Bulgarian Journal of Agricultural Science, 13 (2007), 683-689
National Centre for Agrarian Sciences

Budding and Grafting of the Walnut *(Juglans regia* L.*)* and their Effectiveness in Bulgaria (Review)

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Abstract

GANDEV, S., 2007. Budding and grafting of the walnut *(Juglans regia* L.*)* and their effectiveness in Bulgaria. *Bulg. J. Agric. Sci.,* 13: 683-689

The high biological value of the walnut kernel makes it an indispensable food product and that is why the walnut is on the FAO priority plants list. Therefore, only varieties with proven high nutritive value and good agro-economical indexes have to be propagated. Walnut *(Juglans regia* L.*)* propagation is more difficult, compared to most fruit species, that is why along with the work on new methods for the production of inoculate planting material, old and reliable propagation technologies are being improved worldwide. We compiled a review of the propagation methods, techniques and technologies in the major countries with developed walnut production and analyzed their efficiency in view of climatic and other specific conditions. The most popular inoculation methods for the production of walnut transplants were presented – *budding* and *grafting* as well as the techniques used, such as *patch budding, chip budding, bench grafting, whip, whip-tongue, cleft and omega*. We emphasized on the new methods for the production of inoculated walnut trees, two of which – *hot callus* è *hypocotyl grafting* were tested and adapted in Bulgaria. Heating techniques were described as well – *local heating on the graft union with hot water* and *hot callusing cable*. According to the results of the most recent studies on hypocotyl grafting in temperature controlled greenhouses in Bulgaria, the method is 83% successful and can be implemented in the production of grafted young walnut trees.

Key words: walnut, *Juglans regia* L**.,** propagation, budding, grafting

The high protein and oil content of walnut kernels makes them indispensable for human nutrition. Therefore, the walnut is classified as a strategic species for human nutrition and is included in the FAO list of priority plants. This imposes the necessity to propagate only varieties of good biological and economic value. Due to walnut heterozygosity, propagation by seeds does not lead to inheritance of the characteristics of a certain variety. Unfortunately, the production of grafted walnut trees is a difficult process due to the low rate of callus formation in this fruit

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species (Kuniyuki and Forde, 1985; Coggeshall and Beineke, 1997). According to Rongting and Pinghai (1993), this is due to the presence of high concentration of phenolic compounds in its tissues and their oxidation by wounding, which is the major obstacle for industrial micropro-pagation of this tree. For this reason, different methods of walnut propagation are being investigated all around the world, because propagation with cuttings is difficult (Gautam, 1990) and currently budding and grafting are the most popular inoculation techniques in the production of walnut trees.

Budding in the walnut is done mainly by the technique of ***patch budding***. This is one of the oldest and popular techniques for propagation in a nursery in the open (Kuniyuki and Forde, 1985). Scientific literature reports different data on the efficiency of this inoculation method in different countries (Nedev et al., 1976; Ozkan et al., 2001; Solar et. al., 2001). Solar et al. (2001) reported that the efficiency of patch budding technique in walnut in Slovenia is only 16%. The percentage of successful inoculation in Turkey is higher and is characterized with decrease in time. It is 88.3% after budding, 72.5% in the spring of next year and 41.25% before taking out the young trees from the nursery (Ozkan et al., 2001). Nedev et al. (1976) reported that one month after budding in Bulgaria; inoculation success was about 70-80%. Our unpublished data showed that during the last four years the percentage of successfully propagated plants at the end of the second year (before taking out the trees from the nursery) at the Fruit Growing Institute in Plovdiv, Bulgaria, was within 33% and 47%. Obviously, the success of the method of patch budding depends on the climatic conditions of the respective country. Winter colds and spring frosts reduce successful inoculation percentage but they are not the only limiting factors. Post-inoculation temperature is important as well. According to Lagerstedt and Roberts (1972), transplant in the open may be unsuccessful due to low temperature in the following period that impedes or compromises good callus formation. Gandev and Dzhuvinov (2004) found that growing in the open in South Bulgaria reduces successful transplant percentage due to temperature variation within a 24 h period. That is why the big difference between day and night temperatures in West Europe is not favorable for successful transplants in the open.

Chip budding is another way for walnut propagation in the open. Chandel et al. (2006) reported that the optimum period for chip budding for the climatic conditions of North-western Himalayas mid hills was from mid May to the first week of June and the right period for patch budding was mid June – end of June. In the experiment, budding was done on 1-year rootstocks *(J. regia* L.*)* with buds taken in the current season. Successful inoculation percentage in chip budding was 89.0% and in patch budding – about 50.0% during the mentioned periods of time.

Achim and Botu (2001) reported that chip budding in the open was possible for the climatic conditions of the Carpathian Area of Romania. The authors pointed out that May 15 – June 15 was the best period for budding, using buds collected during the winter dormant period of the trees and kept in a refrigerator at a temperature of 1-4°C. The percentage of successful budding was influenced both by rootstock age and the time of pruning after budding. Rootstock *(J. regia* L.*)* planting in early spring, their forcing and grafting during the

same year in the above mentioned optimal period resulted in 78.0% successful inoculation. In case of rootstocks planted during the previous year and budded within the same period of time, success percentage went down to 40.0%. In both cases, rootstock pruning right after budding reduced the percentage of successful inoculation. It is recommended that rootstock pruning takes place fifteen days after budding. According to the authors, climatic conditions in Romania bear the risk of freezing in propagation in the open and that's why they suggested that walnut propagation in the country took place in controlled temperature conditions in winter.

Porebski (1994) recommended walnut propagation in controlled temperature conditions for the climate of Poland. According to the author, summer chip budding is risky and is possible only in years with average daily temperatures after budding not lower than 18ºC.

Budding can be practiced not only during vegetation but also during the dormant winter period of the trees, budded plants being left in controlled temperature conditions. In this case, patch budding is not recommended due to the difficult separation of buds from the scion (Bayazit et. al., 2005). This problem does not exist in chip budding. Özkan and Gümüs (2001) applied chip budding to 1-year rootstock in January, February and March. The inoculated plants were placed in wooden boxes and covered with wet sawdust. Then they were put in a room at 27°C for a period of 25 days. The authors found out that the highest success percentage was reported for cultivar Tokat in March – 53.0% and the surviving plants were 50.0% in September. Those results seemed good at a first glance but a closer look revealed that the authors had calculated the percentage of survived plants in September on the basis of the successfully budded plants in March. We think that the calculation based on total budded plants would give a clearer picture of the efficiency of this method. Recalculation based on this method gave within 16.0% and 26.0% efficiency of the method in the tested varieties and periods of budding which, to our opinion, is insufficient for its practical implementation. A similar percentage of survival – 26.9% was obtained by Porebski et al. (2002) for chip budding during winter. The authors established that the percentage of survival increased when rootstock was forced and in complete vegetation during the winter chip budding.

Grafting in walnut is less successful compared to most fruit trees, especially in natural climatic conditions. Therefore, different methods are used for the production of transplants in controlled temperature, relative humidity, method and time of grafting and bleeding, etc. The choice and combination of formerly enumerated factors affected the percentage of surviving plants in the different propagation methods in controlled environment. The common parameter for all different methods was the same value of temperature, necessary for callus formation of grafted plants, but there were different ways to achieve it in each separate method. According to Lagerstedt (1979), Millikan (1984) and Wilbur et al. (1998), the optimal temperature for walnut callus formation was 26 - 27ºC.

The most popular and widely practiced method of callus formation in the walnut is to place the plants in woody boxes in a room with controlled temperature. Grafting takes place during winter dormancy with 1-year rootstock and grafts. This

method is known as *bench grafting*. A number of authors (Sen, 1986; Kantarci, 1989; Tsurkan, 1990; Lantos, 1990; Flores et al., 1995 and Ünal, 1995) claim that bench grafting has a number of advantages compared to bud grafting. First of all, the period for grafting is longer and much more work can be done. Second, grafting takes place in winter, i.e. a period with less job opportunities. Third, bench grafting can be mechanized and thus increase labor efficiency. The most popular bench grafting techniques are *whip, whip-tongue, cleft and omega* (Curkan et al., 1975; Nedev, 1976; Korac, 1987; Sen, 1986; Germain, 1999). After grafting, graft unions are plunged into hot paraffin (70-80ºC) and than cooled in cold water. The graft unions are forcing in woody boxes, filled with wet sawdust, in a room under controlled conditions at 26-27ºC and 80% relative humidity. Solar et al. (2001) reported that omega bench grafting technique has resulted in 83.0% plants with callus formation on average for three years, of them, 64.0% survival at the end of the second year with considerable variation of successful grafting during the separate years. The authors do not explain this variation of results. Achim and Botu (2001), using the same technique for the production of transplants, obtained 63.0% successful grafting by whip-tongue grafting technique and 67.2% by cleft grafting technique. Similar results were obtained by Bayazit et al. (2005). The success of grafting depends not only on the grafting technique choice but the month as well. Özkan and Gümüs (2001) compared two grafting techniques - cleft and whip-tongue during the months of January, February and March. They reported the highest percentage of successful grafting in February for both grafting techniques. In Tokat variety – 60% in cleft grafting and 66% in whip-tongue grafts and in variety Yalova – 63% in cleft grafting and 70% in whip-tongue grafting.

In Bulgaria, bench grafting has been tested as well. Grafting was done by whip-tongue technique. The success percentage was 77.9% in 1973 and 88.6% in 1974. Due to the 3-year production cycle and the need for stratification, this walnut propagation method has been replaced by production in the open by the method of patch budding in our country (Nedev et al., 1976).

In the recent years, local heating the graft union with a ***hot callusing pipe*** has been successfully applied (Lagerstedt, 1981; Avanzato, 1997; Avanzato and Tamponi, 1988; Tomas, 1989; Deering, 1981; Achim and Botu, 2001; Avanzato et al., 2006 and Gandev, 2007). The trial methods for local heating of the graft union are different in the different experiments but the purpose is the same – to achieve a temperature of 26 - 27ºC. Achim and Botu (2001) used an installation (***hot callusing pipe)***, where the grafting union was heated with hot water. In whip and tongue grafting the success percentage was 86.0% and in cleft grafting – 89.5%. A possible method of heating is with an electric cable, placed in a plastic U–pipe full of peat. The method is known by the name of ***hot callus*****.** In this technology, the grafted plants are placed horizontally, the graft union being positioned on the heating cable. Gandev (2007) reported 74.2% successful propagation in cultivar Izvor 10 by the methods of cleft grafting and hot callus and Avanzato et al. (2006) obtained 7% to 100% successful plant formation by grafting selected walnut hybrids, success percentage depending on the phytosanitary condition of the mother plants and on the

genotypes. According to Pinghai and Rongting (1993a), the differences among the genotypes were explained by their chemical constituents. The better ripeness and quality of the scions, which contain higher soluble sugars, starch and C/N ratio, the higher the grafting survival percentage. The same authors (Pinghai and Rongting, 1993b) reported in another study that excess bleeding caused formation of anaerobic conditions at the graft juncture resulting in decrease of graft survival. This study triggered new ideas for tying the plastic string. The popular practice in fruit trees is to cover the graft union completely. Obviously, this rule has to be changed for walnut and the plastic string should ensure the access of air to the graft union.

Erdogan (2006) reported that the method ***hot callusing cable*** is used for the annual production of about 20,000 plants in Turkey with an average graft survival of 82%. Grafting takes place at the end of winter. Hartmann et al. (1997) stated that callus proliferation occurs most readily during the late winter and at the time of the year just before or during "budbreak" in the spring. This is due to the reduction in the auxin gradient through the summer and into autumn, and its increase in late winter and through the spring.

During the recent years, ***hypocotyl grafting*** technique was introduced in practice (Atefi, 1997; Vandati and Zareie, 2006; Gandev and Dzhuvinov, 2006). In this method, grafting takes place during vegetation with growing tip of soft wood cutting on cleft. Grafted plants should be kept in about 80-90% relative humidity and temperature of 26 - 27ºC. In Bulgaria, Gandev and Dzhuvinov (2006) obtained 83% graft survival, the grafted plants being kept in a greenhouse under controlled temperature.

Conclusion

Obviously, the new findings in the recent two or three decades allowed the improvement of traditional walnut propagation methods such as patch budding and bench grafting, etc. At the same time, new techniques for walnut transplants production were developed, two of which - hot callus and hypocotyl grafting are very promising. Their application results in high graft survival percentage which is stable throughout the separate years and independent of ambient climatic conditions. The methods hot callus and hypocotyl grafting can be used in the production practice of Bulgaria; moreover, they have been tested and adapted in our country.

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Received June, 2, 2007; accepted October, 23, 2007.