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State-of-the-art and Problems of Walnut Propagation Methods

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Abstract

At present budding and grafting are the most widely used approaches in the production of grafted walnut trees. Poor callus formation in walnut makes it difficult to propagate. Walnut propagation by cuttings is a method difficult to be realized. The presence of high concentration of phenolic compounds in its tissue and their oxidation, is the major reason of using micropropagation as a suitable method. The most commonly used technique is patch budding. Other used methods are bench grafting and hot callus. In the last years hot callus as a technique has been successfully used for propagation of walnut cultivars, but the height of the trees is not enough at the end of the season. A new walnut propagation method is called epicotyl grafting. All the methods of walnut propagation are discussed in the present paper.

Key words: Juglans regia L., budding, scion grafting

Introduction

The high protein and fat content in walnut kernels makes them an essential food for the people. That is why walnut is a strategic species for human nutrition and it was included in the list of FAO as a priority crop to be grown (Gandev, 2007). That necessitates the propagation of only those cultivars that possess good biological and economic properties. Due to heterozygosity of walnut, its propagation by seeds does not result in the inheritance of the characteristics of the chosen cultivar (Sharma et al.,

2003). What is more, seed-propagated trees start bearing fruit later. Those disadvantages could be overcome by vegetative propagation, which, unfortunately, is a difficult process due to the poor callus formation in that fruit species (Kuniyuki and Forde, 1985; Coggeshall and Beineke, 1997).

Analysis

Propagation by budding and scion grafting

At present ***budding*** and ***scion grafting*** are the most popular grafting methods in the production of walnut trees. In walnut, budding is basically carried out by the method of **patch *budding***. That is among the oldest and the most popular techniques of walnut propagation in a nursery in the open (Kuniyuki and Forde, 1985), adapted in our country (Nedev, 1967). The efficiency of that grafting method is different in the separate countries (Nedev et al., 1976; Ozkan et al., 2001). Solar et al. (2001) announced that in Slovenia the success rate of the *patch budding* method applied in walnut propagation is only 16%. In Turkey it is 88.3%, 72.5% in the spring of the following year and 41.25% before taking out the trees from the nursery (Ozkan et al., 2001). Probably the success in that case depends on the climatic conditions in the countries where applied. Winter frosts and spring late frosts reduce the percentage of the tree survival rate, but they are not the only limiting factors. Air temperature after grafting is also important. According to Lagerstedt and Roberts (1972) grafting in the open could be unsuccessful due to low temperatures after grafting, which make difficult or compromise good callus formation. Gandev and Dzhuvinov (2006) established that when growing walnut in the open under the conditions of South Bulgaria, temperature variation during days and nights decreases the percentage of the survival rate. That is the reason why the great difference between the day and night temperatures in the West European countries makes the survival of grafted tree difficult in the open.

The major walnut grafting method in Bulgaria is ***patch budding***. Non-stratified seeds are sown at a depth of 8-12 cm from the middle of November to the first decade of December. During vegetation soil is maintained free of weeds by applying herbicides and by tilling and earthing up the plants in order to form a thinner and tender soil crust at the grafting place. The aim is that the rootstocks reach a minimum thickness of 12 mm at the place of grafting. Hardwood cuttings are used, not less

than 12-14 mm thick. The best time for grafting in the climatic conditions of Bulgaria is from 20 August to 5 September. Grafting is performed at 5-10 cm above the soil surface, using a double knife. One month later the bandage is removed. In the second half of November the patch is covered with 20-30 cm of soil in order to protect the buds from winter cold. In spring (in March) the rootstocks are uncovered and the rootstock is cut off above the grafted bud. Soil is maintained free of weeds to enable the rapid growth of the grafted buds. The ready planting material is taken out after leaf fall.

The disadvantages of that method are: the short period suitable for carrying out the grafting and the dependence on the climatic conditions. In some years the winter cold, the excessive soil moisture during the stage of winter dormancy and the early autumn and late spring frosts could compromise the production of grafted planting material to 100%.

C*hip budding* is another method of walnut propagation in the open. In the climatic conditions of the high himalaya, chandel et al. (2006) announced that the optimal time for grafting is the middle of may till the first week of june and for patch budding – from the middle till the end of june. Grafting is carried out on annual rootstocks (*j. Regia* l.) With buds collected in the same season. In the time mentioned the survival rate is 89.0% for chip budding and about 50.0% for patch budding. Data about the conditions in turkey are controversial. A survival rate of only 13% was reported for chip buding and 43% for patch budding (polat and ördek, 2006).

According to achim and botu (2001), under the climatic conditions of the carpathian region in romania, chip budding could be performed in the open from 15 may till 15 june, using buds collected during the winter dormancy stage of the trees and stored in a refrigerator at a temperature of 1-4°c. Rootstock age and the time of their cutting off after grafting exert an effect on the survival rate. Using common walnut rootstocks (*j. Regia* l.), planted early in spring, forced and grafted in the same year in the period mentioned above, results in a survival rate of 78.0%. When using rootstocks planted in the previous year and grafted in the period mentioned, the survival rate decreases to 40.0%. In both cases, cutting off the rootstocks immediately after grafting leads to a decreased percentage of survival. That is why the rootstocks should be cut off 15 days after grafting. In Romania that method should be applied under controlled temperature in winter months.

Under the climatic conditions of Poland, Porebski (1994) also found out that summer chip budding is risky and it is possible to be used only in seasons when the average daily temperature is not lower than 18ºC.

Chip budding could be performed not only during vegetation, but also in winter months during tree dormancy, however the grafted plants should be kept under controlled temperature. In such cases the patch budding method is not very suitable due to the difficult separating of the bud from the scion (Bayazit et al., 2005). That problem does not exist in the chip budding method. Özkan and Gümüs (2001) applied chip budding to one-year old rootstocks in January, February and March. The grafted plants were put in wooden containers and covered with wet sawdust at a temperature of 27°C for 25 days. In that trial the highest survival rate percentage was obtained in Tokat cultivar in March – 53.0% versus the survival rate of 50.0% in September. The careful analysis shows that the authors calculated the percentage of the survived plants in September on the basis of the successfully propagated plants in March. We think that a clearer picture of the efficiency of that method would be obtained if calculation is done by taking into consideration all the grafted plants. After re-calculating, the method shows an efficiency from 16.0% to 26.0% for the studied cultivars and grafting time, which, in our opinion, is not an efficient method to use. A similar survival rate (26.9%) was reported by Porebski et al. (2002) after winter application of the chip budding method. According to them, the percentage of the plant survival rate could be increased if the rootstocks are forced and if they are in full vegetation at the time of winter grafting. Applying that practice, the authors obtained 81.9% of successfully propagated plants in March.

In Bulgaria the chip budding method is not recommended neither during the dormancy period under controlled temperature, nor during vegetation under natural conditions, due to the unsatisfactory results (Gandev, unpublished data).

Scion grafting of walnut under natural climatic conditions resulted in a worse result compared to most fruit species. In Turkey Demiroren and Buyukyilmaz (1988) obtained 20% of successfully propagated plants after ***cleft grafting*** and improved copulation. The results of Barut (2001) after ***splice grafting*** were similar, i.e. – from 20% to 33% survival rate.

Bark grafting is another approach for walnut propagation by scion grafting. 80% of successfully propagated plants were obtained under the warm conditions of South Africa (Rotondo Walnuts, 2004). The method is

not recommended for industrial-scale production, because rootstocks need to be grown for about 3-4 years before reaching the necessary thickness of 30 mm to 100 mm (Reil et al., 1998; Hartmann et al., 2002).

Due to the above mentioned disadvantages, *scion grafting* is applied in practice basically when walnut is propagated *indoors* (under controlled temperature). According to the different technologies, temperature, humidity, the grafting method and time of grafting are controlled, to provide permanent temperature necessary for the callus formation process.

It is well known that temperature has a definite effect on callus formation in fruit plants, the temperature values varying for the different species (Hartmann et al., 2002). As early as the beginning of the 30s last century, Sitton (1931) established that the optimal temperature for callus formation in walnut is 27°C. Later studies of Rongting and Pinghai (1993) and Reil et al. (1998) showed that the optimal temperature is from 26°C to 27°C, however 22°C is also favourable for the process (Rongting and Pinghai, 1993), while callus formation in walnut is unsatisfactory at temperatures below 20°C (Reil et al., 1998; Hartmann et al., 2002).

Temperatures have an effect on the amount of the callus tissue, as well as on the speed of callus formation. At a temperature of 22°C callus formation begins on the 6th day after grafting, while at 27°C the process starts on the 5th day. When the temperature increases up to 32°C, callus formation begins in 4 days only but at that temperature, less callus tissue is produced (Rongting and Pinghai, 1993).

The temperature of 27°C (± 2°C) has been adopted as a standard used by a large number of researchers and producers of planting material from around the world in their trials to carry out successful walnut grafting under controlled temperature (Zachej, 1976; Lagerstedt, 1981b; Millikan, 1984; Avanzato and Tamponi, 1988; Tsurkan, 1990; Avanzato and Atefi, 1997; Stanisavljević and Mitrović, 1997; Achim and Botu, 2001; Solar et al., 2001; Porebski et al., 2002; Avanzato et al, 2006; Erdogan, 2006; Vahdati & Zareie, 2006).

There is not a common opinion worldwide on the choice of a certain grafting method. *Improved copulation* (Radicati and Me, 1986; Lantos, 1990; Stanisavljević and Mitrović, 1997; Achim and Botu, 2001; Erdogan, 2006; Muzaffar and Kumar, 2011), *cleft grafting* (Pathak and Srivastava, 1975; Gautam, 1990; Atefi, 1997; Qian and Qian, 2000; Achim and Botu, 2001), *omega type grafting* (Lagerstedt, 1982; Ferhatoğlu, 1997; Solar et al., 2001; Dehgan et al., 2010) and *side grafting* (Germain et al., 1999) are all used. In previous studies of Gandev (2007,

2008 and 2009) it was found out that *cleft grafting* results in obtaining a high percentage of successfully propagated plants.

Along with the method chosen, the heating period is also important for the grafting success. There are announcements (Pieniazek, 1972; Avanzato and Atefi, 1997; Özkan and Gümüs, 2001; Karadeniz, 2003, Vahdati and Zareie, 2006; Erdogan, 2006), that the period of heating necessary for good callus production varies from 21 to 33 days. According to Cerny (1965), when the period is shorter than 14 days, the produced callus is insufficient for the good development of the propagated plants.

Studying the phenolic content of walnut, Pinghai and Rongting (1993b) mentioned that the amount of yuglon is different in the studied cultivars and according to Solar et al. (2006) it varies in the different seasons. They admitted that the high content of yuglon is the reason for poorer callus formation. In later studies, Karadeniz and Kazankaya (1997) confirmed those results and they established a reverse correlation between the callus formation process and the content of phenols in nine walnut cultivars. Lantos (1990) reported from 56% to 71% of survival rate after grafting of three cultivars; Stanisavljević and Mitrović (1997) – from 55% to 93% in a study with seven cultivars, while the results of Erdogan (2006) varied for the same cultivars, tested in two consecutive years. According to a number of authors (Pieniazek, 1972; Farmer, 1973; Lagerstedt, 1979; Atefi, 1997; Erdogan, 2006) callus formation in the grafted plants is cultivar specific, however it also depends on air humidity, which should be about 80% (Ferhatoğlu, 1997; Stansavljević and Mitrović, 1997; Germain et al., 1999; Achim and Botu, 2001; Özkan and Gümüs, 2001; Solar et al., 2001). Optimal time for ***scion budding*** is during winter dormancy of both the rootstock and the scion (Lagerstedt, 1979, 1982; Hartmann et al., 2002) and grafting could be carried out at the beginning, in the middle or at the end of the dormancy period. The results of the conducted investigations are controversial. In the US Lagerstedt (1982) recommended to carry out scion budding in the middle of the winter dormancy, i.e. from the middle of December to the middle of January and not in February and March. In the climatic conditions of Israel, Ebadi et al. (2002) obtained a significantly higher percentage of survival when grafting was conducted in December, not in January. Also in Iran, Vahdati and Zareie (2006) preferred to carry out grafting in March, i.e. at the end of the dormancy period, just like Ferhatoğlu (1997) and Erdogan (2006) in Turkey. Our data (FAO project, 2002-2004) showed that *scion budding* in March resulted in successful walnut propagation during the period of winter dormancy.

Various scion budding technologies have been applied indoors in many parts of the world. The most popular and widely used technology for callus formation in walnut is putting the grafted plants in wooden containers and placing them in a room with controlled temperature (Nedev et al., 1983). Throughout the world that propagation method is known as ***bench grafting***. The authors Sen (1986), Kantarci (1989), Tsurkan (1990), Flores et al. (1995) underlined the following advantages of *bench grafting* over *patch* and *chip budding:*

- The period suitable for grafting is extended and a larger number of plants could be produced;
- Grafting is carried out in winter, i.e. in a season of less agricultural work;
- Bench grafting could be mechanized, thus the amount will be increased and the production costs will be reduced.

In Bulgaria *bench grafting* was studied by Anadoliev (1983). One-year old seedlings grown in the open should be used as rootstocks. Scions are collected during the winter dormancy of the mother plants. Grafting is performed by the method of improved copulation from the first decade of February till the middle of March. After grafting the scions are dipped in paraffin at the place of grafting and put in containers. The plant roots are covered with sawdust mixed with perlite at equal amounts. The ready containers are put in a room where the temperature of 26-27°C is maintained for 3-4 weeks. The successfully grafted plants are adapted and planted in the open, in the fields.

Tsurkan (1990) reported that following that technology, he obtained a considerably lower percentage of successfully propagated plants – from 5% to 45% – explaining that the variation in the survival rate depended on the method of grafting. According to Özkan and Gümüs (2001) the percentage varied within 33% – 53% for the separate cultivars. Terziev (personal correspondence) obtained about 40% plants with callus formation in three consecutive years.

Another method of walnut propagation is ***hipocotyl grafting*** (Frutos, 1995; Avanzato, 2001). In the recent years the method gained greater importance (Vahdati and Zareie, 2006; Gandev and Dzhuvinov, 2006; Gandev, 2008). Potted seedling rootstocks are used in that method. Grafting is carried out during the vegetation period. The growing tip of the rootstock is cut off and a growing tip from the propagated cultivar is grafted. The pot is firmly covered with a plastic bag in order to provide high air humidity, necessary for callus formation. Then the plant is placed

for 4 weeks in a greenhouse at a temperature of 26°C (± 1°C). The successfully propagated plants are put for adaptation in a shaded place for 2-3 weeks, after which are taken in the open. Gandev and Dzhuvinov (2006) reported that they obtained 83% of survival rate by that grafting method.

The ***hot callus*** method was described in details by Lagerstedt (1981a, 1981b, 1982, 1983, 1984). Using a heating cable, a temperature of 26°C (± 2°C) is maintained at the place of grafting. The one-year old rootstocks are taken out of the soil, grafted on and put horizontally over the heating source, covering their roots with sawdust. The positive results obtained by Erdogan (2006) who used electric heating cable, confirmed the method efficiency. Avanzato and Atefi (1997) and Avanzato (1999) developed an alternative approach for heating the place of grafting without taking the rootstocks out of soil, using also an electric cable. In that way the stress of the rootstocks is avoided as they are not taken out of soil and the growth of the graftage after the grafting process is favoured.

In Bulgaria the method with electric heating was successfully tested and adapted by Gandev (2007, 2008, 2009). In that case a simple appliance is used in a steel-and-glass greenhouse, placing the electric cable in a groove, covering it with peat. The thermal regulator maintains the necessary temperature. The cleft grafted plants are placed horizontally, perpendicular to the groove, at a distance 10-15 cm from one another and their roots are covered with sand. The place of grafting should be just above the heating cable. The place of grafting is covered with wet foam, firmly fixed above the groove and the grafted plant in order to provide high air humidity. The scions are 12-15 cm long, with 2-3 buds. For 4 weeks high air humidity is maintained at the place of grafting and the temperature should be about 27°C (± 1°C). The plants that have formed callus are adapted and in spring they are planted in the fields. Using radioisotopes, Nacheva and Gandev (2009) studied the transport and distribution of ^{14}C-photoassimilates in walnut plants propagated by the hot callus method. It was established that there was not any negative effect on the movement of photoassimilates in the grafted plants in result of the method applied. The adapted hot callus method of walnut propagation by using an electric cable, described above, has some disadvantages, such as the big energy consumption and the risk of using electricity in humid environment.

Suk-In et al. (2006) announced that along ***hipocotyl grafting***, ***epicotyl grafting*** could also be used for walnut propagation. Taking into con-

sideration the advantages of epicotyl grafting, Gandev and Arnaudov (2011) started the first investigations on the method at the Fruit-Growing Institute – Plovdiv. Similar to the other techniques for winter scion grafting, the parameters in that method are the same: providing a temperature of about 27°C (± 1°C) and high air humidity at the place of grafting for 3-4 weeks. Some elements of the technological process, such as reaching the necessary thickness of the rootstocks before grafting and the types of scions suitable to be grafted, are still understudied.

According to Rodriquez et al. (1989) and Preece et al. (1989) walnut belongs to the group of species, difficult to be cultivated *in vitro*. Rongting and Pinghai (1993a) think that this is due to the high content of phenolic components in the plant tissue and their oxidizing after the injury. The major difficulties in walnut micropropagation are related to setting and stabilizing the tips from matured plants in *in vitro* culture, the low coefficient of multiplication, difficult root-formation, as well as the great losses during plant adaptation. The first announcements about successes in setting and stabilizing in culture are from the beginning of 90s last century (Rodriguez, 1982a,b). Later, a number of studies have been carried out, most of them being based on the nutrient medium for *in vitro* cultivation of *Juglans* spp., developed by Driver and Kuniyuki (1984). It is well known that the genetic type plays an important role at all the stages of vegetative propagation, especially at the rooting stage. In the recent years, successful examples for rooting and acclimatization of different walnut cultivars were announced (Ripetti et al., 1994 and Nacheva, 2012). Unfortunately, there are still unsolved problems in walnut micropropagation and it has not yet found industrial application in the production of walnut planting material.

Conclusion

In many countries the most commonly used technique is ***patch budding***. Other popular methods are ***bench grafting*** and ***hot callus***. In the last years hot callus method has been successfully used for propagation of walnut cultivars, but the tree height at the end of the season is unsatisfactory. The new walnut propagation method is called ***epicotyl grafting***. Depending on the climatic conditions and equipment, each of the methods could be used successfully.

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Stanje i problemi u proizvodnji sadnog materijala oraha

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Sažetak

Danas su okuliranje i kalemljenje kalem grančicom najčešće korišćeni pristupi u proizvodnji kalemljenog oraha. Slabo formiranje kalusa otežava razmnožavanje. Razmnožavanje reznicama je metod koji nije lako realizovati. Prisustvo visokih koncentracija fenolnih jedinjenja u tkivu i njihova oksidacija su glavni razlog za korišćenje mikropropagacije kao odgovarajuće metode. Najčešće korištena tehnika je okuliranje na prozorče. Ostale tehnike koje se koriste su kalemljenje iz ruke i stratifikovanje. Posljednjih godina se stratifikovanje kao tehnika uspješno koristi za razmnožavanje, ali visina stabla ne bude dovoljna na kraju sezone. Nova metoda razmnožavanja oraha je kalemljenje epikotila. U ovom radu se diskutuje o svim metodama razmnožavanja oraha.

Ključne riječi: Juglans regia L., okuliranje, kalemljenje na zrelo

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