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Effects of Some Natural Organic Additives *in Vitro* Plant Propagation

Betül Ayca DONMEZ TELEK[1,2][0000-0002-7631-7384] *,Şule POLAT[3][0009-0005-9131-9694], Awara Muhammad HAMAKHAN[4][0000-0003-2351-4453], and Nesibe Ebru KAFKAS[3][0000-0003-3412-597]

[1]University of Kirsehir Ahi Evran, Faculty of Agriculture, Department of Agricultural Biotechnology, Kirsehir 40100, Türkiye
[2]University of Cukurova, Institute of Natural and Applied Sciences, Biotechnology Department, Adana 01330, Türkiye
[3]University of Cukurova, Faculty of Agriculture, Department of Horticulture, Adana 01330, Türkiye
[4]Bakrajo Technical Institute, Sulaimani Polytechnic University, Protected Cultivation Dept., Ministry of Higher Education, Iraq

betul.donmez@ahievran.edu.tr

Abstract. Tissue culture is a technique that enables the rapid and large-scale multiplication of plants using any parts of them under aseptic conditions. Its success depends on various factors, including the plant species and cultivar, type and age of the explant, culture conditions, and the composition of the culture medium. Determination of specific nutritional requirements of plants can be challenging in tissue culture studies which makes the optimization of culture media components essential. However, the high cost of some medium components has driven the search for alternative, low-cost organic additives that do not compromise plantlet quality. Numerous studies have investigated the effects of organic growth additives on plant development. Commonly used complex organic additives are plant extracts such as coconut water (CW), banana extracts, and a variety of fruit juices; casein hydrolysate (CH), yeast extract (YE) etc. These additives provide natural carbon sources and are rich in vitamins, phenolic compounds, fiber, hormones, proteins, lipids, and minerals. Previous studies have demonstrated that modification of medium composition with natural complex additives can induce cell division, stimulate callus formation, and support both rooting and shoot development. Optimum concentration of these additives is crucial for promoting plant or cell growth and development. This review will focus on the effects of supplementing culture media with coconut water, banana homogenate, and casein hydrolysate as organic additives on *in vitro* plant growth and development. Understanding the effects of these natural additives may prove valuable for plant species that have not yet been investigated in this respect, offering a foundation for future research.

Keywords: tissue culture, organic additives, *in vitro* propagation

1 Introduction

Plant tissue culture is a biotechnological technique that enables the propagation of plants from any explant tissue containing meristematic cells, cultivated under aseptic conditions on a nutrient culture medium. This approach offers wide-ranging applications, including: (1) the large-scale clonal plantlet propagation (micropropagation); (2) the production of disease-free plants through meristem culture; (3) the genetic conservation of endangered and rare species; (4) crop improvement via somaclonal variation and mutation breeding; (5) valuable secondary metabolites production for pharmaceutical and industrial purposes; (6) the creation and regeneration of genetically modified plants through genetic transformation

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techniques; (7) the generation of haploid and dihaploid plants via anther and microspore culture; (8) somatic hybridization through protoplast fusion; (9) long-term germplasm preservation using cryopreservation methods; and (10) virus elimination to produce pathogen-free planting material [1]. This technique also offers an effective application for meeting demand for planting material and afforestation programs to tackle impacts of global climate change and human activities on agriculture-forest-livestock systems [2]. Success of plant tissue culture studies depends on varying factors including plant species and even genotype; types of explants; age and health of the donor plant; physical culture conditions such as temperature, humidity and light; culture media composition etc. Since this technique can be genotype-specific depending on the species used, optimization of the culture medium composition is essential for successful large-scale plant propagation [3].

The culture medium typically consists of macronutrients and micronutrients essential for plant growth, vitamins and minerals that support metabolic functions, amino acids that promote cell division and differentiation, sugars serving as energy sources, gelling agents to solidify the medium, and plant growth regulators (PGRs) that govern morphogenesis and organ development [4]. PGRs are crucial components of the culture medium. They have various effects on plant growth and development depending on their types and concentration. Since plant growth regulators (PGRs) can be effective even in small amounts, determining their optimum concentrations will be beneficial for large-scale micropropagation studies, for enhancing secondary metabolite production in pharmaceutical applications. Although tissue culture techniques have many applications as mentioned above, components of culture medium are expensive. So, it is required for the development of inexpensive options for low-cost tissue culture technology. For this purpose, many studies have been conducted to determine the effect of cost-effective organic additive plant extracts on plant growth and development, ensuring that the quality of the produced plants is not compromised [5]. A variety of complex organic additives are used as supplements in plant tissue culture media, such as natural extracts (coconut water, corn extract, potato extract banana extract), fruit juices (tomato, orange, papaya), protein hydrolysates (casein hydrolysate, peptone, yeast extract) which provide essential nutrients and natural growth regulators to enhance in vitro growth and morphogenesis [6] These additives contain natural carbon sources and are rich in vitamins, phenolic compounds, fiber, hormones, proteins, lipids, and minerals [7].

This review will examine the effects of organic additives such as coconut water (CW), banana homogenate, and casein hydrolysate on in vitro plant growth and development. Understanding their

effects could contribute to future research by providing a scientific basis for plant species that have not yet been investigated in this respect.

2 Some Organic Growth Additives to enhance *in vitro* Culture Techniques

Culture media generally contains basal components (including a carbon and energy source, inorganic salts, vitamins, PGRs) and optional (organic nitrogen compounds, organic acids, and kind of complex natural extracts) components. Optional components can be supplemented into medium depending on purpose of study to enhance *in vitro* plant growth and development. Moreover, types of organic nutrient and their concentrations are critical depending on specific needs of species or tissues and even genotype [4,8].

2.1 Effect of Coconut Water (CW) as an Organic Additive on in vitro Plant Growth and Development

Coconut water is called "Fluid of Life" since it is rich in amino acids, nutrients, minerals, essential electrolytes, vitamins and phytohormones and is low in sugars and calories [9]. It acts as plant hormone sources (auxin and cytokinin), containing compounds such as indole-3-acetic acid (IAA), kinetin, and zeatin which enhance cell division in the roots and shoot systems, adventitious root development, and micropropagation [10].

Many studies have been conducted using coconut water as an organic additive into culture medium to enhance seed germination, shoot multiplication and elongation, and root induction. Although orchids produce numerous seeds, their small size and lack of nutritional reserves result in a low rate of natural reproduction. Furthermore, the seeds of some species have hard seed coats, which can make germination difficult. The addition of coconut water to the nutrient medium showed a markedly positive effect on in vitro seed germination, protocorm formation, and seedling development of Cypripedium macranthos Sw., compared with birch sap, maple sap, banana powder, and peptone [11].There are many studies conducted using endangered and endemic epiphyte orchid species on addition of coconut water on medium and resulted with higher seed germination and protocorm proliferation [12 13; 14] . De Stefano et al. (2022) observed similar result on night scented orchid seeds germination, two times more than control medium [5]. Being a natural carbon source, coconut water has also been determined to facilitate seed germination due to containing amino acids, vitamins, minerals and various organic ions [15].

Peixe et al. (2007) reported that coconut water and BAP can replace zeatin for olive micropropagation [16]. Rate of shoot multiplication enhanced with addition of coconut water to medium, resulting in a cost-effective medium formulation. Study on Asparagus officinalis micropropagation, 20% (v/v) coconut water became the most effective organic additive for shoot induction and root formation in the in vitro plantlets. Moreover, the longest shoot and root lengths were also observed on the same medium. [17]. Conversely, some studies have shown that higher concentrations of CW delayed the development of purple coneflower [18], inhibited shoot elongation, and reduced the shoot length of Dragon Fruit trees [19]. Therefore, there is a need to determine optimal concentrations for different plant species, standardize its use, and further investigate its mechanisms of action. Such studies would facilitate the broader integration of coconut water into plant biotechnology applications.

2.2 Effect of Banana Homogenate (BH) as an Organic Additive on *in vitro* Plant Growth and Development

Bananas are one of the most commonly consumed fruits due to their low cost and high nutrient content containing carbohydrates, proteins, a variety of minerals (Na, Fe, K, Ca, Mg, Ma, Zn) and vitamins (A, B1, B2, B3, B6, pantothenic acid, folic acid and ascorbic acid) [20, 21]. It contains natural auxins and gibberellins, which act as plant growth regulators in vitro studies. It has been used as an organic additive and energy sources for in vitro previous studies to support the plant growth and development especially for heterotrophic plants during the early stages of in vitro cultivation [22].

There are various studies in literature conducted using banana homogenate as an organic additive in medium resulting in enhanced seed germination [23]; protocorm formation and regeneration [24]; improved shoot and root elongation supported shoot length and root growth [25]. Gansau et al. (2016) investigated effect of different types of organic additives (coconut water, tomato juice, banana pulp and peptone) on protocorm proliferation and development for Dendrobium lowii. The highest growth index was obtained from 25 g/L banana pulp treatment, where 100% of the protocorms developed into shoots [26]. Islam et al. (2015) found that effect of different concentration of banana homogenate varied on the Dendrobium sp. var. Sonia protocorm in terms of PLBs multiplication and plantlet regeneration. While 1/2 MS medium supplemented with 100 ml L-1 of banana homogenate was the best for protocorm like body multiplication, shoot and root regeneration was observed from 25 ml L-1 BH treatment [24]. It was considered that excessive BH may inhibit cell growth and development due to high concentration of sugars or calcium or sodium. Lee et al. (2022) also reported that increasing the amount of BH in the

medium led to similar effects, inhibiting the growth and development of Ficus carica cv. Japanese BTM 6 [27].

The highest number of shoots and leaves, as well as the greatest increase in shoot height, was observed in plants cultured on medium supplemented with 10 g/L BH among the four treatments tested. Banana homogenate is one of the most common organic additives in vitro studies. But efficiency of organic additives varies species worked on or even genotype and concentration of additives. The determination of the optimum and efficient concentration of banana homogenate incorporated into the culture medium may contribute to supported plant growth and development and a reduced input cost resulted from plant growth regulators.

2.3 Effect of Casein Hydrolysate (CH) as an Organic Additive on *in vitro* Plant Growth and Development

Casein hydrolysate (CH) is an organic compound containing low molecular weight proteins, amino acids, vitamins, calcium, phosphate, which enhances plant growth by providing a source of reduced nitrogen [28]. Additionally, plant cells can efficiently metabolize and utilize nitrogen from organic sources compared to inorganic sources, highlighting CH as an effective source of nitrogen [29].

Several researches have conducted to show how CH effected the distinct plant species in terms of somatic embryogenesis [30], callus induction [28,31] seed germination and seedling growth [32], shoot regeneration and proliferation [33,34]. Ramakrishnan et al. (2013) found that frequency of callus induction and embryogenic callus formation in Allium cepa L. enhanced with addition of glycine, proline, and casein hydrolysate [31]. Effect of different organic additive also tested were on Barhi Date Palm (Phoenix dactylifera L.) for somatic embryo formation and shoot regeneration. It is determined that casein hydrolysate addition (5.0 g/L and 2.5 g/L) induced secondary somatic embryo formation and enhanced the plantlet regeneration, respectively. However, yeast extraction addition found that not effective as well as coconut milk (30%) and CH (2.5g/l) in term of readings in all assessed concentrations and plantlet regeneration [30]. A study work on okra Abelmoschus esculentus genotype (CoBhH1) reported by Daniel et al. (2018) that CH addition into MS medium supplemented with different type of auxins (2,4-D, NAA) and L-glutamine induced the somatic embryogenesis and improved new plantlet formation from cotyledonary leaf explants. Also, regenerated plants examined by ISSR analysis were determined as morphologically similar to the parent plant [35]. According to Al-Asadi et al. (2024), combination of CH and dicamba (DIC) enhanced the callus development (4.0 mg/L (DIC) + 1.0 g/L

CH), shoot proliferation (4.0 mg/L DIC + 0.5 g/L CH), and biochemical properties of the Barhee date variety [36]. In similar study, Amer et al. (2017) searched on two Egyptian rice cultivars to determine the effect of different concentrations of tryptophan, glutamine, and casein hydrolysate separately. They found that addition of CH encouraged in both callus induction (300 mg/L for both cultivars) and shoot regeneration (100 and 200 mg/L) [28]. There are also various studies conducted to determine effect of casein alone or combined with different types of auxin and cytokines in terms of shoot induction and multiplication. Samiei et al. (2021) stated that CH addition (600 mg/l) into media containing a constant amount of (BAP) and (NAA) promoted shoot proliferation (173%) whereas silver nitrate (100 mg/l) resulted in the formation of the longest (2.5 cm length) and highest quality shoots on in vitro propagation of Rosa canina. [33]. In similar study, Georgieva et al. (2025) reported that addition of CH into medium enriched with BAP and IBA combination increased the shoot length and multiplication of black raspberry cv. Cumberland [34]. Moreover, it is reported that BAP (2.5 mg/L) combined with 600 mg/L CH resulted in the highest root length on Red Barangan banana [37]. As for concerning seed germination and seedling growth, Borbolla-Pérez et al. (2024) established an in vitro asymbiotic germination protocol for Vanilla planifolia seeds due to their low germination rate. They tested different types of medium, different concentrations of zeatin and CH, and their combinations. They observed the highest viability and vigorous seedling growth (74.5%) with 1/4 MS supplemented with 500 mg/L casein hydrolysate, whereas the combination of zeatin with casein hydrolysate (CH = 500 mg/L; zeatin = 0.50 mg/L) resulted in 53% callus formation [32]. However, some researchers found that high amount of casein hydrolysate (200–500 mg/l) usage resulted in shoot tip necrosis and vitrification [35], as well as growth retardation [38,39] in some plant species which means that of casein hydrolyte addition into medium varies to species, even genotype and purpose of study. That's why more studies should be conducted to determine optimum concentration of casein hydrolysate used for plant growth and development each plant species.

3 CONCLUSIONS

Natural organic additives contain carbohydrates, proteins, a variety of minerals and vitamins, essential electrolytes and phytohormones for plant growth and development. Various studies have conducted using different kind of organic additives improved callus induction, somatic embryogenesis, enhanced shoot length and proliferation and root formation. Including those organic compounds into plant tissue culture studies could be sustainable and cost-efficient approach. But it is requisite to determine the

optimum concentration of organic additives in the medium, depending on species, genotype, type of explant, and plant species requirements, to standardize their application in tissue culture protocols and to conduct further studies to better understand how they influence cellular and developmental processes.

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