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Genetic diversity of walnut for horticultural traits under mid hill conditions

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ABSTRACT

Seedling selection and characterization of superior genotypes of walnut in two districts of Himachal Pradesh, Solan and Sirmour were surveyed to identify, select and characterize the prevalent diversity based on growth, flower and nut traits to build a sound database of selected types for future use in crop improvement works. The seedling populations were systematically studied after following UPOV guidelines based on 48 characters. Out of the total, 16 characters were selected to establish a sound and stable database. The quantum of variation observed was categorized in three specific ranges from the total observed variation obtained from evaluation and genotypes classed under each character ranges. Plant height ranged from 4.5-16.5 m, trunk girth 0.5-3.5 m, yield 15-16 kg/tree leaf size (length 20-46 cm, width 20-42 cm), leaflet (length 4-16 cm, width 2-8 cm, leaflet number 4-10, leaf area 250-650 cm^2 catkin number 3.5 - 5.5, nut weight 7-16 g, nut thickness 20-36 mm). Shell thickness 1.25-2. 75 mm, kernel weight 2.5 to 9.25 g, kernel thickness 18-30 mm and kernel percentage 20-65. A database was developed and six promising types were identified and described.

Key words: *Juglans regia*, selection, database, characterization

INTRODUCTION

Among nut fruit crops, walnut has marked potential as a diversifying fruit for its high adaptability to both soil and agro-ecological conditions. Walnut is an ancient nut, belongs to the family Juglandaceae. In recent times, paleontology studies have shown that it is grown in vast regions extending from Carpathian Mountains in Eastern Europe, across Turkey, Iraq, Iran, Afghanistan and southern Russia to north western Himalayas. Crop is called as Carpathian walnut or English walnut but most appropriate name is Persian reflects place of origin, a multipurpose fruit, enriched with high protein (15-20%) and fat (60-75%) contents, besides being the rich source of carbohydrates, phosphoric acid and vitamins.

Its timber is suitable for high class carving, furniture, making of gun butts, bark has medicinal value, nut husk for dying etc. Diversity is evident from 21 species of walnut. Out of which *Juglans regia* L. (Persian walnut) is commercially, known for its excellent nuts. Other species are used in genetic improvement as a potential donor source. In India walnut is grown extensively and commercially in Jammu and Kashmir, Himachal Pradesh, Uttarakhand and to some extent in Arunachal Pradesh.

Varietal improvement has resulted from natural cross followed by selection of superior chance seedlings by human beings. Spontaneously flora in nature is potential source of genetic diversity, provides many opportunities to select desired types with superior traits to be employed in breeding programmes. Morphological markers are visible traits under the control of Mendelian genes that control expression of the trait of interest to identify and select promising ones from varying population (s). Hence, seedlings in growing areas need to be studied in depth to select superior types having large attractive nuts, thin shell, lateral bearing, good kernel quality, high percentage kernel recovery and other worthwhile characters. Local conditions and biotic factors of different areas differ; therefore there is a need to develop cultivars suitable for different areas. Selection of superior genotypes from variable population is a simple and quick method for varietal improvement. In the selected areas for evaluation seedling diversity was assessed on the basis of trunk girth. Quantum of diversity was highly variable especially for nut and kernel traits spread in 30-35 kilometer area. The prime objective of the study was identification and characterization of superior genotypes; establishing database of the better types and developing descriptor of selected types. Valuable types have been marked out and selected in local populations from different walnut growing countries viz. Romania, India, Iran, China USA and several other countries. Associated genetic diversity related to yield, nut and kernel traits, late bud break, flowering, winter hardiness, tolerance to disease has been the hallmark of native genotypes present in Iran. Marked variations in respect of growth and pomological characters in walnut population have been reported by Aleta and Ninot (2) and Sharma and Sharma (18).

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Desired variations for nut and kernel parameters from various surveys and exploration have been reported by several researchers (Khadivi *et al.,* 9; Atefi 2; Khadivi-Khub and Ebrahimi 10; Sutyemez, 21 and Yldrm *et al.,* 25).

MATERIALS AND METHODS

Present investigation was carried out during 2017-2018 on 90 seedling trees selected (Table 1) in two districts (Solan and Sirmour). Plant, foliage, flowering, nut and kernel characters were recorded as per the UPOV (23) using the standard practices. Plant height was measured with Ravi Altimeter from base to the terminal most growing point and expressed in meter. Trunk girth was measured at one feet above ground and expressed in cm as described by Westwood (24). All characters (48) were first subjected to factorial RBD analysis; later the total variation for each character was split in 3-4 range classes and detailed in Table 2. Non metric traits were assessed as per the UPOV guidelines. Out of 90 genotypes, only six promising genotype were selected and evaluated on the basis of fat and Protein percentage in kernel as per the method suggested by Folch *el al.* (6) and Khannizadeh *el al.* (11).

RESULTS AND DISCUSSION

To built an appropriate database of 90 seedling selections from two districts (Solan and Sirmour) in H.P. were studied in detail. Pomologically evaluated characters (out of 48 studied) were taken into consideration in order to establish a sound database. Magnitude of variation was categorized in specific ranges (based on evaluation and the genotypes following under them are listed). The resultant information shall help in further appropriate selection of promising seedling to be used in crop improvement. Characters listed are highlighted in Table 2.

Plant height was classified in three separate groups viz. 4.5 to 8.5 m, 8.6 to 12.5 m, more than 12.5 to 16.5 m and genotypes were displayed as per their height character. Trunk girth varied from 0.5 to 3.5 m. Yield per tree on the basis of two year study was categorized into three groups ranging from 15 to 60 kg/tree and selection which quantified under the ranges are depicted in Table 2. Important traits such as leaf length, width, leaflet size, leaflet number, catkin number were categorized. Leaf area is an important character from the point of view of production and it varied from 250 to 650 cm^2 and genotypes under each range were classified accordingly. Walnut is given high weightage for nut and kernel characters viz. nut weight, nut thickness, shell thickness, kernel weight, kernel thickness and kernel percentage. As for other characters, these prime features were also categorized based on variability assessed and genotypes accomplishing the category ranges (Table 2).

Table 1. List of selected seedlings.

S. No.	Selection Names C-I 0.5-1.5 m (Trunk girth)	Selection Names C- II 1.5-2.5 m (Trunk girth)	Selection Names C-III 2.5-3.5 m (Trunk girth)
1.	Solan S-3	Solan S-5	Solan S-1
2.	Solan S-4	Solan S-7	Solan S-2
3.	Solan S-6	Solan S-9	Solan S-11
4.	Solan S-8	Solan S-10	Solan S-22
5.	Solan S-21	Solan S-12	Solan S-27
6.	Solan S-25	Solan S-13	Solan S-30
7.	Solan S-26	Solan S-14	Solan S-32
8	Solan S-28	Solan S-15	Solan S-33
9.	Solan S-29	Solan S-16	Solan S-35
10.	Solan S-31	Solan S-17	Solan S-37
11.	Solan S-34	Solan S-18	Solan S-39
12.	Solan S-36	Solan S-19	Solan S-41
13.	Solan S-38	Solan S-20	Solan S-43
14.	Solan S-40	Solan S-23	Solan S-44
15.	Solan S-42	Solan S-24	Solan S-45
16.	SS-2	SS-1	SS-12
17.	SS-4	SS-3	SS-15
18.	SS-5	SS-8	SS-17
19.	SS-6	SS-11	SS-21
20.	SS-7	SS-13	SS-25
21.	SS-9	SS-14	SS-26
22.	SS-10	SS-16	SS-27
23.	SS-20	SS-18	SS-30
24.	SS-28	SS-19	SS-34
25.	SS-31	SS-22	SS-36
26.	SS-33	SS-23	SS-38
27.	SS-35	SS-24	SS-41
28	SS-37	SS-29	SS-43
29.	SS-39	SS-32	SS-44
30.	SS-42	SS-40	SS-45

*C- Class *Solan S- Solan Selection *SS- Sirmour Selection

Six promising genotypes were identified based on the above mentioned classification with their brief description (Table 3).

Pomological features of six superior seedling genotypes as indicated in Table 3 are spreading growth habit (Solan S-42 and Solan S-24), erect

Table 2. Detail of each character along with specific genotypes under each range class.

Characters	Genotype
	Plant height (m)
4.5-8.5	Solan S-56, Solan S-8, Solan S-21, Solan S-34, SS-5, SS-7, SS-33 and SS-35.
>8.5-12.5	Solan S-9, Solan S-12, Solan S-14, Solan S-16, Solan S-20, SS-17, SS-35, SS-39, SS-42, SS-13, SS-23, SS-29.
>12.5-16.5	Solan S-37, Solan S-39, Solan S-43, SS-8, SS-15, SS-21, SS-26, SS-27, SS-44, SS-45.
	Trunk girth (m)
0.5-1.5	Solan S-36, Solan S-4, Solan S-21, Solan S-26, Solan S-31, Solan S-42, SS-42, SS-35, SS-9, SS-37, Solan S-42, SS-42
>1.5-2.5	Solan S-5, Solan S-12, Solan S-16, Solan S-19, SS-1, SS-11, SS-23, SS-29, Solan S-24, SS-8
>2.5.3.5	Solan S-1, Solan S-11, Solan S-39 SS-17, SS-27, SS-45, Solan S-45, SS-45.
	Yield (Kg/tree)
15-30	Solan S-4, Solan S-38, Solan S-7, SS-2, SS-4, SS-7, SS-9, SS-11, SS-15.
> 30-45	Solan S-24, SS-37, SS-42, SS-8, SS-44, Solan S-42, Solan S-24
> 45-60	Solan S-44, Solan S-45, SS-42, SS-8, SS-45.
	Leaf size
	Length (cm)
20-28	Solan S-21, Solan S-29, SS-25, SS-17.
> 28-36	Solan S-11, Solan S-37, Solan S-38, Solan S-24, Solan S-5, SS-30, SS-41, SS-42, SS-45, SS-15.
c) > 36-46	Solan S-36, Solan S-16, Solan S-27, SS-28, SS-39, SS-13, SS-19, SS-29.
	Width (cm)
20-25	Solan S-21, Solan S-26, SS-36, SS-34, SS-12.
> 26-32	Solan S-3, Solan S-28, Solan S-38, Solan S-24, SS-5, SS-9, SS-28, SS-43, SS-41.
> 32-42	Solan S-4, Solan S-29, Solan S-27, Solan S-45, SS-10, SS-35, SS-42, SS-11, SS-16.
	Leaflet size
	Length (cm)
a) 4-8	Solan S-34, Solan S-5, Solan S-30, SS-2, SS-4, SS-9, SS-18, SS-36, SS-43.
b) >8-12	Solan S-4, Solan S-25, Solan S-38, Solan S-42, Solan S-20, Solan S-1, SS-35, SS-32
c) >12-16	Solan S-3, Solan S-21, Solan S-36, Solan S-17, Solan S-37, Solan S-44, SS-37, SS-40, , SS-16.
	Width (cm)
a) 2-4	Solan S-14, Solan S-43, SS-2, , SS-5, SS-15, SS-44
b) > 4-6	Solan S-3, Solan S-8, Solan S-24, SS-28, SS-42, SS-37, SS-32
c) > 6-8	Solan S-31, Solan S-9, Solan S-44, Solan S-37
	Leaflet number
a) 4-6	Solan S-1, Solan S-20
b) > 6-8	Solan S-3, Solan S-31, Solan S-20, Solan S-15, Solan S-1, SS-17, SS-41
c) > 8-10	Solan S-26, Solan S-40, Solan S-44, Solan S-35, Solan S-14, SS-33, SS-39 SS-23, SS-32, SS-44
	Leaf area (cm^2)
a) 250-350	Solan S-1, SS-6, SS-17
b) > 350-500	Solan S-8, Solan S-25, Solan S-20, Solan S-17, Solan S-16, Solan S-13, Solan S-37, SS-4, SS-20, SS-42, SS-15, SS-41
c) > 500-650	Solan S-3, Solan S-4 Solan S-28, Solan S-40, Solan S-42, Solan S-12, Solan S-27, SS-37, SS-32, SS-23, SS-14.

Contd...

Table 2 contd...

Characters	Genotype
	Catkin number
a) 3.5-4.5	Solan S-3, Solan S-8, Solan S-36, Solan S-38, Solan S-15, Solan S-30, SS-28, SS-19, SS-11, SS-1, SS-43
b) > 4.5-5.5	Solan S-21, Solan S-25, Solan S-28, Solan S-42, Solan S-7, Solan S-33, Solan S-45, SS-5, SS-33, SS-42, SS-40
c) > 5.5-6.5	Solan S-34, Solan S-10, Solan S-16, Solan S-1, SS-1, SS-4, SS-35, SS-39, SS-13, SS-32, SS-44, SS-27.
	Nut weight (g)
a) 7-10	Solan S-8, Solan S-40, Solan S-22, SS-7, SS-9, SS-19, SS-40 SS-44, SS-26.
b) > 10-13	Solan S-3, Solan S-4, Solan S-44, SS-31, SS-33, SS-37, SS-23, SS-14, SS-11, SS-43.
c) > 13-16	Solan S-21, Solan S-34, Solan S-35, Solan S-7, SS-28, SS-42, SS-41, SS-36, Solan S-42, SS-42, Solan S-24, SS-8, Solan S-45, SS-45.
	Nut thickness (mm)
a) 20-25	SS-26
b) > 25-30	Solan S-30, SS-7, SS-9, SS-39, SS-42, SS-19, SS-38, SS-30.
c) > 30-36	Solan S-3, Solan S-6, Solan S-25, Solan S-14, Solan S-10, Solan S-33, SS-4, SS-31, SS-14, SS-16, SS-41.
	Shell thickness (mm)
a) 1.25-1.75	Solan S-4, Solan S-21, Solan S-26, Solan S-10, Solan S-14, Solan S-27, SS-9, SS-10, SS-31, SS-37, SS-29, SS-18, SS-14, SS-34, Solan S-42,SS-42, Solan S-24, SS-8, Solan S-45, SS-45
b) > 1.75-2.25	Solan S-8, Solan S-28, Solan S-15, Solan S-2, Solan S-22, SS-2, SS-28, SS-39, SS-27, SS-42, SS-40
c) > 2.25-2.75	Solan S-40, Solan S-42, Solan S-33, SS-14
	Kernel weight (g)
a) 2.50-4.75	Solan S-40, Solan S-22, Solan S-32, SS-7, SS-33, SS-19, SS-22, SS-12.
b) > 4.75-7.00	Solan S-3, Solan S-4, Solan S-36, Solan S-20, Solan S-14, Solan S-27, SS-31, SS-42, SS-3, SS-21.
c) > 7.00-9.25	Solan S-10, Solan S-33, SS-2, SS-5, SS-28, SS-38, Solan S-42, SS-42, Solan S-24, SS-8, Solan S-45, SS-45.
	Kernel thickness (mm)
a) 18-22	Solan S-8, Solan S-28, Solan S-34, Solan S-40, Solan S-30,
b) > 22-26	Solan S-3, Solan S-21, Solan S-26, Solan S-18, Solan S-14, Solan S-9, Solan S-27, SS-33, SS-42, SS-29, SS-41, SS-10.
c) > 26-30	Solan S-4, Solan S-8, Solan S-1, Solan S-2, Solan S-39, Solan S-43, SS-4, SS-1, SS-3, SS-8, SS-11, SS-25, SS-44.
	Kernel percentage (%)
a) 20-35	SS-9, SS-27, SS-44
b) > 35-50	Solan S-6, Solan S-8, Solan S-29, Solan S-42, Solan S-18, Solan S-43, SS-7, SS-33, SS-42, SS-8, SS-29, SS-36.
c) > 50-65	Solan S-3, Solan S-10, Solan S-35, Solan S-1, SS-2, SS-6, SS-39, SS-1, SS-45, SS-38, Solan S-42 SS-42, Solan S-24, SS-8, Solan S-45, SS-45.

(SS-45) and semi-erect types (SS-42 SS-8 and Solan S-45). Trunk girth was low/small in Solan S-42 and SS-42, medium in Solan S-24 and SS-8 and large in Solan S-45 and SS-45. Bearing habit was regular in all selected genotypes, nut weight varied from 13.61 to 15.59 g, maximum in SS-45 and minimum in SS-8. Kernel shrivel ranged from less, low and medium to moderate; shell thickness ranged from 1.15 to 1.51 mm. Nut shape was mostly broad ovate except in Solan S-45 wherein it was elliptic. Regarding kernel parameters, weight was maximum in SS-42 (8.16 g) and minimum in Solan S-42 (7.13 g), kernel colour was extra light in SS-8, amber in three selections (Solan S-42, SS-42 and Solan S-24) and dark amber in Solan S-45 and SS-45. Kernel recovery ranged from 50.49 per cent (Solan S-24) to 58.91 (SS-42).

Table 3. Pomological characterization of superior seedling genotypes identified.

Characters \ Genotypes	Solan S-42	SS-42	Solan S-24	SS-8	Solan S-45	SS-45
Growth habit	Spreading	Semi-erect	Spreading	Semi-erect	Semi-erect	Erect
Trunk girth (m)	Low/small (1.48)	Small/low (1.48)	Medium (2.14)	Medium (2.49)	Large (3.29)	Large (3.21)
Yield (kg/plant)	Moderate (44.60)	High (45.60)	Moderate (35.58)	High (47.52)	High (60.55)	High (58.55)
Bearing	Regular	Regular	Regular	Regular	Regular	Regular
Nut weight (g)	14.44	14.66	15.68	13.61	15.02	15.59
Kernel shrivel	Less/well filled	Moderately shrivel	Moderate	Less	Low, medium	Moderate
Shell thickness (mm)	1.35	1.51	1.34	1.15	1.33	1.37
Nut shape	Broad ovate, circular	Broad ovate, ovate	Broad ovate	Ovate, Broad ovate	Elliptic	Broad elliptic
Kernel weight (g)	7.13	8.61	8.31	8.14	8.06	7.41
Kernel colour	Amber	Amber	Amber	Extra light	Dark amber	Dark amber
Kernel recovery (%)	53.09	58.91	50.49	57.65	57.17	54.37
Kernel removal	Easy	Moderate	Moderate	Easy	Easy	Easy

From consumer and market point of view kernel removal was easy in four genotypes (Solan S-42, SS-8, Solan S-45 and SS-45) while it was moderate in SS-42 and Solan S-24.

Fat and protein content of six superior seedling selection identified after systematic evaluation of 90 walnut seedlings are detailed in Table 4.

The fat percentage ranged from 48.43 to 58.24 per cent. It was minimum in selection SS-45 and maximum in SS- 8. In selections Solan S- 45 (51.23 %), Solan S- 24 (53.25 %) and SS- 42 (55.67 %) depicted narrow variation whereas values in SS-45 and Solan S-42 were quite similar. The protein percentage ranged from 11.32 to 15.56 per cent. Solan S-24 (11.32) registered least and SS-8 (15.56) had the highest protein content. Protein percentage in other four selections showed less variation.

Growth characters in present study viz. plant height, trunk girth, TCSA (trunk cross sectional area) and yield of seedling selections showed distinct differences (Table 2). Similar variation in trunk girth was reported by Peter (15). Our findings are in line with the studies of Sharma and Sharma (19). Further Joolka *et al*. (7) indicated marked variation in plant height classed them as moderately vigorous to very vigorous tree and Mosivand *et al*. (14) also highlighted significant variation in plant height.

Yield, the most significant economical character varied due to differences in the age and growing conditions of the studied material. It varied from 16.0-60.0 kg/tree. Recently Sutyemez (21) reported superior fruit yield in genotypes 'Maras18' Sutyemez1' and 'Kaman1' to their parents.

Morphological assessment is of paramount significance that provides base and direction to select appropriate genotype for specific growing areas. Enormous diversity in Iran (Khadivi – Khub and Ebrahimi, 10) suggested systematic evaluation of persisting genetic variation as the fundamental aspect for walnut improvement.

Leaf parameters presented in Table 2 showed narrow differences in the numerical figures. Similar variation in foliage characters were also identified (Attar *et al*., 4, Aleta and Ninot, 2; Lee *et al*., 12; Sharma, 17 and Joolka *et al*., 7). Nut and kernel traits are the determining factors of quality. Nut characters

Table 4. Fat and Protein percentage of six superior selected genotypes

Characters \ Genotypes	Solan S-42	SS-42	Solan S-24	SS-8	Solan S-45	SS-45
Fat (%)	49.32	55.67	53.27	58.24	51.23	48.43
Protein (%)	12.06	14.45	11.32	15.56	13.26	14.84

in the present study showed differences in nut weight (small to large), kernel percentage (low to high), shell thickness (weak to strong) among the seedling selections studied. Kernel characters included are kernel weight, thickness, kernel percentage fat and protein per cent. Findings of the present investigation are in line with earlier studies on seedling population grown under different agroclimatic conditions (Yldrm *et al.,* 25). Keles *et al.* (8), Shamlu *et al.* (16), Khadivi *et al.* (9) identified marked variation in nut characters studied by them at different growing locations. Results revealed closeness as well as distinctness in traits. Some trait features overlapped with the data of present study. Such differences may be due to varying genetic material, tree age, agro-climatic variation in the growing areas, biotic and abiotic stresses.

Protein and fat contents in kernels are also considered very important traits for selection of superior seedling trees from the point of view of human nutrition. The results are in confirmation with the findings of Donno et *al.* (5) and also in agreement with the observations of Sharma (17) where protein contents values varied from 6.47 to 18.77 per cent, although lower value was less compared to that observed in present study. On similar analogy, Thakur (22) reported variation in protein and fat contents of seedling population that ranged from 8.23 to 18.99 and 41.78 to 67.34 per cent, respectively. Protein values did not match with the current observations likewise higher fat content value was due to variation in studied seedling population and location. Acka and Sen (1) and Zhang and Zhang (26) based on evaluation observed higher values of both fat and protein than that observed in the present study but values cited by Mitrovic *et al*. (13) were close to those found in the present seedling populations.

AUTHORS' CONTRIBUTION

Conceptualization of research (Sharma G.); Designing of the experiments (Sharma G. and Sajwan P.); Contribution of experimental materials (Sharma G and Sajwan P.); Execution of field/lab experiments and data collection (Sajwan P. and Thakur K.); Analysis of data and interpretation (Sajwan P. and Yadav A.); Preparation of the manuscript (Sajwan P. and Sharma G.).

DECLARATION

The authors declare no conflict of interest.

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