Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 10, October 2021: 3409-3422

Propagation of Date Palm Using Tissue Technology and the Experience of Its Cultivation in Karbala Governorate

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Abstract:

This research studies the importance of tissue culture for the laboratory propagation of date palm through two methods, the first method is vegetative budding, the second method is embryogenesis .As each method has its own characteristics and importance in terms of production and time spent .It has the experience of the propagation of palm technology textile in the province of Karbala in the project) cultivation and propagation of date palm tissue) This project is one of the Almharia of the Department of Horticulture at the Iraqi Ministry of Agriculture ,was established in (17/10/2010) is located in the Razzazah area of free to spend in the province of Karbala and The reason for choosing this area is because it is far from the palm groves in the governorate, for the purpose of protecting it from diseases and insects that spread in it. The project area is about (32) dunums ,The project land is planned with a distance of (10 x 10) meters, and the good and desirable varieties of local demand such as (Al-Barhi, Al-Maktoum, Al-Khalas (were planted in it ,and the trees are watered using drip irrigation technology.

Introduction:

The date palm tree is of great interest due to its high productivity, the nutritional value of its highly desirable fruits and its adaptation to the conditions of desert regions .In fact, the date palm is the cornerstone of the ecosystem types of agricultural areas due to the favorable microclimate that it creates for the growth of seasonal crops and other fruit trees in the shade produced by the palm trees . [(1)] The date palm reproduces naturally through seeds or cuttings and by culturing plant tissues artificially .The use of seeds always leads to heterozygosity due to its diploid nature, while the use of seedlings for propagation is challenged by the difficult availability of the desired cultivar and is often a source of disease spread if the branches are taken from the affected trees [(2) and] due to the difficulty of providing date palm seedlings in the traditional way for the cultivars Important commercial numbers and the numbers required for horizontal expansion and to meet the need for replacement and renewal that require large numbers of palms . Thinking about the production of shoots through plant tissue culture as one of the most important modern technologies to produce the largest number of shoots in

a limited space and a short time and with the desired specifications to meet the needs required for cultivation [3]

The tissue culture technique was called by this name because the beginnings of this technique were completely dependent on tissues as part of the plant culture In the tubes, botanists mention that the technique on which the tissue culture technique was built came thanks to the accumulation of some knowledge about the events observed on the behavior of plants .From the observations of a French botanist**.** Duhamei Du Monceau During the early eighteenth century, the callus was formed from the wounds of the stems of some plants, followed by that) Schwann 1839 **(**Where he explained in his study that any cell plants can be separated and that this cell can give rise to a whole plant, and noted Haberlandet) 1902 **(**That the cell has the ability to form a plant or several plants if the environmental conditions permit .The features of the tissue culture technique began to become clear when it was possible to grow some plant organs such as corn roots, tomatoes, and asparagus sprouts .The method of micropropagation of plants is one of the most complex and complex tissue culture methods .It represents the stages of tube cultivation, which is characterized by the intensive production of plants inside tubes . [4]

This technology produces disease-free plants that are very suitable for germplasm preservation and are 100% similar to the characteristics of the mother plant .As plant culture in the laboratory (tissue culture) has become an integral part of the topics of physiology, biochemistry , molecular biology and genetic engineering, and this progress extended to palm cultivation .The common method of palm propagation was by cuttings produced from the lateral buds of the palm .As for propagation by seeds, it is an uncommon method because the resulting plants are different from the mother, and the resulting seedlings are approximately half of them are stallions and the other half are females, so the only way is to propagate palm trees by shoots, and in most cases the latter method may not suffice .As required, as in the case of the propagation and circulation of rare varieties of high quality or varieties resistant to diseases and insects because the number of shoots produced from each palm tree is limited . [5]

Discussion and Results :

Most parts of the palm tree are used in tissue culture, such as the nucleus ,the separate peduncle of the plant, the flower parts, the leaf and the roots, as well as the axillary buds and the developing apex .However, the plant part that is widely used for propagation in most laboratories is the heart of the shoot, or the so-called meristem stalk .[6]

There are two ways to propagate palm trees using tissue technology :

1- Palm propagation by vegetative budding) axillary branching (The Axillary bud proliferate)

Among the developed methods of plant micropropagation, axillary bud propagation is the most widely used and is also considered the most suitable for ensuring the genetic stability of the obtained regenerative plants .For rapid plant propagation in vitro, normally dormant axillary buds are stimulated to grow into multiple buds by judicious use of growth regulators. It is often referred to as the most faithful method of in vitro propagation of plants[7] .

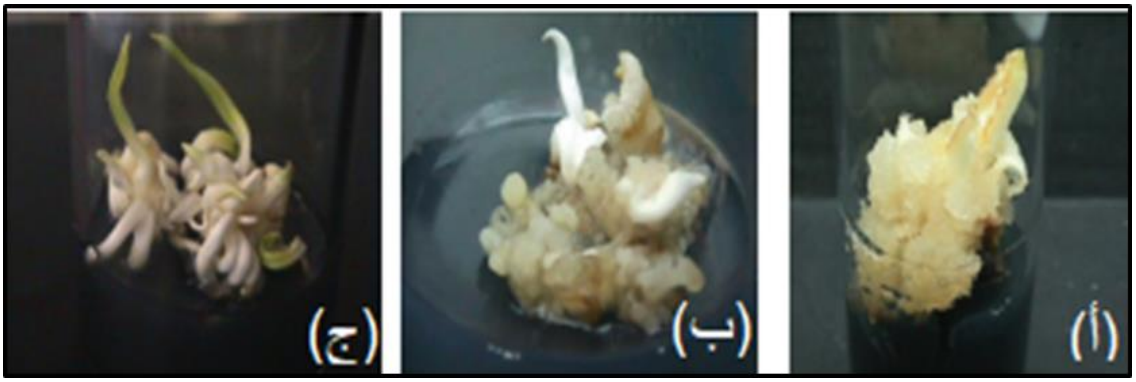
This technique depends on tissues taken from the bases of young leaves located near the growing top of the shoot and placed in nutrient media that help in the formation of buds. The use of this type of tissue is due to the fact that it contains small axillary buds and meristematic tissues with a high ability to form the first buds on which the multiplication process is based . .This method is characterized by the fact that the buds that are multiplied arise directly from the mother's tissue without transforming to the stage of callus ,which makes this technique similar to natural propagation by cuttings .Depending on this feature , the vegetative budding technique preserves the genetic characteristics of the mother palm . (7) The cultivation of date palm through vegetative budding (axillary branching) has important stages that must be followed in sequence to ensure the success of this method, which are as follows :

A- The first stage :Preparation and sterilization of the tissues to be cultured: The process of preparing and sterilizing the tissues to be cultured, selected from young and healthy offshoots carefully separated from well-known and cared for mothers, is of paramount importance during the early stages of the multiplication process, where the basis for obtaining primary samples free of contaminants, fungi and bacteria that depend on It has all the following stages of propagation. The selected shoots are considered as a source of plant parts, so it is preferable to have a weight of (10-15) kg, an age of 2-3 years, and a height of (100-80) cm. The leaves should be removed apically until reaching the separated growing tops. It is preferable to have a diameter of (3-4) cm and a length of (6-8) cm .The area of the plant parts (apical meristem with the inner tender leaves) is preferably 2 cm in diameter , after which it is placed in an anti-oxidant solution, as well as sterilization of the plant parts using commercial minor (sodium hypochlorite) at a concentration of 20 **%** . Also (300) mm of potassium permanganate is added for (12) minutes with a vacuum machine to enable the sterilization solution to reach the leaflets surrounding the apical bud . (8)

B -The second stage is the preparation and formation of the buds : the excised small parts are grown inside completely sterile glass laboratory tubes containing a sterile nutrient medium and a mixture of some growth regulator compounds (cytokinins or auxins) extracted from organic materials and inorganic salts in addition to other elements essential for the growth and development of tissues and to a certain degree) **PH** (ranging from (4-5), picture . (1) These buds are kept inside the tubes for a period ranging from (6-12) months in order to form vegetative buds(**9**).

Picture (1) Preparation and formation of tissue buds

(a) The formation of a callus in the tubules (b) the development of embryos (c) the doubling of embryos .



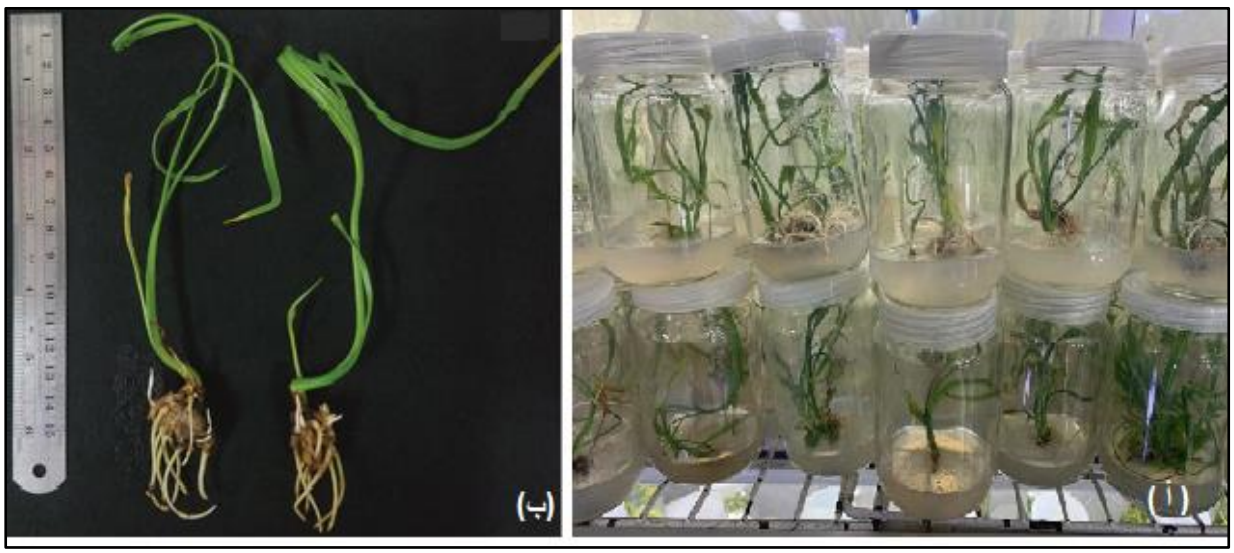
Source: Nasser Bin Saleh Al-Khalifa, Using Tissue Culture Technology in Palm Propagation, National Center for Agricultural Technology, Riyadh, Saudi Arabia, 2011, p. 14.

C-The third stage is the multiplication of the buds :after obtaining the clumps of the first buds, they are divided into groups before being transferred to the rapid multiplication media that differ from the bud preparation media with special growth concentrations, where their concentration is generally reduced to levels ranging between (1.0 and 2.0) mg / liter ,Picture .(3) This stage is considered the first nucleus of commercial propagation of palm trees, where large numbers of buds are obtained by separating the formed groups and transferring them to new nutrient media every eight weeks (**10**).

D- The fourth stage is the elongation and rooting of the buds : After the multiplication stage, they are separated and transferred to media suitable for the growth and elongation of the leaves .It is preferable to transfer the buds from these media after (5-6) weeks so that they can form roots in parallel with the growth of the leaves in media specific for rooting and enriched with auxins especially **)NAA or IBA (to** obtain plants each with a strong stem and crown area containing-2) (3fully formed leaves and roots .(11)

Picture (2) Elongation and rooting offshoots

The elongation of the buds inside the glass tubes (B) Measuring the lengths of the vegetative leaves



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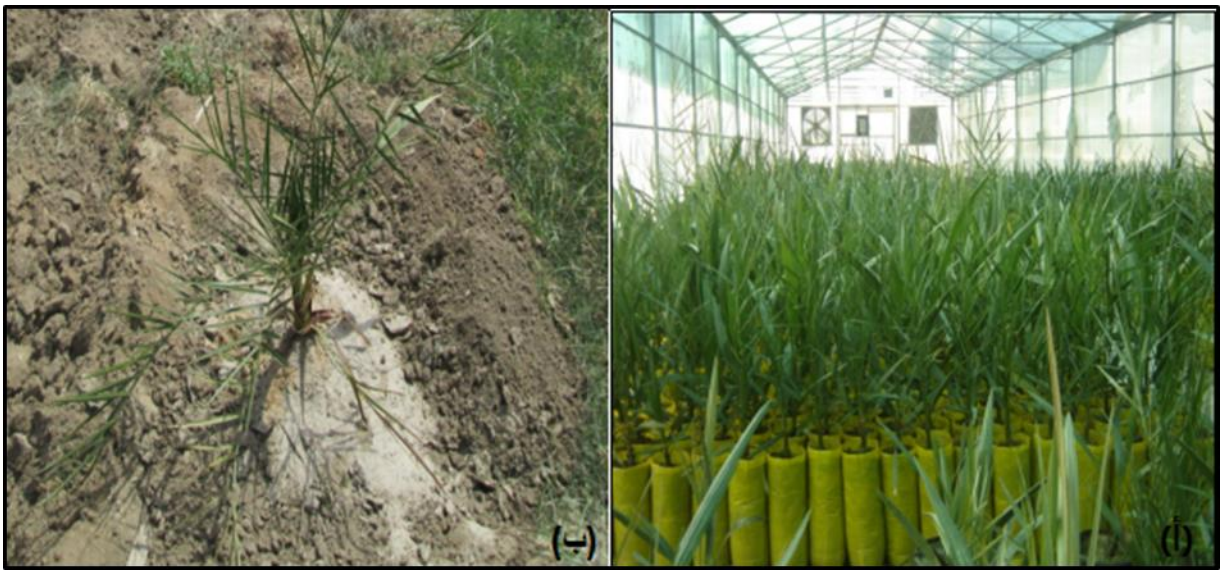
E- Phase V Regionalization :conducting acclimatization process to create plant growth in field conditions by transferring the plant to soils various industrial species and sterile ,taken into account when the process of transportation to maintain the high humidity atmosphere surrounding vegetation where they are placed in a transparent plastic houses or covered by transparent bags allow the passage of light Then the cuttings are acclimatized by a gradual reduction in the ambient humidity by opening the box or the plastic bag for several hours ,gradually increasing until finally the cover is removed for plant growth in normal air humidity , picture .(5) Then it is transferred to the field, which is a dense and strong seedling . [12]

Picture (3) The acclimatization stage

(A) Adaptation of shoots inside greenhouses (b) Adaptation of shoots in soil.



Mushtaque ahmed jatoi et al, effective protocol for micropropagation of some pakistani cultivars of date palm, Article in Pakistan Journal of Botany, 2015, p 1925.

2- **Propagation of palms by creation and formation of the embryonic callus :** Somatic embryogenesis Somatic embryonic development includes the creation of calloids (indirect somatic embryogenesis) or secondary embryonic cultures (direct somatic embryogenesis) .The latter cultures are generated from primary embryos and consist mainly of nodules, each of which has many meristems within the body of parenchymal cells .The meristems give rise to buds . [13] The callus is randomly arranged cells resulting from the cell division of the cells that make up the plant part grown on a nutrient environment . Often the callus is formed in the area where the plant tissue is cut .It is considered a plant material of great importance in plant propagation .There are some main factors that control the success of plant callus formation on a nutritious environment is the selection of the appropriate plant part and the provision of the nutritious environment and appropriate conditions to encourage the formation of callus as well as separation and preservation of the resulting callus .It is worth noting that the series of processes that the embryonic callus goes through in the propagation of

palms inside the laboratory is the same as the processes of propagation of palms using the vegetative budding technique . [14]

This method is considered better than the method of vegetative budding, which is characterized by very slow reproduction and cannot solve the two basic problems, which are the lack of materials and high-quality capabilities for expansion and development, as well as diseases that affect date palms such as egg disease, which is the most deadly disease in date palm caused by a fungus. the soil)**Fusarium oxysporum f. sp. albedinis** (As in southern Morocco and Algeria, a laboratory since 1985 has succeeded in cultivating more than (50) date palm cultivars through physical embryonic development .For about 10 years, our laboratory has been working under a contract with an industrial laboratory now established in the United Arab Emirates: , with a production capacity of more than (100.000)seedlings annually by micropropagation of date palms through physical embryonic development for the purpose of artificial reproduction (**15)**

Somatic embryogenesis is an effective biotechnology tool used in the in vitro mass production of date palm .Moreover, this technique has created an attractive system for studying cell morphology, biochemistry, cellular and genetic factors controlling embryo development and somatic embryogenesis, the induction of somatic embryos is based on the concept of cellular full capacity, that is, the ability of a single cell to reprogram and divide into cells that each constitute an embryo completely .This process is highly feasible if somatic cells are competent and cultured under appropriate induction conditions . [16] This method implies the development of embryos from somatic cells, which undergo a series of morphological and biochemical changes .Somatic embryogenesis goes through a series of stages starting with the formation of the embryonic callus, then the formation of the somatic embryo, the development of the somatic embryo, maturation and the formation of the cotyledon .Embryonic development is the most effective tool for achieving large-scale production . [17]

In general, the technique of tissue culture in the propagation of palm Many advantages, including:

1. Obtaining a large number of offshoots using a small number of date palm mothers, especially the low-production varieties or the perennial productive varieties that do not produce offshoots and are required to be disseminated and expanded in cultivation due to the quality of their fruits and the abundance of their production.

2. Cultivation of the resulting seedlings in permanent land directly without nursery work and waiting for two or three years, as the seedling that is grown in this case has a full root total. [18]

3. The shoots are healthy and free from fungal diseases that are widely spread in many countries.

4. The homogeneity of the shoots resulting from this method and ensuring the speed of growth, as the palm is produced in a period ranging between (2_3) years from the date of planting and the beginning of fruiting in the permanent land.

5. The amount of production with this technology is high compared to the amount of production in traditional agriculture . [19]

This technique, like any technique, has advantages and disadvantages ,as many problems that occur can be summarized as follows: stunting, pollination failure, abnormal fruits ,abnormal tree morphology , bleaching of leaves, inflorescences, twisted branches, and delayed flowering time .However, these deformations in date palm trees derived from tissue culture can be considered as a low occurrence and less than (5%) of the results of palm propagation by this technique . (20)

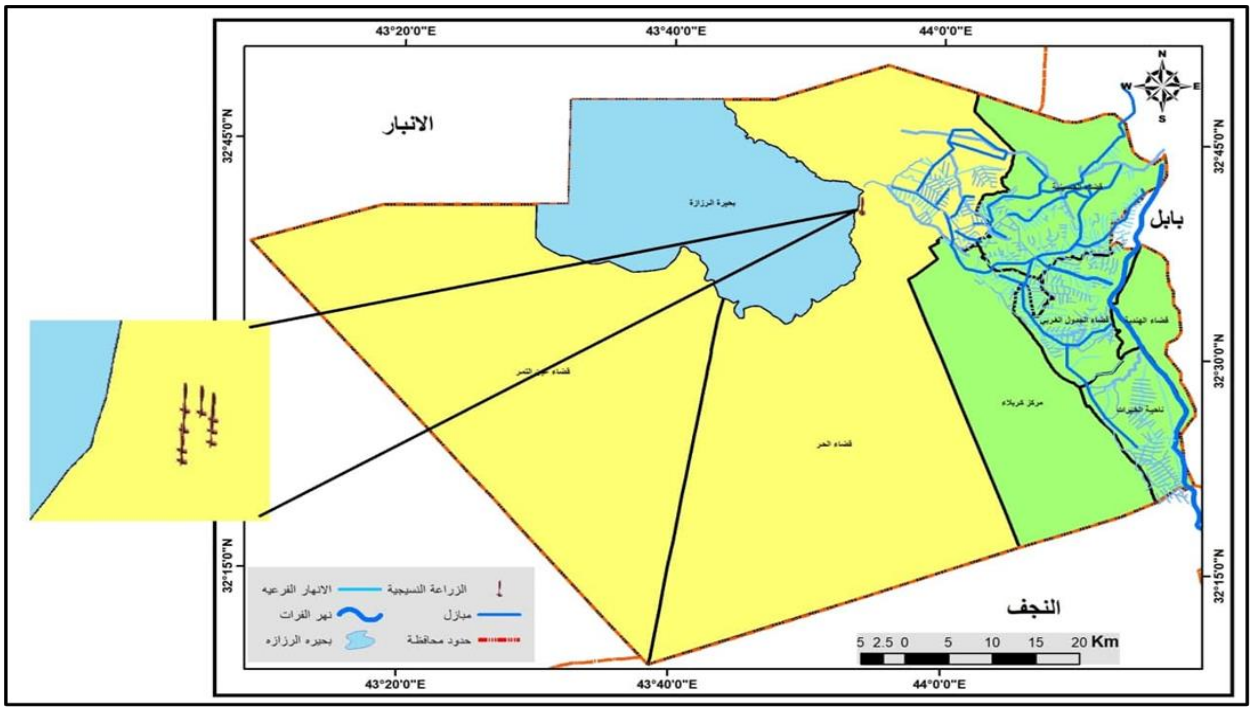
Has Multiplication palm experience textile technology in the province of Karbala in the project (cultivation and propagation of date palm tissue) This project is one of the Almharia of the Department of Horticulture at the Iraqi Ministry of Agriculture, and given the importance of the textile agriculture in the production of free healthy varieties of diseases and pests, the Horticulture Department and the efforts of great the Ministry of Agriculture in Iraq in cooperation with the General Authority for Nakheel (one of the formations and the Ministry of Agriculture) established (26) Station Nakheel distributed (13) province, started working since the beginning of the year (2004) for the propagation of the Iraqi palm varieties ,ran to work on the establishment of orchards mothers palm nurseries at every station .

The source of the tissue shoots was from the United Arab Emirates as a gift to the State of Iraq in order to develop the reality of date palm in the country and in support of the agricultural sector after it was subjected to great neglect over the past decades, as it was cultured in the tissue culture laboratories in the United Arab Emirates, the aim of which is to expand in the propagation of date palm in Iraq ,reaching about 2,500 seedlings down first out (15800) Vecelh palm , including about 1,500 seedlings class Albrahi remaining seedlings of other varieties including) salvation, white hypothesis, Hilali ,unknown , Deglet Noor and stallion Jarvis) which is characterized by its high quality and desire and demand in the local and international markets (21 . (

The project was established on October 17, 2010 and is located in the Al-Razzaza area of Al-Hur District in the Karbala Governorate, map .(. 1) . (The reason for choosing this area is because it is far from the palm groves in the governorate, for the purpose of protecting it from diseases and insects spread in it, in addition to the fact that the area is controlled by the processes that take place inside it, such as sterilization and control, in addition to examinations and evaluation of the shoots and sterilization for the purpose of controlling them from every emergency. The area of the project reached about (32) dunums, including about 4 dunums of this area, which includes project management, a warehouse, and (5) greenhouses ,with a width of (9) meters and a length of (55) meters ,according to international standards, with two water basins and a small basin for cooling the houses plastic, soil this project from the sandy desert soil type which is characterized by high permeability in terms of characteristics and porosity ,derives its water from the river which flows into the Rashidiya Razaza as well as artesian wells drilled into the land of the project (22)

Map(1)

The site of the tissue cultivation project for date palms in Karbala governorate



Source: the researcher ,based on the program Offline Maps and program outputs 10.2.2 Arc GIS 2021

The agricultural operations inside the project are conducted directly by a cadre consisting of about (5) people, including the project officer, the agricultural engineer and the workers. Table (1) shows the number of the job cadre (engineers, workers) according to their scientific level in the project ,

Table(1)

Number of job cadres (engineers, workers) according to their scientific level in the project

For the agricultural season(2021-2020)

the number	Academic achievement	T
1	Reads and writes	1
1	Elementary graduate	2
_	Intermediate graduate	3
1	Prep graduate	4
2	Bachelor's graduate	5
5		Total

Source . From the researcher's work based on the questionnaire form

Seen from the above table that the highest number of staff was class graduates of bachelor 's Faculty of Engineering Agricultural The rest of the categories (reads and writes , a graduate prep elementary graduate (has included one person for each category .This is due to the small number of workers in the project .The impact of this is negatively reflected on the conduct of tissue cuttings service operations It takes several steps.

A-The first step :is to make a mixture of the following materials ,washed sand in a ratio of (1) and moss at a ratio of (1) and perlite at a ratio of (0.5), this mixture is small granules whose benefit is to maintain the moisture of the seedling For the purpose of cultivating the offshoots .

B-The second step : The mixture prepared in the first step is placed in bags of size (30 x 30 cm) and the cutting is placed inside the bag and then planted in a special container (pots) to make room for placing the cutting in it after removing it from the container, as the heart of the cutting should be completely away from Water and moisture for fear of rotting .

C-The third step :the cuttings are irrigated with water with the addition of a fungicide to protect the roots from fungal diseases. Therefore, a sample of the plantings must be taken for the purpose of examination and to ensure that they are free from diseases and insects. The shoot is placed on a wooden scape to raise it from the ground and get rid of excess moisture, then additional holes are made in plastic bags After planting, fertilizing with fertilizers begins .**NPK 20/20/20** (***** For every (10) shoots ,about (5) grams of fertilizer is given to increase their growth speed **.**

D-The fourth step :placing the previously prepared shoots in greenhouses for a period of more than two months, for the purpose of monitoring and protecting them from climatic conditions such as heat, wind and humidity .

E-The fifth and final step : After two months or more of care and follow-up of the tissue shoots ,after this period they are moved to the ground for the purpose of cultivation and fixation in the soil , as the shoots at this age have adapted to the prevailing weather conditions and the nature of the environment in which they are located . These shoots will be distributed to farmers free of charge in order to spread these rare varieties in the country The Ministry has agreed to establish (5) new greenhouses for this project ,bringing the number to , (10) in order to expand the work of this project in the future .

This project is considered one of modern technology applications in the palm cultivation, where it took place agricultural operations from the planning of the project land space (10 × 10) meters, and planted a good and desirable varieties of domestic demand are also grown along with palms and other trees such as olive trees and Alkalpm table (2) .

Table(2)

Types of crops grown in the textile farming project

For the agricultural season(2021-2020)

percentage	**the number**	**type of crop**	**T**

%88.2	**1500**	**Palm trees**	**1**
%7.6	**130**	**Olive trees**	**2**
%4.2	**70**	**eucalyptus**	**3**
100	**1700**		**Total**

Source: From the researcher's work based on the questionnaire form

It is clear from the above table that the highest percentage of the crops planted in the project is for date palm trees ,which amounted to approximately (88.2%), with a number of about (1500) palm trees out of the total planted trees of about (1700) trees, followed by olive trees with a percentage of (7.6) %) with (130) trees out of (1700), and finally eucalyptus trees by (4.2%) with (70) trees .Therefore, the project is mainly concerned with the cultivation of textile date palm trees with various varieties as shown in Table (3), Picture .(4)

Table(3)

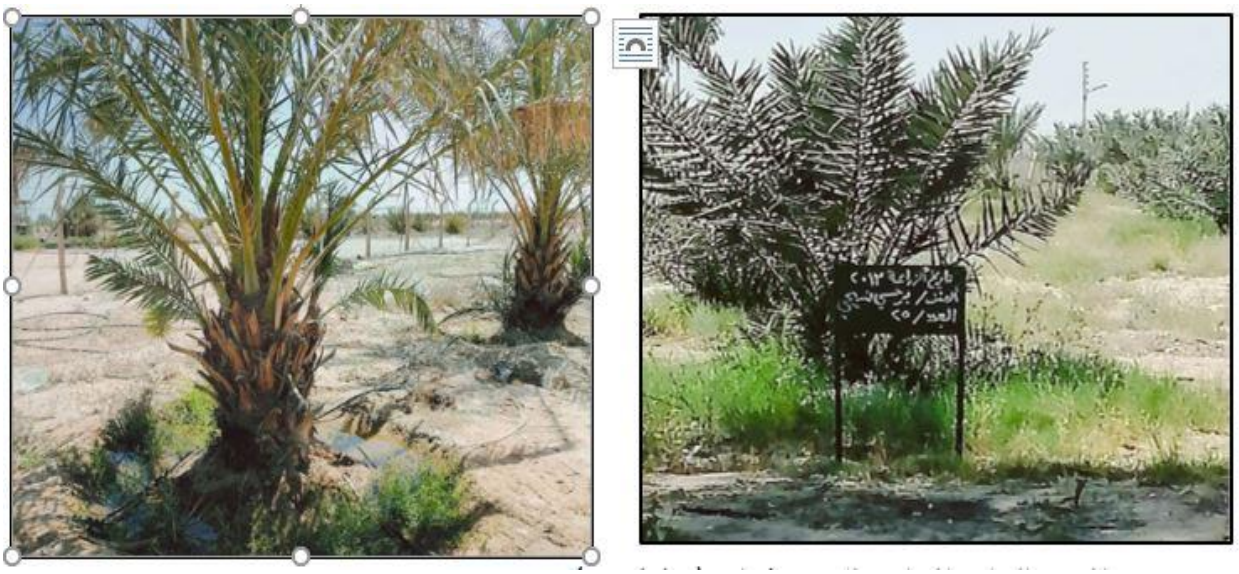
Varieties of textile palms and I prepared them in the project

percentage	the number	item
20%	**300**	**salvation**
46%	**700**	**Barhi**
33%	**500**	**Maktoum**
	1500	**Total**

Source: From the researcher's work based on the questionnaire form

The table shows that the highest percentage is for the Al-Barhi variety, with a percentage of (46%) and a number of nearly (700) palm trees. The origin of (1500) palm trees and finally the Khalas variety (20%) with (300) palm trees out of the total number of (1500) palm trees .

Picture (4) Cultivated palm varieties (A) Al-Barhi variety (B) Khalas variety



Source: field study, for the project on(2021/3/25)

Watering trees is based on the modern irrigation method, which is the installation of a drip irrigation system and its use for watering palm shoots . The system consists of a network of main pipes in which the diameter of the doors is (90) mm, branch pipes with a diameter of (63) mm ,and field pipes with a diameter of (18) mm extending Parallel to the cutting lines, and mounted on them with dots with drainage) ranging between (40-60) liters / hour distributed along the field tubes, where two dots were placed for each seedling with the extension of the tube, and some shoots surrounded by a ring of the field tube , which is the network of tubes linked to two systems ,each system is dedicated It has two pumps to transfer water to the private basins .Their number is only (2), the length of each basin is (10) meters, its width is (10) and its depth is (2) meters , picture) (2).

Picture (5) The drip irrigation system and pumps connected to it in the project

Source: field study, for the project on(2021/3/25) .

The fertilization process is carried out in two ways, the first in the laboratory during the acclimation stage of the cuttings as mentioned, and the second is done manually after the seedlings are planted in the soil by workers who dig a trench with a depth of (50 × 50) cm around each tree in order to put the fertilizer inside, and here fertilizer (urea and humic acid) is used. The project official stated that the recharging technique is not used in this project in order to avoid its recurring problems such as clogging of the dots with impurities and salts carried by water .As for the process of controlling pests and insects, it is carried out using ordinary manual sprayers .

The project suffers from the deterioration of agricultural facilities, including the basins designated for preparing water for irrigation, irrigation systems, and pumps that draw water from wells , which require maintenance and rehabilitation to operate with good efficiency ,image (3) in addition to the damage of large quantities of pipes and drippers that were left in one of the sides of the project, which gives An uncivilized image of such a distinguished project that works with modern technology represented by tissue culture,.

Picture (6) of the water basins that require maintenance and rehabilitation

Source: field study ,dated .(2021/4/10)

Conclusion :

Showed technical micropropagation) tissue culture (palm distinguishes it significantly from the traditional breeding by seedlings or seeds has been tried in many countries of the world were the results of successful experiences among those experiments was the experience of date palm cultivation of tissue in the desert with a soil sandy environment of Vhafezh Karbala As a project was established for the cultivation and propagation of the mother date palm from the seedlings that were grown in the laboratory through tubes, which are of Emirati origin. The project faces it, represented by the neglect and deterioration of agricultural facilities, such as basins designated for the preparation of irrigation water, irrigation systems, and pumps installed on wells . Therefore ,this requires maintenance and rehabilitation for the project to operate with high efficiency .

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23. A personal interview with the engineer Abu Duaa, the official of the textile palm plantation project in Karbala, on the date of .(2021/4/2)
24. Field study project of date palm cultivation histological in Karbala, in the history of .(2021/10/04)
25. **NPK 20/20/20** (Is a compound fertilizer soluble contains balanced proportions of the major chemical elements (such as nitrogen - phosphorus - potassium) in addition to micro - chemical elements, are mixed with water in manure tanks and sprayed on the leaves using machine guns axial or sprays the floor (Boom Asebrer) Or small hand sprinklers, you can also add it to the soil via modern irrigation systems .Please see the source at the following site: **https://www.zra3ah.com/shop/%D8%B3%D8%A7%D8%AF%D8%B1%D8%A8-20-20- 20-npk** /