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In vitro propagation

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Abstract: Biotechnological techniques are currently considered one of the most important means that have helped improve the production of various agricultural crops and the production of food and medicine. They introduce new methods to accelerate plant improvement and have enormous potential to overcome some obstacles that hinder the increase of agricultural production. In recent years, there has been growing interest in practical applications in the field of biotechnology, especially tissue culture technology, which relies on simple methods that do not require complex and costly laboratory equipment and can be practiced without significant investments in equipment and infrastructure. Plant tissue culture has emerged as one of the most prominent technologies of the twentieth century, pushing agricultural sciences beyond their traditional boundaries, as it has become possible to produce plants in the laboratory instead of the field, produce somatic embryos to be used instead of seeds by producing artificial seeds, and produce plant hybrids through protoplast fusion instead of crossbreeding and pollination.

Key words: *In vitro*, induction, multiplication, rooting.

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Introduction

During their growth stages, plants build a set of metabolites that they use for growth and development, including glycosides, phenols, alkaloids , and others . These compounds are important for the plant 's survival and spread in the natural environment , primarily serving as defensive materials against pathogenic agents. They are also pharmaceutical substances, food flavors, dyes, perfumes, or insecticides. Plants are a continuous source of work for producing industrial plant **products (Vanisree et al., 2004 and Tripathi and Tripathi, 2003)**. Natural products are considered key components of many pharmaceutical drugs, and

thei increasingi use and significant trend in recent years towards the use of herbs and medicinal materials , along with the urgent need for them , has begun to pose a major threat to plants and natural resources due to their removal and environmental destruction. Therefore, tissue culture technology and the generation of planti cells and organs outside the living organism are indispensable tools for producing pharmaceutical materials derived from plants . This technology has significant benefits in terms of quality, quantity , and controlled production without being constrained by natural factors such as geographical location , seasonal changes , or environmental stresses **(Zhou and Wu, 2006)**. According to **Al-Kanani (1987)**, Murashige divided the stages of tissue culture propagation into four stages:

1- Initiation.
2- Multiplication.
3- Rooting.
4- Acclimatization.

Initiation Stage

This stage is generally considered the first stage of tissue culture under sterile conditions, aiming to obtain plant cultures free from pathogenic agents by using materials to sterilize plant parts, as well as selecting the plant part (explant) and choosing the appropriate environment, and then planting it under sterile conditions **(Salman, 1988)**. **Hamad and Jassim (2011)** indicated that completely contamination-free seeds of the plant *Atropa belladonna* L. were obtained by soaking in a 4.5% and 6.0% sodium hypochlorite solution for 15 minutes each, followed by washing with sterile distilled water three times . **Iranbakhsh *et al.* (2006)** managed to sterilize *Datura stramonium* L. seeds with 90% ethanol for 2 minutes and then soaking in a 5% calcium hypochlorite solution for 15 minutes , followed by washing with sterile distilled water twice. **Abdel Rahman and others (2008)** stated that the lowest contamination rate was obtained by using 70% ethanol for 5 minutes to sterilize the seeds of the plant *Datura metel* L., followed by immersion in a 50% bleach solution with the addition of drops of Tween-20 for 10 minutes , and then washing with sterilized distilled water several times **Elwan (2009)** mentioned that the best germination rate for the seeds of the pepper plant Capsicum annuum L. was achieved by sterilizing them with 70% ethanol for 2 minutes, followed by immersion in a 20% sodium hypochlorite solution for 20 minutes, and then washing with sterilized distilled water 3 times. Otroshy and others (2011) obtained the lowest contamination rate for the seeds of the pepper plant *Capsicum annuum* L. when sterilized with 70% ethanol for 1 minute , followed by immersion in a 4% sodium hypochlorite solution with the addition of a drop of Tween-20 for 20 minutes , and then washing with sterilized distilled water 3 times. **Aljibouri and others (2012)** achieved the lowest contamination rate from sterilizing the seeds of the plant *Hyoscyamus niger* L. with 70% ethanol for 2 minutes, followed by immersion in a commercial Clorox solution at a concentration of 50%, which contains 6% sodium hypochlorite, with the addition of drops of Tween-20 for 10 minutes , and then washing with sterilized distilled water 4 times. **Ghorbanpour and others (2013)** reported that the lowest contamination rate was obtained by using 70% ethanol for 2 minutes to sterilize the seeds of the plant *Hyoscyamus niger* L.,

followed by immersion in a commercial solution at a concentration of 25%, which contains 6% sodium hypochlorite for 10 minutes, and then washing with sterilized distilled water twice.

Multiplication Stage

This stage is one of the important stages of propagation and has the largest part in the success or failure of the multiplication process . It depends on the total number of plants produced and their quality. It refers to the selection of the appropriate nutrient medium that achieves the highest growth and multiplication rate. There are several approaches, including increasing the number of plants by breaking the apical dominance of branch tips through the growth of lateral branches, or by differentiating the callus into branches **(Al-Rifai and Al-Shubki, 2002; Muhammad and Al-Rais, 1982)**. The interaction between the plant part and the type of cytokinin used plays an important role in the extent of response to multiplication. Many researchers have shown the role that benzyl adenine plays in the multiplication of nodes. **Rodr *et al.* (1991)** demonstrated that culturing nodes of *Datura insignis* derived from tissue culture and grown on MS medium supplemented with 0.004 mg/L NAA and 0.25 mg/L BA resulted in the highest multiplication rate of 14.81 branches per plant part after 4 weeks of cultivation. **Deliu *et al*. (2002)** found that culturing nodes of *Scopolia carniolica* derived from tissue culture and grown on MS medium supplemented with 0.5 mg/L TDZ and 0.1 mg/L NAA led to achieving the highest tissue culture response rate of 100% with an average number of branches of 3-5 branches per plant part after 4 weeks of cultivation. **Loc and Kiet (2011)** indicated that culturing nodes of Solanum hainanense derived from tissue culture and grown on MS medium supplemented with 0.7 mg/L BA resulted in the highest branch length rate of 2.11 cm after 4 weeks of cultivation. **Otroshy *et al.* (2011)** mentioned that culturing nodes of *Capsicum annuum* L. derived from tissue culture and grown on MS medium.

Rooting Stage

This is the stage through which the seedlings are obtained that will develop into the desired plants. This stage focuses on transferring the branches resulting from propagation to a rooting environment, which often contains auxins or is completely free of growth regulators. These are either grown on a full medium with salt concentration or on a medium with half the salt concentration **(AL- Hadedy 2002)**. Several types of auxins are usually used for rooting branch tips, such as IAA and NAA, with IBA being the most common. **Al-Wasel (2000)** mentioned that the rooting percentage of branch tips from Atropa belladonna L. resulting from tissue culture on hormone-free MS medium was 100%. Deliu et al. (2002) reported that branch tips of Scopolia carniolica resulting from tissue culture and grown on MS medium supplemented with 0.5 mg/L BA and 1.5 mg/L IBA achieved the highest rooting percentage of 97%. **Ashrafuzzaman *et al.* (2009)** confirmed that the branch tips of Capsicum annuum resulting from tissue culture on MS medium supplemented with 0.1 mg/L NAA and 0.05 mg.L-1 IBA resulted in the highest average number of roots, 12 roots/branch. **Amiri *et al.* (2011)** indicated that the branch tips of *Datura stramonium* L. resulting from tissue culture and grown on MS medium supplemented with 0.5 mg.L-1 IBA produced the highest average number of roots, 3-6 roots/branch, with a length of 6-9.5 cm. **Loc and Kiet (2011)** showed that the branch tips of

Solanum hainanense resulting from tissue culture and grown on MS medium supplemented with 0.5 mg.L IBA formed the highest average number of roots , 3.29 roots/branch.

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الخلاصة

تعتبر التقانات الإحيائية في الوقت الحاضر من أهم الوسائل التي ساعدت في تحسين إنتاج المحاصيل الزراعية المختلفة وإنتاج الغذاء والدواء، وتضيف طرائق جديدة لتسريع تحسين النباتات، كما إنها ذات إمكانات هائلة للتغلب على بعض العقبات التي تحول دون زيادة الإنتاج الزراعي، زاد الإهتمام في السنوات الأخيرة بالتطبيقات العملية في مجال التقانات الإحيائية وبصفة خاصة تقانة الزراعة النسيجية التي تعتمد على أساليب بسيطة لاتحتاج إلى تجهيزات مختبرية معقدة ومكلفة والتي ممكن ممارستها بدون إستثمارات كبيرة في التجهيزات والبنية التحتية وبرزت زراعة الأنسجة النباتية واحدة من أكثر التقانات في القرن العشرين والذي دفع العلوم الزراعية خارج مجالها التقليدي إذ أمكن إنتاج النباتات في المختبر بدلا من الحقل، وإنتاج الأجنة الجسمية لاستخدامها بدلا من البذور بإنتاج البذور الصناعية، وإنتاج الهجن النباتية بعمليات اندماج البروتوبلاست بدلا من التضريب والتلقيح .