighted Neighbour Joining (UNJ) algorithm.

Molecular profiling using microsatellite SSR marker

Genomic DNA extraction

High-quality genomic DNA was extracted from young leaves using a modified CTAB (cetyltrimethylammonium bromide) protocol described by Doyle and Doyle (18) at the Molecular Plant Breeding Laboratory, ICAR-Indian Institute of Vegetable Research (ICAR-IIVR), Varanasi. Approximately 200–300 mg of fresh young leaf tissue was ground into a fine powder using a pre-chilled mortar and pestle in liquid nitrogen. The powdered tissue was immediately transferred to a microcentrifuge tube containing 750 µL of preheated CTAB extraction buffer (2 % CTAB, 100 mM Tris-HCl, pH 8.0 and 20 mM EDTA) and incubated at 65 °C for 60 min in a water bath. The mixture was gently inverted every 15 min during incubation to ensure uniform mixing and efficient cell lysis. Following incubation, an equal volume of phenol: chloroform alcohol (25 : 24 : 1, v/v/v) was added and the

contents were mixed thoroughly by gentle inversion, followed by centrifugation at 10000 rpm for 15 min. The aqueous phase was carefully transferred to a fresh microcentrifuge tube and further purified using chloroform alcohol (24 : 1, v/v). Genomic DNA was precipitated with chilled isopropanol and incubated overnight at -20 °C. The DNA pellet was recovered by centrifugation, washed with 70 % ethanol, air-dried and finally dissolved in 100 µL of nuclease-free water. The quality of the isolated genomic DNA was assessed by electrophoresis on 0.8 % agarose gel stained with ethidium bromide. Prior to polymerase chain reaction (PCR) amplification, all DNA samples were diluted to a 20 ng µL⁻¹ to ensure consistency among amplification reactions.

Polymerase chain reaction amplification and SSR analysis

The 19 simple sequence repeat (SSR) markers (Table 2) used in the present study were selected based on their reported utility for genetic diversity assessment in *Cucurbita* and their suitability for detecting polymorphism. The PCR amplification was carried out in a Bio-Rad thermal cycler using a 10 µL reaction mixture containing 1× PCR buffer, 0.1 U Taq DNA polymerase, 1 µL dNTP mixture (1 mM each), 0.5 µL each of forward and reverse primers (10 pmol µL⁻¹) and 20 ng of template genomic DNA, with nuclease-free water added to achieve the final reaction volume. The amplification programme consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at the marker-specific annealing temperature (Table 2) for 1 min and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. Amplified PCR products were separated by electrophoresis on 3.5 % high-resolution agarose gel in 1× TBE buffer, stained with ethidium bromide and visualized under a gel documentation system. Although polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis provide higher resolution for SSR fragment analysis, high-resolution agarose gel was considered adequate for resolving the expected fragment size differences of the selected SSR markers and has been successfully employed in previous pumpkin diversity studies. The presence or absence of distinct and reproducible amplification bands was scored in a binary matrix, assigning '1' for the presence and '0' for the absence of a band, which was subsequently used for molecular diversity analysis. Genetic relationships were analysed using DARwin 6.0 software with the UNJ method to generate a dendrogram. The polymorphism of each SSR marker was assessed by calculating the polymorphic information content (PIC) as per (19), providing insights into marker informativeness.

$$PIC_i = 1 - \sum_{j=1}^n p_{ij}^2$$



Table 2. List of primers used in molecular study and their annealing temperatures

Sl. No	Primer Name	Sequence 5'-3'	Tm
1.	CMTm19	F: GCATGGGAGATGAAGGTTAG; R: ATTTCTGGTGGTATGAGATTC	57.30
2.	CMTm61	F: GCCATTATTCCACTCCATGC R: TGCCTGCACCTGTTTTAGC	57.30
3.	CMTm20	F: GTGGGCCATATCGATTCACT R: CGAAAGTCGCAGAGAACACA	57.30
4.	CMTm60	F: TCCTCCAAAGCATACCAACTGT R: GCGCCATTTTATTGATTGGAT	53.97
5.	CMTm52	F: GCTCTCCATTTTCCAGCTTC R: GACGCAGAGGGGAGATTAATGA	57.30
6.	CMTm88	F: CATCGACATTTCGCTCATC R: AGGCAGCTTCCAAATCAGC	56.67
7.	CMTm144	F: ACATGGGCATACCTCGAATC R: CACCTGGCTGTTTTGTCTGA	57.30
8.	CMTm34	F: TGAAACTACACTACATGACCTTGG R: TGGGTTGGTAGACTTGTAGTTGA	59.30
9.	CMTm97	F: AGGGGGAAGTATAGTCATC R: ACCTCACCTTCCATCGTCAC	59.35
10.	CMTp201	F: GTGGAAGTTACTGCGATTG R: GCAAAGAATGTCCTCAGCA	54.51
11.	CMTm48	F: AAGCCTTTGGGGACCTTTAC R: TTGAAACCTTCAAACAAGAAATTG	55.25
12.	CMTm259	F: ACCTCGAGGAAGCAAAAATG R: ATGGAGACGCGCAAGTAGA	56.67
13.	CMTm9	F: TTTTGTGTGCGTGTGTGG R: GCCCAGAAGACAAAAGTTTCG	54.51
14.	CMTm7	F: AACCAAACTCCGGCAAGA R: GTTCTCTCCGTTTCAAGATGG	53.69
15.	CMTm29	F: AGCGCAGCGACAGAAAAAG R: AGCTTCTACGAAGGCGAGGT	59.35
16.	CMTm232	F: AGAAAGAAATAAGGAACCCACAG R: CGTCTCGCAATTCTTCAACT	57.08
17.	CMTp18	F: ACACCTTCGCTTCCGACATC R: TGACATCACTCCGGCAACTC	59.35
18.	CMTm111	F: CTCCATTCCCATGGCTTC R: CCATGAGCTTGAGAGAGGTG	59.35
19.	CMTm91	F: CCCTAGAATTAGTGGGCAAT R: TAGGCCTAAAAAGACCCAAT	55.25

F: Forward primer and R: Reverse primer.

Here: $PI_i =$ Polymorphic Information Content of marker locus i

P_{ij} = Frequency of the j -th allele at the i -th locus

n = Total number of alleles at that locus

3. Results and Discussion

Variance analysis

The analysis of variance indicated that the mean squares due to genotypic differences were significant to highly

significant for all 17 quantitative traits (Table 3). This unequivocally indicates the presence of substantial genetic variability among the assessed genotypes for each trait examined. This variability is essential for effective selection and can be strategically harnessed in breeding programs aimed at genetic enhancement. These findings are in close agreement with the observations reported by previous researchers in pumpkin, further validating the presence of exploitable genetic diversity within the studied population (20).

The assessment of 72 genotypes of *C. moschata* demonstrated considerable genetic variability across 17



morphological and yield-related traits, suggesting significant opportunities for selection and enhancement in pumpkin breeding programs. Cotyledon characteristics, including length (2.17–5.35 cm) and width (1.54–3.44 cm), indicated variability in seedling vigour (Table 4). Vine length varied between 2.80 and 7.94 m, with significant differences noted in leaf blade and petiole dimensions, which influence canopy architecture and photosynthetic capacity. Fruit characteristics exhibited considerable variation, with significant differences observed in fruit length (7.10–30.33 cm), diameter (10.51–31.77 cm) and flesh thickness (1.69–4.84 cm). This variation indicates a diverse market potential, encompassing both fresh consumption and processing applications. Seed traits exhibited variability; however, their main significance pertains to seed quality rather than yield. The seed of pumpkin is highly nutritious and hold promise for economic potential. Several studies were conducted on the development of hull-less seed in pumpkin that can be consumed raw. (21) studied different seed related traits in pumpkin such as SL, SW, ST, number of seeds and 100-seed weight and found that these traits are associated with high heritability and

promise for direct selection and improvement. Yield components exhibited significant variability, with the number of fruits per plant varying from 1.85 to 5.89 and the weight of marketable fruits ranging from 0.59 to 1.88 kg. Total FYP ranged from 1.64 to 7.02 kg, with genotypes VRPK-3019, VRPK-3002 and Azad Pumpkin demonstrating superior performance compared to others (Table 4). The findings underscore the suitability of specific genotypes as parents for developing high-yielding, market-preferred varieties. The variability observed among *C. moschata* genotypes demonstrates a robust genetic foundation that can be utilised for the development of superior cultivars. High-performing genotypes, including VRPK-3019 and VRPK-3002 for fruit yield, KKG/VKS/SKT-243 for early vigour and Narendra Amrit for flesh thickness, should be prioritised in hybridisation and selection programs. The variability in fruit size and type facilitates the development of diverse cultivars designed to meet specific market and processing needs. The study on the North Indian germplasm suggested a sizable diversity among the pumpkin germplasm studied for all the morphological traits (22).

Table 3. Analysis of variance (ANOVA) for quantitative traits in pumpkin germplasm

Characters	Block (Ignoring genotype) Df = 7	Treatment (Eliminating block) Df = 74	Checks Df = 2	Varieties +Checks vs Varieties Df = 71	Error Df = 14
CL	1.197**	0.563**	0.73**	0.559	0.029
CW	1.48**	0.151*	0.393*	0.144	0.044
PLMV	5.44**	1.015*	5.268**	0.893	0.052
LBL	1.693*	1.983**	0.999*	2.011	0.508
LBW	12.139**	5.54**	11.099**	5.381	0.4
PTL	7.266**	3.773**	5.876**	3.712	0.011
PEDL	18.098**	8.594**	0.869*	8.814	0.091
PEDD	21.131**	11.713**	0.094*	12.045	0.018
FL	4.087*	19.687*	29.048**	19.419	9.361
FD	5.231**	19.69**	11.34**	19.928	0.047
FFT	0.536**	0.753**	0.841**	0.75	0.019
FDS	0.265*	0.077**	0.5**	0.065	0.016
SL	0.05*	0.018*	0.02*	0.018	0.009
SW	0.19**	0.093**	0.013*	0.096	0.004
NFP	2.577*	0.843**	0.379*	0.856	0.228
FW	0.338*	0.072*	0.088*	0.072	0.076
FYP	15.097*	1.591*	0.022*	1.635	2.065

* significant at 5 %; ** significant at 1 %, respectively. CL: cotyledon length, CW: cotyledon width, PLMV: plant length of main vein, LBL: leaf blade length, LBW: leaf blade width, PTL: petiole length, PEDL: peduncle length, PEDD: peduncle



diameter, FL: fruit length, FD: fruit diameter, FFT: fruit flesh thickness, FDS: fruit diameter scar, SL: seed length, SW: seed width, NFP: number of fruits per plant, FW: fruit weight at marketable stage, FYP: fruit yield per plant.

Table 4. Mean performance of genotypes for 17 qualitative traits in pumpkin germplasm

Genotype	CL	CW	PLMV	LBL	LBW	PTL	PEDL	PEDD	FRL	FRD	FRTF	FDS	SEDL	SW	NFPs	FW	FYP
VRPK-230	3.99	2.22	4.56	12.04	16.30	12.64	7.18	2.85	14.71	10.51	3.62	1.40	1.66	1.18	4.85	1.20	5.88
VRPK-18-1	4.17	2.54	4.55	14.48	17.48	11.18	7.58	2.87	14.31	14.15	2.48	1.58	1.44	1.20	4.55	1.49	6.85
PUMPKIN-V4	3.77	2.10	4.01	13.18	15.84	14.40	6.90	2.15	15.71	15.57	3.06	1.82	1.19	0.78	3.65	1.78	6.76
VRPK-3002	3.67	1.62	4.37	11.72	15.58	13.40	6.44	2.45	17.37	17.05	2.44	1.72	1.09	0.44	4.55	1.52	7.01
Azad pumpkin	4.33	2.50	4.09	13.04	18.14	14.00	4.96	2.15	15.33	15.27	2.42	1.30	1.26	0.72	4.85	1.41	6.86
Arka Chandan	4.55	3.36	4.37	14.96	19.36	14.46	4.30	2.69	15.45	14.03	2.30	1.76	1.15	0.46	4.55	0.97	4.54
VR Kohra	4.61	3.00	4.29	14.18	19.88	13.26	6.90	1.71	15.99	14.49	3.46	1.56	1.08	0.72	5.45	0.75	4.04
VRPK-3024	3.81	2.40	5.17	10.74	20.26	11.16	4.48	2.65	25.31	15.95	3.68	1.76	1.43	0.44	4.85	0.81	3.98
Anand pumpkin	3.63	2.64	4.50	14.06	19.88	15.08	7.56	2.27	22.33	13.81	2.46	1.36	1.42	0.58	3.95	1.48	6.04
Narendra Agrim	3.21	2.58	3.98	12.02	20.28	11.46	7.24	2.87	12.49	13.79	3.40	1.30	1.09	0.28	3.95	1.08	4.50
Narendra Amrit	3.97	2.54	4.24	12.92	16.86	13.34	6.86	2.09	14.19	19.67	4.84	1.44	1.35	0.40	3.35	1.17	4.24
CM-350	2.33	2.44	5.10	12.90	16.58	13.74	6.46	1.93	16.19	16.31	3.16	1.44	1.34	0.44	2.15	0.71	2.10
Swarna Amrit	3.53	2.82	6.38	13.52	19.14	11.46	7.38	1.47	21.09	22.33	3.98	1.44	1.32	0.30	2.75	1.28	3.95
VRPK-11-6	3.55	2.12	5.30	12.82	17.32	14.00	6.18	2.41	30.33	19.55	4.72	1.46	1.06	0.26	3.65	1.35	5.20
C0-1	4.31	3.00	4.21	12.36	17.14	13.68	6.22	2.41	19.03	18.55	4.58	1.68	1.28	0.32	2.45	1.13	3.25
VRPK-62	4.43	2.42	4.04	15.46	23.10	12.82	6.44	2.41	22.41	14.65	2.48	1.90	1.32	0.36	3.35	1.05	3.86
VRPK-10-1	5.19	2.42	3.86	13.72	18.70	14.04	4.50	2.41	21.35	31.77	2.56	1.94	1.39	0.34	3.05	1.88	6.08
KPS-1	3.81	3.44	7.86	14.56	20.10	12.68	7.50	2.21	16.45	18.55	4.64	1.46	1.74	0.42	2.45	1.17	3.36
VRPK-3001	4.81	2.86	5.12	15.84	18.26	10.52	9.52	2.07	25.43	19.57	2.66	1.72	1.24	0.24	2.75	0.98	3.15
VRPK-310	4.03	2.32	5.21	15.00	21.36	11.96	11.16	2.93	25.53	21.97	3.88	1.68	1.38	0.30	2.15	0.90	2.49
Pumpkin-73	5.01	1.78	5.66	12.40	21.88	11.70	12.82	2.93	25.53	22.45	4.42	1.20	1.38	0.34	2.75	0.91	2.96
VRPK-05-01	2.79	1.86	3.98	12.72	23.30	12.22	14.04	2.77	18.75	23.83	4.06	1.64	1.30	0.34	4.25	1.18	5.19
HARP-10	2.17	1.90	4.54	10.80	14.74	10.02	15.06	2.17	19.31	19.77	4.72	1.62	1.43	0.42	3.05	1.26	4.21
Kashi Harit (C1)	4.80	3.06	4.78	14.12	13.94	12.87	7.16	8.01	20.81	30.86	1.70	1.37	1.33	0.29	3.35	0.85	2.66
Kashi Basant (C2)	4.00	2.94	5.26	13.04	13.04	14.37	6.74	2.33	21.51	24.04	2.72	1.37	1.49	0.25	2.75	1.89	5.29
Pusa Vikash (C3)	4.40	2.88	4.53	12.62	14.20	12.61	7.10	2.87	20.95	23.84	4.24	2.05	1.41	0.29	2.45	0.79	1.75
VRPK-9-01	4.24	2.54	4.61	14.58	14.22	8.86	5.92	3.02	21.89	24.54	2.16	1.49	1.38	0.27	3.95	0.92	3.44
VRPK-22-2	4.28	2.76	5.46	12.90	21.38	12.06	12.34	2.54	17.09	23.90	2.34	1.55	1.34	0.27	3.05	1.10	3.24
VRPK-63	4.00	2.42	6.59	12.98	18.00	12.58	11.20	1.84	17.01	19.86	3.38	1.59	1.60	1.33	4.85	1.01	4.75
VRPK-5-8	4.24	2.10	6.53	14.34	19.34	12.57	14.82	2.28	10.75	19.90	4.06	1.45	1.24	0.37	4.85	1.13	5.35
VRPK-3003	4.74	2.18	6.62	11.88	21.54	13.87	14.14	1.88	9.85	16.46	3.78	1.69	1.24	0.29	5.75	1.00	5.58
VRPK-3004	3.62	2.30	5.98	14.54	17.30	14.07	8.10	2.95	11.01	17.02	3.54	1.73	1.32	0.33	4.55	0.96	4.19
VRPK-3005	3.62	2.42	4.68	13.80	17.56	13.65	7.98	2.97	12.41	15.70	3.72	1.99	1.47	0.37	5.15	1.07	5.34
VRPK-3006	3.12	2.34	6.73	13.58	14.26	14.51	6.98	2.95	12.45	16.58	3.44	1.79	1.53	2.31	4.55	0.92	4.01
VRPK-3007	3.84	2.26	7.01	9.22	14.22	13.05	9.96	2.61	10.21	13.18	2.08	1.45	1.37	0.37	3.05	0.98	2.84
VRPK-3008	3.46	2.88	5.18	9.96	14.82	13.89	9.58	2.35	11.94	15.32	4.12	2.03	1.67	0.29	3.35	0.77	2.39
VRPK-3009	3.44	1.98	7.94	10.66	14.88	17.09	7.94	2.55	11.78	14.56	2.28	1.83	1.37	0.29	3.65	1.04	3.65
VRPK-3010	4.66	2.52	6.76	12.12	16.34	17.69	9.67	2.27	11.64	14.18	4.44	1.83	1.70	0.33	3.05	1.37	4.12
VRPK-3011	4.18	1.54	6.01	12.12	15.92	11.13	8.70	2.27	7.10	16.52	4.40	1.59	1.66	0.29	3.35	1.07	3.46
VRPK-3012	4.82	2.14	5.42	13.04	17.82	16.59	9.68	2.20	14.83	13.62	4.49	1.75	1.55	0.41	3.05	1.28	3.83

VRPK-3013	3.12	2.32	5.40	11.24	14.34	12.14	7.30	2.68	10.32	16.14	3.44	1.79	1.42	0.33	3.35	1.35	4.45
VRPK-3014	3.50	2.06	4.57	12.02	16.62	13.86	8.64	1.46	13.96	19.82	4.44	1.63	1.42	0.37	3.65	1.09	3.87
VRPK-3015	4.84	2.46	4.95	12.54	16.98	11.02	9.68	2.08	15.82	20.24	4.28	2.25	1.33	0.29	4.25	1.35	5.65
VRPK-3016	4.52	2.68	4.56	12.04	20.48	14.05	6.88	2.52	19.13	10.62	1.98	1.89	1.41	0.25	1.85	0.96	1.64
VRPK-3017	4.78	2.34	3.65	12.56	21.16	12.75	6.42	1.87	15.91	11.02	2.78	1.57	1.38	0.25	3.65	1.02	3.57
VRPK-3018	4.02	2.80	2.80	12.56	18.88	15.45	5.00	1.73	17.08	18.34	3.12	1.85	1.40	0.21	4.25	1.37	5.77
VRPK-19-01	5.31	1.90	3.33	12.67	18.79	13.69	7.06	2.41	20.61	17.89	4.54	1.76	1.49	0.27	4.69	1.13	5.28
VRPK-3025	3.17	2.00	4.10	13.71	17.87	13.73	6.56	2.39	22.25	23.39	4.60	1.92	1.47	0.25	4.39	1.24	5.35
PVN/VS/22-26	4.17	2.02	4.08	13.31	16.71	12.29	6.70	1.83	16.37	18.83	4.54	2.14	1.33	0.27	3.19	0.96	2.79
PVN/VS/22-16	4.27	2.76	4.15	12.35	15.93	13.13	6.74	2.49	18.31	18.27	2.52	1.58	1.30	0.29	4.09	1.03	4.09
PVN/VS/22-27	2.47	1.66	4.98	11.27	16.79	13.47	9.10	21.13	8.51	18.07	3.78	1.58	1.20	0.29	3.49	1.18	3.91
PVN/VS/22-28	2.41	1.78	5.16	10.47	16.55	12.57	9.16	21.79	10.49	18.57	2.46	1.86	1.35	0.21	3.79	0.66	2.45
PVN/VS/22-25	2.89	1.76	4.00	12.73	20.83	12.93	17.74	12.69	19.75	12.63	4.66	1.86	1.46	0.29	3.79	1.30	4.72
VRPK-3026	2.35	2.10	3.53	11.03	15.39	10.07	13.16	9.95	12.59	15.41	2.44	1.58	1.28	0.29	3.49	1.07	3.50
VRPK-3027	2.93	1.76	4.01	8.27	13.21	10.87	9.40	2.57	14.29	19.23	2.42	2.04	1.30	0.21	3.19	0.94	2.74
MAYA-75	2.17	1.86	4.34	13.71	19.53	15.45	15.16	1.93	23.29	12.63	3.60	2.04	1.24	0.29	3.49	1.23	4.08
HANNAH 19-03	3.01	2.22	5.09	10.83	15.29	13.65	10.48	2.57	13.57	23.35	3.44	2.12	1.32	0.21	5.89	0.91	5.64
VRPK-17-01	3.69	2.14	6.13	12.43	17.95	13.59	11.44	2.39	11.63	23.57	3.72	1.98	1.36	0.23	4.69	1.14	5.34
PUMPKIN-41	4.01	2.14	5.08	10.47	16.07	13.91	10.30	2.09	16.51	25.37	3.52	1.54	1.46	0.25	4.09	1.04	4.14
VRPK-3019	3.51	2.58	4.42	13.91	17.49	14.17	12.44	1.91	17.99	28.31	3.54	1.98	1.53	0.25	5.29	1.31	7.02
VRPK-3020	3.05	2.32	4.44	13.91	19.59	19.87	13.56	2.47	18.69	27.17	3.94	1.92	1.60	0.15	5.29	0.96	5.23
VRPK-9-01	3.23	1.98	4.18	10.99	16.27	12.69	10.62	1.73	16.77	16.19	2.36	1.56	1.38	0.31	5.29	0.93	5.11
VRPK-3021	3.54	2.46	4.44	9.97	13.69	12.87	12.82	1.55	18.33	16.99	2.72	1.38	1.35	0.31	4.09	0.74	2.96
VRPK-3022	3.24	1.84	3.43	10.83	15.45	13.51	8.86	1.57	16.85	17.39	3.76	1.42	1.41	0.17	5.29	0.59	3.33
KKG/VKS/SKT/-243	5.36	1.96	3.65	10.43	15.25	13.21	7.62	1.53	17.45	17.39	4.42	1.42	1.33	0.33	5.59	0.93	5.39
KKG/VKS/SKT/-303	4.62	2.36	3.58	11.03	15.21	12.01	7.14	1.73	16.65	17.57	4.72	1.42	1.46	0.37	5.29	1.01	5.49
VRPK-3023	4.36	1.94	4.30	12.71	14.41	14.61	7.42	2.07	23.97	15.83	4.48	1.70	1.36	0.29	4.99	0.91	4.63
LSV/21-80	3.76	1.94	5.23	13.09	18.69	20.27	6.26	1.57	16.93	17.89	4.34	1.84	1.26	0.57	5.89	0.87	5.39
Pumpkin-75	5.04	1.98	5.19	12.35	17.89	13.49	7.20	1.43	16.31	17.79	3.80	1.40	1.47	0.29	3.19	1.85	5.49
VRPK-20-02	3.84	1.98	4.91	9.89	19.73	10.89	4.54	2.85	23.27	15.97	3.66	1.78	1.47	0.41	4.99	0.90	4.63
Mean	3.87	2.31	4.88	12.48	17.55	13.20	8.45	3.09	17.36	18.18	3.45	1.63	1.37	0.43	3.95	1.12	4.45
CV (%)	7.62	13.49	4.81	9.02	3.80	2.54	11.83	58.71	9.83	9.29	16.70	6.77	7.30	2.91	13.34	22.92	33.26
CD at 5 %	0.63	0.78	0.84	2.64	2.34	0.39	1.12	0.50	11.33	0.80	0.51	0.46	0.34	0.25	1.77	1.02	5.33

CL: cotyledon length (cm), CW: cotyledon width (cm), PLMV: plant length of main vein (m), LBL: leaf blade length (cm), LBW: leaf blade width (cm), PTL: petiole length (cm), PEDL: peduncle length (cm), PEDD: peduncle diameter (cm), FL: fruit length (cm), FD: fruit diameter (cm), FTF: fruit thickness flesh (cm), FDS: fruit diameter scar (cm), SL: seed length (cm), SW: seed width (cm), NFP: no. of fruits/plant (No.), FW: fruit weight at market stage (kg), FYP: fruit yield/plant (kg).

Variability coefficients for genotype and phenotype

The estimates of genotypic and phenotypic coefficients of variation (genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), respectively) for 17 quantitative traits are presented in Table 5. The PCV values consistently exceeded GCV values, signifying that phenotypic expression was influenced by both genetic

factors and environmental conditions. This trend suggests a moderate to considerable environmental effect on trait expression, as reported previously (23). Thus, selection based solely on phenotypic performance may be inconsistent for specific traits due to erratic nature of environmental variation, a concern similarly highlighted in linseed (24).



The trait with the highest PCV was peduncle diameter (112.18 %), followed by seed width (76.50 %) and PEDL (34.63 %). Other traits exhibiting moderate to high PCV included FYP (31.75 %), FL (25.16 %), FW at marketable stage (24.82 %), FFT (24.49 %), NFP (24.33 %), FD (24.23 %), main vein length (21.01 %), CL (19.83 %) and CW (18.27 %). These values indicate a relatively stable phenotypic performance for these traits, exhibiting a lower degree of environmental influence in contrast to traits with wider PCV-GCV gaps. The GCV ranged from 6.87 % for leaf blade length to a high of 96.07 % for peduncle diameter. A high GCV signifies considerable genetic variability within the population, implying good potential for genetic improvement via direct selection. The relatively narrow differences between PCV and GCV for most traits suggest limited environmental interference, thereby confirming the effectiveness of the underlying genes in trait expression. This observation aligns with the findings of previous researchers, who emphasised the reliability of traits with minimal GCV-PCV disparity for selection-based breeding (25).

Although GCV alone provides preliminary insight into genetic variability, its effectiveness in selection decisions is significantly improved when interpreted in conjunction with heritability estimates. Previous reports asserted that the integration of GCV and heritability provides a more accurate and reliable foundation for selecting superior genotypes, facilitating informed decision-making by breeders in crop improvement programs (26).

Heritability and genetic advancement

Heritability is a crucial genetic parameter that helps plant breeders determine the extent to which genetic factors influence phenotypic traits, allowing them to select

superior genotypes for crop improvement. A higher heritability estimate implies that the observed trait variation is primarily influenced by genetic variance rather than environmental factors.

In the present study, broad-sense heritability and expected genetic advance were estimated for 17 quantitative traits (Table 4). The heritability estimates varied widely, ranging from as high as 99.85 % for SW to as low as 3.20 % for FYP. Genetic advance, which reflects the expected improvement under selection pressure, varied from 52.10 for LBL to 0.075 for FW at the marketable stage. Although several traits had high heritability in the broad sense, the magnitude of expected genetic gain varied significantly. Notably, traits such as SW (99.85 %), PL (97.13 %), main vein length (94.76 %), LBW (92.25 %), PEDL (88.64 %), FD (85.46 %), CL (85.27 %), FL (84.42 %), scar diameter (78.79 %), PD (73.34 %), NFP (69.95 %) and flesh thickness (54.24 %) demonstrated relatively high heritability values, indicating strong genetic control.

Importantly, peduncle diameter and seed width had high heritability (73.34 % and 99.85 %, respectively) as well as high genetic advance as a percentage of mean (169.72 % and 157.59 %, respectively). This suggests that these traits are most likely governed by additive gene action and are highly responsive to selection, making them ideal targets for genetic improvement. Traits with both high heritability and high genetic advance are particularly valuable for breeding programs, as they imply stable inheritance and a greater potential for improvement in subsequent generations. These findings are consistent with the earlier reports, which emphasised the significance of such traits in effective selection and varietal development (27–30).

Table 5. Genetic parameters of genotypic and phenotypic variance, heritability and expected genetic advance in percent of mean for the 17 characters in pumpkin germplasm

Character	σ^2_g	σ^2_p	GCV	PCV	h^2 (bs)	Genetic advance	Genetic advance as % of mean
CL	0.50	0.58	18.30	19.82	85.27	1.35	34.87
CW	0.08	0.17	12.37	18.27	45.90	0.40	17.30
PLMV	0.99	1.04	20.45	21.01	94.76	2.00	41.07
LBL	0.73	2.00	6.87	11.32	36.82	52.10	416.6
LBW	5.29	5.73	13.16	13.71	92.24	4.55	26.09
PTL	3.79	3.90	14.73	14.94	97.12	3.96	29.95
PEDL	7.79	8.79	32.60	34.62	88.64	5.42	63.32
PEDD	9.06	12.35	96.07	112.18	73.33	5.31	169.72
FL	15.77	18.68	23.11	25.16	84.42	7.52	43.82
FD	16.76	19.61	22.43	24.23	85.46	7.80	42.72
FFT	0.39	0.72	18.03	24.48	54.24	0.95	27.40
FDS	0.04	0.05	12.85	14.48	78.78	0.39	23.54
SL	0.01	0.01	7.04	10.12	48.42	0.13	10.11
SW	0.10	0.19	76.44	76.50	99.85	0.65	157.59

NFP	0.64	0.92	20.34	24.32	69.95	1.38	35.10
FW	0.01	0.07	9.01	24.81	13.21	0.07	6.76
FYP	1.80	1.96	24.68	31.75	3.19	4.47	101.42

σ^2_g : genotypic variance, σ^2_p : phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2 (bs): heritability broad sense, CL: cotyledon length, CW: cotyledon width, PLMV: plant length of main vein, LBL: leaf blade length, LBW: leaf blade width, PTL: petiole length, PEDL: peduncle length, PEDD: peduncle diameter, FL: fruit length, FD: fruit diameter, FFT: fruit flesh thickness, FDS: fruit diameter scar, SL: seed length, SW: seed width, NFP: number of fruits per plant, FW: fruit weight at marketable stage, FYP: fruit yield per plant.

Principal component analysis of morphological traits

To decipher the underlying structure of genetic variation among pumpkin genotypes, PCA was conducted on the 17 quantitative morphological and yield-related traits. The result on PCA identified seven principal components (PCs) with eigenvalues greater than one, cumulatively accounting 69.23 % of the total phenotypic variance (Table 6). This dimensionality reduction technique enabled the identification of key traits contributing to the morphological divergence among the evaluated genotypes.

Among the extracted components, PC1 alone explained the largest proportion of variability (13.99 %), followed by PC2 (12.90 %) and PC3 (11.58 %). Together, these three components explained 38.46 % of the total variation, indicating that they captured a substantial portion of the phenotypic diversity present in the

germplasm. The highest positive loadings in PC1 were observed for FYP (0.941), FW at marketable stage (0.800), NFP (0.501), PL (0.260) and SW (0.218), suggesting that these traits were the principal contributors to overall genetic divergence. Hence, these traits can be prioritised during selection for yield improvement. In contrast, CW (-0.417), PLMV (-0.182) and LBW (-0.086) exhibited negative loadings, indicating comparatively lower or inverse contributions to the variation represented by PC1. The predominance of fruit yield and its associated components in PC1 indicates that yield-related traits constitute the major axis of phenotypic differentiation among the studied genotypes. Similar observations have been reported in previous pumpkin diversity studies, where FW, NFP and FYP were identified as the most influential traits governing phenotypic and genetic divergence (11, 12, 31).

Table 6. Principal component analysis (PCA) based on standardised data for 17 quantitative traits of pumpkin germplasm

Eigen values	2.37	2.19	1.96	1.73	1.28	1.15	1.05
% of variance	13.99	12.90	11.57	10.20	7.57	6.76	6.21
Cumulative %	13.99	26.89	38.46	48.66	56.24	63.01	69.22
Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CL	-0.021	0.745	0.014	0.064	0.063	-0.066	-0.205
CW	-0.417	0.395	0.191	0.187	0.167	0.337	0.112
PLMV	-0.182	-0.100	0.661	0.231	-0.082	-0.047	0.269
LBL	0.068	0.154	0.140	0.233	0.248	0.200	0.384
LBW	-0.086	0.025	-0.170	-0.118	-0.117	-0.291	0.053
PTL	0.260	0.060	0.065	0.623	-0.096	0.309	-0.088
PEDL	0.019	-0.518	0.117	0.074	0.097	-0.226	0.104
PEDD	-0.066	-0.779	-0.068	0.036	0.070	0.323	0.320
FL	0.026	0.369	-0.236	-0.378	0.424	-0.262	0.011
FD	0.143	0.011	0.004	0.101	0.752	0.221	0.205
FFT	-0.004	0.232	0.288	0.241	0.027	-0.384	-0.289
FDS	-0.067	-0.033	0.080	0.779	0.140	0.474	-0.237
SL	0.024	0.172	0.741	0.020	0.166	-0.337	0.255
SW	0.218	0.027	0.581	-0.224	-0.459	-0.225	0.506
NFP	0.501	0.157	-0.081	0.333	-0.550	0.013	-0.227
FW	0.800	-0.058	-0.006	-0.073	0.340	0.022	0.314
FYP	0.941	0.067	-0.049	0.187	-0.117	0.020	0.077

CL: cotyledon length, CW: cotyledon width, PLMV: plant length of main vein, LBL: leaf blade length, LBW: leaf blade width, PTL: petiole length, PEDL: peduncle length, PEDD: peduncle diameter, FL: fruit length, FD: fruit diameter, FFT: fruit flesh thickness, FDS: fruit diameter scar, SL: seed length, SW: seed width, NFP: number of fruits per plant, FW: fruit weight at marketable stage, FYP: fruit yield per plant.



Molecular dissection of genetic diversity using SSR markers

The study on genetic divergence among seventy-two pumpkin genotypes was conducted using nineteen SSR markers. Cluster analysis was performed exclusively on SSR marker data using DARwin 6.0 software based on the UNJ algorithm to infer genetic relationships among the genotypes.

Among the 19 SSR markers evaluated, 14 were found to be polymorphic, indicating a high level of allelic variation within the germplasm. The amplified fragment sizes varied between 100 bp (CMTm232) to 300 bp (CMTm19), reflecting the polymorphic nature of the markers used. The PIC values varied from 0.36 (CMTp201) to 0.97 (CMTm34) (Table 7), reflecting considerable differences in marker informativeness. The exceptionally high PIC values of CMTm34 (0.97), CMTm144 (0.96) and CMTm52 (0.92) indicate their strong discriminatory power and potential utility in future marker-assisted breeding and varietal identification programmes. The PIC values obtained in the present study were substantially higher than those reported by previous researchers for *C. moschata*, this may be because of differences in the SSR marker sets and the genetic composition of the germplasm evaluated, which influence allele number and frequency distribution (32). The high PIC value of 0.97 for CMTm34 reflects a highly informative marker within the present pumpkin germplasm, although its performance should be further validated across broader germplasm collections.

Table 7. Allelic frequency and polymorphic information content (PIC) of different molecular markers

Molecular marker	Size Range	Allelic frequency (%)	PIC
CMTm61	300	28.75 %	0.87
CMTm20	200	8.75 %	0.95
CMTm60	100	30.00 %	0.90
CMTm52	250	25.00 %	0.92
CMTm88	100	18.75 %	0.71
CMTm144	100	18.75 %	0.96
CMTm34	100	17.50 %	0.97
CMTm97	100	67.50 %	0.54
CMTp201	100	80.00 %	0.36
CMTm48	100	30.00 %	0.91
CMTm259	200	67.50 %	0.54
CMTm9	100	30.00 %	0.91
CMTm29	100	42.50 %	0.82
CMTm232	100	53.75 %	0.71

In the present study, a total of 36 polymorphic alleles were identified across 14 polymorphic loci, while allele frequencies ranged from 8.75 % to 80.00 %, indicating substantial genetic richness among the evaluated genotypes. The SSR-based cluster analysis grouped all 72 genotypes into three major clusters, with

Cluster III containing 36 genotypes, Cluster II containing 33 genotypes and Cluster I contain 3 genotypes (Fig. 1). Similarly, (33) identified a total of 33 alleles and a mean PIC value of 0.534 in pumpkin genotypes using five SSR markers. The clustering pattern aligns with previous findings which also reported a tri-cluster structure in similar germplasm studies (32, 34).

The SSR markers effectively distinguished genetically related and unrelated genotypes, demonstrating their suitability for varietal identification, germplasm characterisation and parental selection. The high PIC values, broad allele frequency distribution and clear cluster separation collectively confirm that SSR markers provide a reliable and informative tool for molecular diversity analysis in *C. moschata*. Comparable ranges of PIC values and allele frequency distributions have been documented in previous studies, further validating the reliability of SSR-based genetic analysis (31, 32, 34–36). Integration of these molecular results with morphological characterisation enhances the identification of genetically diverse parents and provides a stronger foundation for future breeding programmes aimed at improving fruit yield and other economically important traits while conserving valuable pumpkin genetic resources.

4. Conclusion

An integrative evaluation of 72 *Cucurbita moschata* genotypes, utilising morphological descriptors and simple sequence repeat (SSR)-based molecular markers, revealed significant genetic variability among the assessed genotypes for each trait examined. Significant heritability and genetic gains were noted for traits including peduncle diameter and seed width. Consequently, these traits exhibit a strong responsiveness to selection, rendering them suitable candidates for genetic enhancement. Principal component analysis (PCA) identified fruit yield per plant (FYP), fruit weight (FW) and seed width (SW) as key factors influencing phenotypic divergence. Molecular profiling using 14 SSR markers revealing significant polymorphism (polymorphic information content (PIC) ranging from 0.36 to 0.97) and identifies three distinct genetic clusters among the genotypes. Cluster III contains the highest number of genotypes among the identified clusters. The markers CMTm34, CMTm144 and CMTm52 demonstrated significant discriminatory power, making them strong candidates for marker-assisted selection. The highest PIC value was observed for CMTm34, indicating its significant discriminatory capacity for identifying genetic variability among pumpkin genotypes. The diverse genotypes identified in this study can be utilised as potential parents in hybridisation programmes to develop superior pumpkin lines. Future studies should focus on multi-location evaluation of promising genotypes, validation of informative SSR markers and integration of molecular markers with advanced genomic approaches for precise selection of high yielding pumpkin genotypes.



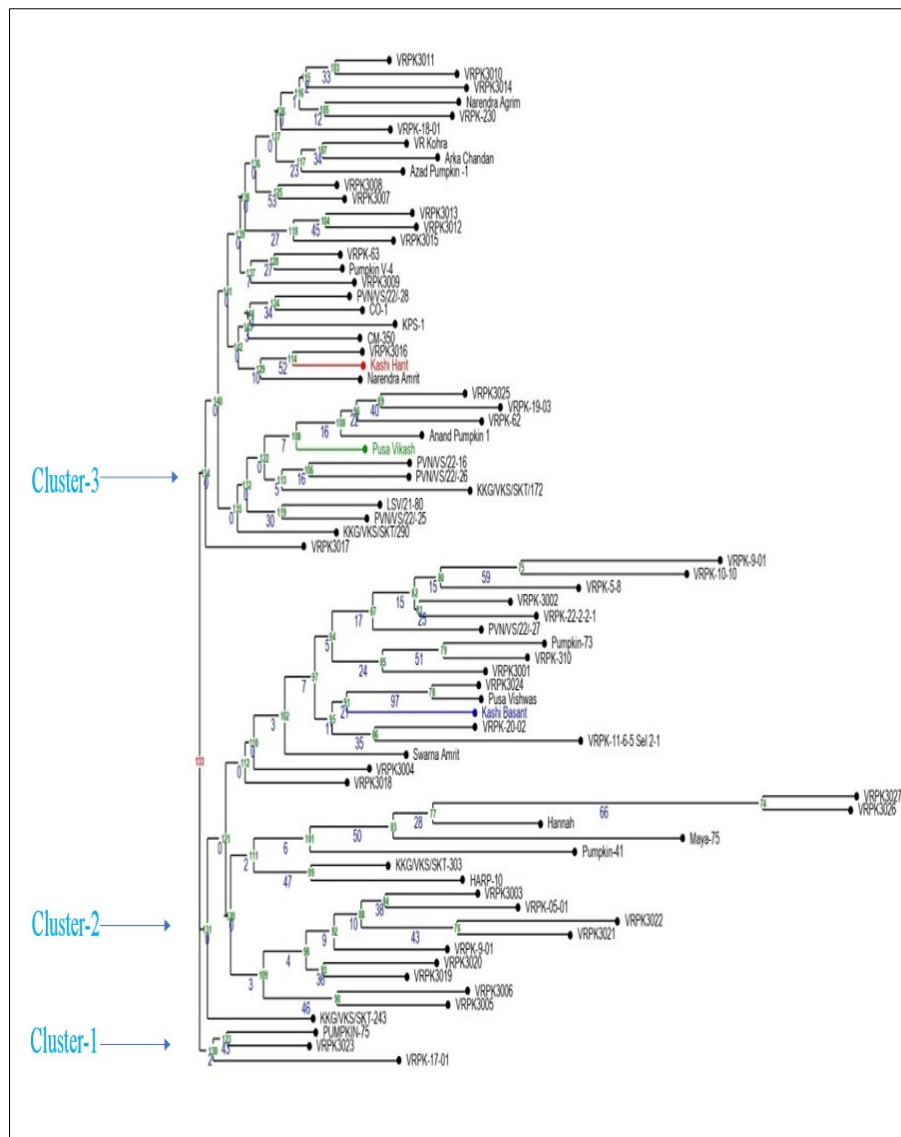


Fig. 1. Molecular dendrogram using unweight neighborhood joining method.

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Authors' contributions

UY conducted the research work, collected the data and wrote the manuscript. NKC conceptualised the research, analysed the data and drafted the manuscript. PG and JD proofread the manuscript. VS guided the researcher, analysed the data and proofread the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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