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Research Article

Pomological and phenological characterization, genetic variability and genetic diversity of walnut (*Juglans regia* L.) genotypes in the Kodaikanal hills of Western ghats

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Abstract
The walnut (*Juglans regia* L.) is one of the most important temperate nut crops grown in India. The present investigation was conducted to study the variations available in the walnut population found in the Kodaikanal hills of Tamil Nadu. Twelve selected walnut genotypes of seedling origin were evaluated for their phenological and pomological characters. The results revealed that among the genotypes, two of them (JR-7 and JR-11) were early in the first leafing (2nd - 3rd April). Genotypes JR-1, JR-3, JR-7, JR-8 and JR-12 were earlier in harvesting (15th – 20th October) than the other genotypes. High variation for nut and kernel characteristics was also observed among the genotypes evaluated. Genotypes, JR-5, JR-7, and JR-8 were identified as promising as they recorded higher kernel weight (6.00 g), nut weight (12.00 g) and kernel per cent (48%) than other genotypes. Diversity analysis (Mahalanobis D^2 statistics method) resulted in a total of five clusters from the 12 walnut genotypes using 10 biometrical traits. More number of walnut genotypes were accommodated in cluster IV and cluster V with three genotypes each whereas cluster I, cluster II and cluster III comprised of two walnut genotypes each. Intra and inter cluster distance of five clusters was assessed and it varied from 1.29 to 10.82 and 7.66 to 21.36. Maximum intra cluster distance was recorded by cluster V (10.82) followed by cluster IV (3.14). Inter cluster distance was high between cluster III and cluster IV (21.36) followed by cluster IV and cluster V (20.55). Principal component analysis of 12 walnut genotypes with 10 quantitative traits resulted in the formation of three principal components *viz*. PC I, PC II and PC III with eigenvalues of 4.06, 1.84 and 1.44, respectively. These superior genotypes could be used for cultivation or as parents in future breeding programmes.

Keywords: *Juglans regia*, walnut, variation, phenological and pomological characters, Kodaikanal

INTRODUCTION

Walnut (*Juglans regia* L.) is the most important nut fruit in the country which has been used globally in human nutrition since ancient times. The high protein and oil contents of the kernels of walnut make this fruit indispensable for human nutrition. Walnuts have a high amount of omega-6 and omega -3 polyunsaturated fatty acids which are essential dietary fatty acids that have a significant role in the prevention of coronary heart disease (Davis *et al.,*2007). Walnut grows well at altitudes of 900 – 3000 m above MSL. In India, walnut is mainly grown in the states of Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh and Arunachal Pradesh. Of these states, Jammu and Kashmir own the highest share in area and production. The total area under walnut is 149.5 thousand hectares with an annual production of 284.4 thousand metric tonnes in 2011-12 (Anon, 2012). Walnuts in India

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are found in different sizes and shapes. The Indian walnuts are categorized into four categories *viz.,* paper -shelled, thin-shelled, medium- shelled and hard-shelled. Most walnut plantations in India are of seedling origin and are in scattered forms which produce nuts of variable quality (Sharma and Kumar, 2001). The average shelling rate of Indian walnut is 40%. The harvest season for walnut in India starts at the end of August and extends up to September with the market arrival at its peak in late October. Breeders over the years have exploited the variation amongst these seedling trees to select superior genotypes with desirable traits.

The high genetic variation in walnut trees is due to their seed based propagation, high heterozygosity, and dichogamy (Aslantas, 2006; Germain, 1997). Local genotypes with high variation are important in any breeding programme (Zeneli *et al.*, 2005). High variability in nut traits *viz.,* nut shape, nut weight, shell thickness, kernel weight, kernel per cent and kernel colour have been reported in walnut in different regions (Khan *et al.,* 2010). The unexploited variability within the *Juglans regia* L. species would be useful to make selections among the natural population or to create new cultivars through hybridization programmes. Knowledge on genetic relationship of the pomological characteristics of walnut genotypes found in the Kodaikanal hills of Tamil Nadu will be of much use in further crop improvement programmes in walnut. With this background, the above study was undertaken to evaluate the promising genotypes within the walnut population of the Kodaikanal hills, Tamil Nadu.

MATERIALS AND METHODS

The promising walnut trees from seedling origin identified during the survey conducted during 2011-13 were used as study material (**Fig. 1**). The study region was at 2300 m above mean sea level located geographically at 10.2^{0}N latitude and 77.5^{0}E longitude. At the end of the preliminary investigation of twenty six numbers of walnut genotypes, twelve of the promising types were selected based on the fruit and nut characters. Phenological parameters like first leafing time, first bud breaking time, time of full flowering and harvesting time were observed and pomological parameters like shell roughness, kernel colour, shell colour, shell removal, fruit weight (g), fruit length (cm), fruit width (cm), nut shape, nut girth (cm), nut length (cm), kernel weight (g), shell nut weight (g), and kernel % (Kernel weight / Nut weight X 100**)** were also studied for evaluation in randomly chosen ten nuts of the twelve genotypes. The data recorded on ten quantitative traits pertaining to fruit and nut parameters were subjected to statistical analysis. Analysis of variance from the ANOVA table is useful to estimate the genotypic and phenotypic coefficient of variations and it was assessed by the following formula which was suggested by Burton, (1952). Sivasubramanian and Madhavamenon (1973) classified the values of GCV and PCV into three different categories *viz.*, low: less than 10 %, moderate: 10 - 20 %, and high: more than 20 %. Estimation of broad-sense heritability was suggested by Lush (1940). Values obtained from heritability estimation were categorized by Johnson *et al.* (1955) as low: less than 30 %, medium: 30 - 60 %, and high: more than 60 %. Genetic advance as per cent of mean was estimated and classified as per formulations of Johnson *et al.* (1955) and denoted in percentage for classifications of genetic advances as a per cent of the mean based on a range of values by low: less than 10 %, medium: 10 - 20 % and high: more than 20 %. Statistical analysis was carried out using the software STAR 2.0. Genetic diversity analysis was carried out by using Mahalanobis (1936) D^2 statistics and clustering of walnut genotypes by Tocher's method as described by Rao (1952). Average inter and intracluster distances were estimated as suggested by Singh and Choudhary (1977) using the statistical software GENRES 7.01 version.

RESULTS AND DISCUSSION

The walnut genotypes of the region were evaluated and considerable genetic variation in pomological and phenological characteristics was observed in all the ecotypes which were of seedling origin. Twenty six genotypes of walnut from seedling origin were evaluated for the phenological characters *viz.,* first leafing time and bud breaking time, full flowering and harvesting time. Ten pomological characters relating to fruit and nut parameters were also examined. The results obtained during the course of the evaluation of the twelve walnut types are discussed in this paper. Walnut productivity depends upon the blooming time and flowering habit. Among the twelve promising genotypes evaluated, there were significant differences in leafing and flowering time (**Table 1**). Date of the first leafing time and full flowering time varied from 2nd - 13th April and 24th April – 2nd May, respectively. Six varieties (JR-1, JR-3, JR-6, JR -7, JR -10, JR-11) exhibited first leafing time in the first week of April between 2nd and 4th. One genotype (JR-8) exhibited first leafing by the end of the 2nd week of April. The flowering time in the selected genotypes was not all the same. The time of first bud breaking and full flowering ranged between 14th - 24th April and 24th April – 30th April. The harvesting time of the selected walnut genotypes ranged between 15th October-5th November. The harvesting time of nine genotypes was in the month of October, whereas three genotypes exhibited harvesting time during the first week of November. A negative correlation was observed between late leafing and full flowering and harvesting time. The results of phenological properties were comparable to those reported by Yargilac *et al.* (2001), Simsek *et al.* (2010) and Vuceta Jacimovic *et al.* (2020). The phenological properties of the selected genotypes in this study were different partly from each other. The difference in the phenological characters might be attributed to the genetic characteristics, climatic conditions and different ecology prevailing in that region.

Fig.1. Map representing the survey area of the walnut genotypes in Kodaikanal region of Dindigul district

Table 1. Phenological characters of the walnut genotypes at Kodaikanal

Genotypes	First leafing time	First bud breaking	Time of full flowering	Harvesting time
JR – 1	3 - 4 April	15 - 18 April	24 - 25 April	15 - 20 October
JR – 2	4 - 6 April	18 - 20 April	27 - 29 April	24 - 30 October
JR – 3	3 - 4 April	14 - 17 April	24 - 25 April	15 - 20 October
JR – 4	6 - 9 April	18 - 20 April	27 - 29 April	24 - 30 October
JR – 5	6 - 8 April	23 - 24 April	29 - 30 April	01 - 05 November
JR – 6	3 - 4 April	18 - 20 April	26 - 27 April	19 - 25 October
JR – 7	2 - 3 April	14 - 15 April	22 - 24 April	15 - 20 October
JR – 8	10 - 13 April	14 - 17 April	24 – 25 April	15 - 20 October
JR – 9	4 - 6 April	15 - 19 April	25 - 26 April	18 - 24 October
JR – 10	3 - 4 April	23 - 24 April	29 - 30 April	01 – 05 November
JR – 11	2 - 3 April	22 - 24 April	29 - 30 April	01 - 05 November
JR – 12	6 - 9 April	15 - 16 April	23 - 25 April	15 - 20 October

In the present investigation (**Table 2**), nut shape of the walnut genotypes was determined as round for seven genotypes, ovate for three genotypes, broad ovate for one genotype and short trapezoid for one genotype. The shell roughness of five genotypes (JR-3, JR-5, JR-6, JR-10 and JR-12) was smooth and that of seven genotypes was medium. The shell roughness is one of the most significant criteria for determining the fruit quality of walnut. It is a desired criterion among the consumers that shell roughness in walnut is smooth.

Besides shell colour, kernel colour is also an important character in the selection of walnut. The shell colour was determined as light in seven genotypes and medium in five genotypes (JR-3, JR-5, JR-7, JR-8 and JR-12). Shell colour of most of the genotypes was light. Shell removal was hard in six genotypes, easy in five genotypes and medium in only one genotype (JR-6). The kernel colour of the seven genotypes was light brown or brown in the remaining five genotypes.

Among the genotypes evaluated, fruit weight was highest in JR-8 (12.74 g) and lowest in JR-4 (8.90 g) with a mean weight of 11.28 g. Fruit length was found maximum in JR-9 (7.52 cm) and least in JR-5 (4.13 cm). The width of the fruit was highest in JR-8 (14.27 cm) and lowest in JR-4 (10.40 cm). JR-8 genotype recorded significantly maximum values with desirable fruit characters (**Fig.2**).

The genotypes also showed high variations for nut and kernel characters (**Table 3**). Nut weight and kernel weight are the two important desirable traits for selection in walnut breeding. Higher nut and kernel weights are desirable for higher yield (Beyhan and Demir, 2006). Nut weight of the selected genotypes varied from 8.04 g (JR-1) and 15.77 g (JR-8) and kernel weight varied between 4.56 g (JR-3) to 9.62 g (JR-8). The results of this study revealed that the highest nut weight (15.77 g) and kernel weight (9.62 g) was recorded in JR-8. The study also indicated a positive correlation between nut weight and kernel weight. Hence, this genotype can be used for further breeding studies to explore its potential as a parental clone. Nut length of the genotypes ranged between 3.20 cm (JR -2) to 6.46 cm (JR – 9), In shell nut weight and a nut width of the genotypes varied from 2.44 g to 4.62 g and 5.06 cm to 12.76 cm, respectively. The genotype JR -8 recorded the minimum shell thickness (0.79 mm) while JR-12 had the maximum shell thickness of 1.56 mm.

Table 2. Pomological characters (shell, nut and kernel) of the selected walnut genotypes at Kodaikanal

Genotypes	Nut shape	Shell roughness	Shell removal	Shell colour	Kernel colour	Shell adhesion
JR – 1	Round	Medium	Easy	Light	Light	Weak
JR – 2	Round	Medium	Hard	Light	Light	Strong
JR – 3	Ovate	Smooth	Easy	Medium	Medium	Weak
JR – 4	Round	Medium	Hard	Light	Light	Strong
JR – 5	Broad Ovate	Smooth	Hard	Medium	Medium	Strong
JR – 6	Ovate	Smooth	Medium	Light	Light	Strong
JR – 7	Short trapezoid	Medium	Easy	Medium	Medium	Weak
JR – 8	Round	Medium	Easy	Medium	Medium	Weak
JR – 9	Round	Medium	Hard	Light	Light	Strong
JR – 10	Round	Smooth	Hard	Light	Light	Strong
JR -11	Round	Medium	Easy	Light	Light	Weak
JR – 12	Ovate	Smooth	Hard	Medium	Medium	Strong

Fig. 2. Fruit, nut and kernel characters of the walnut genotype, JR-8 at Kodaikanal

Shell thickness is an important criteria in walnut breeding (Beyhan and Demir, 2006). Thin shells increase the kernel percentage. In the present investigation, shell thickness ranged between 0.79 mm and 1.56 mm exhibiting a negative correlation between shell thickness and kernel percentage as the genotype JR-8 with minimum shell thickness (0.79 mm) recorded a maximum kernel per cent (9.62%) (**Fig. 2**). Kernel per cent is one of the most important desirable traits in breeding objectives. In the selected walnut genotypes the kernel per cent ranged between 20.01% (JR-4) and 48.23% (JR-8).

Genetic analysis of the economically important walnut characters revealed that all the 12 walnut genotypes available in Kodaikanal significantly differed (**Table 4**) from one another in respect of all the fruit characters. Phenotypic co-efficient of variation ranged from 9.15 to 30.29 per cent. Kernel percentage (30.29 per cent) registered a high percentage of phenotypic coefficient of variation (>-20 per cent) followed by kernel weight (23.69 per cent). The estimates of genotypic coefficient of variation of walnut accessions from different quantitative traits observed in the current study were found to range from 8.75 to 30.17 per cent. A high percentage of the genotypic coefficient of variation (GCV - >20 per cent) was observed for the following traits *viz.,* kernel percentage (30.17 per cent), kernel weight (23.54 per cent) and nut length (21.38 per cent).

Table 3. Fruit, nut and kernel characters of the walnut genotypes at Kodaikanal

Genotype	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Shell thickness (mm)	Nut width (cm)	Nut length (cm)	Nut weight (g)	Inshell nut weight (g)	Kernel weight (g)	Kernel %
JR - 1	11.13	7.02	11.67	1.25	11.36	5.30	08.04	3.25	5.32	24.36
JR - 2	10.52	7.17	12.90	1.23	11.96	3.20	10.28	2.53	6.02	25.48
JR - 3	11.33	7.25	12.11	1.06	10.50	3.80	13.44	3.65	4.56	26.52
JR - 4	08.90	6.12	10.40	1.03	5.06	6.06	12.07	2.48	5.19	20.01
JR - 5	12.00	4.13	13.89	1.17	12.31	5.60	12.92	3.74	6.96	21.12
JR - 6	11.34	7.13	12.93	1.25	10.93	3.80	09.71	3.44	8.54	22.49
JR - 7	10.53	5.84	12.77	1.34	11.86	4.50	13.84	3.65	9.37	20.95
JR - 8	12.74	6.94	14.27	0.79	12.76	5.50	15.77	4.17	9.62	48.23
JR - 9	12.15	7.52	13.46	1.11	11.40	6.46	11.13	4.62	8.62	23.89
JR - 10	12.19	7.21	13.10	0.93	10.60	3.86	10.38	3.31	7.84	21.89
JR -11	11.87	6.23	11.12	1.24	10.87	4.80	11.75	3.48	7.20	25.49
JR- 12	12.09	5.76	12.76	1.56	10.70	4.30	10.63	2.44	6.51	20.89
Grand Mean	11.28	6.63	12.75	1.11	10.85	4.76	11.63	3.39	7.14	25.11
S.Ed	0.60	0.43	0.52	0.32	0.70	0.30	0.63	0.44	0.51	0.89
CD at 0.5%	1.27	0.92	1.10	0.53	1.48	0.63	1.96	0.92	1.08	1.87

Table 4. Genetic analysis of some economically important characters of walnut in Kodaikanal

Genetic statistics	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Shell thickness (mm)	Nut width (cm)	Nut length (cm)	Nut weight (g)	Inshell nut weight (g)	Kernel weight (g)	Kernel %
Mean	11.40	6.53	12.62	1.16	10.86	4.77	11.66	3.40	7.15	25.11
GCV (%)	8.99	14.67	8.75	17.15	18.03	21.38	17.94	19.52	23.54	30.17
PCV (%)	9.37	14.98	9.15	17.42	18.16	21.61	18.06	19.76	23.69	30.29
Heritability (%)	92.19	95.90	91.42	96.86	98.64	97.90	98.75	97.56	98.69	99.24
Genetic advance (% of mean)	17.79	29.58	17.23	34.76	36.89	43.57	36.73	39.72	48.17	61.92

The heritability of all the fruit characters was found to be very high. The highest heritability (> 60 per cent) was reported for the trait, kernel percentage (99.24 per cent), followed by nut weight (98.75 per cent). The genetic advances as per cent of the mean ranged between 17.23 to 61.92 per cent. On the other hand, a high genetic advance was observed for most of the fruit characters except for fruit weight (17.79 per cent) and fruit width (17.23 per cent). Kernel percentage exhibited the highest genetic advance as per cent of the mean (61.92 per cent). High heritability along with high genetic advance as per cent of mean was reported for kernel percentage (99.24, 61.92), nut weight (98.75, 36.73) and kernel weight (98.69, 48.17). High heritability coupled with high genetic advance was observed for all the traits except fruit weight and fruit width. These characters should be used as selection criteria for further improvement of yield and quality in walnut. Similar findings were reported by Kiran Kumar *et al.* (2021) in areca nut and Suresh *et al.* (2020) in turmeric.

Cluster analysis using GENRES software revealed that 12 walnut genotypes were grouped into 5 clusters in such a way that all the accessions within the clusters had smaller values among themselves than those belonging to different clusters. Cluster IV and V had three genotypes each (**Tables 5, 6 & 7**). Intra and inter cluster distance of five clusters was assessed and it varied from 1.29 to 10.82 and 7.66 to 21.36. Maximum intra cluster distance was recorded by cluster V (10.82) followed by cluster IV (3.14). Inter cluster distance was high between cluster III and cluster IV (21.36) followed by cluster IV and cluster V (20.55).

The purpose of principal component analysis is to derive a small number of independent linear combinations (principal components) of a set of variables that capture as much of the variability in the original variables as possible. The results of the PCA of the accessions of walnut are presented in **Table 8.** The principal component analysis revealed five principal components PC1, PC2, PC3,

Table 5. Cluster mean of various characters of walnut genotypes

Traits/ Cluster	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Shell thickness (mm)	Nut width (cm)	Nut length (cm)	Nut weight (g)	Inshell nut weight (g)	Kernel weight (g)	Kernel (%)
I	11.38	7.27	12.90	1.14	11.14	3.67	10.03	3.08	7.50	23.39
II	11.24	6.39	12.07	1.21	11.23	4.38	12.97	3.62	7.00	24.37
III	11.25	6.23	12.30	1.13	9.53	5.22	12.81	3.01	7.14	29.59
IV	11.76	6.22	13.01	1.18	11.69	5.79	10.70	3.87	6.97	23.12
V	11.26	6.62	12.61	1.16	10.48	4.43	12.08	3.25	7.19	25.75

Table 6 . Distribution of walnut genotypes in different clusters

Cluster	Number of genotypes	Name of genotypes
Cluster 1	2	JR – 4 & JR-5
Cluster 2	2	JR – 8 & JR – 9
Cluster 3	2	JR -11 & JR- 12
Cluster 4	3	JR – 1, JR – 2 & JR – 3
Cluster 5	3	JR – 6, JR – 7 & JR – 10

Table 7. Mean intra -cluster (diagonal and bold) and inter-cluster distances among four clusters

Cluster	1	2	3	4	5
1	1.29	8.56	8.93	19.91	7.66
2		2.43	11.23	18.96	9.09
3			2.47	21.36	8.46
4				3.14	20.55
5					10.82

Table 8. Variation among walnut genotypes accounted for principal components

Variables	PC1	PC2	PC3	PC4	PC5
Fruit weight	0.38	-0.27	0.02	-0.14	-0.46
Fruit length	0.05	0.05	0.77	-0.27	0.20
Fruit width	0.41	-0.26	-0.08	0.08	0.04
Shell thickness	-0.23	-0.51	-0.31	-0.08	0.08
Nut width	0.36	-0.44	0.00	0.05	-0.17
Nut length	0.09	0.46	-0.39	-0.54	-0.22
Nut weight	0.27	0.35	-0.26	0.55	0.15
Inshell nut weight	0.40	0.13	-0.05	-0.41	0.05
Kernel weight	0.37	-0.08	-0.13	-0.13	0.76
Kernel percentage	0.36	0.24	0.25	0.32	-0.25
Standard deviation	2.02	1.36	1.20	0.97	0.76
Proportion of variance	0.41	0.18	0.14	0.09	0.06
Cumulative proportion	0.41	0.59	0.73	0.83	0.88
Eigen Values	4.06	1.84	1.44	0.93	0.58

PC4, and PC5 with eigenvalues 4.06, 1.84, 1.44, 0.93 and 0.58, respectively, which have accounted for 0.88% (88.80%) of the total cumulative proportion of variability among accessions. The first three principal components PC1, PC2, and PC3 showed eigenvalues of more than one and cumulatively they explained 0.73% (73.0%) variability. Fruit width 0.41, Inshell nut weight 0.40, fruit weight 0.38, kernel weight 0.37, nut width 0.36 had the highest loadings in PC1, and they had more contribution to the total diversity. So, PC1 is a weighted average of these traits indicating their significant importance for this component. Traits which contributed to higher value in PC2 were nut length 0.46, nut weight 0.35, kernel percentage 0.24 and In shell nut weight 0.13. In PC3, fruit length 0.77, Kernel percentage 0.25 and fruit weight 0.02 had high eigenvectors. The results indicated that there is sufficient variation for the morphological traits observed in the first 3 principal components in the walnut accessions collection that could be used to improve walnut cultivars for these traits. In the present investigation, the walnut genotypes within the seedling population of Kodaikanal had variations in the phenological and pomological characters. JR-8 is identified as a superior genotype based on its nut weight and kernel weight. The variations in the promising genotypes might be attributed to the genetic characters and also the ecological conditions of that region. As a result of adaptation, the identified genotypes can produce improved nut and kernel characters and may contribute much to further breeding programmes.

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