Methods of Blackberry Propagation *in vitro* Condition

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Abstract. Blackberry, one of the most important fruit species belonging to the Rosaceae family, can be grown in different environments due to having wide adaptation ability. Although originated in Europe, today most of the common cultivars have North American origin. Also, expansion of its production last 25 years especially in Europe and the USA, the blackberry has become the fourth berry in the fresh berry market after strawberries, blueberries, and raspberries. Blackberry fruit are rich in vitamins, polyphenols, minerals, and antioxidants, especially gallic acid and routine. Many studies have proven that high nutritional composition has a positive effect on human health in preventing various diseases. It has an important place in the fresh and processed market. Frozen fruits can be used processed such as ice cream, juice, jam, marmalade, cake, and sweet products. Blackberry breeding studies have been ongoing for over 100 years to increase yield and fruit quality, thornless cane, to improve disease insect resistance, and cane management and primocane fruiting. In vitro propagation is an alternative method to introduce new cultivars quickly into the market and to provide disease-free planting material compared with traditional methods. The purpose of this study was to summarize blackberry propagation methods in vitro conditions.

Key words: *Rubus fruticosus*, blackberry, in vitro propagation

1 Introduction

Blackberry is a member of the Rosaceae family. It belongs to the genus *Rubus* (Tourn) L. and subgenus *Eubatus*. Its plant growth ability could be upright, semi-erect, or creeping shrub. The body of blackberry varieties is generally spiny [1,2]. Rosaceae family consists of more than 100 genera. This family contains approximately three thousand species and is the third economically commercial significant plant family [3]. *Rubus fruticosus* is thought to be the species developed from *Rubus armeniacus*. It is a blackberry that is consumed and traded around the world due to its taste, aroma, and nutritional profile. It is widely grown especially in Europe, Asia, Oceania, and North and South American countries [4]. *Rubus fruticosus* is generally consumed as fresh fruit, individually frozen (IQF), made into jam, syrup, wine, tea, juice, concentrate, and puree, and consumed as a further processed product. Blackberries include fiber, some vitamins (C, A, E), some macro elements (potassium, calcium), and phenolic metabolites [5]. It can also be used for health and wellness in traditional and modern medicine [6,7]. Addition to health beneficial, they can be used as decorative landscape ground covers in garden [8].

Blackberry fruits have important antioxidant properties because they are rich in phenolic compounds (phenolic acids, anthocyanins, flavonols, ellagitannins, gallotannins, and proanthocyanidins) [9]. Flavonoids and phenolic compounds in blackberry berries are anticarcinogens that have anti-neurodegenerative and anti-inflammatory properties [10,11]. Blackberry plants are usually propagated very effectively by seed, root cutting, stem cutting, shoot division, grafting, and micropropagation methods [12]. Propagating blackberries from hardwood or stem cuttings has proven not easy [13]. Proliferation of blackberries by seeds, long germination and development times, and low seed germination rate are effective in reducing the production capacity of the seeds [14]. Asexual reproduction also has some problems, such as genetic diversity and the spread of diseases endogenously present in parent plants [15]. The successful application of propagation methods is limited to a certain extent. Propagation in layers requires a large area for the bed due to the problem of weed control between layers. Moreover, not only is propagation by cuttings very simple, but softwood cuttings root easily and require a lot of maintenance [16,17].

In vitro micropropagation is a significant method for breeding programs to multiply and develop suitable varieties in a short time, to add new features, and to multiply elite selections [18]. Propagation of blackberry by tissue culture depends on various factors, such as the physiological

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conditions of the explants, the composition of the culture medium, and plant growth regulators added to the culture medium [19,20,21] The most important part of the propagation of blackberries by tissue culture method is acclimatization and plant growth [22]. In vitro propagation, the operation includes various periods: entry of culture, multiplication, growth of shoots, rooting, and acclimation [23,24]. When the studies carried out in previous years on the propagation of blackberry species by tissue culture were examined, it was seen that blackberry varieties could be propagated in many cultural environments. Murashige and Skoog (MS) medium emerged as the most frequently used option [25,26], followed by McCown's Woody Plants medium[27,28,26] and Gamborg's B5 medium [10,29].

The tissue culture technique in plants is a method of production. Plant tissue culture is propagation of new plantlets, clones of parent plants, from plant's organs such as root, stem, leaf, or apical and meristem tissues. It is the process of culturing these part in vitro in culture media containing various nutrients after sterilization [30,31,32,33] In that way, the aim was to obtain a plant that does not carry microbiological diseases, to produce biochemical substances, to protect the genetic resources of plants, and to overcome the problems that cannot be solved by classical agricultural methods, to provide quality and economical plant production [34,35]. In addition, tissue culture methods in genetic engineering; in the creation of a fast-growing of selected genotypes contributes is used to reveal individuals and to select individuals resistant to abiotic and biotic stress factors for plants [31].

The first study in an in vitro culture environment in plants was carried out by Haberlandt in 1902 [30]. Afterward, tissue culture studies continued based on callus culture and plant regeneration until 1975 [31]. Through plant tissue culture methods, it is possible to grow plants independently of environmental conditions, increase the number of phytochemicals in the plant, create an environment for plant gene transfer, and protect species in danger of extinction [31]. It is also used in the propagation of elite genotypes, and ex situ preservation of gene capacity and plant diversity becomes important [36].

Genetic engineering, synthetic seed production, clonal propagation, unlimited clone production, providing raw materials for the pharmaceutical and nutritional industries, and obtaining biochemical products can be considered as application areas of plant tissue culture [31].

Micropropagation requires few parent plants in a tiny period and in a limited area. It enables the cultivation of a large clonal of plants and is possible to produce in all seasons, regardless of environmental conditions and season if it is done under controlled conditions. [34, 37] This technique is especially suitable for application with recalcitrant (stubborn) seeds, which are difficult to propagate classically, and for vegetatively propagated species

Blackberries are mostly proliferated by tissue culture the explant taken from young axillary buds [37]. The aim of this review was to summarize the propagation methods of blackberries under micropropagation conditions.

1.1 Tissue Culture Stages

1.1.1 Initiation of cultures

Culturing the explants after surface sterilization and then initiating shoot development. Depending on the explant, new shoots from plant meristems. It can be initiated by indirect organogenesis, which consists of callus from shoots, leaves, cotyledons, or explants obtained from the plant. This stage requires 4-6 weeks [38].

1.1.2 Propagation of shoots

It is the placement of shoots in the medium and their continuous reproduction. The proliferating shoots are separated and transferred to a different culture medium [38].

1.1.3 Rooting

This stage includes the rooting from the shoots, acclimatization, and transportation phases [38].

1.1.4 Adaptation (Acclimation)

Also known as the acclimatization phase. It is the stage of transferring regenerated plants to the soil under natural conditions. Plants transferred to natural conditions, leaf structure, and produce changes in morphology. Thus, plants adapt to survive in natural environmental conditions [38,39,40].

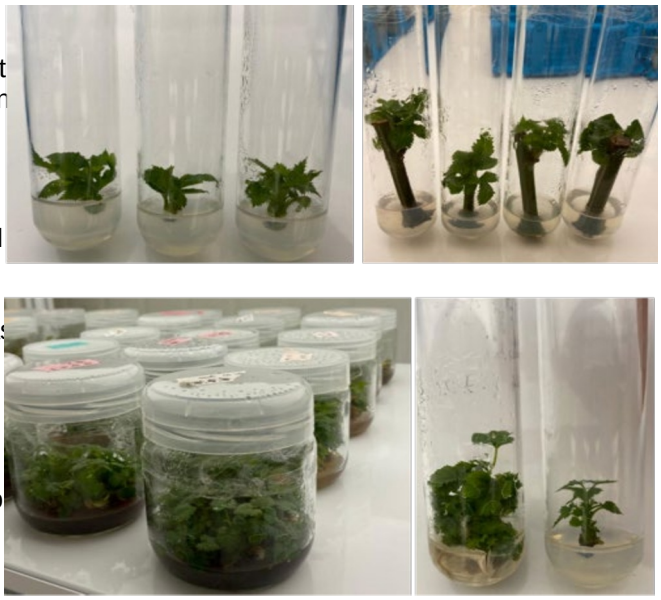

Fig 1. Micropropagation of blackberry

1.2 Applications in the Field of Planting Material Production

1.2.1 Meristem culture

Meristem tissue culture is one of the most common methods to obtain multiple disease-free propagations from a single infected plant in a short time [41,42]. Numerous studies have been conducting since 1952 have revealed that different types of virus-free plants can be produced using the meristem tissue culture method [41]. Viral diseases multiply easily in fruit plants, causing both reduced yield and fruit quality. Therefore, meristem culture is an important method for virus elimination. A biotechnological alternative to obtain large quantities of healthy plants is the isolation of meristematic tissue, which is usually virus-free, as active cell division reduces the differentiation of vascular tissues [43]. Meristems are plant growth centers located in the apical and lateral buds and roots of fruit species, especially in) U D J Dp U [44,45].

1.2.1 Clonal propagation

Axillary bud curing is the most applied method for type appropriate in vitro propagation. Here, an apical bud or node section harboring an axillary bud is cultured to propagate multiple shoots without any intervening callus phase.

1.2.3 Sterilization method and initiation step

Blackberries can be propagated easily by using axillary shoots by initiating existing meristems [37]. As an example, protocols could be like that in this section, explants containing the apical meristem typically range in size from 1 to 5 cm and cut into single nodes, were washed firstly under running tap water for 15-30 minutes for pre-sterilization and then washed in a magnetic stirrer for 5 minutes with 2-3 drops of commercial detergent. They were taken into the sterile cabinet and first kept in 70% lethy alcohol solution for 2 minutes. Afterward, different concentration of chemicals containing a few drops of tween 20 ($NaOCl$, $HgCl_2$, H_2O_2, ClO_2) were used for different periods of time (10, 15 and 20 minutes). Then rinsed three times with autoclaved sterile water [28, 46, 47]

Explants of the blackberry variety were sterilized with chemicals to obtain an aseptic culture. In the initial stage, explants were treated with shoot tips and axillary buds, (0.0, 0.01, and 0.5 mg/L) auxin, NAA and IBA and cytokinin BA, Kinetin, 2-IP and TDZ (0.0, 0.5, 1.0 and 2, 0 mg/l) were cultured in MS, WPM, and DKW medium supplemented with GA3 (between 0.1 and 0.5 mg/l). 3% sucrose and 6 to 7 g/l were added to the agar. MS medium and auxin IBA or NAA (between 0.01 and 0.5 mg/l) and cytokinin BAP (between 0.5 and 1.0 mg/l) showed the best results [37, 47]

The combination of BA and TDZ used in culturing blackberry explants had a great impact on the amount and frequency of shoot induction with a high number of shoot lengths from the initial stage and the highest adventitious bud formation [48].

1.4 Multiplication of shoot-tip and rooting cultures

Fathy et al. (2018) carried out a research to obtain micropropagation of 5 X E X V I U X shoots, Rca lXs induction and increment of bioproduct content in both shoots and calli. In this research, MS, WPM, and B5 medium were used, and results showed that best shoot formation was observed on MS medium including 0.6 mg/l BA; shoots were rooted on MS medium including 2 g/l activated carbon for rooting, and MS medium supplemented with 0.5 mg/l NAA and 1.0 mg/l 2,4-D was obtained in callus induction and growth [27].

Baghdady et al. (2021), the highest shoot number and shoot fresh weight (g), micro shoots of the tested variety were applied in MS medium, three different applications: BA, KIN, and control. As a result of these applications, the best results were obtained from the application with BA at 1.0 mg / l [47].

Topçu (2022), MS basal culture medium, EDDHA, and different concentrations of cytokinin and auxin were used for axillary bud shoot formation, reproduction, and root induction for Rubus spp. The best results in shoot multiplication were obtained with 3.0 mg/l BAP, 0.3 mg/l GA_3, 0.1mg/l NAA, and 1.0 mg/l IBA in root formation [49]

Samaan and Nasser (2022), in their study, aimed to establish an in vitro micropropagation protocol for blackberry "Karaka Black" variety. They tested regeneration ability of root node explants in three different media, WPM, MS, and B5. They also supplied different concentration of BA, Kinetin and TDZ as cytokinin. The results showed that WPM followed by MS medium were showed the highest shoot length and leaf number. During the propagation stage, the most preferred values were BA at 0.4 ppm, Kin at 0.5 ppm, and TDZ at 0.1 ppm, but the one with kinetin gave the healthiest plantlets [50].

Aly et al. (2022) worked on the consequences of gamma irradiation on morphological and biochemical changes in blackberry plants. Gamma-irradiated (20, 40, and 60 Gy) plantlets were sub-cultured using MS medium. They determined the best shoot growth obtaining at as 40 Gy gamma irradiation [51].

Da Silva et al. (2022) evaluated the effect of the consistency of the culture medium for multiplication of shoots using different concentrations of BAP and for root formation using different concentration of IBA for "Ébano" and "Tupy" blackberry cultivars. In this study, explants were cultured on solid, liquid, and double-phase texture medium to determine the best culture medium for shoots. They found that multiplication of "Ébano" and "Tupy" cultivars can be performed in a double-phase MS medium with 5 μM L-1 BAP and rooting in an MS medium with 1.1 μM L-1 IBA [52].

Clapa et al. (2023) evaluated the proliferation capacity of blackberries grown in vitro in wheat starch-gel culture medium beside of classical agar-gel medium. For in vitro shoot propagation, MS medium supplemented with 0.5 mg dm^{-3} BA was tested. To determine the gelling agent, 0.5% plant agar, and wheat starch at 5% concentration was added into media, separately. The results showed that the highest number of shoots/grafts for all blackberry varieties was observed on a wheat starch-gel culture medium [53].

2 Conclusion

In blackberry propagation traditional methods such as sexual (seed) or vegetative methods (layering, cutting, shoot division, grafting) have not significant results leading to heterozygous progenies from seed, requiring more space, insufficient propagules etc. The in vitro propagation technique have many benefits such as rapid plant propagation, preservation of germplasm, elimination of pathogens, genetic manipulations, increasing the cultivation per plant by applying low-cost tissue culture for secondary metabolite production, plant production with sources and resources that will reduce the unit cost of micro propagules, somatic variation, cryopreservation in the genetic manipulation of plant cells, synthesis development facilitates mutations and the progression of genetic transformation

3 References

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