Kirkuk University Journal for Agricultural Sciences
ISSN:2958-6585
https://kujas.uokirkuk.edu.iq

REVIEW ARTICLE https://doi.org. 10.58928/ku24.15110

In vitro propagation of date Palm using bioreactor technology (Article review)

Ali Adil Abdulkareem[1] Anwer Thamer Ghaffoori[2] Ahmed Ali Alsabte[3]

[1,3] *Date palm Research unit, College of Agricultural Engineering sciences, University of Baghdad, Baghdad, IRAQ.*

[2] *Environmental Biotechnology Department, Biotechnogy and Environmental Center, University of Fallujah, IRAQ.*

*Corresponding Author: aliadil.adil@yahoo.com.

Received: 31/12/2023 Revised: 09/02/2024 Accepted: 17/02/2024 Published: 01/03/2024

ABSTRACT

Technological progress has contributed to solving all problems in all fields. In plant tissue culture technology, technology has helped in solve problems Facing traditional agriculture, such as time constraints, the limitations of the resulting plants, and the production of healthy plants free of diseases and overcome obstacles related to the nutrient medium for growing palm embryos. Previous studies have proven that there are some problems in semi-solid or liquid nutrient media, including a high percentage of Ethylene lack of gas exchange, high concentration of nutrients around the cultivated part, the occurrence of vetrivication, and other things that may hinder the success of agriculture. Progress and development in this field helped to solve problems for all media, as it was achieved to innovate the bioreactor system, or what is called the temporary immersion system. It is a precisely working system consisting of containers linked together and connected to sensitive micro devices that allow for gaseous exchange and reduce the percentage of ethylene around the part. Planted and reduces the immersion time of the planted part in the medium, thus preventing the occurrence of vetrivication. Finally, it can be said that this system helped increase the number of palms produced due to the large size of the container that holds many embryos and the nutritional medium. This system is also economically important, as the type of medium used in it is liquid media. Therefore, there is no need to add agar, which is considered an additional cost of media components.

***Keywords*:** Modern technology, bioreactor, Date palm, in vitro.

INTRODUCTION

Date palm (*phoenix dactylliferae* L.) from Arecaceae palm family which cover more than 4000 species and 200 Genus, therefore consider one of the important families after Gramineae. Due to its nutritional and economic importance [1], Fruits have a high nutritional value because they contain a large percentage of nutrients and vitamins such as iron, zinc, phosphorus, and vitamin pantothenic acid B5 and pyridoxine B6, which have a role in treating digestive system problems and muscle spasms. Studies and research also indicate that pregnant women eating dates activates the infant cells and increases its health and immunity. The latest statistics showed there is a significant decline in the rate of palm trees was 17348741 million reached after it was 33 million [2].Various conditions cause this deterioration, most notably the lack of water, the high altitude in the southern regions, and the drought of most of the areas Lands, desertification, pests and diseases, as well as neglect and poor service of palm trees in terms of carrying out various service operations especially plowing, irrigation, fertilization, and weeds control , harmful pests, and diseases that cause the death of palm trees and reduce their production and quality[3][4] , One of the traditional methods used in palm propagation is seeds and offshoots. Despite their success, there are obstacles, including providing heterogeneous plants in the case of seeds, in addition to producing male plants with a high percentage. As for offshoots, the number of shoots that a single palm tree produces is limited or may be almost non-existent during its life cycle. [5] [6]. Therefore, researchers turned to using the technology of plant tissue culture to produce large numbers of plants without being restricted by time or a specific area in producing many plants. This is done by removing the plant apex, axillary buds, or flower buds and cultivating them in sterile semi-solid or liquid media under controlled conditions in terms of temperature, humidity, and sterilization [7] Despite the successes achieved in this technology, some problems have emerged, especially in media, such as lack of ventilation, the occurrence.

vetrification of the cultivated plantlet, or lack of availability of nutrients for the cultivated part, as in semi-solid media.Under the rapid developments in the scientific field, these obstacles were overcome by inventing what is called the bioreactor system or the temporary immersion system, which is a container connected to pumps that open and close simultaneously with timers, and on its sides there are several holes connected to filters to allow gaseous exchange , ventilation and reduce the percentage of ethylene, while the medium inside it rises and descends in contact with the plant part within certain periods to ensure the vetrivication does not occur [8] [9].

Plant tissue culture technology

Plant tissue culture is a technique used to propagate and grow plants in a controlled environment from small tissues or cells of plant parts, like stem segments, leaf explants, or even individual cells. [10] This technology allows researchers and horticulturists to produce identical plants with desirable quality, regenerate plants from a single cell, and perform various experiments and applications in plant biology and agriculture [11]. Here are some key aspects of plant tissue culture:

Explant: Plant tissue culture starts with selecting small plant parts called "explants," which can be pieces of stems, leaves, roots, or other plant organs.

Sterilization stage: The selected explants need to be surface sterilized to remove contaminants such as bacteria, fungi, or other microorganisms.

Initiation stage: The sterilized explants are placed on a nutrient medium containing salts, vitamins, sugar, and plant growth regulators (phytohormones). The choice of medium and its composition varies depending on the specific goal of the tissue culture.

Multiplication stage: In this stage, the plant cells or tissues start to grow and multiply, forming undifferentiated cells called callus.

Differentiation stage: By manipulation the growth regulators in the medium, the callus can be induced to differentiate into various plant organs, such as roots, leaves or shoots.

Rooting and Acclimatization: Once shoots are produced, they can be rooted in a separate medium to form plantlets. These plantlets are then acclimated to the external environment step by step [12] [13].

Date palm propagation

The traditionally propagation of date palm either by seeds or by offshoots, the Sexual method (seeds) It is considered useless because the progeny not similar to mothers. The method of propagating by offshoots is good, but it is criticized because the palm tree produces few shoots throughout its life, especially for rare and desirable varieties [14]. On the other hand, the method of propagation through tissue culture is considered optimal in order to produce large numbers of seedlings in a short period and in a limited area, and the resulting plants are also healthy and free of diseases [15] [16] [17]. This technology consists of eradication buds, shoot apex and flower inflorescences, etc., then cultivating the Excision parts in sterile media, under controlled conditions, in several stages, until new plants are produced. [18] [19] [20]. Obtaining new plants in tissue culture in several ways is the indirect method (callus) or somatic embryogenesis, as well as direct organogenesis [21] [22] [23]. The indirect propagation method (callus) is the most common, as the callus is stimulated to produce somatic embryos using auxins. [24], Subsequent developmental stages, such as elongation and multiplication of embryos, require cytokinin in certain concentrations, and the type of medium (physical status) plays a role in the success or failure of these stages. Many studies have been conducted to increase the efficiency of growth and development of date palm somatic embryos through cultivation in different media components and physical status [25] [26].

Culture Media

The medium is one of the important factors influencing the response of plant parts in vitro, whether for palm trees or other plants. The importance of the nutrient medium in providing the basic nutrients important for the growth and development of the transplanted parts because the transplanted cells and tissues live as Completely dependent on the food provided to them by the nutrient medium and cannot manufacture their food Accordingly, serious attention to the components of the medium and its physical status of the medium is very important, and calculating the need of the cultivated part with great care for each element of the nutrient medium, because of its importance in the growth of the plant part at the specific stage and its subsequent impact in the following stages. The medium consists of inorganic salts, sugar, vitamins, growth regulators, amino acids, and perhaps some complex substances in addition to water, and a hardening material (Agar) that may be used to give a solid texture to the nutrient medium that allows the plant part to be grown and prevents it from submerging in the nutrient medium. Overall, the date palm tissue propagation protocol begins with the process of callus induction, followed by the formation of embryonic callus and then the formation of somatic embryos. These stages take place in media rich in auxin, such as NAA and 2,4-D [27] [14] [28].

Medium physical status.

It is also known that the determinants of the success of the transplanted part are the medium, especially the physical state of the medium, whether it is semi-solid or liquid. In addition, ventilation is another determining factor that affects the growth and progress of the planted part, as in liquid media the plant part is immersed, so gaseous exchange decreases due to immersion, so work Supports like bridges made of filter paper to raise the cultivated part of the liquid medium.

In other hand the moving liquid medium in the shaker machine Although it is easy to provide nutrients to plants, but there are some drawbacks, there are some negatives in this type of media, which is that the cells are breakdown due to their collision with each other when they remain in the medium for a long time in the shaking machine. [29].

Liquid media prepares nutrients for plant parts and are absorbed faster compared to other nutritional media, as well as secretions resulting from metabolic processes, even if they accumulate around the planted part, they do not directly affect growth until after a long period of time, in order to dilute them with the liquid medium. [30]. On the other hand, the semi-solid media, although there are positives in terms of not causing submergence of the plant part and no vitrification, there are some obstacles, including local accumulation of secretions of metabolites around the plant part, lack of ventilation, and lack

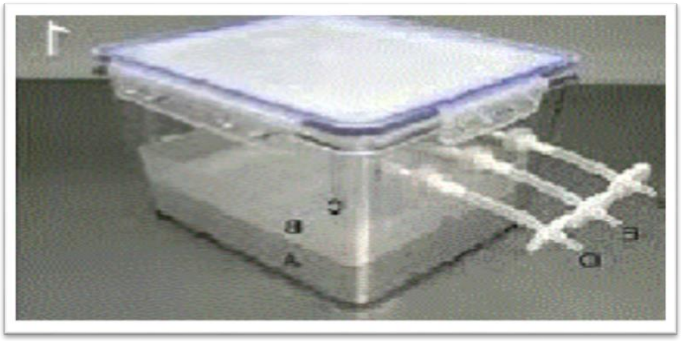
of availability of nutrients to the cultivated part. At the same time, these things are available in liquid media. Some studies have confirmed the success of using semi-solid media for some plants and for certain stages.
The media is hardened by adding agar to it [31], It was concluded that the success rate of parts grown in semi-solid media increased at all stages and did not occur vitrification, in the same side the results obtained [32] It was confirmed that the germination of embryos and the formation of somatic embryos was increased in the semi-solid medium compared to the liquid medium, where the germination of embryos decreased due to the occurrence of vitrification. In the same direction, the researchers obtained unique results, as they confirmed that there was a significant increase in the number of embryos and the wet weight of the callus when using the liquid medium., Were [33] found The highest number of buds 18 buds during 54 days from the callus of phoenix dactyliferae Barhi cv grown in the moving liquid medium in the shaking machine, while the number of buds reached 12.4 buds, in the solid medium for 72 days. recently [34] got positive results the highest number of buds of date palm phoenix dactyliferae in vitro was 75.6 at 4 min /sec cultivated in by using liquid media in (Bioreactor system) with change different immersion duration.
Accordingly, researchers have solved all problems related to media and their physical status by using a bioreactor or temporary immersion system (TIS.)

Bioreactor or Temporary immersion system (TIS)

This system (TIS) was developed and tested in propagating many agricultural crops, and its efficiency was proven, including palm, sugar cane, coffee, bananas, sweet potatoes, blueberries, and pistachios. The researchers resort to using a plant-form bioreactor in plant propagation for several reasons, including those mentioned by [35] [36].
1-Reducing production costs through Using liquid media means dispensing with agar and other solid materials that are used in the usual way for tissue culture propagation, as it is dispensed with this system Also in this system, the size of the containers that make up the system allows for the cultivation and production of large numbers of branches in each of its units, which allows for reducing production costs related to the costs of workers necessary to carry out secondary crops, transportation to new media, electricity expenses, and others. [37]
2- The amount of nutrients can be regulated according to the different stages of reproduction and growth.
3 -Ventilation and Gas exchange are very necessary in tissue culture, as this system lets the crops be enriched with oxygen and releases gases that are harmful to the plant and that prevent its growth, as ethylene. [38[
4 -Improving implants by reducing the phenomenon of vitrification
5 -Improving the success of acclimatization of plants. The temporary immersion system merges the advantages of both liquid and semi-solid media [39]. Which has been proven to increase morphological and physiological performance by supplying nutrition and ventilation [40], as automatic farming systems depend on alternating cycles of temporary immersion of growing plant tissues in liquid media, followed by ventilation periods for the growing tissue. Usually the period of immersion is short, a few minutes, while the period of exposure to air is long. It is necessary to control the time of immersion and the number of times to provide ideal food and air for the planted tissue. The success of temporary immersion reactors is linked to several points, namely their ability to be sterilized, the ease of assembly, disassembly, and sterilization, in addition to the ease of growing tissue in them [41]. The recorded benefits of this system are the reduction of disturbances such as vetrification. This problem is solved by controlling ventilation, reducing the accumulated phenols of the plant part, reducing browning and immersion, and it works to reduce accumulated gases through periodic air exchange. [38]

What are the components of the system?

The system consists of 12 containers for culture. At the base of each container is a perforated basket; the other is a non-perforated container with one hole connected by a tube to one of the filters to push air into the nutrient medium. These two baskets are placed upside down, one opposite the other, and on the side of each container, there are 3 holes (ports) that are connected. It has sterile Millipore (filters) to prevent the passage of fungi and bacteria into the food medium. The first port is for gases to exit the container, the second for pushing air into the nutrient medium, and the third for pushing air over the platform to facilitate the immersion process. It contains a small table placed between the lid and the perforated basket. This table works to stabilize the basket containing the plant parts and prevents it from rising while air is pumped into the container. As shown in the figure below. The basis of its work is to place the liquid nutrient medium in the container at a rate of 500 ml for each container and place the non-perforated basket on top of it. The perforated basket in which the plant parts will be placed, and then tighten the lid of the container well. After that, air is forced into the nutrient medium under the basket and above the basket from an air pump connected to a timer, which is usually turned on for several minutes for every few hours, so that the medium rises and submerges the planted parts, then falls, and so on.



Main parts of Plate form bioreactor system

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التقانات الحديثة بأستخدام نظام المفاعل الحيوي في الزراعة النسيجية للنخيل (مقالة مراجعة)

احمد علي السبتي انور ثامر غفوري علي عادل عبد الكريم

[1,3] وحدة أبحاث نخيل التمر، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق
[2] قسم تكنولوجيا حيوية بيئية، مركز تكنولوجيا الحيوية والبيئة، جامعة الفلوجة، العراق

الخلاصة

لقد ساهم التقدم التكنولوجي في حل كافة المشاكل في كافة المجالات. ففي مجال تكنولوجيا زراعة الأنسجة النباتية ساعدت التكنولوجيا في حل المشكلات والتغلب على المعوقات المتعلقة بالوسط الغذائي لنمو أجنة النخيل. وقد أثبتت الدراسات السابقة وجود بعض المشاكل والمعوقات في الأوساط الغذائية شبه الصلبة أو السائلة، منها ارتفاع نسبة الإيثيلين، وعدم حصول التبادل الغازي، وارتفاع تركيز العناصر الغذائية حول الجزء المزروع، وحدوث التزجيج، وغيرها من الأمور التي قد تعيق نجاح ونمو وتطور الاجنة المزروعة . وقد ساعد التقدم والتطور في هذا المجال على حل كافة المشاكل المتعلقة بالاوساط، اذ تم التوصل إلى ابتكار نظام المفاعل الحيوي أو ما يسمى بنظام الغمر المؤقت. هو نظام عمل دقيق يتكون من حاويات مرتبطة ببعضها البعض ومتصلة بأجهزة دقيقة حساسة تسمح بتبادل الغازات وتقليل نسبة الإيثيلين حول الجزء المزروع مع عمل توقيت معين لغمر الجزء المزروع في الوسط ولمدة معينة لا تتجاوز الدقائق وبالتالي يمنع حدوث التزجيج وأخيراً يمكن القول أن هذا النظام ساعد على زيادة عدد النخيل المنتج نظراً لكبر حجم الحاوية التي تستوعب اكبر كمية من الوسط الغذائي بالتالي يمكن زراعة على عدد اكبر من الأجنة فيها . كما أن لهذا النظام أهمية اقتصادية، حيث أن نوع الوسط المستخدم فيه هو الوسط السائل، وبالتالي لا داعي لإضافة الأجار، وهو ما يعتبر تكلفة إضافية لمكونات الوسط الغذائي.

الكلمات المفتاحية: التقانات الحديثة ، المفاعل الحيوي ، نخيل التمر . خارج الجسم الحي.