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Diversity among Walnut (*Juglans regia* L.) Selections from Himachal Pradesh, India

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Abstract

North-Western Himalayan region of India has significant Persian walnut (*Juglans regia* L.) trees, most of which are of seedling origin. The region has rich morphological, phenological, and carpological walnut diversity because of the large variability of native walnut, resulting from cross-pollination and seedling propagation. Persian walnut (*Juglans regia* L.), also called the English walnut, is the only species widely cultivated for nut production which are being used for table purposes. It has various microelement and nutritious substances and is one of the important nut crops in the world. India has significant Persian walnut trees, most of which are seedling grown. Due to the extensive variability of biological material, the result of cross-pollination and generative propagation prevailed by natural dissemination in Himalayan region of India has rich phenotypic diversity in walnut germplasm. Older orchards in most traditional walnut producing countries consist of mature seedling trees characterized by a long juvenile period and poor, inconsistent bearing habits. Due to the extensive variability of biological material, the result of cross-pollination and generative propagation prevailed by natural dissemination in Himalayan region of India has rich phenotypic diversity in walnut germplasm. In the present study, 10(ten) walnut genotypes were selected from different parts of Himachal Pradesh, India and evaluated to determine phenotypic diversity and to detect superior trees. The seedling plants of walnut normally take 15 to 25 years to come into bearing. The walnut selection from Chamba region of Himachal Pradesh came into bearing in the second year of its grafting that showed precocity. The fruit appears to be borne in lateral position as well as terminally. This is a characteristic of newly evolved early, good quality and heavy bearing walnut cultivars. However, no such cultivar is reported in India so far. The leaf size and annual shoot increment is also differing from other walnut plants collected during the same time. The nut is thin shelled, kernel colour is light yellow and good in taste. It is semi-vigorous in nature. This is also suitable for high density plantation. The Chamba selection is having Lateral bearing and Cluster bearing habit and also having export quality. There was high variability for the measured phenological and pomological characteristics. Among the selections studied, the ranges of 31.24-97.19g for fruit wt., 1.15-1.39 mm for nut shell thickness, 10.2–12.3 g for dry nut weight, 33.3–40.2mm nut size index and 41.3–51.2 % for kernel percentage were observed. The best accessions were Chamba Selection, Jubbal Selection, Kinnaur Selection, Lahaul & Spiti Selection and Soghi Selection which had thinner shell, heavier kernel, more fruitfulness and late flowering. The most promising trees will be used to the benefit of conservation research, breeding and cultivation.

Key words : *Walnut, selection, variability, morphological, phenologocal, horticultural traits, lateral bearing, cluster bearing.*

Introduction

The Juglans genus consists of 21 species and Persian walnut (*Juglans regia* L.) is the only species that widely cultivated for production of edible nuts for table purpose. Walnut is a high energy food, rich in oil including omega-3 fatty acids, vitamins and minerals and valued as healthy snack food and bakery ingredients (1). North-Western Himalayan region of India has significant Persian walnut (*Juglans regia* L.) trees, most of which are of seedling origin. The region has rich morphological, phenological, and carpological walnut diversity because of the large variability of native walnut, resulting from cross-pollination and seedling propagation. Persian walnut (*Juglans regia* L.) is an important and widely distributed cultivated nut species acclaimed for its timber and nutritious kernels (2). It has originated in a wide belt of Asia, stretching from the Balkans to China (3). Walnut is a nutritionally valuable crop in high demand worldwide. It is included in the group of priority plants by food and agriculture organization (4). Furthermore, it has medicinal importance, derived from its high antioxidant capacity and omega –3 fatty acids (5). In India, north-western Himalayan region states comprising Jammu and Kashmir, Himachal Pradesh, and Uttrakhand produces a major portion of the whole walnut in which Jammu and Kashmir produce the highest quantity and best quality walnuts. Walnut is monoecious, bearing male and female flowers separately on the same tree (6). It is self-compatible, but with the most common protandry and sometimes, protogyny (7), the cross-pollination rate remains high (8). Because of its high allelic diversity and Seed propagation, there has been increasing diversity over the years in walnut native areas (9) nuts size, shape, thickness, and colour of the shell, quality, and colour of the kernel and other morphological attributes (6). An ideal walnut cultivar must have late leafing, both terminal and lateral bearing, low incidence of pistillate flower abscission, relatively smooth shell, 50% kernel

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recovery, plump and light-colored kernel, high yielding nuts (6 MT/ha) with large size, and at least moderately resistant to pests and diseases (10). Walnut? breeding is important for developing new cultivars through hybridization, although it is both time consuming and cumbersome. Furthermore, the collection, evaluation, and identification of superior genotypes from existing populations are prerequisites in any walnut-breeding programme (11). Therefore, it is convenient to exploit existing variability by making appropriate selections based on ideal characters such as late leafing, lateral bearing, precocity, high productivity, nut and kernel quality, and resistance to major diseases. Western Himalayan region of India provides agroclimatic conditions suitable for producing high quality walnuts. Jammu and Kashmir State of India produces major share of export quality walnut. The nuts brought to market are generally a mixture of variable size and shape as they are harvested from different seedling tress (12). Based on the observed differentiation of variation, it is concluded that the magnitude of phenotypic variation in the population under study is high and the implication of the results will play a vital role in future walnut breeding programmes.

Persian walnut (*Juglans regia* L.), also called the English walnut, is the most valuable commercial species in its genus belonging to the family Juglandaceae. Persian Walnut (*Juglans regia* L.) is known to be rich in oil, vitamins, minerals and proteins (13) and is mostly produced in orchards in temperate zones like Turkey, China, Iran and USA (4). Different Juglans species originated on several continents, while the Persian walnut is native to temperate regions in mountainous Eastern Europe and central Asia, extending from Turkey, Iran and western China eastward to the Himalayan regions in India and Nepal (14). All Juglans species are monoecious, with catkins being borne laterally on one-year-old wood, and pistillate flowers borne terminally or laterally (newer cultivars) on current season's wood. Although walnuts are genetically self-fruitful they exhibit the phenomenon of dichogamy, being either protandrous or protogynous depending on cultivar. Hence, walnuts are mostly cross-pollinated by wind (15). The walnut is regarded as highly valuable by an increasingly health conscious world due to various health benefits, including a great source of vitamin E and omega-3 fatty acids, as well as the ability to lower the level of cholesterol in human bodies (16).

Older orchards in most traditional walnut producing countries consist of mature seedling trees characterized by a long juvenile period and poor, inconsistent bearing habits. This is due to the fact that the Persian walnut is more difficult to propagate vegetatively than any other tree nut species (17). For a long time in the past, propagation through seed was only method available for walnut multiplication though this practice resulted into plants of great variability (18). Generally, walnut does not respond favourably to the vegetative propagation techniques under normal conditions, the way other temperate fruits do.

Seedling trees of walnut (*Juglans regia* L) are found growing in all parts of North Western Himalayan Region at 1200-2200 meters elevation and trees exhibit considerable variation in respect of vegetative growth and fruit characters. Use of this variability in the selection of superior seedlings with desirable traits will be of paramount importance in the expansion of walnut cultivation in this country. These seedling selections will not only produce nuts of desirable quality, but are also well adapted to the local climatic and soil conditions. Persian walnut is one of the most important nut crop grown in temperate region and produces edible nuts having higher nutritional value. In India there is no systematic orchards of walnut and trees of seedling origin are grown which exhibit wide intra specific diversity for tree, foliage, floral, nut and kernel characters. The variation provides better opportunity for selection of unique walnut tree (s). Breeding new walnut cultivars through hybridization is both difficult and time consuming. Therefore, it is convenient to exploit existing variability by making appropriate selections based on characters like climatic adaptations, precocity, high productivity, good quality of nut and kernel and resistance to major diseases. Anthracnose disease affects all leaves, leaf petioles, shoots,nuts and peduncles, and has been reported to infect several cultivars of Persian (*J. regia*) as well as black (*J. nigra*) walnuts severely (19). Interestingly, the major share of walnut production comes from the trees of seedling origin, which has a lot of variability in nut size, shape, shell thickness and kernel quality (20).

Materials and Methods

Survey of the walnut germplasm was carried out in different districts of Himachal Pradesh, India. Out of the total population of seedling trees, Ten genotypes viz. Soghi Selection, Kullu Selection, Chamba Selection, Lahul and Spiti Selection, Kinnaur Selection, Kotkhai Selection, Shimla Selection, Rohru Selection, Jubbal Selection and Theog Selection were identified on the basis of nut and kernel characteristics and yield potential and bearing habit. Trees were selected after evaluation on the basis of regular fruit production according to interviews with orchard owners and observed phenotypic diversity and plants multiplied through grafting. The selected trees were healthy, mature and had a full crop. Diverse horticultural practices, fertilizer application, irrigation and other cultural practices were applied at regular intervals each year after planted at IARI research farm, Shimla.

Observations on various vegetative and nut characters were recorded following the standard guidelines (21). Thirty nuts were selected randomly from the tree after these were sun dried to edible stage. Fat content was estimated by the method as suggested by (22).

Different characteristics were used to assess the range of variation among the accessions during two successive years (Table-1). Measurements of each nut and kernel trait were based on 30 replicates and the mean values were used. Some variables were measured by laboratory equipment. Nut dimensions (length and width) were measured using a digital Vernier caliper. The weight for nut and kernel was measured using electronic balance with 0.01 g precision. Also, some characteristics such as bearing habit, tree vigour, fruit bearing type, dichogamy and disease tolerant were determined based on observations.

Results and Discussion

The tree characteristics of walnut selections represented in Table-1. The nut characteristics and bearing habit of the walnut selections from different districts of Himachal Pradesh represented in Table-2. Nut size index varied from 33.4mm to 57.2mm. Maximum recorded in Jubbal Selection whereas minimum exhibited in Lahaul and Spiti Selection. Moderate nut size recorded in Kinnaur Selection, Kotkhai Selection and Chamba Selection. Dry nut weight ranged from 10.3g to 20.2g. Jubbal Selection recorded maximum followed by Chamba Selection (12.3g) whereas minimum was observed in Soghi Selection. Kernel ratio (%) also varied 41.4 % in Shimla Selection to 53.3% in Jubbal Selection followed by Chamba Selection (51.2%). Fruit weight varied from 97.20g to 31.24g. Maximum Fruit weight recorded in Jubbal Selection where as minimum was exhibited in Rohru Selection. Moderate Fruit weight recorded in Kotkhai Selection, Kinnaur Selection, Lahaul and Spiti Selection and Chamba Selection. Nut Shell Thickness (mm) ranged from 1.15mm to 1.39mm. Maximum recorded in Kotkhai Selection and Lahaul and Spiti Selection where as minimum in Soghi Selection followed by Chamba Selection (1.20mm). Both Lateral and Terminal bearing habit recorded in Chamba Selection only. Also Cluster bearing habit observed in Chamba Selection only (Plate-1 and Plate-2).

Chamba selection came into bearing in the second year of its grafting. The seedling plants of walnut normally take 15 to 25 years to come into bearing. The fruit is borne in lateral position as well as terminally. Terminal bearing is associated with initiation of new growth early in the season which culminates in a mixed bud (with both floral and vegetative primordial). This is a characteristic of newly evolved early, good quality and heavy bearing walnut cultivars. However, no such cultivar is reported in India so far. The nut is thin shelled. Kernel colour is light yellow and good in taste. The oil per cent as well as shelling per cent recorded 55 and 50, respectively. An ideal nut should weigh between 12-18 g has a clean, strong and thin shell with tight seal and easily removable light kernel, clean and plump kernel weighing at least 50 per cent of the in-shell-nut (6). According to (23) selection of clones that produce pistillate flowers on lateral buds has resulted in significant yield increases in Persian walnut, *Juglans regia*. Lateral bearing Persian walnut cultivars tend to be more precocious and are better suited to high yielding, high density plantings. Some eastern black walnut cultivars possess a characteristic known as lateral bud bearing (24). These cultivars produce pistillate flowers on a profusion of short, spur type branches distributed along main scaffold limbs. With this growth pattern, both leaves and nuts are born throughout the tree canopy resulting in increased yield potential (25). The heritability of the lateral bearing characteristic is unknown for black walnut but (26) found this trait to have a moderate level of heritability in Persian walnut. An ideal walnut cultivar must have late lea?ng, both terminal and lateral bearing, cluster bearing, low incidence of pistillate ?ower abscission, high yielding nuts with large size, relatively smooth,50 % kernel recovery, plump and light colored kernel and at least moderately resistant to pest and diseases (10).

In India, the maximum walnut trees are grown on own roots i.e. seedling origin. The evaluations conducted for all the 10(ten) walnut selections showed that 4 of them are vigorous in tree size, 5 selections have medium vigor and only one selection has low vigor (Table-1). Phenological stages of the walnut selections have been recorded during the study period.

The dichogamy was not all the same in the selected trees. But, protandry was the most common among the studied trees. Most of walnuts have genetically protandry (27). Ecological condition may effect on dichogamy in walnut (28). A high variation of adaptation characters such as earliness and length of the flowering period and dichogamy offer the possibility of adapting the crop to diverse agro-ecological environments. Out of 10, 9 selections were terminal fruitfulness, so that all shoots from terminal generated female flowers and fruits. The Chamba Selection was mixed and had both terminal and lateral fruitfulness. Yield capacity is an important trait for economic walnut production. Productivity of walnut depends on flowering time and habit, fruit number on lateral and terminal shoots, lateral fruitfulness, nut and kernel weights, and kernel percentage (29).

Table-1 : Nut and Bearing habit characteristics of the walnut selections from Himachal Pradesh, India.

No.	Selection	Nut Size Index (mm)	Dry Nut weight (g)	Kernel ratio (%)	Fruit wt. (g)	Nut Shell Thickness (mm)	Both Lateral and Terminal Bearing	Cluster Bearing
1.	Soghi Selection	34.6	10.2	48.2	42.07	1.15	No	No
2.	Kullu Selection	34.4	10.6	45.4	42.17	1.28	No	No
3.	Chamba Selection	36.5	12.3	51.2	43.71	1.20	Yes	Yes
4.	Lahul & Spiti Selection	33.3	10.9	48.6	51.24	1.39	No	No
5.	Kinnaur Selection	40.2	10.6	47.2	54.03	1.29	No	No
6.	Kotkhai Selection	39.6	11.2	46.0	56.25	1.39	No	No
7.	Shimla Selection	34.4	11.5	41.3	37.63	1.38	No	No
8.	Rohru Selection	35.3	10.3	46.2	31.24	1.37	No	No
9.	Theog Selection	34.6	10.4	48.2	39.92	1.36	No	No
10.	Jubbal Selection	57.1	20.1	53.2	97.19	1.25	No	No

Table-2 : Tree characteristics of walnut selections from Himachal Pradesh, India.

No.	Selection	Tree vigour	Fruit bearing type	Fruit yield	Resistance to Blight and Anthracnose	Dichogamy
1.	Soghi Collection	Medium	Terminal	High	Less susceptible	Protandry
2.	Kullu Collection	Medium	Terminal	High	Less susceptible	Protandry
3.	Chamba Collection	Low	Mixed Lateral & Terminal	High	Less susceptible	Homogamy
4.	Lahul& Spiti Collection	High	Terminal	High	Less susceptible	Protandry
5.	Kinnaur Collection	High	Terminal	Medium	Less susceptible	Protandry
6.	Kotkhai Collection	Medium	Terminal	Medium	Less susceptible	Protandry
7.	Shimla Collection	High	Terminal	High	Less susceptible	Protandry
8.	Rohru Collection	High	Terminal	Medium	Less susceptible	Protandry
9.	Theog Selection	Medium	Terminal	High	Less susceptible	Protogyny
10.	Jubbal Selection	Medium	Terminal	Medium	Less susceptible	Protandry

Nut size is the determining factor for the market. Nut size index varied from 33.4 to 57.2 mm. Previous research (30) in 109 walnut accessions of seedling origin growing naturally in Oltenia region of Romania showed the diversity of the fruit: nut length (28.20–49.70 mm) and nut diameter (25.70–40.60 mm). Nut weight is one of the most common important parameters influencing the quality. Dry Nut weight varied from 10.3 to 20.2 g. The highest value of nut weight among the studied accessions here (20.2 g) was less than nut weight reported by (28) for walnuts in Adilcevaz, Turkey (23.81 g); and more than nut weight by (31) for walnuts in Kamal-Abad, Iran (20 g); (32) for Himachal Pradesh, India (18.60 g); (33) for east Anatolia, Turkey (17.04 g); (34) for North-eastern Anatolia, Turkey (16.01 g) and (30) for Oltenia region, Romania (18.40 g). Desirable nut and kernel weight should range from 12 to 18 g and 6–10 g, respectively, or kernel weight should be at least 50 % of the entire nut weight, and the kernel should have a light color (31). Percentage of kernel is a feature of great importance in setting the number of selections and an important character for improvement. This character is related to nut and kernel weight. Accordingly, kernel percentage varied from 41.4 to 53.3 %. The higher the kernel percentage, the lower the nut weight, while the ratio kernel/nut is higher and increases the value of the fruit. The highest kernel percentage (83.88 %) was lower than the data reported by (20) (63.80 %), (34) (67.14 %), (31) (79.60 %) and (30) (71.70 %). Among all of the studied accessions, 204 accessions proved to be promising for new selections owing to higher kernel percentage i.e. [50 %. Of them, accession no. 573 had the highest kernel percentage (83.88 %), followed by accessions no. 95 (71.82 %), 309 (68.91 %), 374 (68.91 %) and 539 (68.75 %). Walnut accessions with a kernel percentage higher than 48–50 % are more desirable (27). It has been reported that fruit characteristics are not affected by tree age (35). In the present study, kernel color in all accessions was very light to light. Generally, the most interesting accessions are those whose kernels can be easily removed from the shell and those whose kernels have a light color (Jubbal Selection,Chamba Selection, Kinnaur Selection and Soghi Selection. These traits have been used for selection of superior walnut genotypes (20, 35).

Out of 10 walnut selections, 9 have terminal bearing type, while those of Chamba Collection have intermediate bearing. Fruit size and weight varies very much from one selection to another. Walnut nut size is one of the main determinants for international trade (Table-2). Nut Size Index (N.S.I.) as an average of the widest fruit width (D), narrow width (d) and height (h) have been used (Table-2). The good quality walnut fruits of 9.0 to 10.0 g weight can

Plate-1

Plate-2

be used for shelled market while those exceeding 11 grams can be used also for in-shell market. Another important element for defining the walnut quality is the percentage of kernel. In case of selections, the percentage of kernel ranged from 41.2% to 51.1%. Of the 10 selections, only 2 (Chamba Selection and Jubbal Selection) yields more than 50% kernel. Nut shell of the walnut selections is thin or medium (1.2 mm to 1.39mm in thickness). Taking into account the growth and fruiting characteristics of the walnut genotypes Chamba Selection, Jubbal Selection, Kinnaur Selection, Lahaul and Spiti Selection and Soghi Selection have been considered for further evaluation and direct use in the walnut farms, but only after clonal propagation.

Conclusions

Several factors are important to consider when selecting a walnut cultivar including local climate and pest conditions. Walnut requires a period of winter chill to break dormancy and initiate leaf and flower production. Because pollination is required to set a crop, growers should select a cultivar with overlapping male and female flower maturity or, if a suitable pollen source is not nearby, plant a few trees of a pollinizer variety. Early leafing and flowering varieties are more exposed to spring rains that contribute to bacterial blight.

High development costs for establishing a walnut orchard demand the planting of cultivars that bear large crops at an early age. Cultivars displaying lateral bud fruitfulness generally come into production well in advance of non-lateral bearing cultivars. Shell thickness and structure are the most important determinant of percent kernel and nut crack-ability. The highest quality walnuts have a thin outer shell with no internal convolutions protruding into the nut meat. The inner shell partition between kernel halves should be very thin to allow easy removal of kernel pieces. An ideal walnut cultivar must have late leafing, both terminal and lateral bearing, Cluster bearing, low incidence of pistillate ?ower abscission, high yielding nuts with large size, relatively smooth, 50% kernel recovery, plump and light-colored kernel and at least moderately resistant to pest and diseases. The Chamba Selection appear to meet this standard.

The north-western Himalayan region particularly Himachal Pradesh, India have important biodiversity of the *Juglans regia* L. species, wide variability, but increasingly subject to genetic erosion and genetic vulnerability pressure. Chamba Selection, Jubbal Selection, Kinnaur Selection, Lahaul and Spiti Selection and Soghi Selection have greater agro biological value similar to commercial cultivars and can be popularized after clonal propagation, into the new walnut orchards to be established. The best selections were Chamba Selection, Jubbal Selection, Kinnaur Selection, Lahaul and Spiti Selection and Soghi Selection which had thinner shell, heavier kernel, light coloured kernel, more fruitfulness and late flowering. The most promising trees will be used to the benefit of conservation research, breeding and cultivation. More over, Chamba Selection could be used in high-density orcharding as well as export potentiality point of view.

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