Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 2, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IN VITRO CULTURE OF KIWIFRUIT SPECIES (*ACTINIDIA* SP.) - A REVIEW

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Abstract

Actinidia deliciosa, known as kiwifruit, has gained a great popularity and demand due to its nutritional and medicinal value. Actinidia genus has at least three cultivated species with an important economic role. In vitro culture of kiwifruit species was an important tool for plant micropropagation and for unconventional breeding (direct organogenesis, callogenesis, somatic embryogenesis, cells culture, etc). The actual paper presents a comprehensive image of the main results that were achieved in different in vitro cultures of kiwifruit species. New alternative developments are being proposed in order to obtain efficient protocols of the species for production of clonal planting materials.

Key words: *callus, culture media, micropropagation, organogenesis, somatic embryos.*

INTRODUCTION

Actinidia deliciosa, commonly known as kiwi fruit and belonging to the *Actinidiaceae* family, is a dioecious woody climbing shrub. It thrives in temperate and cold regions as well and is cultivated for its flavourful fruit. Among the various species within the *Actinidia* genus, *A. deliciosa, A. arguta* and *A. chinensis* are the three specifically grown for their fruits.

Rugini and Gutierrez-Pesce (2003) note that kiwifruit is notable for its high vitamin C content, with *A. deliciosa* providing 140 mg per 100 g of fresh weight. The fruit also contains pretty high levels of minerals, mainly potassium and magnesium, as well as trace elements such as copper, zinc, and manganese. While kiwifruit is commonly consumed fresh within 1 to 6 months post-harvest, it is also processed for various food products, including jams, juices, and syrups.

In Romania, kiwi is recognized as an imported fruit and is predominantly available in supermarkets at a relatively high cost. The experimental orchard belonging to the Faculty of Horticulture in Bucharest, Romania, has kiwi fruits planted since 1993, and since then, efforts have been made to develop optimal techniques for cultivation, management, and micropropagation, resulting positive outcomes. (Stănică et al., 2007)

The successful marketing of *A. deliciosa* cultivars has motivated the initiation of breeding programs with the goal of enhancing the species value. Nevertheless, the species' dioecious nature, prolonged juvenile period, and inadequate rooting, are constraints to these breeding initiatives.

Kiwifruit plants needs approximately 25 weeks from flower bloom to achieve physiological maturity (Mardiana et al, 2018). Consequently, obtaining large quantities of kiwi seeds out of seeds would require a considerable amount of time.

The most cost-effective technique for capturing genetic gains in horticulturally important plants is the production of clonal planting material from vegetative tissue through conventional methods. However, this method is often limited by the poor rooting of cuttings. In contrast, tissue culture techniques provide a fast and reliable alternative for producing genetically uniform clonal material in a shorter time frame, making them highly effective for the propagation and conservation of species with reproductive challenges.

Actinidia sp. is commonly propagated through grafting and stem cuttings, but the limited

cuttings rooting success restricts the production of clonal planting materials.

The initiation of an *in vitro* culture, using plant materials sourced from the field is a crucial step in establishing a clonal propagation process. The effective establishment of *in vitro* cultures is influenced by factors such as genotype, health of the mother plant grown in the field, preparation of the explant, surface sterilization procedures, medium composition, and growth conditions, etc.

The purpose of this paper is to provide a brief overview of the achievements and progress made in the recent years regarding the *in vitro* micropropagation of *Actinidia* species.

HISTORY

Tissue culture with its *in vitro* manipulation of plant cells, tissues, and organs, stands as a prominent biotechnological technique. Several tissue culture methods have been applied to overcome the challenges associated with traditional reproduction in *Actinidia*. Harada (1975) was the first to propose a micropropagation protocol for *Actinidia*, which was later refined by Standardi (1983), Wessels et al. (1984), and Monette (1986). The Murashige and Skoog (MS) method has become widely used for preparing the culture medium, demonstrating benefits for both regeneration and callus formation. Additionally, other media such as Gamborg B5 and N6 have demonstrated success in this context.

STERILIZATION

The sterilization of explants is a critical stage in plant tissue culture, as it is necessary to eliminate all microorganisms, including bacteria and fungi. This step is essential for achieving successful initiation, growth, and development of cultured tissues in vitro. Without proper sterilization, the cultured tissues would be susceptible to contamination and compromised in their development. Deb et al. (2019) described a successful sterilization protocol involving the collection of nodal segments from branches, which were then wrapped in fresh banana leaves and moistened with sprinkled water to retain humidity. The scales and other impurities were carefully removed, followed by washing under running tap water. The segments were stored at 4°C in a refrigerator until needed. For surface sterilization, 2 cm nodal segments were gently scrubbed with a soft brush using "Labolene" (a commercial laboratory detergent at 1:100, v/v). This was followed by washing under running tap water for 10-15 minutes, sterilization with an aqueous solution of $HgCl_2$ (0.3%, w/v) for 3-5 minutes, and subsequent washing 4-5 times with sterile water.

In a study done by Zhong et al (2021), the stem segment containing a single bud from the kiwifruit underwent disinfection using 70% alcohol and 15% NaClO. The outcomes demonstrated that the rates of contamination and browning could be maintained below 20%, and the survival rate could surpass 70% when the single bud stem segment of the kiwifruit was sterilized with 70% alcohol for 30-60 seconds and 15% NaClO for 15 minutes, respectively.

MULTIPLICATION STAGE

Various vegetative explants, such as leaves, bulbs/pseudo-bulbs, roots, axillary buds, nodal segments, and inflorescences have been utilized for the propagation of various agronomical significant species. Deb et al. (2019) identified nodal segments with axillary buds as the most effective method for producing clones among various propagation techniques.

Based on the previously achievements in what is currently referred to as *A. deliciosa* (mainly in cv. 'Hayward') and *A. chinensis*, plant regeneration has been achieved in *A. arguta* using young leaves (Zhang and Qian, 1996; Zhu, 1997) and mature embryos (Xiao et al, 1990). Similarly, in *A. deliciosa,* regeneration has been accomplished from mature endosperm (Xiao et al, 1990), cell suspensions (Zhang et al, 1991), long-term stored stems (Ding et al, 1997), and in interspecific hybrids of *A. deliciosa* x *A. arguta* (Famiani et al, 1997).

Factors beyond traditional plant growth regulators and hormones that impact plant regeneration have been investigated. Coconut milk has been demonstrated to enhance axillary shoot growth (Boase et al, 1993), while vermiculite is noted to help in the rooting of

regenerated shoots, either alone or in combination with agar *in vitro* (Chen 1997, Wang 1997). Feito et al (1994) discovered that the metabolic activity of BA and endogenous cytokinins is influenced by the physical composition of the culture medium.

Pedroso's et al. (1992) discovered that inoculating nodal segments and shoot tips on Murashige and Skoog (MS) media without growth regulators, it results in a good plant development at a reduced cost and minimal manipulation.

In recent years, increased efforts have been directed towards comprehending the mechanism of plant growth regulator uptake, particularly cytokinins. The absorption and metabolism of 6-benzyladenine (BAP) were investigated in *A. deliciosa* explants cultivated in ventilated cultures, a method employed to prevent the formation of hyperhydric shoots during propagation in liquid medium. Cañal et al. (2000) reported that 65% of the initial amount of BAP disappeared within the first half-hour of culture, converting into seven distinct glucosides. Moncaleon et al. (2001) further demonstrated that 6-benzylaminopurine (BAP) plays a critical role throughout various phases of micropropagation and influences the development of regenerants. The highest quality shoots, measured by multiplication index, weight, length, and callus formation, were achieved after one day of cultivation with 4.4 mg L^{-1} of BAP. Additionally, zeatin was identified as the most effective cytokinin for inducing shoot regeneration (Akbaş et al., 2007). Herrera et al. (2005) obtained optimal results in shoot multiplication and elongation using Cheng K medium supplemented with 0.05 µM NAA, 22 µM BA, and 1.4 µM GA3 (Prado et al., 2002). In an experiment conducted by Akbaş et al (2007), the use of BAP resulted in a more favourable outcome for shoot production compared to the kinetin treatments. Optimal results for kiwifruit shoot production were achieved with 0.5 mg L^{-1} BAP. Consistent with these studies, Moncaleon et al. (2001) noted that BAP significantly affects multiple stages of micropropagation and also plays a role in regulating the development of regenerants. A comparison is summarized in Table 1. In analogous findings concerning cv. Hayward kiwifruit, zeatin appears to be the most effective in inducing shoot regeneration from callus, while BAP is typically employed for shoot proliferation (Rugini et al., 1991). Marino and Bertazza (1990) found that BAP caused hyperhydricity in older leaves, an effect that was not seen with zeatin, even though zeatin resulted in higher proliferation rates. A significant increase in shoot proliferation was documented when employing a liquid medium containing 8.9 µM indole butyric acid (IBA), with chilled lateral buds proving to be the most effective for multiplication (Lionakis & Zirari, 1991). Pais et al. (1987) reported successful proliferation by employing a combination of 50% MS macro- and micronutrients, full-strength MS vitamins, and 5 mg L^{-1} ascorbic acid as an antioxidant, along with 2.3 µM zeatin and 0.3 µM IAA during a 4-week subculture period. The most favourable average root number and length per shoot were achieved with media supplemented with 1.0 mg L^{-1} NAA (Akbaş, et al. 2007).

Table 1. Plant growth regulators and hormons aiming successful shoot multiplication for *Actinidia* sp.

Type of growth regulator	Author	Year
BAP	Rugini et al.	1991
BAP	Moncaleon et al.	2001
Zeatin	Moncaleon et al.	2001
Cheng K+NAA+BAP+GA_3	Herrera et al.	2005
0.5 mg L^{-1} BAP	Akbaş et al.	2007

Mardiana et al. (2018) demonstrated the significance of gelling agents in influencing the growth of kiwi shoots. Based on the findings of this study, the results indicated that the treatment with Swallow Globe Agar at 4 g/l combined with Nutrijell Agar at 4 g/l resulted in the highest average number of shoots, as well as the highest number of leaves and roots. This substantiates that the combination of Swallow Globe and Nutrijell agar creates favorable conditions for the growth of kiwi shoots.

Deb et al. (2019) successfully established an efficient *in vitro* propagation protocol, utilizing nodal segments as *in vivo* sources, implementing an effective rooting technique, and conducting primary hardening with cost-effective substrates as an alternative to agar.

Fortified with 3% (w/v) sucrose, Polyvinylpyrrolidone (300 mg/L), and benzyl adenine (BA) (6 µM), 7-week-old nodal segments were established on MS medium, resulting in an average formation of 4.2 shoot buds in 75% of cultured segments. Additionally, using MS medium supplemented with 3% sucrose and 3 µM BA produced 2 micro shoots in 58.3% of cultures per cycle. The activation of axillary buds relies on a comparatively high concentration of cytokinin, which inhibits apical dominance and facilitates the growth of buds (Pierik, 1987). Velayandom et al. (1985) and Monette (1986) identified this method, which uses nodal segments and shoot tips, as simpler than other micropropagation techniques. It consistently generates a substantial number of uniform shoots from a single explant in a short period, maintaining genetic stability. Zuccherelli (1981) noted that for *Actinidia* species, particularly in large-scale cultivation, this approach is the most practical propagation method. Standardi (1981, 1983) and Wessels et al. (1983) emphasized the importance of carefully avoiding the propagation of shoots derived from basal callus, as *Actinidia* species easily regenerate from undifferentiated cells and are prone to somatic variation. For root induction, various techniques are utilized, many of which rely on indole-3-butyric acid (IBA) as an effective root inducer. A common method involves briefly immersing the base of the shoot in a high-concentration IBA solution (50 mg/L) to promote root formation. An alternative method includes the overnight immersion of the shoot's basal part in solutions with low IBA content (0.3, 1, 2 mg/l), resulting in a high rate of successful rooting (adapted from Standardi, 1981; Wessels et al., 1983).

CONCLUSIONS

In vitro propagation through the development of axillary buds is quicker than conventional methods and needs a small quantity of starting material, the *in vitro* plants propagated in this way in many species have proved to be true to type (Murashige, 1974; Navarro et al., 1975; Styer, 1983). Therefore, it is the best way for propagation for new cultivars or clones for fruit production. Before this, *in vitro* propagation technique is to be used for propagation of *Actinidia sp.*, the mother plants of this origin must be evaluated, but can be used immediately for breeding purposes.

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