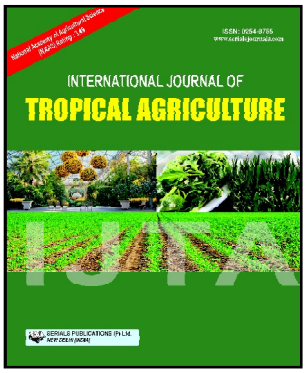

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Selection of ideal Walnut (*Juglans regia L.*) and its multiplication

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Abstract: Persian walnut (*Juglans regia* L.), also called the English walnut, is the most valuable commercial species in its genus belonging to the family Juglandaceae. 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. In fact, there is an urgent needto standardize the suitable techniques forclonal multiplication of walnut in order toensure supply of quality plant material forexpansion of area, achieve increase inproduction and productivity of superior nutsand meeting the international standards ofquality characters of nut and kernel. A unique walnut plant,Sel-3 (Pusa Khor) is being evaluated at the ICAR-IARI Regional Station Shimla, Himachal Pradesh, India. It was collected from Chamba region of Himachal Pradesh which came into bearing in the second year of its grafting. The known varieties of walnut normally take 10 to 15 years to come into bearing. Sometimes it takes even more. 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 survey.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. Among the selections studied, the best wasSel-3 followed by Sel-5,Sel-4 and Sel-1 which had thinner shell, heavier kernel, light kernel, more fruitfulness and late flowering. The most promising trees will be used to the benefit of conservation research, breeding and cultivation.

INTRODUCTION

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 (Ozcan, 2009, Özcan, İman, & Arslan, 2010and is mostly produced in orchards in temperate zones like Turkey, China, Iran and USA (Erturk & Dalkilic, 2011;FAO, 2013. 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 (Leslie & McGranahan, 1998). 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 (Polito, 1998). 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 (Savage *et al*., 2001).

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 (Lagerstedt, 1979; MacDonald, 1987; Reil *et al*., 1998; Hartmann *et al*., 2002). For a long time in the past, propagation through seed was only method available for walnut multiplication though thispractice resulted into plants of greatvariability (Sharma *et al*., 2003). Generally, walnut does not respond favourably to the vegetative propagation techniques under normal conditions, the way other temperatefruits do. Various methods of vegetative propagation in walnut have been reportedto give varying degree of success underdifferent climatic conditions in India andabroad. The variations are dependent on different environmental conditions to whichthe plants are subjected before and after propagation (Chase, 1947, Ibrahim *et al.,* 1978, Awasthi *et al.,*1982, Qureshi and Dalal, 1985). In fact, there is an urgent needto standardize the suitable techniques forclonal multiplication of walnut in order toensure supply of quality plant material forexpansion of area, achieve increase inproduction and productivity of superior nutsand meeting the international standards of quality characters of nut and kernel.

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 intraspecific diversity for tree, foliage, floral, nut and kernel characters. The variation provides better opportunity for selection of unique walnut tree (s). The multiplication of these elite trees through vegetative propagation will play an important role in broadening the genetic base of nut crops.A unique walnut plant (Pusa Khor) is being evaluated at the ICAR-IARI Regional Station Shimla, Himachal Pradesh, India.

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, Nine genotypes viz. Soghi Selection, Kullu Selection, Chamba Selection, Lahul & Spiti Selection, Kinnaur Selection, Kotkhai Selection,Shimla Selection, Rohru Selection and Theog Selection were identified on the basis of nut and kernel characteristics and yield potential. Trees were selected after evaluation on the basis of regular fruit production according to interviews with orchard owners and observed phenotypic diversity. The selected trees were healthy, mature and had a full crop. Diverse horticultural practices, fertiliser application, irrigation and other cultural practices were applied at regular intervals each year.

Observations on various vegetative and nut characters were recorded following the standard guidelines (UPOV,1988). 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 Folch et al. (1957). Different methods of propagation followed for multiplication viz. grafting, budding, stooling, cuttings, top working, other methods (hot callus) and micro-propagation.

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 nut shape, kernel traits (filled, plumpness, shriveling and color), shell traits (color, seal, texture and hardiness), kernel removal from nuts were determined based on rating.

RESULTS AND DISCUSSION

A unique walnut plant is being evaluated at the IARI Regional Station, Shimla. It was collected from the Chamba region of Himachal Pradesh. This plant came into bearing in the second year of its grafting. The known varieties of walnut normally take 10 to 12 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 (Table-1). 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 (McGranahan and Leslie,1990). According to Ramos et al. (1984) 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 (Sparks, 1982). 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 (Reid, 1986). The heritability of the lateral bearing characteristic is unknown for black walnut but Hansche et al. (1972) found this trait to have a moderate level of heritability in Persian walnut.

However, the healthy and disease free, hard shelled, big nuts should be selected for seedling rootstock.

Walnut propagated vegetatively by different methods discussed below and their success rate was given in Table 2.

1. Grafting

Grafting is an age-old horticultural technique that can be defined as attaching a twig from one tree to the stem of another in such a way that the twig continues to grow and become a permanent part of the tree. All of the branches that grow from that twig will have the identical characteristics of the tree from which the twig was taken. Grafting a twig (the scion wood) from a tree that produces high-quality nuts onto a seedling tree (the stock) is the only way to ensure that your tree will produce desirable nuts.

Tongue or whip grafting, cleft and veneer grafting during February and early March have given good results. Epicotyl grafting has also given encouraging success in tile propagation of walnut. The best period for grafting of Pusa khor is January -February. For propagating tile plants through veneer grafting, 5-6 month old scion wood of 15 cm is grafted on the rootstock of same thickness. The selected scion wood should be defoliated 15 days prior to its detachment from the scion cultivars. The optimum time for veneer grafting under mid -hill condition is January-Mid February. One year old seedlings of hard shelled walnut or black walnut can be used as rootstock. Scion for tongue grafting should always be selected from the tree which has already started fruiting.

One year after grafting, prune the growth on the graft to a central leader. If more than one bud grows from the bud stick, leave only the growth coming from the strongest bud to form the new top of the tree. During this same time, prune off about one third of the lower limbs to force more of the trees energy to the graft. In subsequent years, continue pruning the top of the tree to a central leader and remove limbs below the graft.

2. Budding

Once the rootstock is established, growing well in the nursery and has a diameter matching scion wood, the scion variety can be grafted or budded on the rootstock. Trees are typically propagated in the nursery by fall-budding the rootstock between late August and mid-September (patch or T-bud). A finished tree will be grown by the next fall if the buds heal over, remain dormant until the spring and grow out in the following summer. The rootstocks that were initially too small, or had failed fall buds, are whip and tongue grafted in the spring and the scion is encouraged to grow immediately following grafting. Both techniques create a finished tree in two years.

A more recent technique, June budding. Rootstocks to be used in June budding are grown in very fertile conditions and typically reach budding size by June, coinciding with the time current season scion buds develop to a condition suitable for use as bud wood. The budded trees are then managed intensively for the rest of the summer to create a tree.

Patch & T-budding: Patch budding is the most common budding method used for walnuts. However, T-budding can also be employed successfully. Collect bud sticks when bark is slipping on both the rootstock and scion bud stick. To ensure the best take, the bud wood can be prepared by removing the leaves while still on the tree a few weeks before use. Patch budding is generally practiced to propagate walnut plants vegetatively. The best period for budding is July -August. Scion should always be selected from the tree which has already started fruiting.

Patch budding is done using a double bladed knife to cut a square piece of bark from the rootstock which is replaced with the same size patch from the bud stick containing a well-developed bud. T-budding uses a single bladed knife to slit the rootstock bark in the shape of a T and a shield shaped piece

Table 1
Fruit 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)*
1	Sel-1	34.8	10.1	48.0	42.05	1.13
2	Sel-2	34.3	10.5	45.2	42.12	1.29
3	Sel-3	35.1	12.2	51.1	43.72	1.20
4	Sel-4	33.2	10.8	48.5	51.22	1.38
5	Sel-5	40.1	10.5	47.0	54.01	1.28
6	Sel-6	39.6	11.2	46.0	56.25	1.39
7	Sel-7	34.3	11.3	41.2	37.61	1.37
8	Sel-8	35.1	10.2	46.0	31.22	1.36
9	Sel-9	34.5	10.3	48.1	39.91	1.35

Table 2
Propagation methods and their success on different walnut collection

No.	*Selection*	*Grafting (%)*	*Budding (%)*	*Stooling (%)*	*Cuttings (%)*	*top working (%)*	*other methods (%)*	*micro-propagation (%)**
1	Sel-1	65.05	69.91	09.01	09.95	70.01	50.05	80.92
2	Sel-2	67.08	70.45	09.91	10.01	70.25	50.92	81.13
3	Sel-3	71.01	75.12	10.05	10.10	75.55	52.01	83.03
4	Sel-4	65.91	68.92	09.92	10.02	69.23	50.57	80.51
5	Sel-5	66.11	70.27	11.12	10.97	70.57	50.21	81.39
6	Sel-6	62.19	65.15	12.27	10.78	70.25	51.32	80.28
7	Sel-7	60.05	65.25	11.18	10.59	70.01	50.47	81.74
8	Sel-8	64.56	68.97	10.91	10.07	70.58	51.73	80.85
9	Sel-9	68.72	70.08	09.89	09.97	70.79	50.08	81.09

• Hardening of tissue cultured plant is really a challenge to take to farmer's field

of stem including a bud from the bud stick is cut and placed into the opening. After placement, the bud should be covered with budding tape to prevent desiccation. The tape is removed when the bud has healed, usually after a few weeks.

3. Stooling

Stool layering is a suitable method of walnut propagation and is useful for the multiplication of true to type rooted plants from a rootstock bed. One-year-old seedlings of a known cultivar are planted in a nursery bed at I sq. m. distance and headed back from 6 .8 cm above the ground in March before bud swelling. All the cut ends are painted with Chaubatia paste. In April, buds start swelling and 3 -4 shoots come out from the stock. In July, 2 -5 cm bark is removed and the ringed portion is treated with IBA 6000 ppm in lanolin base. After a week the upper end of the ringed part swells and development of root primordia is initiated. The treated shoots are

then earthed up, covering the shoot even beyond the ringed portion. Fortnightly irrigation is given to the stool bed to keep the moisture constant. During the second fortnight of February the shoots are unearthed. These shoots show rooting and are detached from the mother plant and planted in the main field in the month of March.

4. Cuttings

Although hardwood and semi-hardwood cuttings can also be used for rootstock propagation, they are not commonly used because success rates are often low. Such cuttings have rooting percentages from 30 to 80% and often have poor initial survival. Use cuttings from only vigorously growing shoots for propagation and root them in individual liners to promote uniform and deeper root branching. The bases of cuttings are treated with KIBA at 8-12,000 ppm before planting. Semi-hardwood cuttings are rooted in greenhouses on bottom heated mist benches in mid to late summer and hardwood cuttings on bottom heated mist benches in late fall and winter outdoors. The use of broadcast flats yields lower rooting percentages, shallower roots and poor root development. Plant liner-sized rooted cuttings in the field in late February or early March. Direct field rooting is not commercially successful.

5. Top working

Top working is a very useful choice for walnut production in the hilly tracts of India since a large number of young seedling trees are found producing inferior quality of nuts. Top -working is usually carried out by modified cleft grafting or bark grafting late in the spring season or when new growth occurs. The dormant scion wood should be removed from the parent tree in advance and stored in refrigerator after proper packing. Bleeding is a problem in walnut top-grafting especially when it is done in early spring which can be avoided by heading back the stock two weeks before actual operations. After grafting, the open wounds must be covered by grafting wax and if required re-waxing may be done. White washing of the stem may be done to protect them from sunburn. As the rootstock is already well established, the scion makes rapid growth and bears earlier than the transplanted trees.

6. Other methods

Omega grafting is conducted using one-year old rootstocks and scions. Graft unions were plunged into hot paraffin (70-80^0 C) and then cooled in cold water. The graft unions were forced in woody boxes, filled with wet sawdust in a room under controlled conditions at 27^0 C and 80 % relative humidity (Nedev *et al.*, 1976).

Hot callus method. The experiment was set in an unheated green-house. Cleft grafting was carried out with one-year old rootstocks and scions. The grafted plants were tied with a plastic band and the grafting place was covered with foil. Thus prepared, the plants were placed horizontally, the place of grafting being located above an electric heating cable buried in a plastic U-shaped pipe filled with peat. After that their roots were covered with wet sand and wet foam was put over the place of grafting, pressed with weights to the plastic pipe in order to save the heat and to maintain the temperature of 27^{0}C at the place of grafting. Periodically the roots were watered and the foam was wetted (Avanzato *et al.*, 2006).

7. Micro-propagation

Micro-propagation has increased in popularity because walnut is difficult to propagate clonally from cuttings. To micro-propagate walnut, surface-sterilize individual stem segments containing one bud (no leaves) to remove surface bacteria and fungus. Next, is the placing of basal portion of the stem segments into an agar medium containing a mixture of plant hormones, nutrients and sugars to promote growth of bud. Many clonal micro shoots can be produced from a single bud. These micro shoots can be used

for rooting (clonal rootstock or own-root trees). For rooting, the bases of micro shoots are treated with potassium indolebutyric acid (KIBA) in vitro for 5 to 7 days and then stuck in a peat:perlite medium on a fog bench in a greenhouse (Vahdati *et al.*, 2004). The walnut micropropagation is one of the methods largely studied (Driver and Kuniyuki, 1984; Gruselle *et al.*, 1987; Gruselle and Boxus, 1990; Marques Silva and Dias 1997; Navatel and Bourrain, 2001; Lopez, 2004; Britton *et al.*, 2007; Vahdati *et al.*, 2009). Driver and Kuniyuki (1984) demonstrated the feasibility of the tissue culture approach for mass propagation of Paradox (*J. hindsii*x *J. regia*) and to apply this process on a larger scale sufficient to satisfy commercial requirements. They obtained a good rate of shoot multiplication by using DKW medium with 1.0 mg/l benzyladenine (BA) and 0.001 mg/l indolilbutyric acid (IBA).

A properly proliferation rate of walnut shoot was obtained by using of MS modified medium with 1 mg/l BAP and 0.03 mg/l IBA (Gruselle *et al.*, 1987; Gruselle and Boxus, 1990). Marques Silva *et al.* (1997) have obtained good results in shoots multiplication rate with DKWC medium and 1 mg/l BAP. Navatel and Bourrain (2001) have used DKW medium with BA (0.2 mg/l) and IBA (0.05 mg/l) for establishment of cultures. For shoot multiplication they have increased the concentration of BAP to 1 mg/l and have decreased the IBA concentration to 0.01 mg/l. Vahdati *et al.* (2009) studied the micropropagation of some dwarf and early mature walnut genotypes and for shoot multiplication was used DKW medium with 4.4 µM BAP and 0.05 µM IBA. In addition, acclimatization of *in vitro* grown shoots from mature walnut trees was realised (Vahdati *et al.*, 2004). But the problem is hardening of tissue cultured plants.

In India, the maximum walnut trees are grown on own roots i.e. seedling origin. The evaluations conducted for all the 9(nine) walnut selections. Nut size is the determining factor for the market. Nut size index varied from 34.3 to 41.2 mm. Previous research (Cosmulescu and Botu 2012) 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 10.2 to 12.3 g. The highest value of nut weight among the studied accessions here (12.3 g) was less than nut weight reported by Sen and Tekintas (1992) for walnuts in Adilcevaz, Turkey (23.81 g); and more than nut weight by Atefi (2001) for walnuts in Kamal-Abad, Iran (20 g); Sharma and Sharma (1998) for Himachal Pradesh, India (18.60 g); Yarilgac *et al.* (2001) for east Anatolia, Turkey (17.04 g); Aslantas (2006) for North-eastern Anatolia, Turkey (16.01 g) and Cosmulescu and Botu (2012) for Oltenia region, Romania (18.40 g). Weight of kernel ranged from 1.32 to 10.00 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 (Arzani *et al.* 2008). Percentage of kernel is a feature of great importance in setting the amount of selections and an important character for improvement. This character is related to nut and kernel weight. Accordingly, kernel percentage varied from 41.3 to 51.0%. 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 higher than the data reported by Zeneli *et al.* (2005) (63.80 %), Aslantas (2006) (67.14%), Arzani *et al.* (2008) (79.60%) and Cosmulescu and Botu (2012) (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 (Germain

1997). It has been reported that fruit characteristics are not affected by tree age (Sharma and Sharma 2001). 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 (Chamba Collection, Kinnaur Collection and Soghi Coolecton. These traits have been used for selection of superior walnut genotypes (Sharma and Sharma 2001; Yarilgac *et al.* 2001; Zeneli *et al.* 2005; Aslantas 2006; Arzani *et al.* 2008; Cosmulescu and Botu 2012).

Out of 9 walnut selections, the fruit characteristics exhibit variability in both of the selection areas due to a range of morphological and biochemical elements. Fruit size and weight varies very much from one selection to another. Walnut fruit size is one of the main determinants for international trade (Table 1). Fruit weight varied from 30.92gm in Sel-8 to 56.05 in Sel-6.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 1). The good quality walnut fruits of 9.0 to 10.0 g weight can 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 41.3% to 51.0%. Of the 8 selections, only 1 (12.3) yields more than 50% kernel. Nut shell of the walnut selections is thin or medium (1.14 to 1.39 mm in thickness). Taking into account the growth and fruiting characteristics of the walnut genotypes Sel-3,Sel-5, Sel-4 and Sel-1 have been selected for further evaluation and direct use in the walnut farms, but only after clonal propagation.

CONCLUSION

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, low incidence of pistillate flower 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 (Cosmulescu *et al.* 2010; Botu *et al.* 2010; McGranahan and Leslie 2012). The nuts of the tree collected by scientists of ICAR-IARI, Regional Station, Shimla from Chamba District of Himachal Pradesh, India appear to meet this standard.

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