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Effect of Adding Some Substances in Media for Induction and Development of Callus

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Abstract: Callus is a mass of unspecialized parenchyma cells, and its size and growth increase depending on the type and quantity of growth regulators present in the nutrient medium. This tissue is commonly used for plant propagation through tissue culture by inducing and growing it on media containing auxins, cytokinins, amino acids, sucrose, glutamine, salicylic acid, and sodium chloride, and the effect of these additions on enhancing callus induction.

Key words: Callus, Salicylic acid, Amino acids, Sucrose.

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Introduction

Research studies have shown that the basic structure of alkaloids, particularly the tropane group, is derived from certain amino acids and other biological molecules **(Al-Shahat, 2006)**. To increase the production of secondary metabolites, it is essential to add the intermediate compounds that produce them, including amino acids. It has been observed that their addition has increased the production of tropane alkaloids **(Silvestrini *et al.*, 2002; Whitmer *et al.*, 1998; Moreno *et al.*, 1993)**.

Tryptophan

Tryptophan is one of the important amino acids involved in the biosynthesis of alkaloids, which are primary metabolic products from which alkaloid groups are formed, providing the carbon skeleton and nitrogen components for alkaloids **(Sarin, 2005; Al-Saadawi and Younis, 1991)**. This was confirmed by **Dmitruk (1973)** in his study on the effect of amino acids on the production of tropane alkaloids from the plant *Scopolia* sp. It was

found that the amino acid Tryptophan was the most effective in producing the alkaloid Hyoscyamine and the alkaloid Scopolamine.

Glutamine

Glutamine is one of the amino acids that contribute to the structure of cell proteins and thus the construction of enzymes that play an important role in the biosynthetic processes of plants. It also helps regulate the acid-base balance of the cell to produce ammonia and is an important source of cellular energy or a carbon source. Additionally, it provides nitrogen in many biological processes, including the synthesis of purines, which are involved in the construction of nucleic acids, as well as being a source of carbon and an important transporter of ammonia **(Yuneva *et al*., 2007; Hall and Guyton, 2006; Aledo, 2004; Brosnan, 2003)**. **Taha *et al*. (2009)** cultivated callus from the plant Catharanthus roseus L. on MS liquid medium supplemented with five types of amino acids and found that cultivating the callus on the medium supplemented with 300 mg/L of glutamine increased the alkaloid content in the cultivated callus, producing the highest amounts of Vincristine and Vinblastine.

Salicylic Acid

The role of SA is embodied in plant growth and development, as it affects photosynthesis, transpiration, and the absorption and transport of nutrients within the plant **(Sakhanokho and Kelley, 2009; Hayat and Ahmad, 2007)**. It also induces or causes distinctive changes in the anatomy of the plant leaf and the structure of chloroplasts. It plays an important role in inducing plant resistance against pathogens by stimulating plant cells to produce Pathogenesis-Related Proteins **(Verberne *et al*., 1999; Yalpani *et al*., 1993)**. Furthermore, salicylic acid is involved in the acquired systemic resistance that occurs as a result of a pathogen attack in one part of the plant, and this signal can be transmitted to neighboring plants, prompting them to develop resistance by converting salicylic acid into the volatile ester methyl salicylate **(Hayat and Ahmad, 2007; Molinari, 2007). Eskandari *et al*. (2012)** studied the effect of adding SA at different concentrations on the alkaloids hyoscyamine and scopolamine from genetically modified hairy root cultures of the belladonna plant (*Atropa belladonna* L.) in MS medium, obtaining the highest amount of the mentioned alkaloids from the culture at 34 mgÂ·L^-1 SA, which were 1.6 mg/gram hyoscyamine and 1.3 mg/gram scopolamine. **Pitta *et al*. (2000)** demonstrated that the use of SA at different concentrations led to an increase in the accumulation of hyoscyamine and scopolamine alkaloids from the hairy root culture of *Brugmansia candida* in MS medium. **Al-Muhari (2014)** managed to obtain the highest amount of callus, 0.584 g, from the leaf culture of the periwinkle plant (*Catharanthus roseus* L.) in MS medium supplemented with 0.75 mgÂ·L^-1 SA and 0.75 mgÂ·L^-1 2,4-D after 50 days of culture.

Sodium chloride

Sodium chloride is a salt that increases the negative osmotic potential of the cell, thereby enhancing the exchange of solutes within the cell, which plays a role in increasing the process of photosynthesis and consequently the production of secondary metabolites. The composition

of the nutrient medium supplemented with sodium chloride has profound effects on cell growth and the accumulation of secondary metabolic products, although many of these products are not associated with cell growth. The term "Elicitation" refers to the treatment of plant cells with biotic or abiotic stress factors, which is an effective means of improving the production of secondary metabolites in plant tissue culture. This strategy is based on the premise that this accumulation is part of the defensive reactions of plants against pathological infections and environmental stresses **(Zhou and Wu, 2006)**. Numerous experiments have demonstrated the effect of adding sodium chloride to the culture medium on biological processes. **Aljibouri *et al*. (2012)** indicated that the addition of 200 mg/L sodium chloride to the MS nutrient medium for the callus induced from the plant *Hyoscyamus niger* L. resulted in a significant increase in the amount of Scopolamine and a decrease in the Hyoscyamine compound compared to the control treatment. **Abdel Rahman *et al*. (2013)** in a study on the production of Atropine and Scopolamine in the plants *Datura metel* and *Datura stramonium* found that the addition of 7 mg/L sodium chloride to the MS nutrient medium led to an increase in the compounds Atropine and Scopolamine in the induced callus compared to the control treatment without sodium chloride after one month of cultivation. **Al-Muhari (2014)** indicated that the highest amount of callus, 0.659 g, was obtained from the cultivation of leaves of the plant Catharanthus roseus on the MS nutrient medium supplemented with NaCl at 1.0 mg/L.

Sucrose

Sucrose is the most commonly used sugar as an energy source in the propagation of plants outside the living organism. It is a non-reducing ketonic disaccharide composed of one glucose unit and one fructose unit **(Al-Bahr *et al*., 1999)**. **Laszol (2003)** found that the highest fresh weight of the callus was when the root hairs of the *Datura innoxia* plant were cultured on MS nutrient medium supplemented with 10 and 20 g/l sucrose. **Iranbakhsh *et al*. (2006)** indicated that the highest production of alkaloids from the *Datura stramonium* plant was from the callus induced on MS nutrient medium supplemented with 20 g/l sucrose after 5 weeks of cultivation. **Aljibouri *et al*. (2012)** showed that culturing the induced callus of the *Hyoscyamus niger* L. plant on MS medium supplemented with 50 g/l sucrose resulted in a significant increase in the amount of Scopolamine and a decrease in the amount of Hyoscyamine compared to the control treatment.

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الخلاصة

يعد الكالس تجمع من خلايا برنكيمية غير متخصصة ويزداد حجم هذا الكالس ونموه وفق نوع منظمات النمو وكميتها الموجودة في الوسط الغذائي وعادة يستخدم هذا النسيج في إكثار النباتات بالزراعة النسيجية من خلال استحداثه وتنميته على الأوساط الحاوية على الاوكسينات والسايتوكاينينات والاحماض الأمينية والسكروز والكلوتامين وحامض الساليسليك وكلوريد الصوديوم ومدى تأثير هذه الإضافات في زيادة استحداث الكالس.