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Characterization of Indian walnut germplasm for nut and kernel traits

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ABSTRACT

In the present study, a total of 44 Indian walnut germplasms were evaluated for nut and kernel morphometric traits to characterize the seedling selections and study the genetic variability among the accessions. A wide range of variability for nut and kernel quantitative traits was observed. Nut length, nut diameter, nut weight, shell thickness, and the thickness of the dividing membrane, ranged from 30.42-50.04 mm, 28.93-47.87 mm, 8.74-21.68 g, 1.40-2.73 mm, and 0.16-1.42 mm, respectively. The kernel percentage among the studied Indian walnut germplasm ranged from 30.43 to 61.42%. The Bhushan genotype excelled with a shell whiteness index of 51.60, kernel size (similar to CITH-W-1), kernel weight of 12.99 g, and a kernel content of 61.42%. Overall, twelve selections, namely, Bhushan, PAD 1-23, CITH-W-6, CITH-W-8, CITH-W-12, CITH-W-16, CITH-W-17, CITH-W-22, Pusa Khor, W-1, W-2, and Gumma Selection-3, were identified as the most promising genetic resources for the crop improvement programmes.

Key words: *Juglans regia* L., genetic variability, morphology, kernel percentage, browning index.

INTRODUCTION

Walnut (*Juglans regia* L.) is the most important nut crop in terms of global production, acreage, and trade value (Bernard *et al*., 2). It is a rich source of proteins, vitamins (such as niacin, tocopherols, B6, and folate), minerals (potassium, phosphorus, calcium, magnesium), fatty acids (omega-3 and 6 fatty acids), and other phytochemicals, especially phenolic compounds which play an essential role in several health benefits (Bernard *et al*., 2).India produced 329 thousand metric tonnes of walnut with an area of 109 thousand hectares during 2022-23 (MoA& FW, 13). Indian walnut industry is primarily based on the crop harvested from various seedling-origin trees, which leads to variable size, quality, food values, and low productivity, which remains a major bottleneck for the walnut industry in India. In contrast, in the leading walnut-producing countries, cultivation is dominated by improved hybrid/cultivars(Bernard *et al*., 2). Being the centre of origin for the Persian walnut, a large diversity of walnut exists in the Western Himalayan region which was attributed to the predominant cross-pollination monocliny (monoecism) and dichogamous reproduction behaviour (Bernard *et al*., 2; Poggetti *et al*., 16).

Characterization of germplasmfor nut and kernel quality traits in the existing diversity is of utmost importance for identifying the uniqueness of each genotype and selecting suitable parents with the desired trait combinations for hybridization. However, comprehensive comparative information concerning relative horticultural traits for the Indian walnut growing region is lacking, which is important for the effective management and utilization of genetic variability by the breeders. Furthermore, enforcing intellectual property rights (IPRs) underpins the need for detailed comparative characterization of available genetic resources with Distinctness, Uniformity, and Stability testing. Therefore, this investigation was carried out to comprehensively evaluate and characterizethe Indian walnut germplasm from different regions for various nut and kernel traits.

MATERIALS AND METHODS

In the present investigation, a total of 44 distinct walnut genotypes were analyzed for nut and kernel characteristics during the year 2023. Uniform-size, healthy, injury-free nuts at optimum maturity stage were harvested (25 nuts from each genotype, five per replication) from the tree, covering the entire major walnut growing area of India in the Jammu and Kashmir and Himachal Pradesh (Table 1). The harvested fruits were analyzed at the Division of

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Table 1. Nut quantitative traits and shell colour attributes of Indian walnut genotypes.

Genotype	Nut length (mm)	Nut diameter (mm)	Nut weight (g)	Shell thickness (mm)	TDM (mm)	*L**	*a**	*b**	Whiteness index	Browning index
Kainthal Selection	42.01	37.76	14.84	1.62	0.65	55.58	12.17	27.04	46.58	80.27
Lara	33.23	37.62	15.46	2.17	0.41	52.00	13.03	28.16	42.78	92.58
SH-24	38.73	38.63	16.94	1.81	0.32	52.53	12.01	25.25	44.89	80.20
Kashmir Selection	38.84	33.50	11.64	1.98	0.24	52.20	11.12	24.64	45.05	77.25
Mandi Selection	42.82	41.29	21.30	2.66	0.73	52.37	9.38	21.13	47.02	63.41
KNR	38.76	33.01	14.80	2.54	0.18	48.23	11.42	22.64	42.25	79.46
Sirmour Selection	45.10	37.68	18.05	2.13	1.08	51.19	11.81	25.74	43.52	84.02
SR-16	38.72	39.28	15.06	1.53	0.49	48.50	12.19	23.45	42.01	82.17
SR-6	42.99	38.99	18.35	2.59	0.36	41.62	11.09	18.95	37.51	78.63
Gobind	36.76	38.57	18.98	2.28	0.32	51.81	11.87	24.82	44.46	79.53
CITH-W-5	48.41	38.55	17.83	2.05	0.66	51.85	12.97	27.73	42.92	91.98
CITH-W-8	40.58	37.45	12.50	1.50	0.30	57.82	10.82	25.29	49.64	69.25
K-9	43.51	37.63	17.11	1.71	0.77	55.90	10.63	24.22	48.54	69.09
W-1	34.41	36.19	14.95	1.62	0.29	59.14	10.08	24.38	51.34	64.03
W-2	37.72	34.53	15.18	1.63	0.69	56.22	12.09	27.74	46.72	81.24
W-3	43.36	36.06	16.94	1.83	0.32	50.72	10.73	23.85	44.17	77.03
Bhushan	44.12	44.53	19.90	1.40	0.22	61.32	10.99	26.89	51.60	69.36
PAD 1-23	37.92	34.16	15.28	1.62	0.25	58.01	11.50	27.24	48.57	75.51
GL 1-23	38.39	41.02	20.27	2.08	0.45	53.69	10.04	22.31	47.61	66.40
Gumma Selection-1	38.91	38.08	19.15	2.06	1.01	46.04	10.78	21.15	41.02	76.81
Gumma Selection-2	36.89	37.82	17.37	1.95	0.93	50.84	9.64	21.06	45.59	66.61
Gumma Selection-3	38.76	36.63	16.60	2.05	0.48	48.46	10.96	22.76	42.57	77.76
Pusa Khor	38.37	29.68	10.62	2.09	0.38	52.33	10.77	23.99	45.54	74.21
Walnut Seedling	30.42	32.25	9.96	1.71	0.42	47.02	10.73	22.11	41.57	77.93
Kullu Selection	32.11	29.92	9.96	1.79	0.31	48.01	11.52	23.64	41.69	82.65
Shoghi Selection	32.45	28.93	10.19	1.95	0.42	49.02	9.17	20.34	44.26	66.14
Amartara Selection-1	37.99	35.92	15.20	2.01	0.35	47.76	9.87	21.01	42.82	71.29
Amartara Selection-2	38.57	33.18	12.45	2.12	0.75	45.50	10.17	20.03	41.02	72.59
CITH-W-1	48.49	41.61	21.68	2.57	0.62	53.18	11.90	26.40	44.91	82.13
CITH-W-2	41.11	38.45	18.88	2.73	1.13	55.35	10.71	24.89	47.76	71.92
CITH-W-3	35.40	35.22	12.21	2.72	1.34	57.89	9.78	23.52	50.77	62.84
CITH-W-4	48.12	33.90	15.80	2.68	1.23	55.15	11.52	25.56	47.07	75.82
CITH-W-6	39.27	34.13	14.27	2.07	0.16	57.22	11.88	27.87	47.50	79.17
CITH-W-7	50.04	35.34	15.02	1.96	1.29	55.06	9.84	22.48	48.66	63.76
CITH-W-10	33.35	30.85	9.69	2.23	1.42	53.53	8.88	19.15	48.95	55.22
CITH-W-11	35.00	35.00	8.74	2.08	0.36	52.62	11.89	25.68	44.71	81.12
CITH-W-12	42.51	41.44	16.39	1.92	1.02	55.53	11.06	25.26	47.62	73.32
CITH-W-13	35.64	37.76	9.30	2.32	1.33	55.95	12.64	27.80	46.37	82.36
CITH-W-15	46.38	46.98	11.31	2.18	0.65	56.43	12.33	28.10	46.67	82.02

Contd...

Table 1 contd...

Genotype	Nut length (mm)	Nut diameter (mm)	Nut weight (g)	Shell thickness (mm)	TDM (mm)	*L**	*a**	*b**	Whiteness index	Browning index
CITH-W-16	47.87	47.87	15.82	2.14	0.60	60.15	10.34	25.80	51.41	66.80
CITH-W-17	45.88	45.88	15.15	2.33	0.54	56.05	10.62	25.90	47.88	73.77
CITH-W-19	40.64	40.64	16.38	1.46	0.63	56.05	11.55	26.88	47.17	77.98
CITH-W-20	47.12	47.12	13.76	1.95	0.55	44.31	10.06	19.45	39.92	72.11
CITH-W-22	46.41	46.41	15.07	2.45	0.59	55.63	13.26	28.18	45.79	85.43
Range	30.42-50.04	28.93-47.87	8.74-21.68	1.40-2.73	0.16-1.42	41.62-61.32	8.88-13.26	18.95-28.18	37.51-51.60	55.22-92.58
LSD (p≤0.05)	4.16	2.25	2.07	0.33	0.29	4.00	1.13	2.25	3.50	9.56

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Nut size (length and diameter), thickness of the shell and dividing membrane, and kernel size (length and width) were measured using a digital vernier caliper. The morphological traits of the nut and kernel were analyzed following the standard Walnut Descriptor (IPGRI, 8; PPV & FRA, 15). Nut and kernel weight was taken with the help of digital electric balance and the kernel percentage (w/w) was estimated.Shell and kernel colours were recorded following the CIE system, using a LabScan XE HunterLab colourimeter as described by Amin *et al*. (1).

The experimental data on nut and kernel traits were subjected to General Linear Model analysis with SAS® 9.3 software (SAS Institute Inc., Cary, NC, USA). Hierarchical cluster analysis was also attempted to investigate the intrinsic relationships among the genotypes using R-Studio Version 4.2 software.

RESULTS AND DISCUSSION

The results of the present study revealed significant genetic variability for the nut and kernel quality traits in India's walnut germplasm, indicating a strong baseline for selection among the promising genotypes and breeding with a complimentary set of traits. The wide variations observed for quantitative traits may be attributed to the genetic makeup in the studied genotypes, which is also supported by the fact that existing diverse seedling populations arise from predominant cross-pollination (Houmanat*et al*., 7). The results of our study indicated that most of the Indian walnut germplasm had ovate-shaped nuts, followed by round-shaped nuts (Table 2). Of the three types of nut shapes in cross-section (round, oblate, and elliptic), the maximum number of genotypes had a round nut shape, followed by an oblate shape (Table 2). The shell surface was observed to be smooth in the maximum number of genotypes, followed by rough and moderately smooth surfaces in some of the accessions (Table 2). The morphological parameters shape of the apex and base perpendicular to the suture were characterized as truncate, emarginated, pointed, and rounded, and the prominence of the apical tip was noted as strong to weak (Table 2). These findings agree with the walnut studies in different countries (Manthos and Rouskas, 11; Mirmahdi and Khadivi,

Table 2. Kernel quantitative traits and colour attributes of Indian walnut genotypes.

Genotype	Kernel length (mm)	Kernel width (mm)	Kernel weight (g)	Kernel percentage (%)	*L**	*a**	*b**	Whiteness index	Browning index
Kainthal Selection	30.03	27.81	6.40	43.02	33.22	12.54	21.89	28.49	128.35
Lara	26.98	28.25	6.35	41.03	39.24	11.43	23.35	33.45	109.07
SH-24	30.18	31.81	7.96	47.01	23.43	10.03	13.73	21.26	112.85
Kashmir Selection	26.77	21.87	3.55	30.43	30.74	8.86	16.07	27.80	88.80
Mandi Selection	30.48	31.79	8.76	41.02	28.30	10.47	17.71	25.36	122.52
KNR	27.81	23.75	5.91	39.92	29.36	9.65	19.04	25.62	120.86

Contd...

Table 2 contd...

Genotype	Kernel length (mm)	Kernel width (mm)	Kernel weight (g)	Kernel percentage (%)	*L**	*a**	*b**	Whiteness index	Browning index
Sirmour Selection	34.77	30.08	8.30	45.90	31.20	11.30	21.40	26.84	132.94
SR-16	29.25	31.45	7.08	46.73	31.46	9.52	18.59	27.40	102.36
SR-6	30.50	29.00	5.74	31.04	35.38	9.80	19.28	31.76	109.91
Gobind	34.17	29.65	6.33	33.81	31.05	11.72	23.45	25.84	160.99
CITH-W-5	35.33	29.79	8.27	49.27	22.11	9.67	14.83	20.00	133.18
CITH-W-8	31.10	28.64	6.79	54.33	31.31	10.61	20.39	27.50	121.66
K-9	33.31	30.86	8.52	49.64	29.72	11.86	18.43	26.23	118.94
W-1	26.69	30.78	7.74	51.80	35.87	11.15	25.66	29.95	136.07
W-2	30.26	28.26	8.08	53.25	48.59	9.66	30.84	39.15	110.81
W-3	32.01	28.63	7.97	46.93	22.29	8.92	12.20	20.28	101.65
Bhushan	31.40	32.60	12.19	61.42	36.76	12.19	24.02	31.20	124.10
PAD 1-23	32.60	28.69	8.35	54.69	46.71	10.42	29.30	38.22	108.96
GL 1-23	30.38	31.97	9.69	47.78	36.62	11.45	23.56	31.33	119.03
Gumma Selection-1	25.27	26.17	8.08	42.31	28.21	11.66	18.37	24.61	124.39
Gumma Selection-2	28.85	28.57	7.83	45.30	22.70	8.88	9.91	21.48	85.19
Gumma Selection-3	27.76	28.97	6.52	39.29	43.22	9.30	24.87	36.93	101.33
Pusa Khor	27.93	23.95	5.51	51.96	39.88	11.85	23.92	33.87	108.34
Walnut Seedling	21.74	23.43	4.36	44.44	33.62	10.35	18.96	29.90	101.91
Kullu Selection	24.96	23.13	4.61	46.17	35.12	11.54	22.31	30.19	116.92
Shoghi Selection	24.76	23.73	4.86	47.67	31.91	11.13	20.16	27.90	117.46
Amartara Selection-1	30.41	28.39	7.37	48.45	31.13	11.85	17.85	27.79	109.99
Amartara Selection-2	25.26	22.89	5.61	44.44	27.05	9.78	15.90	24.63	109.53
CITH-W-1	36.36	30.77	10.24	47.47	40.14	10.06	24.72	34.24	108.44
CITH-W-2	31.90	31.38	9.25	48.78	45.56	9.40	23.62	39.87	87.47
CITH-W-3	26.08	25.39	5.42	44.43	39.03	10.74	24.90	32.76	116.64
CITH-W-4	34.47	26.02	7.36	46.73	38.43	10.82	23.27	32.68	107.69
CITH-W-6	30.87	28.70	7.58	53.13	43.14	11.19	27.03	35.59	112.10
CITH-W-7	34.87	28.45	7.30	48.83	29.34	11.08	18.60	25.52	117.53
CITH-W-10	24.94	24.08	4.06	41.24	35.13	8.91	19.33	30.85	90.86
CITH-W-11	25.44	22.06	3.96	45.25	39.66	11.62	24.29	33.46	109.20
CITH-W-12	33.40	28.41	8.29	50.57	24.39	9.06	10.71	22.98	84.51
CITH-W-13	25.43	22.79	3.77	40.30	24.28	8.66	12.23	22.54	90.17
CITH-W-15	34.32	22.72	4.88	43.25	38.97	10.61	25.34	32.99	117.19
CITH-W-16	31.54	30.70	7.55	47.73	42.50	10.64	26.04	35.93	109.48
CITH-W-17	35.90	27.89	7.71	50.46	33.93	12.12	21.58	29.23	120.21
CITH-W-19	30.86	25.94	6.49	39.58	37.23	9.31	22.49	32.44	108.00
CITH-W-20	34.28	27.31	6.81	49.50	35.35	10.53	22.78	30.20	117.62
CITH-W-22	33.46	27.58	6.16	40.47	32.90	10.57	21.29	28.55	119.66
Range	21.74-36.36	21.87-32.60	3.55-12.19	30.43-61.42	22.11-48.59	8.66-12.54	9.91-30.84	20.00-39.87	84.51-160.99
LSD (p≤0.05)	2.47	2.85	1.22	6.49	8.56	2.74	6.65	6.68	30.85

12; Einollahi and Khadivi, 4). The wide variations for the nut and kernel morphology were observed in the Indian walnut accessions, providing the growers and breeders with the opportunity of choice as per the consumer demand in the international or domestic market.

The nuts of various walnut genotypes were found to differ significantly concerning nut size (length and diameter) and weight (Table 1). Of the 44 genotypes, CITH-W-7 tended to show the longest nuts (50.04 mm) having similarity statistically with CITH-W-1, CITH-W-4, CITH-W-5, CITH-W-15, CITH-W-16, CITH-W-17, CITH-W-20 and CITH-W-22 (45.88-48.49 mm), while shortest nut size was registered in Walnut Seedling (30.42 mm) with no significant difference with the nuts of Kullu Selection, Shoghi Selection, W-1, Lara and CITH-W-10 (32.11-33.35 mm). The highest nut diameter was recorded in the nuts of CITH-W-16 (47.87 mm) without showing any significant difference with CITH-W-15, CITH-W-17, CITH-W-20, and CITH-W-22; however, Pusa Khor, Kullu Selection, Shoghi Selection, and CITH-W-10 were found to bear nuts with a lesser diameter (28.93-30.85 mm). The heaviest nut was recorded in CITH-W-1 (21.68 g), proving similarity statistically at par with the nut weight of Mandi Selection, Bhushan, GL 1-23, and Gumma Selection, while it was lightest in CITH-W-11 (8.74 g) with no significant difference with the nut weight of Walnut Seedling, Kullu Selection, Shoghi Selection, and CITH-W-13 genotypes. The thickness of the shell and dividing membrane of the tested walnut genotypes varied significantly, ranging from 1.40-2.73 mm (Table 1). The nuts of Bhushan had the thinnest shell (1.40 mm) followed by CITH-W-19 (1.46 mm) with no significant difference, while the nuts of CITH-W-2 had the thickest shell (2.73 mm) having similarity statistically with Mandi Selection, KNR, SR-6, CITH-W-1, CITH-W-3, and CITH-W-22 genotypes. The dividing membrane inside nut was the thinnest in CITH-W-6 (0.16 mm), proving similarity statistically with the nuts of Kashmir Selection, KNR, SR-6, Gobind, CITH-W-8, W-1, Bhushan, PAD 1-23, Pusa Khor, Walnut Seedling, Kullu Selection and Amartara Selection-1, while in CITH-W-2, CITH-W-3, CITH-W-4, CITH-W-10 and CITH-W-13, it ranged from 1.13-1.42 mm without showing significant difference (Table 1). The lightest shell colour was observed in Bhushan (61.32 *L** value), having statistical similarity with CITH-W-8, PAD 1-23, CITH-W-3, and CITH-W-16. In terms of *a**, Lara, Kainthal Selection, SR-16, CITH-W-5, CITH-W-13, and CITH-W-22 proved superior with no significant difference; however, the lowest *a** value was found in CITH-W-10, which was found statistically at par with CITH-W-3, Shoghi Selection, Gumma Selection-2, and Mandi Selection genotypes. As far as the whiteness index is concerned, the Bhushan (51.60) genotype excelled; however, it proved statistically at par with CITH-W-3, CITH-W-7, CITH-W-8, CITH-W-10, CITH-W-16, K-9, and W-1 genotypes (Table 1).

The kernel weight in the tested genotypes ranged from 3.77 g to 12.99 g, registering its highest value in Bhushan (Table 2). The longest kernel size was recorded in the nuts of CITH-W-1 (36.36 mm) and the Walnut Seedling had the shortest kernel (21.74 mm) statistically. The highest kernel width was recorded in the nuts of Bhushan (32.60 mm). The kernel weight of tested genotypes differed significantly, registering the heaviest nuts in Bhushan (12.19 g) statistically, while it was lighter in Kashmir Selection (3.55 g), which proved similar statistically with the kernel weight of Walnut Seedling, Kullu Selection, CITH-W-10, CITH-W-11 and CITH-W-13 genotypes (Table 2). Kernel percentage among the tested genotypes varied significantly from 30.43 - 61.42%. The highest kernel percentage was recorded in the nuts of Bhushan (61.42%), while it was lowest in Kashmir Selection (30.43%), with no statistical difference with the nuts of SR-6 and Gobind genotypes. The kernel percentage in the present study ranged between 30.43 and 61.42% in various Indian walnut genotypes, and the highest was noted in the Bhushan (61.42%) genotype. A desirable walnut variety should have a kernel percentage above 50% (Sharma *et al*., 17; Khadivi-Khub *et al*., 10). Notably, out of the 44 genotypes evaluated, only nine walnut genotypes had a kernel percentage ≥50%, which underscores the need for improvement of the existing selection available to the growers. Yield-related parameters (nut weight, kernel weight, kernel size, and kernel %) are of significant economic importance, serving as target traits for plant breeders and growers.

Colour is among the most important characteristics, affecting marketability and even the biochemical composition of the walnut kernel and light amber-coloured kernels with the least browning are the most preferred in the domestic as well as international markets, as darker colour generally represents a higher degree of rancidity (Warmund *et al*., 20; Amin *et al*., 1; Gama *et al*., 5). The genotypes W-2, PAD 1-23, CITH-W-2, Gumma Selection-3, CITH-W-6, CITH-W-16, and CITH-W-1 had the light-coloured kernel (Table 2 and Fig. 1). The kernel and nut parameters measured in the present study were more or similar in the range to the previous findings for walnuts from different regions of the world (Khadivi *et al*., 9; Mirmahdi and Khadivi, 12; Shah *et al*., 16; Soveili and Khadivi, 18; Chatrabnous *et al*., 3; Einollahi and Khadivi,

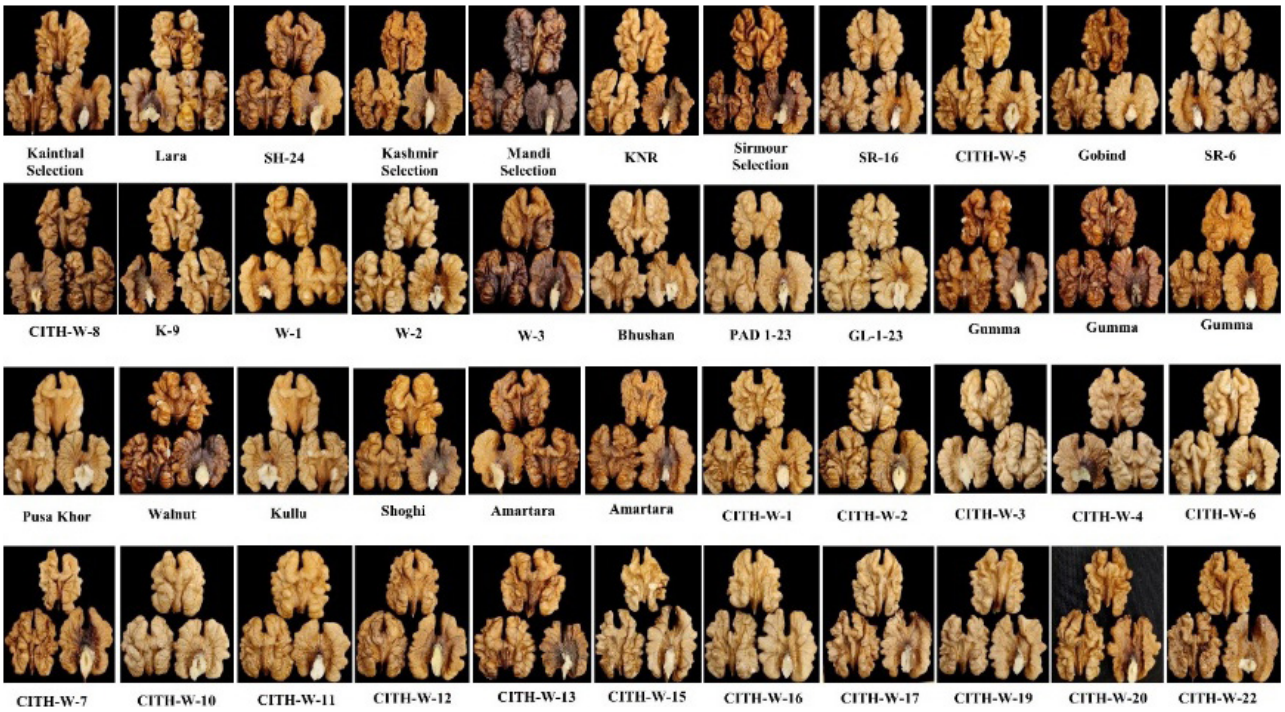

Fig. 1. Variability of kernel among the Indian walnut genotypes.

4; Hakimi *et al*., 6). The external kernel and nut morphological traits such as shape, size, and colour are important considerations for consumers apart from their role as key traits for identifying genotype/ variety as descriptors in addition to influencing the packaging, transportation, and grading operations (Verma *et al*., 19). These traits are considered heritable, *i.e*., depending on the genetic background of individual genotypes, and remain unaffected by edaphoclimatic conditions (Verma *et al*., 19).

Hierarchical cluster analysis (wards D2) was carried out to group the 44 walnut genotypes studied based on studied traits, and a total of four clusters were obtained (Fig. 2). The largest cluster *i.e.,* Cluster-III comprised 12 genotypes; Cluster-I and Cluster IV comprised 11 genotypes; while Cluster-II contains

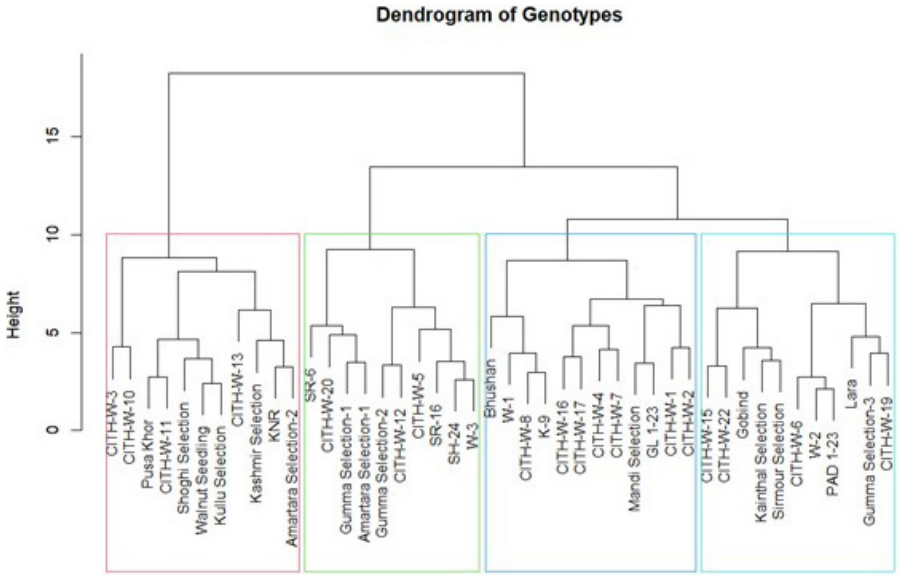

Fig. 2. Hierarchical clustering depicting the relationship among Indian walnut genotypes based on quality traits.

ten genotypes. The hierarchical clustering and PCA could not separate the Indian walnut diversity based on their geographical location, which indicates that walnuts grown in each region are not distinct from other regions, *i.e.,* lack of regional preference and genotypes were selected independently from seedling variability. The cluster mean values for important traits indicated that genotypes in Cluster III were promising for higher nut weight, kernel weight, and kernel percentage, while Cluster IV exhibited the least shell thickness in addition to the higher kernel browning index. The genotypes from the different clusters can be selected as parent material based on breeding objectives, and the results of the present study suggested that hybridization between genotypes in clusters III and IV may provide ample scope for isolating elite hybrids in the segregating generation with a desirable nut quality trait. The hierarchical cluster analysis helps to understand the genetic makeup of the walnut population and offers useful insights for breeding programmes.

The current investigation confirmed that a wide variation exists in Indian walnut germplasm, as evidenced by differences in nut and kernel morphological and physical attributes. The genotypes Bhushan, PAD 1-23, CITH-W-6, CITH-W-8, CITH-W-12, CITH-W-16, CITH-W-17, CITH-W-22, W-1, W-2, Pusa Khor, and Gumma Selection-3 excelled with the higher kernel percentage (≥50%) and whiteness index (>35). These genotypes were found to have the potential to be used as parents for Indian walnut breeding programme in addition to their promotion to bring more area under export-oriented walnut production. Future research on the comprehensive evaluation of individual phenolics, carotenoids, flavonoids, and sugars should be conducted to understand these genotypes' nutritional value better.

AUTHORS' CONTRIBUTION

Conceptualization of research (RMS); Collection of experimental materials (S, KKM, OCS, NS, KK, DPS, MKV); Lab analysis (S, GPS, VS, SS); Data analysis (AS, RRK. NK); Manuscript preparation (S, AKG, NS).

DECLARATION

The authors declare no conflict of interest.

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