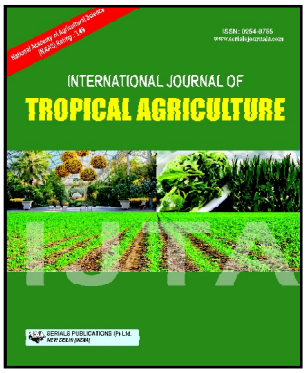

INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE

ISSN : 0254-8755

available at http: www.serialsjournals.com

Volume 37 • Numbers 3 • 2019

Techniques for Unique walnut (*Juglansregia L.*) 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 need to standardize the suitable techniques for clonal multiplication of walnut in order to ensure supply of quality plant material for expansion of area, achieve increase in production and productivity of superior nuts and meeting the international standards of quality characters of nut and kernel.A unique walnut plant (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 at par with other walnut plants collected during the same survey. But they are also not yet in bearing stage. The nut is thin shelled, kernel colour is light yellow and good in taste. This is also suitable for high density plantation. This walnut contains 55 % oil and shelling per cent is 45-50 and dry nut weight is 12gm and above which are the desirable characteristics for choosing a walnut cultivar for successful cultivation even for export purposes. Veneer grafting and chip budding found to be successful methods of propagation.

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. 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 methodavailable 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 vegetativepropagation in walnut have been reportedto give varying degree of success underdifferent climatic conditions in India andabroad. The variations are dependent ondifferent environmental conditions to whichthe plants are subjected before and afterpropagation (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 ofquality characters of nut and kernel.

A unique walnut plant (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 at par with other walnut plants collected during the same survey. They are also not yet in bearing stage. The nut is thin shelled, kernel colour is light yellow and good in taste. This is also suitable for high density plantation. This walnut contains 55 % oil and shelling per cent is 45-50 and dry nut weight is 12gm and above which are the desirable characteristics for choosing a walnut cultivar for successful cultivation even for export purposes. There is a great demand for the planting material. In fact, there is an urgent need to standardize the suitable techniques for clonal multiplication of unique walnut in order to ensure supply of quality plant material for expansion of area, achieve increase in production and productivity of superior nuts and meeting the international standards of quality characters of nut and kernel.

CONSTRAINTS FOR WALNUT CULTIVATION

Since the entire walnut plantation is of seedling origin, there is considerable variation in nut crop production. The development of walnut cultivation has in addition faced a number of constraints, including:

- non-availability of plant material of known pedigree and characteristics produced by vegetative propagation
- an absence of proper classification of local varieties
- non-availability of good rootstock
- a long gestation period
- low tree density per hectare
- low productivity.

Rootstocks

Rootstocks are bred to grow in different soil types and conditions, and provide the best anchorage, vigor, and resistance or tolerance to soil borne pests and diseases. However, no individual rootstock is tolerant of all factors that impact walnut production. The strengths and weaknesses of each rootstock should be considered in the context of a specific orchard location. Paradox (*Juglans hindsii* x *J. regia* hybrid) a dwarfing and precocious, tolerant to drought is used as a rootstock. In India, seedling rootstock is prevalently used for walnut.

Propagation

Walnut can be propagated either by seed or by vegetative methods and very recently by tissue culture techniques. These methods are described in the following

A. Seed propagation: Healthy and disease free nuts should be selected for sowing.The big nuts should be selected with bright brown colour having good cracking quality of the shell, good in taste and flavour of the kernel.

Seed Stratification: Walnut seed will not germinate until a low temperature treatment is given which is called stratification.

(i) Stratification under controlled conditions: Seeds are placed between 2-3 cm thick layers of moist sand in wooden boxes or polythene bags and kept in refrigerator at temperature 4-7^{0}C for 60-90days.

(ii) Stratification under field conditions: areas with night temperature below 7^{0}C can be stratified in the field. A piece of land should be identified which shows frosting during winters. A furrow 2 inches deep should be made and filled with wet sand and maintaining the moisture condition by giving irrigation at regular interval. They may be sown in lines 50 cm apart and the nut to nut distance should be kept at 25 cm. Sowing is done during mid-November to mid-February depending on the altitude and low temperature.

Higher germination rates in walnut have been achieved by sowing seed in a vertical position at a depth of 7 cm. The beds should be covered with grasses after sowing and irrigation. While preparing the beds, proper drainage facilities should be provided. However, it is advisable to avoid propagation by seed (nut) because the plants which are grown by this method take a very long time (10 to 12 years and even more) to begin fruiting and the plant characteristics may also not be true to type as desired due to variability.

Growing trees in situ (place): Prepare a fine seed bed in the area intends to plant nut tree seed. During early spring, plant three to five stratified nuts, 2 inches deep, at each tree location. Be certain to mark the area where nuts are planted and to keep the area weed-free. During the first year, select the strongest growing tree and remove the others by

cutting them oû below the root collar. Water trees when conditions become dry.

Establishing a nursery choose a deep, sandy loam soil for a nut tree nursery to facilitate digging and transplanting. Prepare a fine seed bed for the nursery area in early spring. Plant stratified seed 2 inches deep, 2 feet apart, in rows at least 4 feet apart. Keep the nursery weed-free and well-watered. Nursery-grown trees should be dug for transplanting in March of the following year. When digging 1-year-old seedlings, be sure to dig at least 16 to 18 inches deep and preserve as many fibrous roots as possible.

Growing trees in containers: There are several sizes and shapes of containers that can be used for growing walnut seedlings. Choose a container with an open bottom. Use a potting soil mixture that allows free movement of water through the pot. Mix in a fertilizer that can provide both macronutrients (primary elements essential for plant growth: nitrogen, phosphorus and potassium) and micronutrients (trace elements essential for plant growth: boron, chlorine, copper, iron, manganese, molybdenum and zinc). Plant a single, stratified nut in each pot. Walnut tree seedlings grown in containers need daily watering and the careful attention demanded by all containerized nursery plants. Container-grown stock can be transplanted into the field in the early winter. The winter planting season starts three to four weeks before the first killing frost and continues until mid-November. Containerized stock can also be planted during the traditional spring planting season. However, if you are holding container trees over the winter, you must protect tree roots from sub-freezing temperatures during the winter. After trees become dormant in the winter, cover the trees with an insulating cover or hay mulch.

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

B. Vegetative propagation

Scion selection: 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

Walnut can be propagated vegetatively by different methods discussed below

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. Success rate observed more than 80% (Fig 1). Scion for tongue grafting should always be selected from the tree which has already started fruiting.

Figure 1: Multiplied through Chip Budding and Veneer Grafting

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 (Chip 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. Success rate observed more than 70% (Fig.1)

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 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 27^0C at the place of grafting. Periodically the roots were watered and the foam was wetted (Avanzato*et al.*, 2006).

C. 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 indole butyric acid (KIBA) in vitro for 5 to 7 days and then stuck in a peat:perlite medium on a fog bench in a greenhouse (Vahdat i*et al.*, 2004). The walnut micro-propagation 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 micro-propagation 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).

CONCLUSION

Fruiting at an early stage is much required as the establishment cost of walnut orchard is very high. Lateral bearing cultivars generally come into production well in advance of non-lateral bearing cultivars. The highest quality walnuts have a medium-thin outer shell with no internal convolutions protruding into the nut meat. 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. This selection (Pusa Khor) can meet the increasing demand for an early bearing, high yielding of good quality, good shelling per cent , attractive kernel colour and thin shelled having export quality which

could be suited to high density walnut orchard in India. There is an urgent need to standardize the suitable techniques for clonal multiplication of walnut in order to ensure supply of quality plant material for expansion of area, achieve increase in production and productivity of superior nuts and meeting the international standards of quality characters of nut and kernel. Veneer grafting and chip budding found to be successful methods of propagation.

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