Integrated morphological, phenotypic, and biochemical profiling of *Juglans regia* genotypes within agroforestry systems of the north-western Himalaya

Asima Gazi[1], Megna Rashid[1]*, G.M. Bhat[1], M. Maqbool Rather[1], J.I Mir[2], Iqbal Jeelani[1], Nazir A. Pala[1], Mansha Manzoor[1] and Sufiya Shabir[1]

ABSTRACT: *The present investigation, entitled "Morpho-phenotypic and biochemical characterization of Juglans regia genotypes in the agroforestry systems at Benhama, Ganderbal" was conducted to evaluate growth, flowering behavior, yield, and biochemical traits of walnut genotypes grown under an agroforestry system. The study was carried out on an eight-year-old walnut plantation spaced at 7 m × 7 m and intercropped with forage crops. Five walnut genotypes CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, Sulaiman, and Hamdan were evaluated using a replicated experimental design. The results revealed pronounced genotypic variation in morpho-phenotypic, yield, and biochemical characteristics, indicating the influence of genotype and environment. Flowering across all genotypes occurred during early to mid-April. Among the genotypes, CITH-Walnut-1 exhibited superior growth and yield performance, whereas CITH-Walnut-3 showed enhanced biochemical properties, including higher phenolic content, antioxidant activity, and oil content. GC–MS analysis confirmed the presence of important fatty acids, particularly linoleic and palmitic acids, across all genotypes. Principal component analysis explained a substantial proportion of total variability, and cluster analysis grouped the genotypes into distinct categories, reflecting considerable genetic diversity. Overall, the findings highlight the potential of specific walnut genotypes for improving productivity and nutritional quality in agroforestry systems, supporting sustainable walnut-based land-use practices in the temperate Himalayan region.*

Research Communication

ARTICLE INFO

Received: 06.10.2025

Accepted: 10.12.2025

Keywords:
Juglans regia genotypes,
GC–MS analysis,
Fatty acid profiling,
Morpho-phenotypic variation,
Biochemical traits,
Walnut-based agroforestry productivity

1. INTRODUCTION

Juglans regia L. (English or Persian walnut) is a high-value temperate nut crop cultivated worldwide for its premium kernels, nutraceutical properties, and timber (Pollegioni *et al.,* 2017). In India, walnut cultivation is primarily confined to the temperate Himalayan regions, with Jammu and Kashmir accounting for nearly 90% of the national production (Taufique and Khursheed, 2018). Despite this dominance, walnut productivity in the region remains low, primarily due to reliance on seedling-origin plantations, limited access to elite planting material, and inadequate orchard management practices (Shah *et al.,* 2017). These constraints have resulted in wide variability in tree growth, yield stability, and nut quality across plantations. In the Kashmir Himalayas, walnut cultivation is increasingly integrated into agroforestry systems, where trees are grown alongside forage or agricultural crops to enhance land-use efficiency, farm income, and ecological sustainability. Such systems are particularly relevant in mountainous regions with fragmented landholdings, where diversification and year-round income are critical for livelihood security. However, the performance of walnut genotypes under agroforestry conditions can differ markedly from monoculture orchards due to altered microclimate, interspecific competition, and management practices. Despite the growing adoption of walnut-based agroforestry systems, scientific evaluations of genotype performance under such systems remain limited. Walnut exhibits substantial genetic variability in growth habit, flowering behavior, nut and kernel characteristics, and yield potential, making genotype selection a critical factor for improving productivity and quality. In agroforestry systems, selecting genotypes with suitable canopy architecture, stable yield, and compatibility with intercrops becomes particularly important. Alongside agronomic traits, walnuts are increasingly valued for their biochemical composition, including phenolics, antioxidants, and polyunsaturated fatty acids that contribute to their nutraceutical importance (Davis *et al.,* 2007; Amarowicz *et al.,* 2016). While biochemical profiling of walnuts has been reported, comparative evaluation of locally important genotypes using advanced analytical tools such as GC–MS, particularly under agroforestry systems, is scarce. The five genotypes evaluated in the present study CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, Sulaiman, and Hamdan

✉ Megna Rashid
megnarashid@gmail.com

[1] Faculty of Forestry, SKUAST-Kashmir

[2] ICAR-Central Institute of Temperate Horticulture

represent a combination of improved selections and widely cultivated local genotypes in the Kashmir region. These genotypes are commonly preferred by farmers for their adaptability and economic potential; however, their relative performance in terms of morpho-phenotypic traits, yield attributes, and biochemical quality under agroforestry conditions has not been systematically assessed using integrated analytical approaches.

Research gap

There is a lack of integrated studies combining morpho-phenotypic, yield, biochemical, and multivariate analyses of walnut genotypes grown under agroforestry systems in the temperate Himalayan region.

Experimental methodology

The experiment was conducted during the 2022–23 growing season in an eight-year-old walnut-based agroforestry plantation established at 7 m × 7 m spacing at the Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Benhama, Ganderbal (Jammu and Kashmir, India). Five walnut (*Juglans regia* L.) genotypes CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, Sulaiman, and Hamdan were selected for evaluation.

Floral phenotypic and Identification of different bioactive molecules and antioxidant activity of *Juglans regia* genotypes.

Floral and morpho-phenotypic traits were recorded following the DUS guidelines for walnut as prescribed by the Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA, 2012). Observations on flowering behavior, vegetative growth, nut, and kernel traits were recorded at appropriate phenological stages using standard field measurement procedures. (DUS guidelines, 2012).

Identification and extraction of different bioactive molecules of 5 walnut genotypes was carried out in harvested walnuts at Kashmir University (KU) and SKUAST-K Srinagar.

Reagents and chemicals and Preparation of Extracts

Methanol, Ethanol, Gallic acid, Ferrous sulphate, Sodium Carbonate, petroleum ether, Aluminium chloride, potassium acetate, TPTZ (2,4,6-tripyridyl-s-triazine), Folin–Ciocalteu, Ferric chloride, Ascorbic acid, DPPH (1,1- diphenyl-2-picryl-hydrazyl). Quercetin was obtained from Sigma, Germany; 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., USA, Hydrogen chloride. All chemicals and reagents utilized in the experiments were of analytical grade.

Fully matured walnut kernels were harvested, shade-dried, and finely ground. Methanolic extracts were prepared by extracting 2.5 g of powdered sample with 15 mL methanol using sonication at 4°C for 60 minutes, followed by centrifugation at 8,000 rpm for 11 minutes. The supernatant was filtered through a 0.2 µm membrane filter and stored at 4°C until analysis.

Determination of total polyphenolic content (TPC) and total flavonoid and flavanols content

The modified Folin-Ciocalteu technique (Omoruyi *et al.,* 2012) was used to obtain the total phenol content of walnuts. 1 ml of each extract (1 mg/ml), 5 ml of the Folin-Ciocalteu reagent (previously diluted 1:10 v/v) with distilled water, and 4 ml (75% w/v) of sodium carbonate (Na_2CO_3) were combined. After that, the tubes were vortexed and left at 40°C for 30 minutes to allow the colour to develop. In order to quantify absorbance, a spectrophotometer was used at 765 nm. The standard curve was calibrated using gallic acid at concentrations between 0 and 400 mg/l. The results were expressed as mg of gallic acid equivalents/g of walnut sample.

The method of Chang *et al.,* (2002), which is based on the flavonoid-aluminum complex formation, was used to determine the total flavonoids in the walnut extract. 1 ml of extract (1 mg/ml) or quercetin (a standard flavonoid compound) was combined with 1.5 ml of 60% methanol, 1 ml of 2% aluminum chloride, and 6 ml of 5% potassium acetate. The mixtures were then left to stand at room temperature for 40 minutes in order for the yellow colour to develop, signifying the presence of flavonoid. A spectrophotometer was then used to measure the absorbance at 415 nm. To calibrate the standard curve, quercetin was added to methanol at concentrations of 0, 25, 50, 100, and 200 ppm. Total flavanols were also expressed in terms of quercetin equivalent (mg/g), which was the common reference compound.

Antioxidant activity, Oil Content and GC–MS analysis of bioactive compounds

With a few modest adjustments, the Benzie and Strains method (1996) was used to perform the Ferric Reducing Antioxidant Potential (FRAP) experiment. The FRAP reagent was made by combining 20 mmol/l $FeCl_3$ solution, 10 mmol/l TPTZ solution in 40 mmol/l HCl, and sodium acetate buffer (300 mmol/l, pH 3.6) in a ratio of 10:1:1 (v/v), respectively. Before use, the FRAP reagent was freshly formed and heated to 37^0C in a water bath. 1.8 ml of the FRAP reagent was mixed with 200 ml of extract. After 40 minutes, the absorbance was measured at 594 nm. To calibrate the standard curve, $FeSO_4$ solution (0, 40, 80, 160, 320, 640 lmol/l) was utilized.

Using the Soxhlet equipment and extraction procedure, the oil content of nuts was ascertained. Using a Soxhlet apparatus, 5 g of walnut kernels will have their oil content extracted using petroleum ether (b.p. 40–60°C). The residual solvent will be eliminated using vacuum distillation. The oil content of the tubes will be determined by comparing their weights before and after the experiment.

GC–MS analysis was performed using a Varian GC system coupled with a Varian 4000 mass spectrometer equipped with a VF-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1} with a split ratio of 1:10. The oven temperature was programmed from 40°C (3 min hold) to 280°C at 15°C min^{-1}, with a final hold of 15 minutes. Injector and detector temperatures were maintained at 250°C. The MS was operated in electron impact (EI) mode at 70 eV, with a scan range of m/z 40–400. Compound identification was carried out by comparing mass spectra with the NIST and Wiley mass spectral libraries, along with retention index matching and literature data. Instrument calibration and tuning were performed prior to analysis following manufacturer guidelines (Matlawska *et al.,* 2015).

Yield estimation

The total yield obtained from walnuts gave out the total yield obtained. The fruit yield was observed during harvesting by weighing the total number of fruits on each tree and was expressed in tonnes per hectare.

Statistical Analysis

The experimental data were analyzed using analysis of variance (ANOVA) appropriate for a Randomized Block Design (RBD), following the procedures described by Gomez and Gomez (1984). Genotypes were treated as fixed effects, while replications were considered random effects. Prior to ANOVA, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Where necessary, appropriate data transformation was applied to meet ANOVA assumptions. Mean separation among walnut genotypes was performed using Tukey's Honestly Significant Difference (HSD) test at a 5% level of significance ($p \leq 0.05$). Statistical analyses were conducted using SPSS (version XX) and GraphPad Prism (version XX) software. To explore relationships among genotypes and to identify patterns in biochemical and phenolic attributes, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were performed. PCA was used to reduce data dimensionality and identify key variables contributing to total variability, while HCA was carried out using Euclidean distance and Ward's linkage method to assess genetic similarity among genotypes. Multivariate analyses were conducted using XLSTAT/R software.

Results and Discussion

Tree characteristics of different walnut genotypes

Table-1, represents tree characteristics of walnut genotypes pertaining to tree vigor revealed that high vigor was observed in CITH-Walnut-1 and Hamdan exhibited high vigor, whereas CITH-Walnut-3 showed comparatively low vigor. With respect to tree growth habit, CITH-Walnut-1 and CITH-Walnut-2 displayed a semi-erect growth habit, while CITH-Walnut-3, Sulaiman, and Hamdan exhibited a spreading growth habit. Branch density varied among genotypes, with dense branching observed in CITH-Walnut-1 and Hamdan, whereas CITH-Walnut-2, CITH-Walnut-3, and Sulaiman showed intermediate branch density. All evaluated genotypes exhibited a terminal bearing habit, and shoot colour was uniform (brown) across genotypes, indicating no observable variation for this trait. Variations in tree growth and development are caused by a combination of physiology and heredity, according to Mir *et al*., (2018) in *Juglans regia* and Zhebentyayeva *et al*., (2012) in Apricot.

Tree height showed moderate but consistent variation among genotypes. CITH-Walnut-1 recorded the maximum tree height (3.51 m), whereas CITH-

Table 1: Tree characteristics of walnut genotypes

Genotype	Tree height (m)	Tree diameter (cm)	Tree vigour	Tree growth habit	Density of branches	Bearing habit	Shoot colour
CITH-W-1	3.51	12.81	High	Semi erect	Dense	Terminal	Brown
CITH-W-2	3.15	10.70	Intermediate	Semi erect	Intermediate	Terminal	Brown
CITH-W-3	2.78	9.95	Low	Spreading	Intermediate	Terminal	Brown
Sulaiman	3.00	11.22	Intermediate	Spreading	Intermediate	Terminal	Brown
Hamdan	3.22	11.86	High	Spreading	Dense	Terminal	Brown
$CD_{p=0.05}$	0.06	1.10	-	-	-	-	-
$W_{p=0.05\ Shapiro\text{-}Wilk}$	0.09	0.54	-	-	-	-	-

Walnut-3 exhibited the minimum average height (2.78 m). Likewise, significant differences were observed in trunk diameter, with CITH-Walnut-1 recording the highest trunk diameter (12.81 cm) and CITH-Walnut-3 the lowest (9.95 cm). The findings imply that genetic variants, resource competition, and other factors may contribute to variations in height and diameter even in trees of the same age and growing in the same conditions. According to Pathak *et al*., (1984), a tree's physical characteristics are determined by its genetic composition and environmental conditions in which it grows. Bernard *et al*., (2018) in walnut and Vishal *et al*., (2015) in *Melia composita* and have also revealed that morphological characteristics of trees are influenced by genetic factors.

Leaf characteristics of different walnut genotypes

Table-2, represents leaf characteristics of different walnut genotypes showed maximum leaflet length in CITH-Walnut-1 (17.68 cm), followed by Sulaiman (16.49 cm) and Hamdan (16.35 cm), whereas CITH-Walnut-2 (9.62 cm) and CITH-Walnut-3 (8.19 cm) showed comparatively shorter leaflets. With respect to leaflet shape, CITH-Walnut-1, Sulaiman, and Hamdan exhibited a broad elliptic shape, while CITH-Walnut-2 and CITH-Walnut-3 showed an elliptic leaflet shape. All genotypes uniformly exhibited an entire leaflet margin, indicating no variation for this trait. Leaflet colour also varied among genotypes. CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, and Sulaiman displayed dark green leaflets, whereas Hamdan exhibited green-coloured leaflets. The rachis colour was uniform (green) across all genotypes. Regarding phenology, Sulaiman showed early leaf fall, while CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, and Hamdan exhibited mid leaf fall.The observed variation in leaf morphological and phenological traits among genotypes grown under similar environmental conditions suggests a strong genetic control, possibly modulated by phenotypic plasticity. Comparable genotypic variability in leaf traits of Juglans regia. Same results were shown by Mir *et al.,* (2018), Lone (2017), Khan *et al.,* (2020) and Angmo *et al.,* (2015).

Flower characteristics of different walnut genotypes

Table-3, represents flower characteristics of walnut genotypes all of which were protandrous, and warm spring temperatures accelerated the flowering process and shortened the overlap period between male and female flowering. reported by Cosmulescu *et al*., (2010), Chandra and Tomar (2012) and Kumar and Sharma (2013) in walnut. The initiation of female flowers begins early in all the five varieties. The average number of male catkins per cluster ranged from 2.5 to 4, with the highest number observed in CITH-Walnut-1 (4.0), followed by CITH-Walnut-2 (3.25), Sulaiman (3.0), CITH-Walnut-3 (2.75), and Hamdan (2.5). The average number of female flowers per cluster ranged from 1.50 to 3.00, with CITH-Walnut-3 showing the maximum (3.0), followed by CITH-Walnut-1 (2.5), CITH-Walnut-2 (2.0), Hamdan

Table 2: Leaf characteristics of walnut genotypes

Genotype	Leaflet length (cm)	Leaflet shape	Leaflet margin	Leaflet colour	Rachis colour	Time of leaf fall
CITH-W-1	17.68	Broad elliptic	Entire	Dark green	Green	Mid
CITH-W-2	9.62	Elliptic	Entire	Dark green	Green	Mid
CITH-W-3	8.19	Elliptic	Entire	Dark green	Green	Mid
Sulaiman	16.49	Broad elliptic	Entire	Dark green	Green	Early
Hamdan	16.35	Broad elliptic	Entire	Green	Green	Mid
$CD_{p=0.05}$	0.92	-	-	-	-	-
$W_{p=0.05\ Shapiro\text{-}Wilk}$	0.09	-	-	-	-	-

Table 3: Flower characteristics of walnut genotypes

Genotype	Dichogamy	Initiation of 10% female flowering	Stigma colour	Number of male catkins per cluster	Number of female flowers per cluster
CITH-W-1	Protoandrous	Early	Green	4.00	2.5
CITH-W-2	Protoandrous	Early	Green	3.25	2.00
CITH-W-3	Protoandrous	Early	Green	2.75	3.00
Sulaiman	Protoandrous	Early	Green	3.00	1.5
Hamdan	Protoandrous	Early	Green	2.5	1.75
$CD_{p=0.05}$	-	-	-	0.15	0.11
$W_{p=0.05\ Shapiro\text{-}Wilk}$	-	-	-	0.07	0.09

(1.75), and Sulaiman (1.5). All genotypes displayed green-colored stigmas. Minor variations observed among trees within the same genotype likely reflect microclimatic differences and inherent genetic variability, in agreement with Milatovic *et al.* (2020) for *Juglans regia* and Wilkie *et al.* (2008) for mango and apple. These findings highlight the genetic control of flowering traits, which is critical for optimizing cross-pollination and nut set in agroforestry systems.

Fruit characteristics of different walnut genotypes

Table-4, represents the fruit characteristics of walnut genotypes, showing clear genotypic differences in fruit phenology, nut morphology, and kernel traits. The onset of hulling occurred earliest in CITH-Walnut-3, followed by CITH-Walnut-1, while CITH-Walnut-2, Sulaiman, and Hamdan exhibited comparatively late hulling, indicating variation in maturity behavior among genotypes. Nut shape varied distinctly across genotypes, with CITH-Walnut-1 producing long trapezoid nuts, CITH-Walnut-2, CITH-Walnut-3, and Hamdan showing ovate nuts, and Sulaiman producing round nuts. Among the genotypes, CITH-Walnut-1 consistently recorded the largest nut size, with maximum nut diameter and length, whereas CITH-Walnut-3 produced the smallest nuts. Correspondingly, average nut weight was highest in CITH-Walnut-1, followed by Hamdan, Sulaiman, and CITH-Walnut-2, while CITH-Walnut-3 recorded the lowest nut weight. Shell characteristics also varied among genotypes. CITH-Walnut-1 and Hamdan exhibited smooth shell surfaces, whereas CITH-Walnut-2, CITH-Walnut-3, and Sulaiman had rough shells. Shell colour was predominantly light in most genotypes, except Sulaiman, which showed a medium shell colour. Although shell strength remained intermediate across all genotypes, shell thickness differed significantly, with CITH-Walnut-2 exhibiting the thickest shell, followed by CITH-Walnut-3, while Hamdan recorded the thinnest shell.

Table-5, represents the fruit parameters of walnut genotypes, where kernel traits showed marked genotypic variation; CITH-Walnut-1 recorded the highest kernel weight, followed by CITH-Walnut-2 and CITH-Walnut-3, while Sulaiman exhibited the lowest kernel weight. Kernel colour varied from extra-light (CITH-Walnut-1 and Hamdan) to light (CITH-Walnut-2, CITH-Walnut-3, and Sulaiman). The observed positive association between nut size and kernel weight aligns with earlier reports in *Juglans regia* by Ebrahimi *et al.,* (2011), Cerovic *et al.,* (2010) and Khadivi-Khub *et al.,* (2015a) in Juglans regia L. genotypes and the findings revealed that nut dimensions and weight were in significant positive correlation with kernel weight.

Since all genotypes were grown under identical environmental conditions, the variation in fruit and kernel attributes can be primarily attributed to genetic differences. Additionally, genotypes with lighter shell colour tended to produce heavier nuts and kernels, corroborating observations by Atefi (1990) in elevated regions and Amiri *et al.,* (2010) in walnut.

Total polyphenolic content (mg/g GAE)

Table-6, represents the bioactive molecules and antioxidant activity of walnut genotypes, revealing that dried kernels of CITH-3 exhibited the highest phenolic content (47.89), followed by Sulaiman

Table 4: Fruit characteristics of walnut genotypes

Genotype	Time of hulling	Nut shape	Shell surface	Shell colour	Shell strength	Kernel colour
CITH-W-1	Mid	Long Trapezoid	Smooth	Light	Intermediate	Extra light
CITH-W-2	Late	Ovate	Rough	Light	Intermediate	Light
CITH-W-3	Early	Ovate	Rough	Light	Intermediate	Light
Sulaiman	Late	Round	Rough	Medium	Intermediate	Light
Hamdan	Late	Ovate	Smooth	Light	Intermediate	Extra light

Table 5: Fruit parameters of walnut genotypes

Genotype	Nut diameter (mm)	Nut length (mm)	Nut weight (g)	Shell thickness (mm)	Kernel weight (g)
CITH-W-1	44.66	46.44	24.73	0.91	13.61
CITH-W-2	42.84	43.07	16.78	1.72	12.31
CITH-W-3	32.66	35.94	15.91	1.56	11.16
Sulaiman	35.24	41.07	17.56	1.05	6.45
Hamdan	39.12	45.42	19.18	0.73	9.35
$CD_{p\leq0.05}$	0.97	1.10	0.89	0.17	0.92
$W_{p\leq0.05\ Shapiro\text{-}Wilk}$	0.11	0.09	0.07	0.04	0.15

(44.63), CITH-1 (42.61), and Hamdan (40.55). The lowest phenolic content (38.34) was found in CITH-2. Similar results were reported by Oliveira (2008), Shah *et al*., (2018) and Jan *et al*., (2022) in *Juglans regia* L. The differences in total phenolic content may be due to genetic structure, fruit ripeness and ecological conditions as reported by Komur *et al*., (2023).

Total flavonoid and flavanols content (mg/g QE)

Table-6, represents the bioactive molecules and antioxidant activity of walnut genotypes, revealing that among the five genotypes, dried kernels of CITH-3 exhibited the highest flavanols and flavonoid content (10.07), followed by Sulaiman (8.82), CITH-1 (6.81), and Hamdan (5.92). The lowest flavanols and flavonoid content was found in CITH-2 (5.21). Similar results were reported by Ghasemi (2011), Shah *et al*., (2018) and Jan *et al*., (2022) in *Juglans regia* L.

Antioxidant activity (µM Fe^{2+}/g FW)

Table-6, represents the bioactive molecules and antioxidant activity of walnut genotypes, showing that the highest antioxidant activity was recorded in CITH-3 (752.42), followed by Sulaiman (433.18), CITH-1 (414.69), and Hamdan (384.19). The lowest antioxidants were found in CITH-2 (355.94). Similar results were observed by Shah *et al*., (2018) and Jan *et al*., (2022) in *Juglans regia* L.

Oil content (%)

Table-6, represents the bioactive molecules and antioxidant activity of walnut genotypes, illustrating that among the genotypes, the maximum oil content was recorded in the kernels of CITH-3 (52.14%), followed by Sulaiman (51.86%), CITH-1 (50.23%), and Hamdan (50.14%). The minimum oil content was present in the kernels of CITH-2 (50.02%). Similar results were found by Kodad and Socias (2008) in *Prunus amygdalus,* Li *et al*., (2014), Poggetti *et al*., (2018) and Shah *et al*., (2018) in *Juglans regia* L. Since, the nuts of CITH-3 variety are smaller in size but the biochemical activity is highest, due to the reason that major portion of nutrients and minerals are used up in building the secondary metabolites than primary ones.

GC/MS analyses of the extracts

Table-7 represents the compound summary of CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman, and Hamdan, where GC–MS analysis of walnut kernel extracts revealed that fatty acids were the dominant class of compounds across all five genotypes, with linoleic acid (9,12-octadecadienoic acid) and palmitic acid (n-hexadecanoic acid) consistently representing the major constituents, indicating a broadly conserved lipid profile among the evaluated walnut varieties. Minor variation in the relative abundance of these compounds was observed among genotypes, with CITH-Walnut-3 and Hamdan showing comparatively higher representation of linoleic acid, while palmitic acid predominated in CITH-Walnut-2 and Sulaiman. In addition, 2,4-decadienal was detected in Sulaiman, contributing to genotype-specific compositional differences. The predominance of unsaturated fatty acids, particularly linoleic acid, is consistent with earlier reports in walnut and other nut species. Similar fatty acid-dominated profiles have been reported in almond were observed by Sathe *et al*., (2008) in almonds, Gupta *et al*., (2012) in wild apricot and Loo and Foo (1998) in apple seeds that consist mainly of fatty acids (80.19%) with linoleic acid as the most dominant one (51.2%), Ahad *et al*., (2020) and Poggetti *et al*., (2018) in walnut kernels where major phenolic component identified was linoleic acid.

Comparative yield/productivity of walnut varieties in agroforestry systems.

Table-8 represents the yield of walnut genotypes, showing that the productivity of walnut genotypes grown under the agroforestry system varied significantly among the evaluated varieties. According to the data, there are variations in the production of several genotypes of walnuts in the agroforestry system, with CITH-Walnut-1 having the maximum output (0.71 tonnes per hectare) followed by CITH-Walnut-2 (0.63 tonnes per hectare), Hamdan (0.60 tonnes per hectare), Sulaiman (0.52 tonnes per hectare) and CITH-3 (0.37 tonnes per hectare). In terms of nut production, CITH-1 continuously

Table 6: Bioactive molecules and antioxidant activity of walnut genotypes

Genotype	Phenols	Flavonoid and flavanols	Antioxidant activity	Oil content (%)
CITH-W-1	42.61	6.81	414.69	50.23
CITH-W-2	38.34	5.21	355.94	50.02
CITH-W-3	47.89	10.07	752.42	52.14
Sulaiman	44.63	8.82	433.18	51.86
Hamdan	40.55	5.92	384.19	50.14
$CD_{p\leq0.05}$	1.11	0.52	18.77	0.18
$W_{p\leq0.05\ Shapiro\text{-}Wilk}$	0.06	0.09	0.06	0.07

surpassed other kinds, demonstrating its potential as the best option for walnut farming given the local climate and soil conditions. The reduction in yield is due to the competition between the roots of the trees and the shading as observed by Newman *et al*., (1998) and Rao *et al*. (1998). Zalac *et al*. (2021) reported that a lack of water inhibits tree growth, which might postpone and lower fruit production.

Table-7: Compound Summary of CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman and Hamdan

Compound	Formula	Samples Detected	Retention Time Range	Max Area %	Importance
n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman, Hamdan	19.99-20.94	100.00	Major fatty acid; antimicrobial, antioxidant
9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	$C_{18}H_{32}O_2$	CITH-W-1, CITH-W-2, CITH-W-3, Hamdan	23.11-25.24	100.00	Essential PUFA; anti-inflammatory
9-Octadecenoic acid, methyl ester (Oleic acid ester)	$C_{19}H_{36}O_2$	CITH-W-1, CITH-W-2, Sulaiman	22.68-22.78	17.83	Lipid metabolism, bioactive ester
Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman, Hamdan	24.09-25.31	16.60	Saturated fatty acid
γ-Sitosterol	$C_{29}H_{50}O$	CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman, Hamdan	40.36-40.47	16.24	Phytosterol; cholesterol-lowering
γ-Tocopherol	$C_{28}H_{48}O_2$	CITH-W-3, Sulaiman, Hamdan	37.16-37.20	6.22	Vitamin E; antioxidant
Eucalyptol (1,8-Cineole)	$C_{10}H_{18}O$	CITH-W-1, CITH-W-2, CITH-W-3	4.15-4.16	8.31	Volatile monoterpene; antimicrobial
Naphthalene	$C_{10}H_8$	CITH-W-1, CITH-W-2, CITH-W-3, Sulaiman, Hamdan	5.78-5.83	14.15	Aromatic hydrocarbon
2,4-Decadienal (E,E)	$C_{10}H_{16}O$	CITH-W-1, Sulaiman	7.51-7.56	26.31	Flavour compound; bioactive aldehyde
5β-Cholestane-3α, 7α, 12α, 24α, 25-pe	$C_{42}H_{88}O_5Si_5$	CITH-W-1, CITH-2	31.68	6.66	Steroidal compound
9,19-Cyclolanost-24-en-3-ol (β-form)	$C_{30}H_{50}O$	CITH-W-2, Sulaiman	29.83-29.89	8.08	Triterpenoid; bioactive

Table 8: Yield of walnut genotypes

Genotype	Average yield per tree (kg)	Yield (tonnes per hectare)
CITH-W-1	3.51	0.71
CITH-W-2	3.12	0.63
CITH-W-3	1.85	0.37
Sulaiman	2.54	0.52
Hamdan	2.96	0.60
$CD_{p=0.05}$	0.33	0.07
$W_{p=0.05\ Shapiro\text{-}Wilk}$	0.09	0.12

Principal component analysis

Table-9 represents, the principal component analysis of parameters of walnut genotypes, where the first principal component (PC1) explained 63.70% of the total variance and was strongly associated with yield-related traits, showing high positive loadings for total yield (0.999), average yield (0.996), and nut length (0.990). This indicates that PC1 primarily represents productivity potential under agroforestry conditions. The second principal component (PC2) accounted for 17.42% of the total variance and was mainly defined by kernel weight (0.914), followed by number of female flowers per cluster (0.746) and shell thickness (0.655), reflecting variation in reproductive efficiency and nut quality traits. Comparable patterns of trait contribution have been reported in walnut germplasm evaluations, where the first few principal components explained the majority of phenotypic variation (Ara, 2023; Liu *et al.,* 2020), supporting the robustness of the present multivariate analysis. (Figure 10). Hierarchical cluster analysis further classified the five walnut genotypes into four distinct clusters, highlighting clear genetic and phenotypic differentiation. CITH-Walnut-1 formed an independent cluster, reflecting its distinct superiority in yield and growth attributes. CITH-Walnut-2 constituted the second cluster, while Sulaiman and Hamdan grouped together, indicating similarity in their morphological and yield traits. CITH-Walnut-3 formed a separate cluster, which is consistent with its contrasting biochemical profile and comparatively

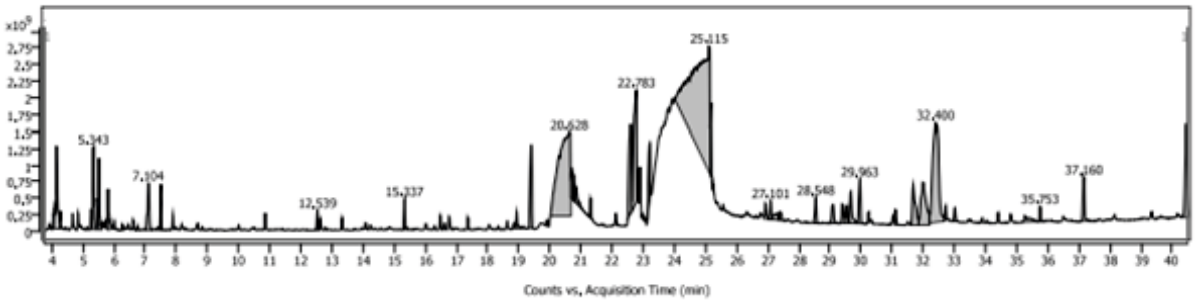

Figure-1: Chromatogram of CITH-W-1

Figure-1, represents GC–MS chromatogram of methanolic extract of walnut (Juglans regia L.) kernel genotype CITH-1 showing the presence of major phenolic and bioactive compounds identified based on retention time and mass spectral matching with NIST library. Peak intensities indicate relative abundance of detected compounds.

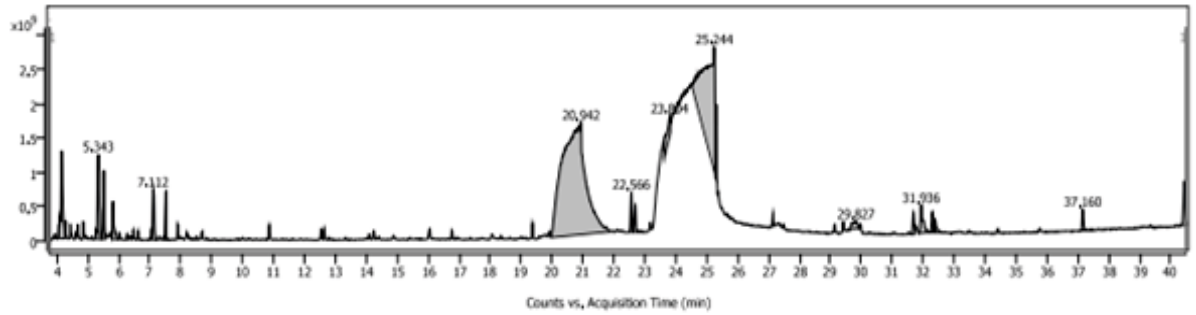

Figure-2: Chromatogram of CITH-W-2

Figure-2, represents GC–MS chromatogram of methanolic extract of walnut kernel genotype CITH-2 illustrating the qualitative and quantitative distribution of phenolic and related bioactive constituents. Differences in peak number and intensity reflect genotypic variation in phytochemical composition.

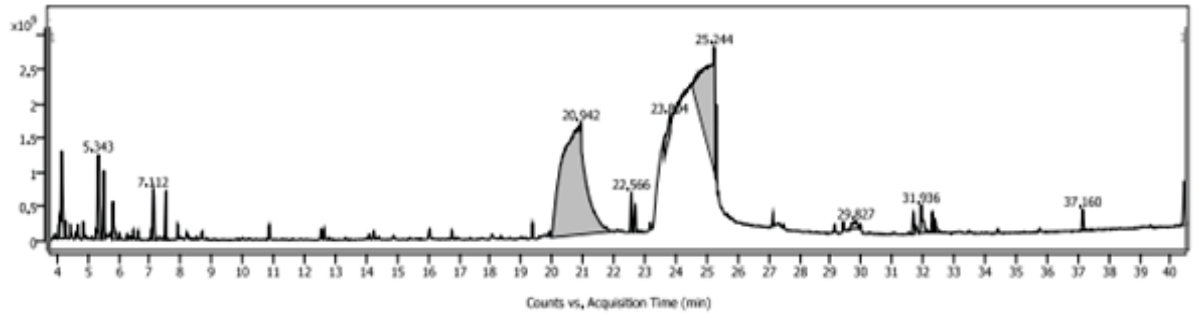

Figure-3: Chromatogram of CITH-W-3

Figure-3, represents GC–MS chromatogram of walnut kernel genotype CITH-3 depicting dominant phenolic and lipid-derived compounds. Higher peak intensities of selected compounds indicate comparatively greater accumulation of phenolics in this genotype.

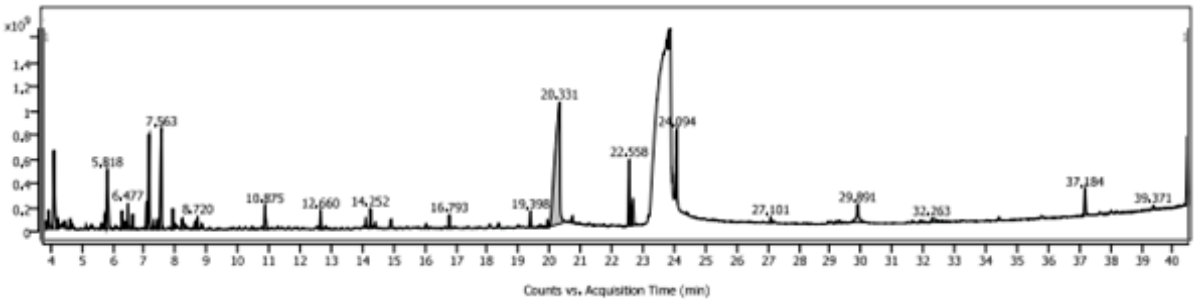

Figure-4: Chromatogram of Sulaiman

Figure-4, represents GC–MS chromatographic profile of walnut kernel genotype Sulaiman showing multiple phenolic and antioxidant-related compounds identified by their retention times and mass spectra, suggesting moderate to high phenolic richness.

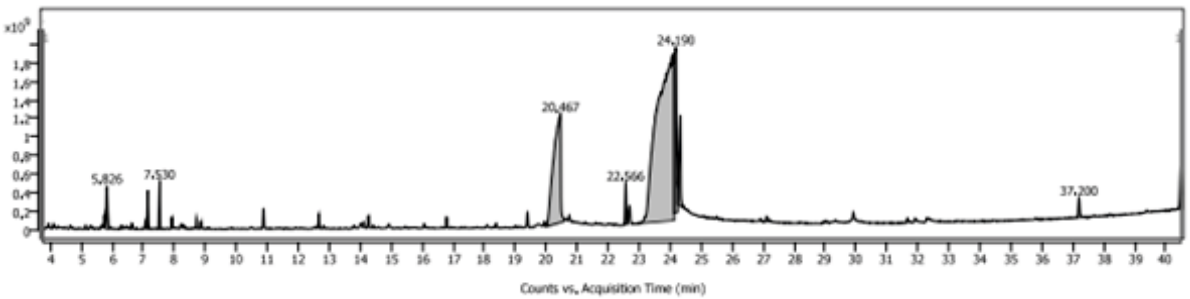

Figure-5: Chromatogram of Hamdan

Figure-5, represents GC–MS chromatogram of walnut kernel genotype Hamdan indicating the presence of several phenolic acids, fatty acids, and sterol compounds. The chromatographic pattern highlights genotypic differences in compound abundance and diversity.

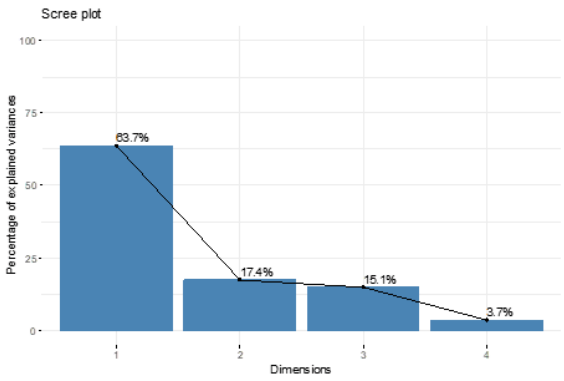

Figure-6 represents, the scree plot illustrates the eigenvalues of principal components derived from the analyzed walnut genotype parameters. The first principal component accounts for a substantially higher proportion of variance compared to the subsequent components, indicating its dominant contribution to data variability. The sharp decline after PC1 suggests that only a few principal components are sufficient to explain most of the variation among the genotypes.

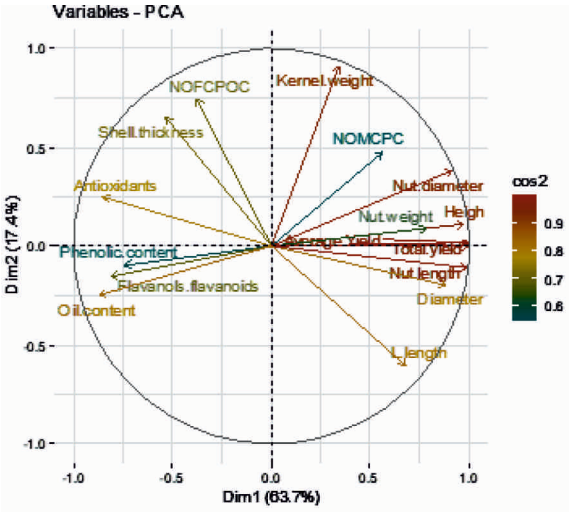

Figure 7: The component pattern plot illustrates the relationships and contributions of different yield, morphological, and biochemical parameters to the principal components of walnut genotypes. Yield and nut size traits show strong positive loadings along the first principal component, indicating their major role in explaining variability among genotypes. In contrast, biochemical attributes such as phenolic content, flavanols, flavonoids, antioxidants, and oil content load in different directions, reflecting their distinct and sometimes contrasting influence on genotype differentiation.

lower yield performance. Similarly, Arzani *et al.,* (2008) reported three clusters in *Juglans regia* genotypes and other perennial fruit crops such as apple Gong *et al*., (2015).

CONCLUSION

The present study demonstrates that all five evaluated walnut genotypes CITH-Walnut-1, CITH-Walnut-2, CITH-Walnut-3, Sulaiman, and Hamdan are capable of successful establishment and growth under walnut-based agroforestry systems in the degraded lands of Benhama, Ganderbal. However, clear and consistent genotype-specific differences were observed in growth, yield, and biochemical attributes. Among the genotypes, CITH-Walnut-1 exhibited superior vegetative growth, reproductive performance, nut and

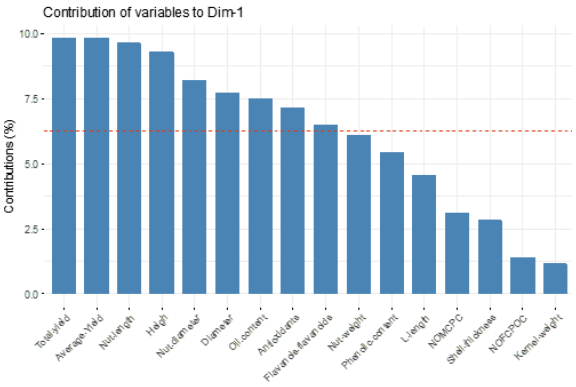

Figure-8, illustrates the contribution of individual variables to Dimension 1, highlighting the relative importance of different traits in explaining overall variability among walnut genotypes. Yield-related and nut morphological traits, including total yield, average yield, nut length, height, and diameter, show the highest contributions, indicating their dominant influence on this dimension. In contrast, biochemical attributes such as phenolic content, shell thickness, and kernel weight contribute comparatively less, suggesting a secondary role in structuring the variation along Dimension 1.

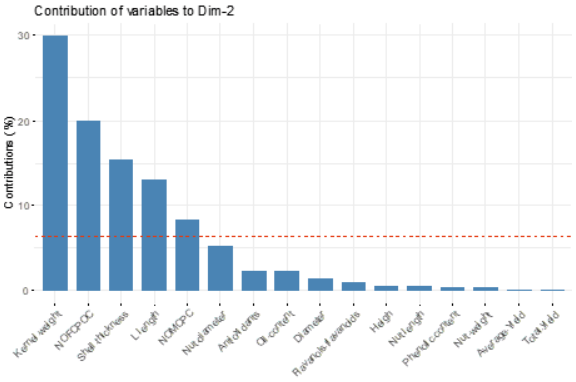

Figure-9, shows the relative contribution of variables to Dimension-2. Kernel weight, number of filled pods, and shell thickness contribute the most, indicating their strong influence on this dimension. Biochemical and minor morphological traits contribute less, falling below the reference line, showing minimal impact on Dimension-2.

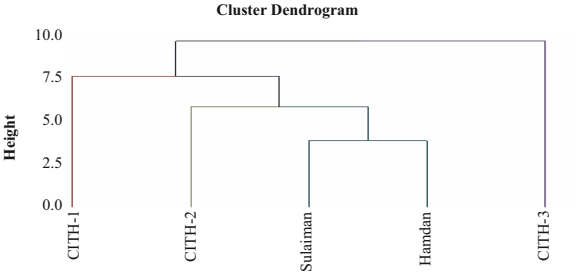

Figure-10: The cluster dendrogram illustrates the hierarchical relationships among the different samples. The vertical axis represents the distance or dissimilarity between clusters, while the horizontal branches indicate the clustering of individual samples. In this dendrogram, the samples Sulaiman and Hamdan form a closely related cluster, suggesting high similarity between them. CITH-W-2 joins this cluster at a higher level, indicating moderate similarity, while CITH-W-1 and CITH-W-3 are more distantly related, forming separate clusters. This hierarchical clustering provides a clear visualization of the relative similarities and differences among the samples.

kernel traits, and overall yield, indicating its suitability for productivity-oriented agroforestry systems where higher nut yield and farm income are primary objectives. In contrast, CITH-Walnut-3 consistently recorded higher levels of phenolics, flavonoids, antioxidant activity, and oil content, highlighting its potential for quality- and nutraceutical-oriented production systems. Multivariate analyses (PCA and cluster analysis) further supported these findings by clearly differentiating genotypes based on yield-related and biochemical traits, thereby validating the robustness of genotype selection under agroforestry conditions. The distinct grouping of CITH-Walnut-1 and CITH-Walnut-3 emphasizes their contrasting but complementary strengths. From a practical perspective, the results provide clear guidance for growers, extension agencies, and agroforestry planners. CITH-Walnut-1 is recommended for large-scale adoption in walnut-based agroforestry systems aimed at enhancing nut yield and livelihood security, while CITH-Walnut-3 may be promoted in systems targeting value addition, nutraceutical markets, and diversification of farm income. Overall, the study supports informed genotype selection as a key strategy for improving the productivity, quality, and sustainability of walnut-based agroforestry systems in the temperate Himalayan region.

Acknowledgement

We acknowledgement the financial assistance provided by CAFRI, AICRP-AF during the entire study.

References

Ahad, T., Gull, A., Nissar, J., Masoodi, L. and Rather, A. H. 2020. Effect of storage temperatures, packaging materials and storage periods on antioxidant activity and non-enzymatic browning of antioxidant-treated walnut kernels. *Journal of Food Science and Technology* 57: 3556-3563.

Amarowicz, R., Gong, Y. and Pegg, R. B. 2016. Recent advances in our knowledge of the biological properties of nuts. *Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications*, pp: 377-409.

Amiri, R., Vahdati, K., Mohsenipoor, S., Mozaffari, M. R. and Leslie, C. 2010. Correlations between some horticultural traits in walnut. *HortScience* 45(11): 1690-1694.

Angmo, S., Diengngan, S. and Hasan, M. A. 2015. Characterization of walnut (*Juglans regia* L.) genotypes in Ladakh region of India. *International Journal of Agriculture, Environment and Biotechnology* 8(2): 265-270.

Ara, T., Shafi, S., Ghazwani, M., Mir, J. I., Shah, A. H., Qadri, R. A. and Wahab, S. 2023. In Vitro Potent Anticancer, Antifungal, and Antioxidant Efficacy of Walnut (*Juglans regia* L.) Genotypes. *Agronomy* 13(5): 1232.

Arzani, K., Mansouri-Ardakan, H., Vezvaei, A. and Roozban, M. R. 2008. Morphological variation among Persian walnut (*Juglans regia*) genotypes from central Iran. *New Zealand Journal of Crop and Horticultural Science* 36(3): 159-168.

Atefi, J. 1990 Preliminary research of Persian walnut and correlation between pair characters. *Acta Hort* 284 :97-104.

Benzie, I. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry* 239(1): 70-76.

Bernard, A., Lheureux, F., and Dirlewanger, E. 2018. Walnut: past and future of genetic improvement. *Tree Genetics and Genomes* 14(1):1.

Cerovic, S., Gološin, B., Todorovic, J. N., Bijelic, S. and Ognjanov, V. 2010. Walnut (*Juglans regia* L.) selection in Serbia. *Horticultural Science* 37(1): 1-5.

Chandra, P. and Tomar, C. S. 2012. Growth, flowering fruit set and yield in some cultivars/selections of walnut (*Juglans regia*). *Indian Journal of Agricultural Sciences* 82(5): 402-404.

Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. 2002.Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10(3): 178-182.

Cosmulescu, S., Baciu, A., Botu, M. and Achim, G. H. 2010. Environmental factors' influence on walnut flowering. *Acta Horticulturae* 861: 83-88.

Davis, L., Stonehouse, W., Loots, D. T., Mukuddem-Petersen, J., van der Westhuizen, F. H., Hanekom, S. M. and Jerling, J. C. 2007. The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome. *European Journal of Nutrition* 46(3): 155-164.

DUS guidelines, 2012. Guidelines for the conduct of test for Distinctiveness, Uniformity and Stability on walnut (*Juglans regia* L.). Protection of Plant Varieties and Farmer's Rights Authority (PPV and FRA) MOA, Government of India, New Delhi.

Ebrahimi, A., Fatahi, R. and Zamani, Z. 2011. Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia* L.) using morphological traits and SSRs markers. *Scientia horticulturae* 130(1): 146-151.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical procedures for agricultural research*, 2nd edn. John wiley and sons Inc., New York, pp 680.

Gong, L., Meng, X., Liu, N. and Bi, J. 2014. Evaluation of apple quality based on principal component and hierarchical cluster analysis. *Transactions of the Chinese Society of Agricultural Engineering* 30(13): 276-285.

Gupta, A., Sharma, P. C., Tilakratne, B. M. K. S. and Verma, A. K. 2012. Studies on physico-chemical characteristics and fatty acid composition of wild apricot (*Prunus armeniaca* Linn.) kernel oil. *Indian Journal of Natural Products and Resources* 3(3): 366-370.

Jan, R., Ara, T. and Mir, J. I. 2022. Quality profiling and estimation of total phenols, flavonoids, flavonols and antioxidative potential of walnut kernel (*Juglans regia*) from Kashmir Valley. *Journal of Nuts* 13(3): 211-225.

Khadivi-Khub, A., Ebrahimi, A., Mohammadi, A. and Kari, A. 2015a. Characterization and selection of walnut (*Juglans regia* L.) genotypes from seedling origin trees. *Tree Genetics and Genomes* 11: 1-10.

Khan, W., Hussain, M., Ali, K., Ali, M. and Nisar, M. 2020. Distribution and phenotypic variation in *Juglans regia* L. growing in Hindu Kush ranges of Pakistan. *Acta Ecologica Sinica* 40(5): 363-372.

Kodad, O. and Socias i Company, R. 2008. Variability of oil content and of major fatty acid composition in almond (*Prunus amygdalus* Batsch) and its relationship with kernel quality. *Journal of agricultural and food chemistry* 56(11): 4096-4101.

Komur, Y. K., Karakaya, O., Sutyemez, M., Dirim, E., Yaman, M., Say, A. and Ayaz, I. B. 2023. Characterization of walnut (*Juglans regia* L.) hybrid genotypes; fatty acid composition, biochemical properties and nutrient contents. *Genetic Resources and Crop Evolution* pp: 1-21.

Kumar, A. and Sharma, N. 2013. Protoandrous protogynous dimorphism in indigenous selections from North Western India and some exotic cultivars of Persion walnut (*Juglans regia* L.). *Adv Hortic Sci* 61-66.

Li, X., Zhao, Y., Gong, X., Zhao, C., and Zhou, X. 2014. Quality evaluation of walnut oil through HPLC and in vitro antioxidant activity. *Journal of Food and Nutrition Research* 2(5):244–249.

Liu, B., Zhao, D., Zhang, P., Liu, F., Jia, F., Jia, M., and Liang, J. 2020. Seedling evaluation of six walnut rootstock species originated in China based on principal component analysis and cluster analysis. *Scientia Horticulturae* 265 :109212.

Lone, I. A. 2017. Diversity for leaflet shape in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley. Advance *Research Journal of Crop Improvement* 8(1):62-65.

Lu, Y. and Foo, L. Y. 1998. Constitution of some chemical components of apple seed. *Food Chemistry* 61(1-2): 29-33.

Matlawska, I., Bylka, W., Widy-Tyszkiewicz, E., and Stanisz, B. 2015. Determination of the juglone content of *Juglans regia* leaves by GC/MS. *Natural Product Communications* 10(7).

Milatovic, D., Nikolic, D., Jankovic, S., Jankovic, D. and Stankovic, J. 2020.Morphological characteristics of male reproductive organs in some walnut (*Juglans regia* L.) genotypes. *Scientia Horticulturae* 272: 109587.

Newman, S. M., Bennet, K. and Wu, Y. 1998. Performance of maize, beans and ginger as intercrops in *Paulownia* plantations in China. *Agroforestry Systems* 39: 23-30.

Omoruyi, B. E., Bradley, G. and Afolayan, A. J. 2012. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. *BMC Complementary and Alternative Medicine* 12(1): 1-9.

Pathak, P. S., Gupta, S. K. and Roy, R. D. 1984. Autecology of *Leucaena leucocephala* Linn. *Journal of Tree Sciences* 3(1/2):15-19.

Poggetti, L., Ferfuia, C., Chiaba, C., Testolin, R., and Baldini, M. 2018. Kernel oil content and oil composition in walnut (*Juglans regia* L.) accessions from North-Eastern Italy. *Journal of the Science of Food and Agriculture* 98(3): 955-962.

Pollegioni, P., Woeste, K., Chiocchini, F., Del Lungo, S., Ciolfi, M., Olimpieri, I. and Malvolti, M. E. 2017.Rethinking the history of common walnut (*Juglans regia* L.) in Europe: Its origins and human interactions. *PloS one* 12(3): 1-24.

Rao, M. R., Nair, P. K. R. and Ong, C. K. 1998. Biophysical interactions in tropical agroforestry systems. *Agroforestry Systems* 38: 3-50.

Sathe, S. K., Seeram, N. P., Kshirsagar, H. H., Heber, D. and Lapsley, K. A. 2008. Fatty acid composition of California grown almonds. *Journal of Food Science* 73(9): C607-C614.

Shah, R. A., Bakshi, P., Wali, V. K., Jasrotia, A. and Gupta, R. 2017. Status, challenges, opportunities and approaches for walnut production in Jammu and Kashmir, India. *Biotech Articles.*

Shah, U. N., Mir, J. I., Ahmed, N., Jan, S. and Fazili, K. M. 2018. Bioefficacy potential of different genotypes of walnut *Juglans regia* L. *Journal of Food Science and Technology 55*: 605-618.

Taufique, M. and Khursheed, V. 2018. Walnut industry in Jammu and Kashmir: A geographical analysis. *Research Journal of Humanities and Social Sciences* 9(4): 793-798.

Thakur, A. 2011. Juglone: A therapeutic phytochemical from *Juglans regia* L. *Journal of Medicinal Plants Research* 5(22): 5324-5330.

Vishal, J., Dhillon, S. R., Bangarwa, K. Gupta, A. 2015. Phenological behaviour and reproductive biology of *Melia composita*. *Indian Journal of Agroforestry* 16(1): 62-67.

Wilkie, J. D., Sedgley, M. and Olesen, T. 2008. Regulation of floral initiation in horticultural trees. *Journal of Experimental Botany* 59(12): 3215-3228.

Zalac, H., Burgess, P., Graves, A., Giannitsopoulos, M., Paponja, I., Popovic, B. and Ivezic, V. 2021. Modelling the yield and profitability of intercropped walnut systems in Croatia. *Agroforestry Systems* 1-12.

Zhebentyayeva, T., Ledbetter, C., Burgos, L. and Llacer, G. 2012. Apricot. In: *Fruit Breeding* (Eds. Marisa Luisa Badeness, David H. Byrne). 8: 415-458. *Springer.*