Effect of Culture Method, Some Treatments and Transgenic Technique on Callus Growth and Development of Date Palm (*Phoenix dactylifera* L. cv. Barhi).

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(Plant Tissue Culture)

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بسم الله الرحمن الرحيم

وَهُزِّي إِلَيْكِ بِجِذْعِ النَّخْلَةِ تُسَاقِطْ عَلَيْكِ رُطَبًا جَنِيًّا ﴿٢٥﴾

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IN THE NAME OF GOD

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Dedication

To who delivered the message…perform the secretariate ... and advised the nation ... to the prophet of mercy and light of the worlds

Our Messenger and beloved of our hearts , who chosen by Allah, Muhammad bless on him and his pure family

To who taught me success and patience ... To the reason of my existence in life ... To who I miss when facing the obstacles ... when anxiety surround me, I swim in their kindness sea to relieve my pain ... My father and mother, may Allah give them his mercy

To who sacrificed themselves and martyred for the nation "my brothers "

To my brothers .... My supporters and partner in my joys and sorrows.

To my dear wife ... Whoever took care of me with her tenderness ... my pearl and sun of my life ... my eyeball…

To my children ... the fruit of my life, my hope, and the pulse of my heart

dedicate the fruit of my effort to everyone

Ahmed 2021

In the name of Allah , the most gracious and the most merciful

Acknowledgment

Praise be to Allah for his benevolence and thanks him for the success and gratitude, we bear witness that there is no god except Allah, who has no partner and we bear witness that Muhammed is his servant and his messenger

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summary:

The experiment was carried out at the Palm Research Center affiliated to Basra University during the period from December 2017 to August 2019.The success of micropropagation of date palm tissue with high genetic stability is a challenge sought by researchers, so, this experiment was aimed to verify TDZ (0,0.5 and 1.0)and PG(0 ,25 and50) And the physical state of the medium's efficacy in the micropropagation of *Phoenix dactylifera.* L in the bioreactor system and prove its success in genetic matching and GM technology transfer salt-resistant gene *SeNHX1* to callus. Results showed the following:

1- liquid medium system (bioreactor) was superior in the weight of fresh callus, buds, vegetative growth, the average number of buds, and vegetative growth. Additionally, auxins, gibberellins, cytokinins, nitrogen, phosphorous, magnesium, manganese, carbohydrates, and potassium were significantly higher in the liquid medium system (bioreactor) in most of the growth stages. In contrast, the solid medium showed superiority in the content of the cultured tissues of abscisic acid.

2- The addition of Thidiazuron (TDZ) and Phloroglucinol(PG) to the medium separately affected most of the physical, chemical, and physiological traits, including TDZ at a concentration of 0.5 mg. L^{-1} and the treatment 50 mg. L^{-1} PG increased the average weight of callus, buds, vegetative growth, the number of shoots and vegetative growth, the addition of these substances of the same concentrations showed the highest content of callus, buds and vegetative growth of auxins, cytokines, and gibberellins, also it increased in the percentage of nitrogen, phosphorous, potassium, magnesium and manganese, These treatments were also more

effectively increasing the content of shoots and vegetative growth of carbohydrates.

3- an interaction of the type of medium with TDZ also significantly affected the treatment TDZ at a concentration of 0.5 mg. L^{-1} with the liquid medium gave a significant increase in most of the traits. On the other hand, the treatment of 50 mg.L^{-1} PG with the liquid medium was more effective than the other concentrations in most of the studied traits.

4- The interaction between (TDZ and PG) affected the physical, chemical, and physiological traits. It was observed that the interaction between 0.5 mg. L^{-1} TDZ and 50 mg. L^{-1} PG resulted in a significant increase in the mean of studied traits compared to the other concentrations.

5- The interaction among 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG with the liquid medium showed superiority as compared to the other treatments. Such treatment improved the average weight of callus, buds, vegetative growth, the number of shoots and vegetative growth.The addition of these substances of the same concentrations showed the highest content of the nitrogen percentage and phosphorous, potassium, magnesium, carbohydrates and manganese, as well as the content of callus and buds of auxins, cytokines, and gibberellin. While the two treatments (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG in liquid and solid medium) were superior in the content of vegetative growths of auxins, cytokines and gibberellins, while The treatment outperformed 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid medium in the content of buds and vegetative growth. It was observed that the interaction between (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG in liquid and solid medium) resulted in the highest vegetative growths content of auxins, cytokines, and gibberellins, while the combined

application between 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid medium caused affected increasing the content of buds and vegetative growth, while the treatment of 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the solid medium gave the highest content of the elements potassium and magnesium, also the highest content of vegetative growth of manganese was recorded, while the control treatment with the liquid medium showed significant increased in abscisic acid content in the callus and buds

6- The results of the genetic stability study with the two techniques ISSR (814, 844a, HB9, HB10, ISSR6) indicated that the treatments did not affect on the DNA pattern, genetic matching was noted in all ISSR markers, as well as it was observed that there was a high genetic match between treatments.

7- The study demonstrated the success of transferring the gene *SeNHX1* responsible for plant resistance to salinity of callus tissue.

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List of abbreviations

abbreviations	Full name
ABA	Abscisic acid
BA	Benzyl adenine
BAP	6-benzilaminopurine
CRD	Completely Randomized Design
CTAB	Cetyl Trimethyl Ammonium Bromide
DMSO	Dimethyl sulfuoxide
EDTA	Ethylene Diamine Tetra Acetic acid
GA_3	Gibberelic acid
IAA	Indole acetic acid
IBA	Indole butyric acid
ISSR	Inter simple sequence repeat
KIN	Kinetin
LB agare	Sold medium
LB	Liquid medium
MES	2-(N-Morpholino)ethanesulfonic acid
MS	Murashige and Skoog
NAA	α-Naphthalene Acetic Acid
NOA	Beta naphthoxyacetic acid
PCR	Polymerase chain reaction
PG	Phloroglucinol
PVP	Poly Vinyl Pyrrolidone
RAPD	Randomly amplified polymorphic DNA
RT PCR	Real time Polymerase chain reaction
TBE	Trisboric acid EDTA
TDZ	Thidiazuron
TIS	Temporary immersion system

Tris base	Tris [hydroxymethyl] aminomethane
Tris - HCl	Tris [hydroxymethyl] aminomethane hydrochloride
WPM	Woody plant medium
ZEA	Zeatin
ZR	trans-Zeatin-riboside
2iP	Iso Pentenyle Adenine
2-4-D	2,4-Dichlorophenoxyacetic acid

1- Introduction :

Date palm *Phoenix dactylifera* L.is one of the evergreen fruit trees with monocotyledons. It belonged to the family Arecaceae and was called in some ancient texts the tree of life; each part of the date palm tree has been used in human life. In addition to it is nutritional,values its fruits are an important as energy-rich source because they contain the main nutrients (Al-Badri, 2010). it is believed that this plant originated in the Arabian Gulf region,and, spread to other regions (Al-Jubouri, 2002).

Date palm propagation is carried out by two methods, either vegetatively by offshoots or sexually by seeds (Al.Khalifah and Shanavaskan, 2012). Offshoot propagation is the preferred and common method of propagating date palms. Because generation plants are genetically identical to the mother plant. However, it faces many difficulties, including the low number of offshoots formed on the mother palm for some cultivars during the period of life, where the number of productive offshoots is between 1-33 offshoots depending on the cultivar and service processes (Matar, 1986), in addition to the fact that the offshoots propagation are slow. The offshoots must remain attached to the mother tree for a period ranging between 2-3 years in order to be able to form an integrated and developed root system before separating it from the mother tree. Also, separating the offshoots from the mother tree is a costly and stressful process that requires great care to preserve the separation area from contamination (Sudhersan and AboEl-Nil, 2004).

The decrease in date palm numbers in Iraq in general and Al-Basrah city,in particular, is due to many reasons, wars, agriculture neglecting, and bad inviroumental conditions which there were influential aspects of the country's economy (Al-Yasiri, 2018). So it became among the necessary proceedings for the propagation of date palms, is to benefit from new applications in the field of biotechnology and plant tissue culture.

The plant tissue culture technique is one of the modern technologies characterized by its high efficiency in productive plants and their homogeneity at short periods. The resulting plants are identical in genetic composition with the true- to -type from which the plant tissue was taken and free from pathogens and insects. It can also be multiplied throughout the year (Zaid *et al*. 2011).Date Palm propagation by tissue culture is carried out in tow pathway either by direct adventicious bud formation through organogenesis or indirectly by callus (Al-khateeb and Al-Dinar, 2002), or somatic embryogenesis, either directly from the explants (Karam *et al.*, 2017), or indirectly by callus (Al-Kharyi, 2005).

Researchers resorted to the use of modern culturae systems, including culture in a liquid medium, where the cultivation is in containers more extensive than those used in solid media, and culture in these includes the temporary immersion system (TIS) and bioreactors, and this technique is considered a way to provide direct contact the explant with the medium, in addition to providing sufficient opportunity for gas exchange, it has also been successful

in commercially propagating many economically important plants (Welander *et al.,* 2007)

Researchers were interested in using chemical compounds to stimulate the generation of plant cells, among those used compounds Thidiazuron (TDZ) and Phloroglucinol (PG)1,3,5 tryhydroxy benzene. The TDZ gained great attention because it is one of the most efficient and effective synthetic cytokinins in tissue culture for woody plants (Huetteman and Preece,1993). TDZ has been widely used to stimulate and propagate the shoots in many plant species, including the date palm (AL-Mayahi, 2014). As for PG, adding it to the medium caused an increase in the rate of induction of shoots (Kumar *et al*., 2005), As well as the inclusion of the medium with the PG compound encouraged induction and production of shoots and their multiplication (Buthuc-keul and Deliu, 2001).

One of the most important challenges facing the tissue culture researchers is the resulting plants' genetic stability. There are several techniques for detecting matching genetic, as genetic fingerprinting techniques have been introduced to verify the genetic stability of the resulting plants and their genetic compatibility with mothers (Gabriel, 2005 and Al-Khatib, 2001).

After the advancement of plant biotechnologies, researchers sought to harness them in treating some problems that facing plants propagation, and the most important of these problems is salt stress, that gene transfer is the most successful and fastest method in transferring the genetic trait to increase plant resistance to salt

stress, but this type of research is almost very little in the success of the transfer the genetic for date palm because of it is one of the monocotyledons plants, and there are many difficulties facing researchers in cultures this is the first reason and the second, gene transfer is difficult in such this plant .The study sought to achieve some of the most important aims wich in :

1. In tissue culture research, there is a continuous need to search for compounds that can lead to better and more efficient growth *in vitro* culture

2. Testing the efficiency of culture in a liquid media by the bioreactor system and comparing it with culture in solid medium and their effect on some of the physical and chemical characteristics of different growth stages.

3. Study the effect of different concentrations of TDZ and PG and their interactions on some of the physical and chemical characteristics at different stages of growth.

4. Detection of plants' genetic stability resulting from tissue culture using some DNA indicators based on the DNA polymerase chain reaction (PCR) by using ISSR, RAPD technology.

5. Study the possibility of transferring the salt-tolerant gene (*SeNHX1*) to the date palm callus and follow-up the success of the transfer with the possibility of increasing their tolerance to environmental stresses, especially salinity through genetic transmission.

2: literature review

2-1: The history of proliferating date palm *in vitro*

One of the essential methods of palm propagation *in vitro* is propagating through adventitious buds from callus tissue. (Bekheet and Saker, 1998). It is known that there are sites where buds appear naturally are called according to the location of their appearances, such as being apical (terminal) buds or auxiliary buds (lateral), but when buds appear from other sites than their natural sites they are called adventitious buds (Salman, 1988). The first attempts to promote the emergence of adventitious buds to the last century fifties in which plant growth regulators were used to stimulate the non-specialized tissue to differentiation and specialization (Mahdi, 2002).

The differentiation mechanism into adventitious buds is achieved by transforming some fast-dividing meristematic cells into semi-meristematic cells, which are called Meristemoid. These cells can develop into Meristemes that can give root or stems initiators that later develop into real roots and stems (Al-Maari and Al-Ghamdi, 1998).). The method of induction of adventitious buds has received considerable attention from researchers in many studies in this aspect (Sudhersan and Abu-El-Nil, 2004; Al-mayahi, 2016; 2018; 2019).

Adventitious bud regeneration in date palm *in vitro* is important, mostly in creating new plants. Al-Mayahi, (2008) showed the possibility of date palm propagation by tissue culture using

different explants (apical buds, axillary buds, and leaf primordia) taken from offshoots 3-4 years old cultivars for Khusab, Um-Aldehin, Sharifi, and Al-Awaidi.

2-2: The effects of Phloroglucinol (PG) and Thidiazuron(TDZ).

2-2-1: Phloroglucinol (PG)

Phloroglucinol (1,3,5-trihydroxy benzene) (Figure 1), which abbreviates PG and the chemical symbol C6H6O3, is one of breaking down Phloridzin, which is a phenolic compound found in xylem sap in many wood trees such as apples,PG contributes to many physiological processes and their role in tissue culture, it is enhanced for growth through its role in increasing the multiplication of shoots , somatic embryos formation and enhancing callus growth, form adventitious shoots more effectively and increase the rate of shoot proliferation in certain shoot cultures, additionally, it can improve rooting in many horticultural and field crops when added with auxin due to their synergism mechanism ,another mechanism of PG is the physiological activity of cytokines and auxin-like substances,it also acts as a compound with an activity similar to the action of cytokines, Phloroglucinol can also act as an initiator in the bioconstruction pathway to synthesize lignin by controlling the activity of sap during the accent process, thus increasing the rate of division in woody plants and other types that difficult to propagate by traditional methods, Recently it has been used in a wide range of histopathology studies (Jaime , *et al*.2013).

Murali *et al.,* (1996) noted the addition role of PG might enhance the somatic embryogenesis in the cultures of roses (*Rosa*

hybrida c.v"Arizona"). Reis *et al*. (2008) reported that PG addition at low concentrations increased the number of somatic embryos, reversing high concentrations of PG. The results were consistent with the addition of phloridzine or PG into oil palm culture medium (*Elaeis guineensis*), which caused an increase in the numbers of somatic embryos (George *et al*. 2010).

To demonstrate the effect of shoot induction and proliferation, one of the most followed methods of producing plants on a large -scale and the production of plants identical to the mother plant. Another independent study on the effect of medium supplementing with PG on early bud break with enhanced shoot regeneration in nodal explants of *Vitex negundo*, commonly known as the Chinese chaste tree, found had significant difference with control (Steephen *et al.,* 2010). Also, the medium supplementation with PG in the bud induction of *capsicum annuum* with 400 µM PG increased the bud induction response by 17–18% (Kumar *et al.*, 2005). As the other effects of PG on rooting, De Klerk *et al.,* (2011) noted increased rooting apple stem slices, reasoning the physiological effect that PG protects auxins from oxidation.

Sharifian *et al.*, (2009) reported that the influence of PG on rooting parameters of three cultivars of Juglans regia, when using PG at concentrations 0.5 and 1.0 mM gives a high percentage. However, Ainsley *et al.,* (2001) found that the PG effect on rooting of almond (*Prunus dulcis* Mill.) varied according to treatment. On the effect of (PG) on the banana multiplication (*Musa sp*), *in vitro*, it was observed that the concentration (200mM) of PG was optimal and

significantly superior to the control treatment and other treatments (600, 800, 1000mM) in the buds length, root number, and its length, while there was no significant difference in the buds number (Londe *et al*., 2017)

Figure (1). Phloroglucinol (PG)

2-2-2: Thidiazuron (TDZ)

TDZ is one of the compounds that researchers have gained wide attention during the past decades for its active role in plant cell and tissue culture in many plants *in vitro*. It has been observed that there are many physiological responses to the effect of TDZ on plants, where studies have shown that its effects are similar to the effect of Auxin and Cytokinins, although completely different in their chemical composition (Figure 2). TDZ also has another physiological role in stimulating and enhancing biological activity, the mechanism of TDZ work is not accurately diagnosed until now, but several studies deal with its morphological mechanism. On the other hand, other research indicated its a direct or indirect role in regulating internal hormones, which works to increase the division and reproduction of cells as well as its effects in the cell membrane, nutrients absorption, and transport (Guo *et al.,* 2011)

TDZ is combared to the many other plant hormones in terms of physiological and morphological responses and contributes to stimulating reproduction for a wide range of different plants. The TDZ's organizational ability has made its use necessary in tissue culture because of its physiological effect on cultivated plant tissue (Matand and Prakash, 2007).

Verma and Bansal (2014) have also demonstrated that TDZ has a significant role in promoting tissue growth due to its effective performance in plant tissues. Although the use of TDZ alone without other growth regulators in the culture medium had a significant effect in stimulating plant cells to re-differentiate as in the petals of Whitesim or other types of plants, additionally adding it with other growth regulators gave positive results in stimulating the production of shoots and formation of somatic embryos (Frey and Janick, 1991; Ricci *et al.,* 2001). The availability of TDZ and NAA in nutritional medium further stimulated growth and development of shoots (Hutchinson *et al* ., 1996). The study conducted by AL- Mayahi, (2014) showed the role of TDZ in multiplying the shoots multiplication of date palm c.v Helawi propagation by tissue culture, where the response rate was 66.66% in the food medium provided with 0.5 mg. L^{-1} TDZ + 1 mg. L^{-1} BA compared to other treatments.

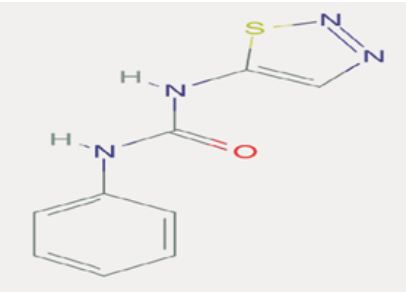


Figure **(2).**Thidiazuron **(**TDZ**)**

2-3:Type of Culture medium

2-3-1: Solid medium

Several media have been used in tissue culture. Murashige and Skoog (1962) MS is one of the most used media in plant tiusse culture, as it contains inorganic salts known as MS salts and other materials that include growth regulators, vitamins, amino acids and other organic materials which are added in specific and different concentrations to the nutritional medium, according to the stage of growth and plant type. The composition of the nutritional medium dramatically influences the nature of growth and development of cultivated explant and the division and specialization of plant cells. In addition to the growth of shoots and roots directly affected by the nature of the chemical medium (Al-Kinani, 1987).

Mineral salts and sugars are the main of energy sources, water, organic compounds, and growth regulators have an effective role in determining the type of media used in culture as well as the agar material that gains the nutritional medium the solid strength of the medium (Gamborg and Phillips, 1995). The effect of growth regulators, the study by Mater (1986) showed that adding growth

regulators to the culture medium depends on the type of growth regulator used and its concentration as well as, the purpose of culture, as well as the state of the physical medium (Zouine and EL-Hadrami, 2007). The addition of auxins with relatively high concentrations 2,4, D to the medium (MS) intended for multiplication of date palm led to stimulation of callus formation, either if the callus is transferred to a medium without growth regulators or with low concentrations leading to the formation of somatic embryogenesis (Othmani *et al*. , 2009).

Also, the availability of the energy source is necessary for the plant parts to grow and differentiate; therefore, sucrose was used for this purpose as it was used in the media prepared for the propagation of many plant species (Murashige, 1974). The study by Badawy *et al.*, (2005) showed that the optimal treatment was observed in the medium containing 30 g. L^{-1} sucrose showed significant superiority in stimulating growth and differentiation of date palm tissue compared with other treatments. The physical condition of the medium has a direct effect on the explants cultured *in vitro* in terms of tissue growth and development, so it found it necessary to add "agar" to the culture medium in the early stages of culture to avoid the sinking of the explants, which leads to death because of it is inability to breathe (Aslam and Khan, 2009).

2-3-2: Liquid Medium

Liquid media differs from solid media by the absence of a solidifying substance, whereby the absorption of nutrients by

cultured tissue is easier than that in the solid medium, Moreover, the liquid medium is less susceptible to pollution other than the solid medium as a result of pollution that may occur to the agar. In order to avoid the sinking of tissues and to provide a large amount of air and gases for the grown tissue, the shaker device was used, Furthermore, it provides movement or vibration what the tissue needs of the nutrients and air, as well as to protect the tissue from immersion in the liquid medium (Othmani *et al*. , 2009). The use of the liquid medium also stimulated the development of tissue that requires the highest rate of multiplication of Barhi, Dagalat Noor, and Qantar cultivers. as compared to the solid medium (Zoine *et al*. , 2005; Mazri, 2014). Khieralla and Bader, (2007) explained the superiority of the moving medium over the solid medium significantly in the number of shoots. the temporary immersion system (TIS) one of the most important methods of culture in the liquid medium is the method of culture, where research dealt with the role of the plantform bioreactor in the multiplication of date palm *in vitro* and the use of several methods including the production of somatic embryos (Al-Mayahi and Al-Musawi, 2017; Okere *et al*., 2010; Al-Myahi, 2015). The temporary immersion system TIS is defined as immersion of plant tissue grown in a liquid medium for certain periods through a timer, to is also supplied with air pumps connected to special, sterile, and precise filters to prevent contamination (Watt, 2012).

The use of the temporary immersion system in the late nineties of the last century was limited as was only used as biological reactors for the production of some secondary

compounds, as was used in some physiological and biological studies, researchers were able to develop this system to benefit from it in the multiplication of many horticultural crops such as bananas, coffee, pistachios and sugar cane (Arencibia, *et al*. 2013).The bioreactor was used in the cultivation system with temporary immersion as it depends on the air being pushed through an air pump into the planting container. That will cause pressure on the liquid medium and thus raises it to the place that contains the tissues. To protect the medium and the tissues the bioreactor was provided with special and accurate filters to purify the air entering from any pollutants. The method of sterilization is conducted in the same way as sterilizing the vessels of agriculture, where it is done by the "autoclave" sterilizer, after placing the medium in it (Welander and Sayegh, 2012).

The study carried out by Al-Mayahi and Al-Mousawi, (2017) showed significant superiority of the temporary immersion system after an immersion period of 2 minutes per 8 hours in the number of embryos induced from callus and the speed of formation of embryos, as well as an increase of their length and its diameters compared to the embryos growing in the solid medium, in addition to the liquid medium significant superiority the solid medium in the embryos content of starch throughout the study period.

A study conducted by Naif, (2019) on date palm cv. Barhi culture in the liquid medium (TIS), was increased the number of shoots, the fresh weight, and the content of the shoots of cytokinins. As well as in their content of nitrogen elements, phosphorus, iron,

potassium, and sodium compared to the solid medium as well as the liquid medium in the number and length roots, The hormones (auxins, gibberellins, and abscisic acid), the length of the shoots may exceed the solid medium.

2-4: Effect of growth regulators on some physical properties of tissues

2-4-1: The effect of growth regulators on callus growth

Thomas and Katterman (1986) showed that TDZ has a role in stimulating cell division and callus formation from tissue taken from Sohybean and has also encouraged the formation of adventitious shoots in the Tobacco plant.Also AL-Taha *et al* (2018)showed that callus initiation from apical of arrole of christmas cactus plant when supplemented MS medum different conceutrations of TDZ.

The study that was carried out by Sidky (2014) on the date palm cv. El-Sewy showed that the highest stimulating rate for callus was 1.66g when treating TDZ at a 1 mg .L^{-1}concentration, while the combination between 1 mg. L^{-1} TDZ with 10 mg. L^{-1} picloram application was more effective in the callus initiation rate of 2.00 g ,the study conducted by Zayed and Abd Elbar (2015) on the regeneration of the date palm through the use of immature femal inflorescences of the Sawi cultivar showed through somatic embryos by using a nutrition medium provided with 0.5 mg. L^{-1} TDZ,other researchers showed that adding TDZ to the nutritional medium stimulated the growth and formation of embryogenesis in a

nutritional medium containing cytokinin,the anatomical sections indicated that the callus consists of two types of cells: cells with small vacuoles that are not involved in the formation of the embryos. At the same time, the other is the masses of compact cells that possess the ability to form somatic embryos.

The a study carried out by Hassan, *et al*. (2017) to the efficacy of TDZ with IBA on of callus formation from immature female inflorescence of date palm El-Sawi cv, that the TDZ treatment at 0.5 mg.l^{-1} had a significant increase of callus induction, at 31.7%, while the combined application (0.5 mg. L^{-1} TDZ with 4 mg. L^{-1}IBA) increasing callus induction at 35.2%.

Fayek *et al*., (2017) reported in the study conducted on date palm using immature female inflorescences that the percentage of callus was affected significantly by adding different concentrations of TDZ, NAA and BA to the medium, where the highest significant value of callus 22% achieved by TDZ treatment at 2.0 mg. L^{-1} and 0.5 mg. L^{-1} NAA, then TDZ at 1 mg. L^{-1} with NAA at a concentration of 0.5 mg. L^{-1} gave 21%, while the control treatment and treatment with 1.0 mg. L-1 BA with 0.5 mg. L^{-1} NAA had the lowest values in the percentage of callus at 0.0%.

Various phenols, including PG can be added to the medium to enhance callus growth. PG supports other plant hormones' activity and increases the callus (Jaime, *et al*., 2013). Murali *et al*. (1996) reported the induction of somatic embryogenesis in callus cultures of rose. Manjula *et al*., (1997) showed that PG is essential when the addition to medium because it promotes callus formation in

Aristolochia indica. These results agree with George *et al*., (2010) that the addition of PG to the medium can increase the number of somatic embryos produced from embryogenic callus of oil palm.

2-4-2: Effect of growth regulators on buds and shoots growth

The study conducted by Hussain *et al*., (2001) on several cultivars of date palm cvs.Asil, Hussain, zaidi used MS medium with different concentrations of TDZ with 2iP at a concentration of 0.5 mg. L^{-1}. The highest rate of multiplication of buds was at interaction treatment was 0.5 mg. L^{-1} TDZ with 1 mg. L^{-1}2 iP. Asil cv. has a significant effect on cvs Hussian and Zaidi as (10-15, 3-5, and 2-7) respectively, simultaneously, the lowest rate was observed with control treatment for all cultivars which reached only one bud.

Hassan *et al,*. (2017) study conducted on date palm showed the cultivar of El-Siwi that the culturing on several media supplemented with different concentrations of TDZ, the TDZ at 1 mg. L^{-1} showed the highest response rate of buds formation compared to two concentrations (2 and 0.5) mg L^{-1} TDZ, as well as, the treatments (1 mg. L^{-1} TDZ with 2 mg L^{-1} IBA) showed a significant difference in the percentage formation of direct buds compared to other treatments.

The effect of using TDZ alone or with NAA and NOA on the formation of somatic embryos of date palms, where the addition of TDZ alone at two concentrations (5 and 10) mg. L^{-1} to obtain the highest response rate in embryo induction directly from the shoot tip, where it reached (50, 80)%, respectively, compared to other medium

containing (NAA and NOA)at a 1 mg.L^{-1} concentration with TDZ in all concentrations (Ali and Abdulzhara,2018).

AL-Mayahi (2014), in his study on date palm cultivar Hillawi, showed that the highest response rate for bud formation was 66.67% when the MS medum supplemented with (0.5 TDZ +1 BA) mg. L^{-1} while the number of buds was reached (4.2 and 18.2) bud After 16 and 24 weeks respectively. High concentrations of TDZ recorded a significant decrease in the response rate and the number of buds. The lowest response rate at treatment 2 mg. L^{-1} TDZ with 1 mg. L^{-1} BA which reached 16.67 with an average number of buds of 1.0 and zero after 16 and 24 weeks sequentially.

Londe *et al*., (2017) studied the multiplication of Grand naine banana plant *in vitro*, by using several levels of PG and one level of BA were used. After 30 days of culturing, it was noted that PG treatment at a concentration of 200 uM and BA at a concentration of 13.2 uM a significant superiority on the control treatment and all other levels of PG. These study concluded that the possibility of using PG in the banana plant propagation medium, especially at a concentration of 200µM PG, which showed superiority over all treatments except for the treatment of 13.2mM BA, while the others of the traits, showed a significant increase at the treatment of PG 200µM in the average of length vegetative branches reached 2.84 cm, the number, and lengths of roots (4.17 and 4.04cm), respectively, while the treatment of 1000 µM PG, it gave the lowest number shoots and roots reached 1.66 and 1.33 respectively and recorded the lowest shoots lenth 1.66 cm and roots 0.84 cm

Laboratory results indicated an increase in organs' production from the apple shoots in the medium provided with PG and GA compounds, The vitrification phenomenon decreased in the medium provided with PG, as the plants were better, the leaves were broad and green radiant and tender than what was in the control treatment, as is the case in bud production, has increased in both medium in response to the addition of PG or GA, or both together, and the appearance of shoots in the medium containing PG and GA in apple plant together more frequently compared to other treatments, however, adding PG to the MS medium stimulated growth and caused a high increase in the number of internal nodes, As for the interference between the medium type and the addition of compounds, the study showed a significant increase in the length and number of the shoots compared with control treatment and treatment with active charcoal. (Rustaei *et al.,* 2009)

Jani *et al.,* (2015) in a study on the effect of different concentrations of PG (39.7, 79.4 and 198.5)µM on stimulating buds in the cultures of the plant (*Tinospora Cordifolia*) showed that the highest response rate was in both treatments (79.4µ M PG + 4.7µM Kin)and (79.4µMPG +7.0 µM Kin) was 84.4% for both treatments, respectively, while the lowest response rate was recorded at 39.7µM PG at 34.4%. The study also indicated the superiority of treatment 7.0 µM Kin + 79.4 µM PG in the percentage response by giving it the highest percentage of 60.3%. In contrast, the lowest percentage of 7.3% was recorded at the control treatment. The study showed the significantly superior treatment of 7.0 µM Kin + 79.4 µM PG in the

number of buds formed as it reached 7.5 buds. In contrast, the control treatment gave the lowest number of buds and reached 6.2 buds.

2-5: Browning

There are some obstacles associated with *in vitro* propagation of date palm, where the cultured tissue usually produces toxic substances that are concentrated in the medium and thus cause poisoning and death of the tissue due to absorbing these materials, one of the methods of treating this phenomenon was by adding Activated charcoal or (Polyvinyl pyrilidone). The study also showed that the rate of browning discoloration is more in the axillary buds and the Jamar tissues than in the primordial of the leaves and shoot tip(EL-Shafey, *et al.,* 1999; EL-Bellaj and EL-Hadrami, 2004).

Mohsen *et al*., (2015) explained in his study on four cultivars of date palm (Halawi, Barhi, Khudrawi and Sayer), The Sayer cultivar was significantly superior with the lowest browning percentage reaching 20%, while no significant differences occurred between the Halawi and Barhi cultivars, while the highest percentage for browning in Khadrawi cultivar 70% with no significant difference between it and Barhi cultivar.

Sidky (2014) reported in a study of date palms of Sewy cultivar that the browning percentage varies according to the added concentrations of growth regulator (picloram), where the lowest browning percentage was recorded at the treatment of 5 mg. L^{-1} of picloram reached 20%, and the treatment is 1.0 mg. L^{-1} of picloram

gave the highest percentage of browning percentage, reaching 46.66%.

Salekjalali (2012) showed that adding the PG compound to the medium influenced the ratio of browning percentage in damask rose (*rosa damascene*) using various concentrations of (0, 50,100) mg. l^{-1} of PG, whereas the treatment with a concentration of 50 mg. L^{-1} has recorded the highest rate of browning percentage was 5.41 compared to the control treatment 4.67. The study showed that the highest rate of browning percentage in the medium was supplied with 100 mg. L^{-1} PG with 1 mg. L^{-1} BAP, while the mean number of cultures in which browning occurred was 5.44 at treatment 50 mg. L^{-1} PG with 2 mg. L^{-1} BAP.

2-6-: Effect of growth regulators on some chemichal properties

2-6 -1: Effect of growth regulators on plant hormones

Growth regulators such as auxin and cytokinins play an important role in plant tissue culture, so it must be added to the medium to affect cell division and elongation and reveal plant organs. Researchers reported that increasing the ratio of auxin to cytokinins in the medium stimulates the induction of the roots while increasing the ratio of cytokinins to auxin it leads to the regeneration of buds and in a state of balance between them occurs continuous cell division .

The effect of TDZ on the callus levels from internal hormones (Auxin and cytokinins), it was found that adding TDZ to the medium helped to increase the content of callus tissue from IAA and trans-Zeatin-riboside (ZR) during the first five days. The peak of the increase in the concentration of IAA was on the fourth day,As for the ZR, it increased rapidly on the second day, then decreased on the third day, then gradually increased again on the fourth and fifth days. The reason is that TDZ enhances the accumulation of IAA and internal ZR directly in callus tissue (Kou *et al.*, 2016).

The metaplasm of endogenous geowth regulators in the plant has a direct relationship with the presence of TDZ in media during formation and regulation of endogenous growth, as the role of TDZ in morphology is closely related to metaplasm of endogenous growth regulators, where studies have shown that there is an increase in the levels of IAA and ABA in response to the suppliment the medium in the compound TDZ (Hutchinson *et al*., 1996).

Pai and Desai, (2018) showed the physiological effect of the TDZ compound, mentioned in Figure 3, which illustrates the combined effect of TDZ with different plant hormones, as it is similar to the physiological effect of cytokinins. It also contributes to stimulating the construction of auxin by sharing cytokines by increasing tryptamine in the cytoplasm .TDZ interferes with gibberellin's action in stimulating growth of dwarf plants by

stimulating growth of short Internodes

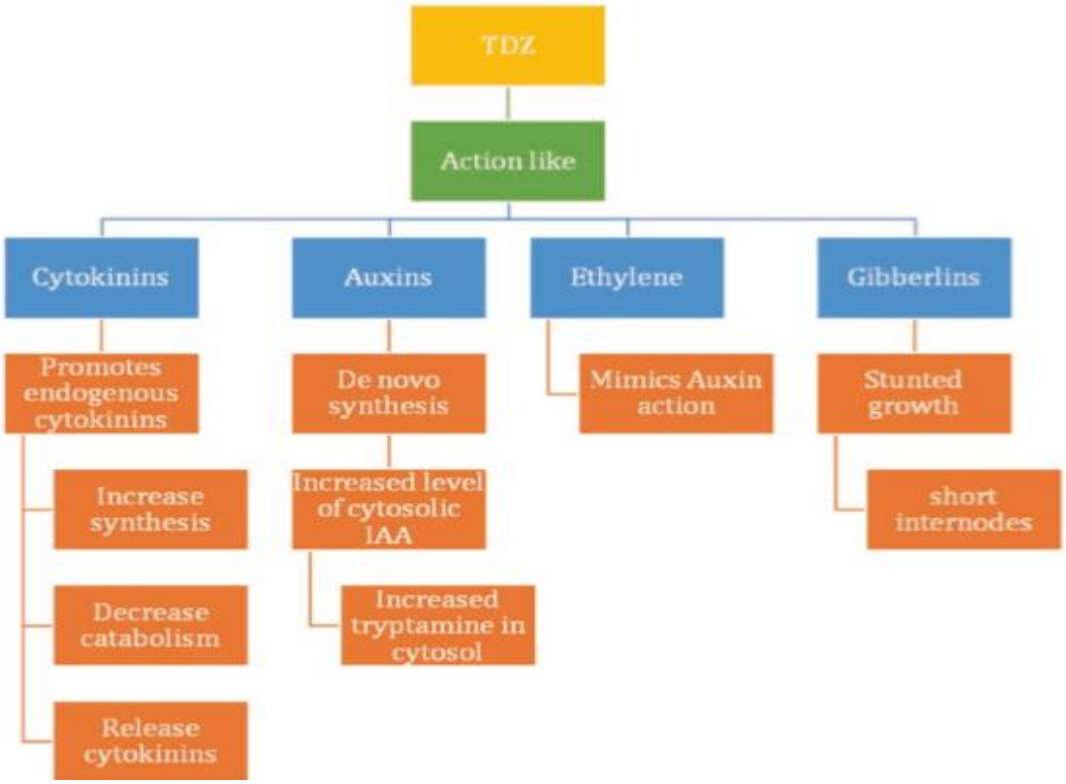


Figure (3) the physiological effect of TDZ and its relationship to plant hormones (Pai and Desai, 2018)

2-6-2: Effect of growth regulators on plant content of chlorophyll

In a study by Ferrante *et al*. (2009), growth regulators were used to prevent yellowing of the cut flowers.Plants were placed in different environments: distilled water and a solution containing 5, 10 µM of TDZ. The effect of TDZ in the presence of light worked on delaying the yellowing of the leaves during 30 days compared to the effect of TDZ in the absence of light, while the treatment at concentration 5 µM TDZ significantly superior on all treatments.

Olivrira *et al*., (2010) show in a study conducted on *Annona glabra L*. using the woody plant medium (WPM) and supplied with some cytokines, the study showed that adding 1 mg. L^{-1} of TDZ to the WPM medium led to a decrease in the content of chlorophyll compared to the control treatment.

Perez *et al*., (2015) show in their study on the effect of PG compound on chlorophyll content in Papaya (*Carica papaya L*.) leaves, using several levels of PG with IBA with a concentration of 9.8 µM, PG at 158 µM exhibited a significant increase in their content of chlorophyll a, b, reached (175, 171) mg. g^{-1} respectively compared to the other treatments.

2-6-3: Effect of growth regulators on plant content of carbohydrates

The importance of carbohydrates in living cells is an important as energy source and a carbon structure for the organic building process. It is also necessary as an osmotic factor (Jergensen, 2009).

Al-Samer (2017) explained the significant difference in the concentration of carbohydrates in the pro callus of date palm cultivars in a medium supported by Auxin 50 mg. L^{-1} of 2,4, D and fungicide Beltanol where the concentration of carbohydrates was 34.25 mg. g^{-1} compared to the standard treatment in which carbohydrates' concentration reached 11.83 mg. g^{-1} and significant differences were observed in the concentrations of carbohydrates in the developing pro callus in the 10mg.l^{-1} Dicamba-supported medium

and the fungicide Beltanol by giving it the highest carbohydrates concentration as it reached 40 mg g^{-1}.

Naif (2019) found in a study of date palm Barhi cultivar that there were significant differences in the content of total soluble sugars between the 2iP concentrations added to the medium, where it was observed that the rate of the total sugar increased as the concentration of 2iP increased and that the highest accumulation rate of total soluble sugars was at treatment 0. 4 mg. L^{-1} of 2iP and reached 2.527 mg. g^{-1}, significantly higher than the other 2iP concentrations, while the control treatment recorded the lowest total soluble sugar content of 0.432 mg. g^{-1}

2-6-4: Effect of growth regulators on plant content of mineral elements

The accumulation of major elements in plant tissue grown *in vitro* is greatly affected by the availability of growth regulators in the medium, where it was noted that cytokines were mainly effective on the accumulation of some elements necessary for the plant such as nitrogen, phosphorus, potassium, calcium, and magnesium in the callus tissue of the *Viscum album* plant (Barberaki and kintzios, 2002).

Olivreira *et al.* (2010) indicated that the content of apple buds from nitrogen, potassium, calcium, manganese, iron and zinc elements was influenced by the type of cytokinin that was supplied to the medium (Kin, ZEA, BAP and TDZ), the results indicated that all cytokinins led to an increase in nitrogen component levels, when the

treatment reached 1 mg. L^{-1} TDZ (47.13) g. Kg^{-1}, and a significant difference from the control treatment, which amounted to (25.26) g. Kg^{-1}, while an increase was recorded in the potassium and zinc elements levels, which amounted to (20.30 g. Kg^{-1}, 112.33 mg. Kg^{-1}) Consecutively, however, the differences were not significant compared to the control treatment. Simultaneously, there was a decrease in phosphorous, magnesium, manganese, and iron elements without a significant difference from the control treatment.

Naif (2019) found that the content of the date palm of Barahi cultivar type of nitrogen was higher in treatment 0.4 mg .L^{-1} 2iP, which amounted to 1.51% and a significant difference from the control treatment, which reached to 0.327. The study also showed that all concentrations of 2iP were significantly superior on the control treatment; as for the proportion of phosphorus, it also increased by increasing 2iP concentrations in the used medium, as did potassium wire behavior itself, as the content of the higher potassium, increased significantly with increasing concentrations of 2iP in the medium, where the highest percentage of potassium ions at the treatment was 0.4 mg.L^{-1} 2iP as it reached 0.9274 mg. L^{-1} while in the control treatment, it was 0.3181 mg. Kg^{-1}. The study also indicated that there were significant differences in the higher iron content of all the 2ip concentrations used, and the treatment 2iP was recorded at the concentration of 0.3 mg. L^{-1} The highest iron content was 0.050 mg. Kg^{-1}, while the lowest value when treatment was 0.4 mg. L^{-1} and it reached 0.039 mg. GM^{-1}, as the study showed, treatment with 2 iP at a concentration of 0.2 mg. L^{-1} superior on the

other treatments at its high content of sodium, as it reached 0.075%, while it was the lowest percentage when treating 2 ip at a concentration of 0.1 mg. L^{-1}, which reached 0.0034%

2-7: Molecular heterogeneity

Molecular indicators are an effective and important means in genetic improvement programs, whether in setting up programs for breeding, which include selecting the necessary primary materials for breeding, as well as evaluating the results of breeding operations or raising the efficiency of breeding programs through early detection of the presence of desired traits in the genetic source, in addition to To that is verification of genetic originality or verification of the degree of mixing, as it can be used in many agricultural applications or in creating a database of genetic information (Ashter, 2009).

The development of molecular biology techniques has caused a significant qualitative shift at the genetic level of the plant, as the use of molecular methods in the classification and breeding of plants has witnessed significant progress that has been exploited to distinguish sex and genetic fingerprint, after it was dependent on phenotypic characterization and biochemical characterization followed by accuracy in characterization being less than in molecular technology, plant characterization began using molecular indicators at the beginning of the eighties of the last century, where these indicators are characterized by the appearance of genetic variants directly on DNA, and DNA is the genetic material that cannot change with environmental influences as is the case with biochemical and phenotypic effects (Baumung *et al*., 2004)

Paterson *et al.,* (1991) also indicated that DNA markers are an important genetic marker for studying the genetic relationship between individuals and the knowledge of the genetic fingerprint that shows the difference in genetic information, as well as its ability to detect genetic differences between the closest individuals, no matter how slight.

The wide applications of DNA indicators in various fields, especially in finding the DNA fingerprinting, characterizing strains of cultivars and distinguishing sex early, and identifying kinship among them enabled plant breeders to crossbreed, strike and develop new cultivars by knowing the level of the existing variations (El-Itriby, 2004).

Imranul Haq, (2016) explained that the development in biotechnology has helped in the emergence of many molecular markers of DNA of great importance that differ in the type and level of information obtained by which they can form maps that relate to genetic association as well as the identification of gender or DNA. Genetic diversity can be known

Abu Al-Jadayel (2014) also stated that the confirmation of the genetic stability of the perennial plants resulting from propagation in vitro, such as palm and fruit trees, is done through the use of several indicators of DNA to detect and verify the genetic stability and issue a certificate of genetic conformity with the mother plant.

2-7-1: Determination of molecular variations using technology

2-7-1-1 : Randomly Amplified Polymorphic DNA (RAPD)

Molecular biology techniques that are based on the Polymerase chain reaction (PCR) are considered among the new tools used in genetic analyzes and mapping, and the RAPD technique has been used for random amplification of DNA, which enables Identification of individual species and differentiation between species (Liu *et al*. 2005)

A study by Al-Moshileh *et al*. (2004) in which the suitability of the random amplification method of DNA fragmentation using RAPD technology as genetic evidence to analyze the genetic fingerprint of five cultivars of date palms, was used 12 prefixes where he gave 64 packages, that the RAPD technique was quite successful in distinguishing between the cultivars, as the five cultivars showed a difference in the level of DNA. The study also showed that the genetic similarity ratios between the studied cultivars ranged between 70% to 80%, and the diabetic cultivar was the most distant in terms of genetic similarity from other cultivars.

The analysis of the RAPD index using four prefixes showed that only three prefixes succeeded in doubling the DNA sequence of the first callus date palm *in vitro* in a medium supported by different growth regulators (Primer 650, OPAR8, OPAR3). It was observed that there was a difference in the relative efficiency between the prefixes. Whereas the Primer650 initiator was better than the other prefixes, with a relative efficiency rate of 38%, and the electrical

relay results showed a doubling of 68 total beams, at a rate of 22.67% for each initiator. The study also indicated the results of the similarity coefficient analysis that the highest rate of genetic similarity was when control treatment and treatment of 2,4, D at a concentration of 50 mg. L^{-1}, which recorded 96%, while the lowest value for the similarity coefficient, when compared to the control treatment with high concentrations of 2,4-D treatment, was 27% (Al-Samer, 2017).

Sakr *et al.*, (2012) indicated in a study to classify eight cultivars of date palm and one male cultivar of date palm using RAPD technique, where 20 random prefixes were used to examine the genetic forms. But only five prefixes gave one packet, reaching 179 packs, i.e. 36 packs for each initiator, including eleven multiple genotypes. The initiator gave OPK1 the highest percentage of genetic variation, was 9.52, while the lowest percentage of the genetic variation was at the beginning of OPK4, which amounted to 4.25. The study showed that both the initiator OPK4 and OPK5 gave a genetic similarity in its enlargement in all-female cultivars. In contrast, the prefixes OPK1, OPK3, OPK7 gave a little phenotypic difference. The analysis showed that all packages are similar, and there was a clear contrast between the female and male cultivars.

Moghaleb *et al*., (2011) showed in a study to test the genetic similarity between the parent plants of date palm for the cultivar, the cultivar is unknown and with a number of plants resulting from tissue culture, which have been re-cultivated several times the similarity of both cultivars was studied by RAPD technique and using ten prefixes

Where the data indicated that the plants that produced them from the medjol and ferhi cultivars showed a genetic difference of 36.2 and 37.8%, while the genetic similarity was between 63.8 and 62.2% in succession with the mother plant, that the genetic similarity between the mother plants and the resulting plants revealed that through the RAPD technology, it led to the use of modern research to replant the culture *in vitro*. It can be used as a product of genetically modified date palm plants, such as expression, to produce economically important genes.

2-7-1-2: Inter Simple Sequence Repeats (ISSR)

ISSR is a semi-random marker in which the target portion of DNA is amplified between two sites where only one initiator is used with target microsatellite and PCR dependent (Zietkiewicz *et al*., 1994). ISSR technology is characterized by speed and ease, unlike RAPD technology. This technology is less expensive than the rest of the techniques as it was used with many plants in a large and wide way (Abate and Tesfaye, 2017).

During the past years, the employment of molecular markers such as ISSR contributed to the evaluation of the genetic stability of many types of plants that have been renewed by the tissue culture technique, where the frequency of the formation varies according to the type of plant as well as the conditions of culture. However, it is recognized that physical differences or variations are a common phenomenon associated with tissue culture histology. The selection of the explant, the tissue's age, the genetic structure, culture

conditions, and the regeneration method are fundamental aspects of genetic stability (Rout *et al*., 2006).

Thummar *et al*., (2015) in an attempt to discover the genetic diversity between plants used in tissue culture using PCR-based techniques, which could be used to improve the genetic palm genetics, the ISSR technique was used to know the genetic variances between the original plant and the plants resulting from tissue culture, after re-cultivation twice in succession. It was observed that the packages formed in the first and second group did not have any genetic variation when replanting for two consecutive periods

Ahmed *et al*, (2012) used five csllus of cultivars of date palm that were replanted eight times to find out the changes during micropropagation operations, where 780 batches of DNA were obtained using ISSR technology by 16 primers, giving 12 initiators packages with genetic differences between callus and plants. Resulting from re-cultivation in one or more cultivars, while four prefixes did not show a genetic difference between callus and plants resulting from re-culture, this difference was observed by the presence of a bundle in the resulting plants. It did not appear in callus as in the of Khuneizy cultivar that showed a genetic difference at the beginning BT9. The bundle may be found in callus and not present in propagated plants as in the cultivar, which showed a genetic variation of five prefixes.

The study carried out by Hazaa, (2019), to determine the gender and genetic diversity of a number of cultivars of date palms

and to find the genetic fingerprint using some molecular marker techniques, showed that the results of the ISSR technique using seven prefixes, which was the number of packages produced by 1908 packages where the starting factors were registered (ISA02 and ISA71) The highest number of packages in male and female cultivars, which reached (188 and 185) in succession, and the two parameters (Hb12 and ISA02) showed the highest genetic formation, as the number of packages (37 and 33) in succession, so the initiators (H B10 and Hb12) gave the highest value of the individual packages In the female cultivar and ISA02 in the male cultivar, the initiators (Hb12 and ISA02) recorded the highest diagnostic strength (19.17% and 19.64%) for the male and female cultivar.

2-7-2 Inserting the gene

In order to increase the salt tolerance of the alpha alfalfa plant, Zhang *et al.,* (2012) transferred the *SeNHX1* gene to the plant by Agrobacterium bacteria from the *Salicornia europaea* L. plant, which is one of the salinity tolerant plants to the alfalfa plant where success was achieved in transferring the gene of five plants exposed to 6% NaCl for 21 days after being tested by Real-Time PCR as the amount of chlorophyll and some oxidative enzymes was examined. Some researchers confirmed that the genetic expression of some genes that have characteristic of salt tolerant (*SsNHX1, TaNHX1, AhBADH*) increased the salinity tolerance of genetically transformed plants by transferring the gene compared to the plant Al-Birri (Jin *et al*., 2010; Li *et al*., 2011; Zhang *et al*., 2012). The transfer of the *SeNHX1* gene into three types of tobacco plant stimulated the plant's

tolerance to salinity up to 200 Mm. The rate of photosynthesis in genetically modified plants by transferring the gene was less affected by salt stress than wild plants (Zhou *et al*., 2008).

The rose plant was used to study the optimum conditions for transferring the *SeNHX1* gene to the callus. Results showed that the best medium used to stimulate the gene transfer process was MA + 0.5mg.l^{-1} 2,4-D + 0.5 mg.l^{-1} TDZ, the carrier used in this process is the Agrobector, which showed its ability to transfer the target gene to the callus using the blue fingerprint upon transfer (Hui-chao *et al*., 2010).

Jha *et al*., (2011) observed that when the *SPNHX1* gene was transferred from the *Selecornia brachiatn* halophyte to the tobacco plant, it succeeded in increasing the salt tolerance of the tobacco plant where analysis of the gene expression process and using RT PCR showed its maximum value when the plant was exposed to NaCl. A concentration of 0.5 M within 48 hours also showed that this gene contains a sequence of amino acids that are very similar to some halophyte. It is also recommended that this gene is important in the process of saline tolerance of the plant.

3- Materials and methods:

This study was conducted at the plant tissue culture laboratories of the Date Palm Research Center at the University of Basrah for the period from December 2017 to September 2019. The part of the chemical and molecular analyses has been conducted in the laboratories of Horticulture and Landscape Design department , College of Agriculture, University of Basrah. while another part has been done in the Laboratories of Ramen University of Genetic Engineering , Islamic Republic of Iran.

3-1- Medium preparation

The medium was prepared from a combination of inorganic salts (MS salts) , according to Murashige and Skoog, (1962) as shown in Table (1). Steps below to prepare one liter of the medium:

1- The(500) ml of Double distilled water in 1000 ml volumetric flask was heated in heat heat-resistant glass Pyrex

2-The (4.33) gm of MS salt was added

3- The the chemical substances were added that shown in Table (2) according to (Tisserate, 1981).

4- The plant growth regulators and fixed concentrationswere added to both the solid and liquid mediums according to the stage of growth and the objective of the experiment.

the Auxin was Dissolve in (5 ml) of 0.1N NaOH, and cytokines in (5 ml) of 0.1N HCl. as well as dissolve TDZ in Dimethyl sulfuoxide

(DMSO) solution, and PG was dissolved in (5 ml) of 95% ethanol alcohol.

5-The pH of the medium at 5.7 was Adjusted by using the Digital pH-Meter type 7740 after the calibration.

6- The 7 g of agar was added to the solid media, and preheat to 90 ° C.

7- The 35 ml of medium was distribute to the jars , cover with aluminum foil , and then close with metal plugs. The bioreactors were prepared with 450 ml of liquid medium (free agar) and closed with tight plugs which prepared for this purpose.

8- Medium and tissue culture tools were sterilized by using autoclave type Sanshengi liaegixie Model 7X-4507 at 121 ° C and under 1.05 kg. Cm^{-2} pressure for 20 minutes.

9- The medium containers were shaked several times for homogenization and left until getting cool. Some growth regulators was added to both types of mediums (liquid and solid). TDZ at concentrations 0, 0.5,1,0 mg. L^{-1} was added with PG to the medium at the following concentrations 0.25,50,100 mg. L^{-1} as well as the substances in Table 2.

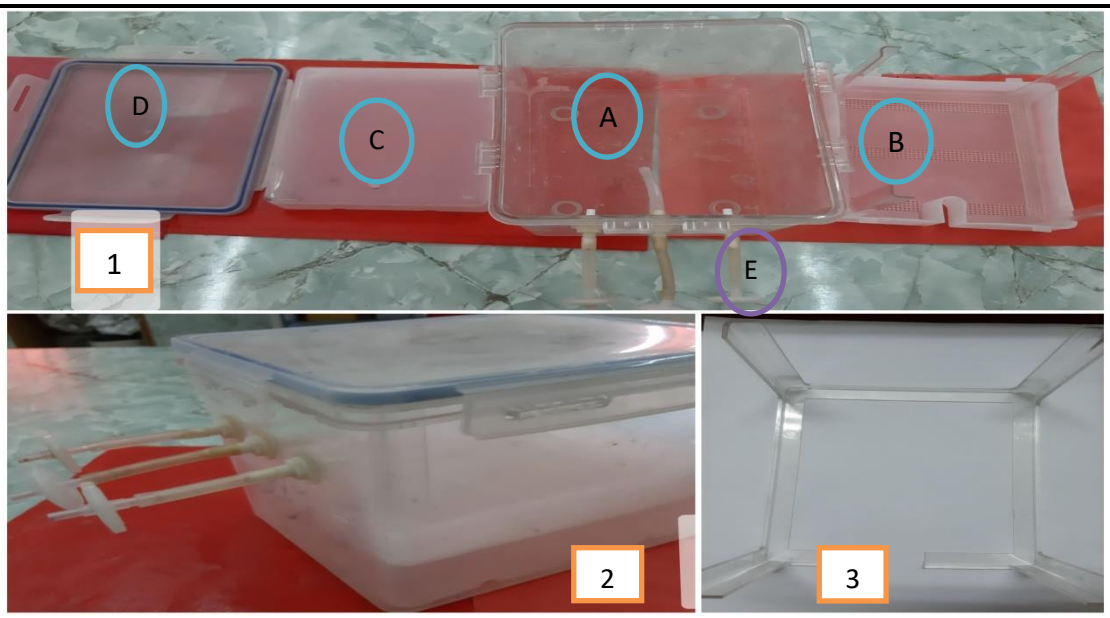
The Bioreactor system consist of two-parts (two containers), The first container was perforated. The second container at the bottom contains an aperture that allow for air entry , help to raise the liquid medium and reache to the plant parts that planted in the upper perforated container.

Table (1) Concentrations of inorganic salts of MS medium.

Group	Name of substance	Chemical symbol	Quantity mg. L^{-1}
Nitrate	Ammonium nitrates	NH_4NO_3	1650
	Potassium nitrates	KNO_3	1900
Sulphates	Magnesium sulphates hydrated	$MgSO_4.7H_2O$	0370
	Manganese sulphate hydrated	$MnSO_2.H_2O$	16.9
	Zinc sulphate hydrated	$ZnSO_4.7H_2O$	8.6
	Cupric sulphate hydrated	$CuSO_4.5H_2O$	0.025
P.B.MO	Potassium di Hydrogen Phosphate	KH_2PO_4	170
	Boric acid	H_2BO_3	6
	Sodium molybdate hydrated	$NaMoO_4 2H_2O$	0.25
Halides	Calcium Chloride hydrated	$CaCl_2.2H_2O$	440
	Potassium Iodide	KI	0.83
	Cobalt chloride hydrated	$COCl_2.6H_2O$	0.25
Cheated iron	Ferrous Sulphate hydrated	$FeSO_4.7H_2O$	27.84
	Chelating substance in the form of disodium salt	Na_2 EDTA	37.24

Murashige and Skoog, (1962)

The upper perforated vessel was connected by a tube to one of the air filters. The bioreactor system contained a plate with four arms that placed between the cap and the perforated vessel, this plate works to prevent the raising of plant parts container due to the high level of the liquid medium, The process of pumping air to the medium was carried out by sterilized filters connected to an electrical regulator (Timer) that work according to the required time of immersion, as described in picture 1, 2 and 3. It is operated five times per day for two minutes, so tthe medium raised and submerged the cultivated parts. The recultuer was done every four weeks.



Figure(4)The bioreactor parts (A: The culture container B: The base of the container, C: The lifting plate, D: The container cover, and: The air intake and purification system. Air filters and tubes Figure (5)The bioreactor plate Figure (6) : explants fixation plate

Table (2) concentrations of additive substances to the medium

Substance	Quantitative mg. L^{-1}
Sucrose	30000
Sodium hydrogen ortho phosphate	170
Adenine sulphates	40
Meso-Inositol	100
Thiamine-HCl	0.5
Neutralized activated charcoal	3000
Agar	6000

3-2- culture Stage

3-2-1- Effect of different combinations of TDZ and PG on callus growth:

Induced callus from the apical buds of date palm cv. Barhi was cut and cultured on the solid and liquid MS medium with the substances as mentioned in Table (2). The medium was prepared with different concentrations of TDZ (0.0, 0.5 and 1.0) $mg.L^{-1}$ and PG (0.0, 25, and 50) mg. L^{-1}, with 6 and 1.5 $mg.L^{-1}$ of NAA and 2iP respectively as a fixed treatment, in a Factorial Experiments, to determine the appropriate concentrations with culture systems (Bioreactor and the solid medium) and their interaction in the growth

of callus. The propagation process continued for (10) weeks, and the callus was incubated in the growth room at 27 ± 1 ° C under (1000) Lux of illumination intensity for (16) hours daily, and the recultuer process was carried out every four weeks.

3-2-2- Effect of different combinations of TDZ and PG in stimulating the development of adventitious buds from callus tissue.

Several concentrations of TDZ (0.0, 0.5 and 1.0) mg.L^{-1}, and PG (0.0, 25, 50 and 50) mg.L^{-1}were examined with 0.5 mg. L^{-1} of naphthalene acetic acid (NAA), 0.5 mg. L^{-1} of 6-benzylad¬enine (BA) and 0.5 mg. $L^{-1}$of N6-furfuryladenine “kinetin” as a fixed treatment, in a factorial experiment, to determine the appropriate concentrations for each with cultivating systems (The bioreactor and solid medium) and their interactions in stimulating the formation of adventitious buds. The medium consisted of MS salts in addition to the substances mentioned in Table 2 with the experimental treatments. The implants were incubated in the growth chamber at 27 ± 1 ° C under (2000) Lux of illumination intensity for 16 hours daily, the re-culture process was carried out every four weeks.

3-3: Physical characteristics

3-3-1- Fresh weight of the callus formation:

The fresh weight of the primary callus and buds of ten replicates was measured after (8) weeks of cultivation, using a sensitive Balance (Sartorius type).The calculation was done according to the Saad (2001).

3-3-2- Number of buds

The number of adventitious buds was calculated after 12 and 20 weeks of callus culture.

3-3-3- Number of shoot regeneration

The number of formed branches was calculated after 28 and 32 weeks of callus culture .

3-3-4- Percentage of browning

The percentage of browning was calculated by the formula

browning percentage% = (number of brown explant / (total number of explant) X100

3-4- Plant hormones

3-4-1- Auxin, gibberellins and Abscisic acid- like substances extraction

The extraction process was carried out according to the method that described by Abbas *et al*., (1995) 50 ml of methanol 80% was added to 5 g of fresh weight of tissue of callus, buds and vegetative growths , incubated at 4 ° C for 24 hours. The leachate was taken and repeated the incubation on the remaining of botanical sample. Total volume of extract (100 ml) was evaporated by rotary evaporator (Type RE-120) until reaching to the aqueous phase, the sample was centrifuged for 5 min after addition of 3ml of lead acetate 45%, then 3 ml of 22% of potassium oxalate was added to the suspension and centrifuged for 5 min , The suspension was

collected and the volume completed to 50 ml by distilled water. The pH was adjusted to 2.5 by HCl acid (1N). The sample was placed in separating funnel and 50 ml of the diethyl ether solvent was added. and shacked manually for 10 minutes .The process was repeated three times and mixture collected in 150 ml flask. By rotary evaporator device, ether was evaporated until the volume reached to 3 ml, and , the extract was transferred to a small 10 ml vials and placed in the dark until the ether was completely evaporated. 5 ml of methanol was added to the sample and preserved in a freezing degree until the estimation process was performed.

3-4-2: IAA-like substance determination

Auxin-like substances were estimated based on the Crozier *et al*., (1980) method by the Spectrophotometer Visible Shimadzu type. The samples were measured at 280 nm wavelength. The concentrations were calculated based on a standard curve in which the natural Auxin was used (IAA indole) and the results were estimated in $\mu g.g^{-1}$ fresh weight.

3-4-3: GA_3-like substances determination

Gibberellins-like substances were estimated based on Abbas *et al*., (1995). Samples were read at 205 nm wavelength, and the compositions were calculated by using a standard curve in which Gibberellic acid was used, the reading was estimated in $\mu g.g^{-1}$ fresh weight.

3-4-4: ABA-like substances determination

ABA-like substances were estimated based on the standard curve in which ABA was used at 254 nm wavelength the result was estimated in µg. g^{-1} according to (Horgan, 1981).

3-4-5: Extraction of cytokinin-like substances:

The extraction process was performed according to Abbas and Fandi (2001), the extraction was processed similarly as in Paragraph (3-5-1) and the pH of aqueous solution adjusted to 8.5 by using sodium hydroxide (2N).

The partitioning was performed using finale separation method as described earlier for three times to transfer the organic material to the ethyl acetate solvent, and then the organic phase extracts were collected. The extract was concerted to 10 ml by the vaporization process, the extract was transferred (10 ml) to small tubes and left in the dark and cool place until ether acetate solvent evaporate and dispose completely, then 2 ml of methanol was added to each tube and kept freezing until the measurement process.

3-4-6- Cytokinin-like substances estimating:

The cytokinin-like substances in the samples were estimated by UV absorption method at 265 nm according to the Abbas and Fandi (2001) method. The result was calculated depending on Benzene Adenine curve and expressed in µg.g^{-1} fresh weight.

3-5- mineral content

3-5-1: Percentage of nitrogen

0.2 g of dry weight callus, buds or vegetative growths were digested according to the Cresser and Parsons (1979) method. The nitrogen was estimated in the digested samples using the Micro Kjeldhal apparatus described in Page *et al*. (1982).

3-5-2: Percentage of phosphorous

Phosphorus was measured in tissue samples using the Ammonium Vanadomolybdate method, the intensity of the yellow color was measured in the spectrometer at 470 nm wavelength according to the Jakson (1958).

3-5-3: Potassium determination

Potassium element was estimated using the Flame photometer method as described by Cresser and Parsose (1979).

3-5-4: Magnesium and Manganese determination

Magnesium and manganese elements were estimated by the Atomic absorption device and according to Black (1965).

3-6: Physiological characteristics:

3-6-1: Carbohydrates contents in buds and vegetative shoots (mg . 100g $^{-1}$ dry weight)

The total soluble of carbohydrates was estimated by the phenol - sulfuric acid method that described by Dubius *et al*. (1956) .

1- 0.2 g of dry vegetable sample per experimental unit was weighted and put it in a glass jar.

2- 75 ml of distilled water was added to the sample and placed in a hot water bath at 70° C for 60 minutes.

3 The extract was filtered through Whatman No1 filter paper,and 25 ml of distilled water was added to 5 ml of the extract, and then add 1 ml of Phenol 5% and 5 ml of concentrated sulfuric acid to 1 ml of diluted extract and cooled into room temperature.

4- Absorption was measured at 490 nm wavelength with the Spectrophotometer.

5- The total dissolved carbohydrates were estimated according to a standard curve of used glucose.

3-6-2: Total chlorophyll contents (mg.g^{-1} fresh weight)

Vegetative samples were taken randomly from five samples for each treatment, and they were washed and dried by the air, 8 ml of acetone 80% was added to 0.5 g of sample and crushed gently by mortar and then centrifuge, A spectrometer was used to measure the optical absorption at 645 and 663 nm, the amount of chlorophyll (mg of g^{-1}) was calculated by the following equation (Howrtiz,1975),:

Total chlorophyll = (20.2 x D (645) + 8.02 x D (663)) x (V / (W x 1000))

D = Optical Density

D (645) = optical absorption reading at 645 nm wavelength

D (663) = optical absorption reading at 663 nm wavelength

V = final volume of the extract (10 ml)

W = tissue weight

3-7- Molecular indicators

3-7-1- Preparation of plant samples

The botanical tissue was selected from culture containers and washed well with distilled water, sterilized by alcohol 70% and cut off into small parts. The samples was placed in a sterile ceramic eyelid and crushed well with liquid nitrogen until turning into white powder, transferred the powder in a 10 ml vial a with labeling and kept in -80 C for later analyzes

3-7-2- DNA Extraction

For the purpose of DNA extraction, the following solutions were used

(Cetyl Trimethyl Ammonium Bromide) CTAB

(Tris [hydroxymethyl] aminomethane hydrochloride) Tris - HCl

(Tris [hydroxymethyl] aminomethane) Tris base

(Ethylene Diamine Tetra Acetic acid) EDTA

(Poly Vinyl Pyrrolidone) PVP

(sodium chloride) NaCl

DNA was extracted using CTAB method, according to Aitchitt *et al*. (1993) and Doyle and Doyle (1990) with minor modifications, as in the following steps:

1- 0.1g of ground tissue was taken and placed in a 2 ml Eppendorf tube with labeling.

2- 600 µl of CTAB Buffer solution was added(preheated in a water bath at 65 ° C).

3- 8 µl of mercapto ethanol (ME)was added and vortex by Vortex electrostatic mixer to mix the solution with the sample well.

4- The samples were placed in a 65 °C water bath for 60 minutes with shaking every 10 minutes.

5- 600 µl of chloroform was added: Isoamyl alcohol solution (24: 1)ml was added with handshaking forward and backward for 5 minutes.

6- The samples at 13,000 rpm to 10 min was centrifuged

7- The upper layer by Micropipette was transfer to 1.5 ml Eppendorf tubes, 600 µl of cooled Isopropanol solution was added to the samples, the tubes for 5 minutes were shaked gently until homogeneous, and then at 20 ° C for 20 minutes were incubate.

8- The samples were placed in a ready-made centrifuge radiator at 13,000 cycles .minute $^{-1}$ for to 10 min.

9- The suspension was discarded and the samples left in the air to dry for 15 minutes.

10- 90 µl of 4 mM of sodium acetate(pH 5.2) was add, then 1 ml of 99.9% of ethanol was add and mixed slowly several times to precipitate the DNA and then incubate in 20 ° C for 20 minutes.

11- The centrifugation process was carried out at a speed of 13,000 cycles .minute $^{-1}$ for 10 min

12- The suspension was discarded and a precipitate was washed with 600 µl of 70% ethanol and the tube was shaked several times by hand.

13. Centrifuge at 4 C with 6000 rpm for 5 minutes and then DNA was left in the air for 30 minutes.

14. 100 µl of TE buffer solution was added to each sample and storage at -20° C until use.

3-7-3: DNA concentration and purity estimating:

The concentration and purity of DNA were estimated by the Nano-Spectrophotometer. The device was calibrated to zero by placing a drop of distilled water and then samples were read at wavelengths 260 and 280 nanometers.

3-7-4: Agarose gel Preparation

Electrophoresis device, power supply, and special comb were prepared for drilling formation (Wells), 0.3 g of agarose was dissolved in 20 ml of 1X of Tris –Boric acid EDTA (TBE), heat the mixture with continuous stirring until the gel melts totally and left until reached to (50-55) ° C, 1 µl of the gel ethidium bromide dye was

added, the gel gently was pour and continuously to prevent the formation of air bubbles in the sterile casting mold with sterile water treated with chlorine 10% and left for 30 minutes until it solidifies. Then, lift the comb gently to keep the pits (Williams *et al*., 1990).

3-7-5- Agarose Gel Electrophoresis and gel documentation

After preparing the gel, it was transferred to the electrophoresis chamber and immersed with a TBE (1X) buffer. The electrophoresis process was performed for the PCR reaction products.The samples were distributed over the hole, the electrodes were connected to the power supply, the wattage was fixed to 80 volts for half an hour and the electrical relay was directed towards the positive electrode. The gel was then transferred from the electrophoresis device to the documentation UV-Transilluminator to observe the beams that appeared in ethidium bromid (Sambrook *et al*., 1989).

3-7-6 - Determination of DNA fingerprint by RAPD and ISSR using PCR technique

Five primers of RAPD and five primers of the ISSR were used to identify and determine the DNA fingerprint and to detect the presence of genetic variances as shown in Tables 3 and 4.

Table(3)Primers used to determine the DNA fingerprint RAPD technique

NO.	Primers	Primers Sequence	GC%
1	OPB-07	GGTGACGCAG	70
2	OPO-07	CAGCACTGAC	60
3	OPD-10	GGTCTACACC	60
4	OPA-2	TGCCGAGCTG	70
5	OPA-12	TCGGCGATAG	60

Table (4) Primers used to determine the DNA fingerprint by ISSR DNA technique.

NO.	Primers	Primers sequence	GC%
1	844A	CTCTCTCTCTCTCTCTAC	50
2	814	CTCTCTCTCTCTCTCTTG	50
3	HB10	GAGAGAGAGAGACC	57
4	HB9	GTGTGTGTGTGTGG	57
5	ISSR6	GACAGACAGACAGACA	50

A- DNA amplification by using RAPD-PCR technology.

The DNA was doubled by PCR technology according to the program setting that used in the presence of five RAPD primers and as shown in Table (5). The reaction mixture was papered from the reactants that show in table (6) with 50 ng of DNA template. .The samples were mixed well by vortex for 10 seconds, and then were transferred to the thermocycler PCR (Cleaver Scientific).

Table (5) RAPD-PCR Program

Stage	Steps	Temperature	Time	No. of Cycle
1	1	Denaturation 95C°	5 min	1
2	1	Denaturation 95C°	1 min	35
	2	Annealing 32-34C°	1min	
	3	Extension72C°	2min	
3	1	FinalExtension72 C°	10 min	1

Table (6) The components included in the RAPD-PCR reaction

No.	Materials	Volume
1	RAPD Primer	1 µL(1.0 µM)
2	Buffer	2.5 µM(1X)
3	DNTPs	8µl (1.25µM)
4	$MgCL_2$	1µl (1.5mM)
6	DNA Taq Polymerease	1µl(1Unit.μl^{-1})

The volume was Completed to 25 µl with sterile distilled water

B- DNA amplification by using ISSR –PCR technology

DNA segments were amplified using PCR technology using five ISSR primers according to the program setting as shown in Table (7). The reactions reagents that described in Table (8) were papered in the Eppendorf tube to the final volume 25 µl with 50 ng of a DNA template, mix well for 10 seconds, and then transfer to the Thermocycler PCR(Cleaver Scientific) to start the reaction according to (Hamza *et al*. 2013).

Table (7) ISSR-PCR Program setting

Stage	Steps	Temperature	Time	No. of Cycle
1	1	Denaturation 94C°	5Min	1
2	1	Denaturation 94C°	1Min	35
	2	Annealing 44-54C°	1Min	
	3	Extension72 C°	1Min	
1	1	Final Extension72 C°	7Min	1

Table (8) The components included in the ISSR-PCR reaction

No	Materials	Volume
1	ISSR Primer	1 µL(1.0 µM)
2	Buffer	2.5 µM(1X)
3	DNTPs	8µl (1.25µM)
4	$MgCL_2$	1µl (1.5mM)
5	DNA Taq Polymerease	1µl(1Unit.μl^{-1})

The volume was Completed to 25 µl with sterile distilled water

3-7-7- Data analysis

3-7-7-1- Determination of the molecular weights of the replication bands

Molecular weights of the bands were estimated using photo capture program MW. 10.01V based on the standard marker (1300 bp)

3-7-7-2- Indicators of the bands

Number of generating bands from the electrophoresis of the agarose gel was calculated for each primer, the obvious polymorphic and monomorphic bandS were calculated after converted to configuration tables in Microsoft Excel by using (1) when a band exists and (0) when a band does not show . The following factors were estimated for each primer according to (Khierallah *et al*., 2011; Alansari *et al*., 2014).

To prepare a liter of the solid medium, add 25 g of LB Agar, Sterilize the medium by Autoclave at 120 ° C and 1.04 kg. 2 cm pressure for 20 minutes, after that, 400 µl of Garamycin antibiotic was added for every 100 ml of LB Agar solid medium under sterile growth chamber which supplied with special and sterile filters. For The liquid medium preparation, 25 g of LB Broth is added to 1 liter of distilled water, then the sterilization is carried out as mentioned earler.

3-7-7-3- Gene transferring to bacteria:

The gene transformation carried out based on Saker *et al.,* (2009) as below.

1- Place the plasmid pCAMBIA3301 containing the *SeHNX1* genes and hygromysin phosphor transferase gene (*hpt*) with rice adapted 35SCaMv and bacteria (*Agrobacterium tumefacines* strain AGLI) in a 1.5 ml Eppendorf tubes in the ice box.

2- 10 µl of plasmid was added to the bacteria in ice box and incubated at 4 C for 30 min

3- The bacterial containing the plasmid were placed for 2 minutes in warm water 42 ° C, then the bacteria were placed in ice box for 15 minutes and then incubate at 4 C.

4- The bacterial containing the plasmid was transferred to the liquid medium LB (1000 µl).

5- The tubes was Shaked by the shaker (encompasser) for one hour.

6- The sample was centrifuged for 2 minutes at 4 ° C in 5000 rpm and . the suspension was discarded and the precipitate which was the bacterial plasmid keep

7- Bacteria was cultured on the solid medium by using sterilized loop.(Picture 4:A)

8- The media was incubate with bacterial plasmid for 24 hours at28 ° C. until the appearance of white spots of bacterial growth Picture (4: b).

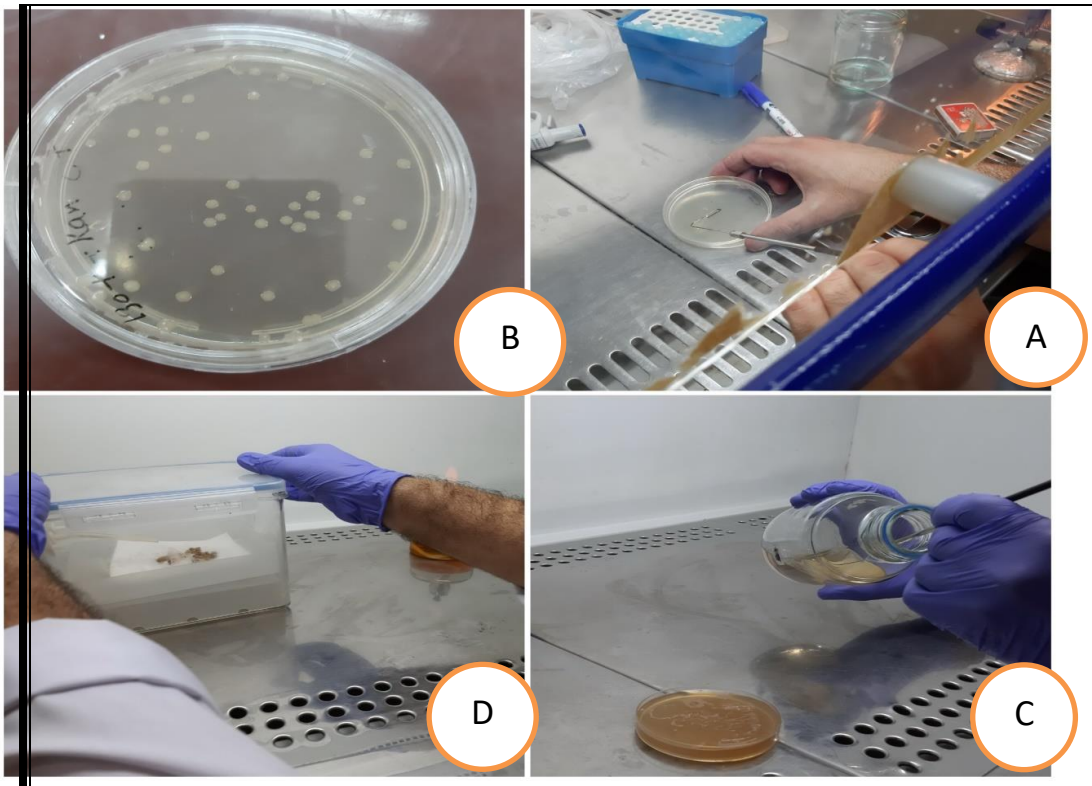


Figure (7) a: the bacterial culture process on the solid media; b: the bacterial growth on the medium; c: the process of transferring the bacteria to the liquid medium; d: the callus in the bioreactor

3-7-7-4- Gene transferring from bacteria to callus

3-7-7-4-1: Liquid medium and the liquid medium for callus preparation:

1- the liquid medium was prepared by adding 25 g of liquid LB to 1 liter of distilled water

2- The nutritional medium was prepare for callus planting by adding macroelements and microelements with half-strength, 60 g. L^{-1} sucrose, 10 g. L^{-1} glucose, 200 mg. L^{-1} KH_2PO_4, 1 ml per liter of crystalline acid, as well as 10 mg L^{-1} Auxin 2,4-D and 3 mg. L^{-1} cytokinin 2ip .

3- All components were placed in the sterilization device, as mentioned previously.

4- The medium was left to be cool down, then 100 µM of acetocercone was added and 200 mg. L^{-1} of MES, take two parts of the medium equal to the volume of the liquid LB. Steps were done under sterilized conditions.

3-7-7-4-2- Solid medium LB Agar preparation.

The solid medium was Prepared by adding 25 g. L^{-1} of LB Agar and 400 ul. 100 ml^{-1} of the Kanamycin antibiotic and 1000 µl. 100 ml^{-1} of Rifambecin, after sterilization, medium were distributed in Petri-dishes and left for 15 minutes until be solid.

The bacteria was cultured on the solid medium (where it was liquid in the tube), incubate for 2-7 days at a 28 ° C. Then the gene was transferred to the callus as follows:

1. Swabs was taken from grown bacteria on the solid medium and cultured in the liquid LB medium, picture (4: c). incubate the samples at 28 ° C for 18 hours, with a continuous shaking.

2. The sample was divided into tubes and centrifuge for 10 minutes at a 4000 rpm and 4 ° C.

3. The suspension was discarded and the precipitate only was taken in the sterile hood.

4. the bacteria was transfered to the medium of the callus by besmearing a little part of callus medium with precipitate of bacteria with shaking and returning it to the callus medium again.

5. The container of both the medium was closed and bacteria with aluminum foil, and then incubated with continuous shaking at 28 ° C for 3-4 hours.

6. The callus was prepared in the sterile cabin an hour before.

7. The callus was placed in the Bioreactor medium, and incubated for 2 hours at 28 ° C.

8. Medium was discarded and keep only the callus, the callus was washed by a second part of media (without bacteria).

9. Mhe media discarded and , re plant the callus in a new medium has 0.5 g. L^{-1} active charcoal and 0.5 mg. L^{-1} NAA. Leave callus for 3 days in the dark.

10- the callus cultur was repeated after 3 days by washing it thoroughly with sterile water inside the planting cabin in a selected medium has previous elements and0.5 g. L^{-1} active charcoal, 0.5 mg. L^{-1} NAA, 500 mg antibiotic Cefataxin, and 3 ml. L^{-1} of phosphenotelicien and incubate for 21 days

11- Callus was recultured as mentioned previously with 200 mM NaCl for each of the standard and gene transfer treatment.

3-8: Experimental design and statistical analysis

The experiments were carried out as factorial experiment by three-factor for each experiment using Complete Randomized Design (CRD) (Al-Rawi and Khalaf Allah, 2000). Statistical program analysis system Gensat under the Windows system was used to perform statistical analyzes. The means were compared using the Least Significant Differences L.S.D at 0.05 probability level to test the significant differences between the treatments.

4- Results

4-1- Effect of medium type, TDZ and PG on physical traits:

4-1-1- Callus weight.

According to the results obtained in Table (9), the callus tissues grown at liquid medium showed significantly superior in the callus weight 5.61 g . compared to the callus grown on a solid medium, which was (5.16 g) .

Data Table (9) showed there was a significant effect of TDZ on the mean callus weight. The highest callus weight was obtained on the media supplemented with TDZ at a concentration of 0.5 mg L^{-1}, where it reached 6.24 g. which has significantly superior on the two concentrations (0and 1 mg L^{-1}), while the lowest weight of callus was at the control treatment 4.74 g . additionally, TDZ treatment at 1mg.L^{-1} showed significant superiority compared with control treatment.

Table (9)showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority compared with other concentrations (0 and 25 mg. L^{-1})in the mean weight of callus after 8 weeks of culture, where the highest value of callus weight was recorded 6.07 g . The lowest weight of callus was at the control treatment (4.80 g).

Data in Table (9) indicate that the treatment between TDZ and medium type had a significant effect on callus's weight after 8

weeks from culture. TDZ treatment at concentration 0.5 mg. L^{-1} with the

Table (9) Effect of medium type TDZ, PG, and their interactions on mean fresh weight of callus(g) after 8 weeks of culture

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	3.53	5.34	5.69	4.85
	25	5.05	6.40	5.55	5.67
	50	6.23	6.94	5.75	6.31
solid medium	0	3.11	5.64	5.49	4.75
	25	4.46	6.27	4.01	4.91
	50	6.03	6.87	4.58	5.83
					Mean of medium
TDZ and medium	Liquid	4.94	6.23	5.66	5.61
	Solid	4.53	6.26	4.96	5.16
					Mean of PG
TDZ and PG	0	3.32	5.49	5.59	4.80
	25	4.75	6.34	4.78	5.29
	50	6.13	6.91	5.16	6.07
Mean of TDZ		4.74	6.24	5.18	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.34	0.42	0.42	0.59	0.59	0.72	1.03

solid medium significantly superiority on the other treatments except for treatment 0.5 mg. L^{-1} of TDZ with the liquid medium, which recorded the highest value reached 6.26 g. while the control treatment recorded the lowest average weight of callus reached (4.53 g).

The results in Table (9) indicate that the PG treatment at 50 mg L^{-1} and the liquid medium was significant superior on other treatments except for the treatment 50 mg.L^{-1} PG and the solid medium, which recorded the highest weight of callus 6.31 g . Comparted to the control treatment and the solid media recorded the lowest average weight of callus where it reached (4.75 g).

According to the results obtained, a combination between 0.5 mg. L^{-1} of TDZ and 50 mg.L^{-1} of PG application had the highest weight of callus 6.91 g compared to the other treatments after 8 weeks of culture.The control treatment recorded the lowest value reached (3.32 g).

As for the triple interaction, Table (9) indicates the superiority of the treatment 50 mg. L^{-1} of PG and 0.5 mg. L^{-1} of TDZ in the liquid medium, which recorded the highest value reached 6.94 g, compared the treatments' others. In contrast, the control treatment with the solid medium recorded the lowest value reached (3.11g) (Fig 5).

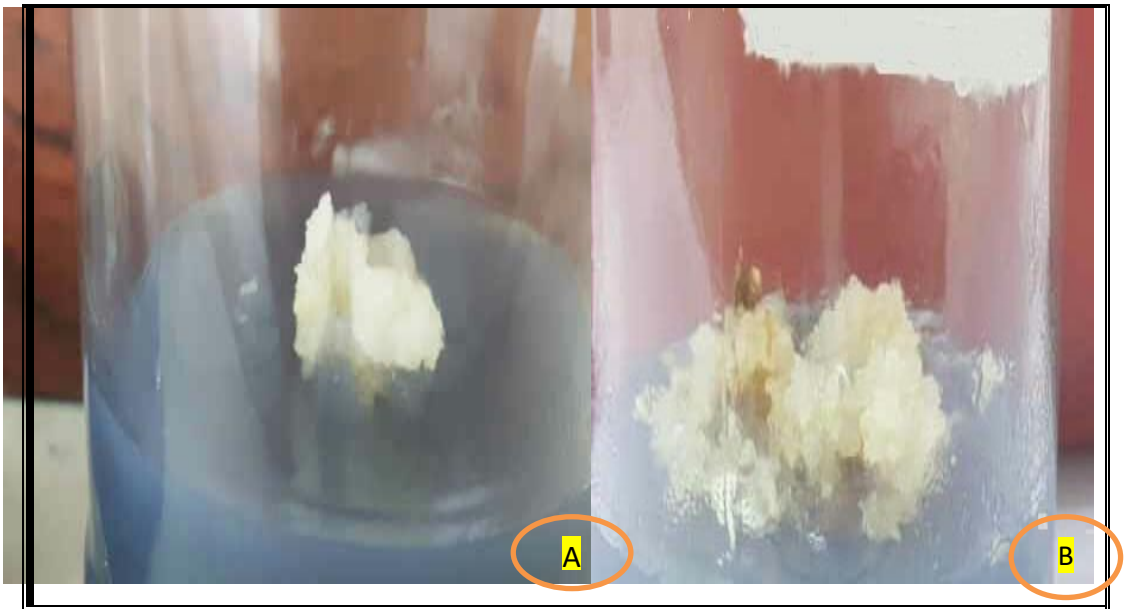


Figure (8) Callus multiplication on MS medium with (A) control treatment. (B) 50 mg.l^{-1} PG with 0.5 mg.l $^{-1}$ TDZ.

4-1-2- Bud weight

The cultures that grown in liquid medium showed significantly superior in the mean weight of buds(11.41g), compared to the cultures were grown in the solid medium was (10.83 g), (Table 10).

The same table(10) showed there was a significant effect of TDZ on the mean weight of buds. The highest weight on buds was obtained on the media supplemented with TDZ at a concentration of 0.5 mg L^{-1} where it reached 11.96 g , which has significantly superior on the other two concentrations (0 and1 mg L^{-1}), while the lowest weight of buds was at the control treatment (10.43 g).

Table (10) showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant

superiority compared to the other concentrations (0 and 25 mg. L^{-1}) in the weight of buds after 12 weeks of culture, while the highest value of buds weight was recorded 12.39 g. The lowest weight of buds was at the control treatment (10.08g) .

Data in Table (10) indicated that the treatment interaction between TDZ and medium type had a significant effect on buds weight after 12 weeks from culture. TDZ treatment at concentration 0.5 mg. L^{-1} and the liquid medium showed significant superiority on the other treatments except for treatment 0.5 mg. L^{-1} of TDZ and the solid medium, which recorded the highest value reached 12.31 g, while the control treatment in the solid medium recorded the lowest average (10.25 g).

The results are given in Table (10) indicate that the PG treatment at 50 mg L^{-1} with the liquid medium had the highest mean weight of buds 12.62g, as compared with other treatments after 12 week from culture, while the lowest value of the mean weight of buds 9.83gm., was detected at the control treatment and solidmedium.

Table (10) showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ and 50 mg. L^{-1} of PG, which showed a significant superiority compared with other treatments in the mean weight buds after 12 weeks of culture, which recorded the highest value reached 14.54 g , In contrast, the control treatment recorded the lowest value reached (8.64 g), (Fig 6).

Table (10) effect of medium type, TDZ, PG, and their interactions on bud weight (g) after 12 weeks of culture

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid Medium	0	8.91	11.25	10.82	10.33
	25	11.47	10.75	11.61	11.82
	50	11.43	14.92	11.51	12.62
solid medium	0	8.37	10.58	10.54	9.83
	25	10.50	10.10	10.90	10.50
	50	11.88	14.17	10.46	12.17
					Mean of medium
TDZ and medium	Liquid	10.60	12.31	11.31	11.41
	Solid	10.25	11.62	10.63	10.83
					Mean of PG
TDZ and PG	0	8.64	10.92	10.68	10.08
	25	10.98	10.42	11.25	10.89
	50	11.66	14.54	10.98	12.39
Mean of TDZ		10.43	11.96	10.97	

RLSD 0.05						
Medium	PG	TDZ	TDZ and Medium	PG and Medium	TDZ and PG	TDZ, PG and Medium
0.75	0.92	0.92	1.31	1.31	1.60	2.27

Table (10) indicated the significant superiority of the treatment 50 mg. L^{-1} of PG and 0.5 mg.L^{-1} of TDZ in the liquid medium in the mean weight buds compared the other treatments except for 50 mg. L^{-1} of PG and 0.5 mg. L^{-1} TDZ in the solid medium, which recorded

the highest value reached 14.92 g .after 12 weeks of culture, while the control treatment with the solid medium recorded the lowest average reached (8.27 g).

Figure (9) Bud regeneration on MS medium with (A)control treatment (B) 50 mg.l^{-1} PG with 0.5 mg.l $^{-1}$TDZ.

4-1-3- Shoots weight

Table (11) showed the significantly superior of the liquid medium on the solid medium in the average weight of the shoots reached 10.53 g , compared with the shoots grown on a solid medium, which was 8.77 g , after 12 weeks of culture.

The same table showed there was a significant effect of TDZ on the mean shoot weight. The highest mean shoots weight was obtained on the medium supplemented with TDZ at a concentration of 0.5 mg L^{-1}, where it reached 10.12g , which has significantly superior on the control treatment, while the lowest weight of shoots

was at the control treatment 9.03 g , which did not show significant differences between it and treatment 1 mg. L^{-1}

Table (11) showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority compared with the control treatment in weight of the shoots after 8 weeks of culture. The highest value of shoots weight was recorded 10.32 g . The lowest weight of shoots was at the control treatment (8.98 g).

According to the results obtained, the interactions between 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG had the highest mean weight of shoots 11.01g . compared to the other treatments after 8 weeks of culture, the control treatment recorded the lowest value reached (8.75 g).

Data in Table (11) indicated that the treatment interaction between TDZ and medium type significantly effected in weight of hoots after 12 weeks from culture. TDZ treatment at concentration 0.5 mg. L^{-1} with the liquid medium showed the significant superiority on wight of shoot compard to the other treatments except for treatment interaction between 1.0 mg. L^{-1} of TDZ the liquid medium, which recorded the highest value reached 11.12 g. The control treatment in the solid medium recorded the lowest average (8.09 g).

The results are given in Table (11) indicated that the PG treatment at 50 mg L^{-1} with the liquid medium had the highest mean weight of shoots 11.11 g , as compared to other treatments after 12

weeks of culture, while the lowest weight at the control treatment in the solid medium was (8.04 g).

Table (11) effect of medium type TDZ, PG, and their interactions on shoots weight (g).

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	9.64	10.43	9.69	9.92
	25	9.81	11.00	10.85	10.55
	50	10.48	11.93	10.93	11.11
solid medium	0	7.87	8.07	8.20	8.04
	25	7.83	9.19	9.15	8.73
	50	8.57	10.10	9.92	9.53
					Mean of medium
TDZ and medium	Liquid	9.98	11.12	10.49	10.53
	Solid	8.09	9.12	9.09	8.77
					Mean of PG
TDZ and PG	0	8.75	9.25	8.95	8.98
	25	8.82	10.10	10.00	9.64
	50	9.52	11.01	10.42	10.32
Mean of TDZ		9.03	10.12	9.79	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.75	0.92	0.92	1.30	1.30	1.60	2.26

As for the triple interaction, Table (11) indicated that the treatment 50 mg. L^{-1} PG and 0.5 mg.L^{-1} TDZ in the liquid medium had the highest mean weight of shoots 11.93 g , compared to other

treatments after 8 weeks of culture. In contrast, the solid medium control treatment recorded the lowest value reached (7.87 g).

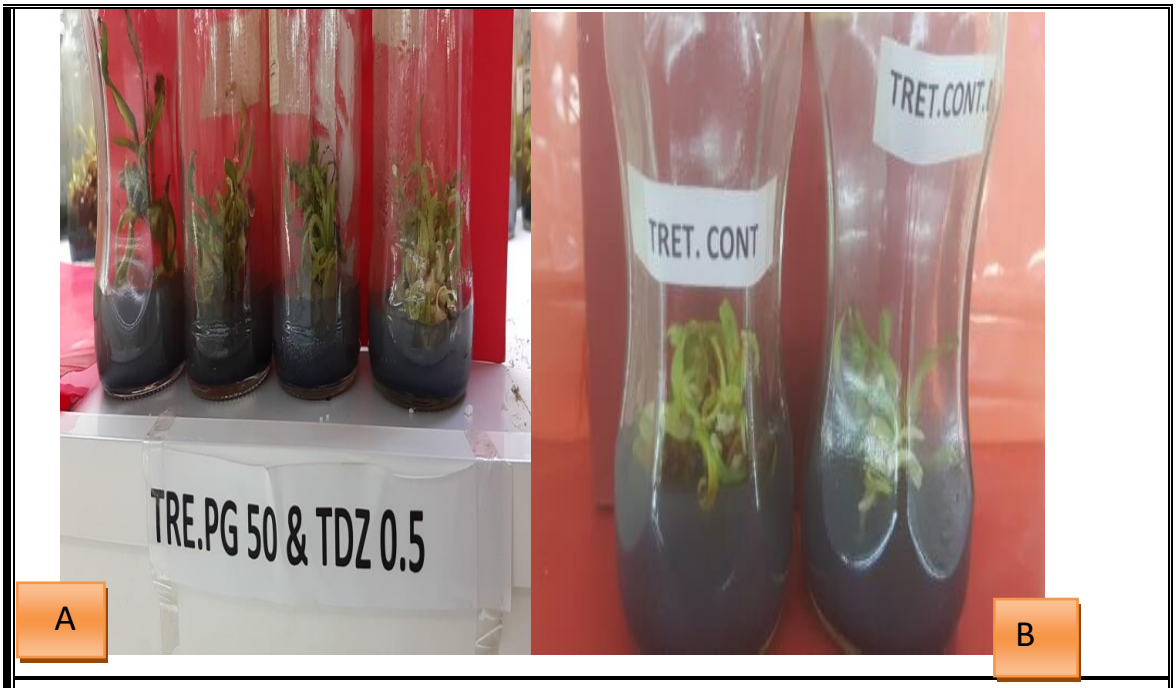


Figure (10): Shoots regeneration on MS medium with (A)control treatment (B) 50 mg.l^{-1} PG with 0.5 mg.l $^{-1}$ TDZ

4-1-4- Number of buds

The results in Table (12) showed the liquid medium's superiority to the solid medium in the number of buds. The number of buds in the liquid medium reached 18.00 buds after 12 weeks of culture, while the number in the solid medium 16.15 buds .

The same table showed there was a significant effect of TDZ on the average number of buds. The highest mean of buds number was obtained on the medium supplemented with TDZ at a concentration of 0.5 mg L^{-1} where it reached 18.83 buds., which has significantly superior on the two concentrations (0 and1) mg L^{-1}, while the lowest

number of buds was at the control treatment 15.44 buds. The table also shows the superiority of treatment PG at a concentration 50 mg. L^{-1}, which recorded19.94 buds, while the lowest value was at the control treatment reached 15.33 buds after 12 weeks of culture.

Table (12) effect of medium type, TDZ , PG and their interactions on bud number number of buds.

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	14.33	18.00	16.67	16.33
	25	14.33	18.33	19.00	17.22
	50	18.67	23.00	19.67	20.44
solid medium	0	12.67	16.67	13.67	14.33
	25	14.67	15.67	13.67	14.67
	50	18.00	21.33	19.00	19.44
					Mean of medium
TDZ and medium	Liquid	15.78	19.78	18.44	18.00
	Solid	15.11	17.89	15.44	16.15
					Mean of PG
TDZ and PG	0	13.50	17.33	15.17	15.33
	25	14.50	17.00	16.33	15.94
	50	18.33	22.17	19.33	19.94
Mean of TDZ		15.44	18.83	16.94	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
1.03	1.27	1.27	1.80	1.80	2.20	3.11

Table (12) showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG, which showed a significant superiority compared to the other treatments in the average number of buds after 12 weeks of culture, which recorded the highest value reached 22.17 buds. In contrast, the control treatment recorded the lowest value reached 13.50 buds.

Data in Table (12) indicated that the treatment interaction between TDZ and medium type had a significant effect on the average number of buds after 12 weeks of culture. TDZ treatment at concentration 0.5 mg. L^{-1} with the liquid medium showed a significant superiority on the other treatments except for treatment interaction between 1.0 mg. L^{-1} of TDZ in the liquid medium, which recorded the highest value reached 19.78 buds. The control treatment in the solid medium recorded the lowest average 15.11 buds.

The same table also indicated the PG treatment at 50 mg. L^{-1} in the liquid medium has significantly affected compared to the other treatments in the average number of buds except for the 50 mg.L^{-1} PG in the solid medium, which it was recorded highest value 20.44 buds, while the lowest value was at the control treatment in the solid medium, which reached 14.33 buds.

As for the triple interaction, Table (12) indicated the significant superiority of the treatment 50 mg. L^{-1} of PG and 0.5 mg. L^{-1} of TDZ in the liquid medium in the mean number of buds compared to the other treatments, which recorded the highest value reached (23.00) buds after 12 weeks of culture. While the control

treatment with the solid medium recorded the lowest number reached 12.67 buds.

4-1-5- Number of shoots

The results in Table (13) indicated the liquid medium superiority over the solid medium in the average number of shoots. The number of shoots in the liquid medium reached 36.61 shoots. In contrast, the solid medium gave the lowest rate in the number of shoots 33.56 shoots after 12 weeks of culture.

The same table also showed a significant effect of TDZ on the average number of shoots, . The highest mean of shoots number was obtained on the media supplemented with TDZ at a concentration of 0.5 mg $.L^{-1}$ where it reached 37.39 shoots , which has significantly superior to control treatment, while the lowest number of shoots was reached 32.58 shoots at the control treatment.

The cultures were grown at PG (50 mg. L^{-1}) showed better results in a number of shoots 36.00 shoots compared to the cultures grown at “control treatment” and 1 mg $.L^{-1}$),which were 34.42 and 34.83, respectively. However,the differences between 50 mg. L^{-1} PG and the other two concentrations(0 and1)mg.L^{-1} are not significant.

Table (13) showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG, which showed significant superiority compared to the interaction treatments (0 mg. L^{-1} of PG with 0, 0.5 mg. L^{-1} of TDZ, and 25 mg. L^{-1}PG with 0 mg. L^{-1} TDZ) in the average number of shoots after 12 weeks of culture, which recorded the highest value reached 38.83

shoots. In contrast, the control treatment recorded the lowest value reached 29.92 shoots.

Table (13) The effect of medium type, TDZ, PG, and their interactions on shoots number

Medium type	PG ($mg.L^{-1}$)	TDZ ($mg .L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	33.50	39.00	36.00	36.17
	25	35.33	36.33	36.33	36.00
	50	35.67	41.00	36.33	37.67
solid medium	0	26.33	37.00	34.67	32.67
	25	32.00	34.33	34.67	33.67
	50	32.67	36.67	33.67	34.33
					Mean of medium
TDZ and medium	Liquid	34.83	38.78	36.22	36.61
	Solid	30.33	36.00	34.33	33.56
					Mean of PG
TDZ and PG	0	29.92	33.00	35.33	34.42
	25	33.67	35.33	35.50	34.83
	50	34.17	38.83	35.00	36.00
Mean of TDZ		32.58	37.39	35.28	
RLSD 0.05					
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
2.12	2.60	2.60	3.68	3.68	4.51	6.37

Table (13) showed the significant superiority of the treatment of 0.5 $mg.L^{-1}$ TDZ with the liquid medium in the mean number of shoots compared to the interaction treatments (0 mg. L^{-1} TDZ with the solid

and liquid media, 1 mg. L^{-1} TDZ with the solid medium) reached 38.78 shoots, after 12 weeks of culture, while the lowest average number of shoots was recorded at the control treatment in the solid medium reached 30.33 shoots. Also, the table shows the PG treatment in the liquid medium at 50 mg. L^{-1} was the highest number of shoots 7.67 shoots. In contrast, the lowest average number of shoots was recorded at the control treatment in the solid medium reached 32.67 shoots.

As for the triple interaction, the treatment of 50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the liquid medium was a significant superior on the number of shoots reached (41.00) shoots after 12 weeks of cultivation, while the lowest number of shoots was recorded at the standard treatment with the solid medium of (26.33) (Fig 8, Index Fig3)

Figure (11) Multiple shoot regeneration of date palm cv. Barhee in the container of the bioreactor system at 1 mg.l-1TDZ.

The resnt showed Tables (9, 10, 11, 12, and 13) that TDZ treatment has a significant effect on the studied physical characteristics, including the weight of callus and buds, the shoots' weight, the number of buds and number of shoots formed. The reason may be that TDZ is one of the pheny urea substituted compounds that It is known at present with the activity of cytokinins, which are characterized by their high efficacy, superior to most other types of cytokines, including Zeatin. The natural product in the plants (Huetteman and Preece, 1993).

The use of TDZ led to the formation of new callus cells and achieved the highest rate of cell multiplication compared to other growth regulators,the stimulation of callus induction at low levels is an indication of endogenous vital activity, as Murthy *et al*., (1998) mentioned the stimulation of the development of callus from the axillary buds is due to the effect of TDZ at a very high rate. The use of TDZ has stimulated at low concentrations (0.1-10) µmol, lateral shooting, and formation of adventitions buds and direct or indirect shoots grown *in vitro* (Guo *et al*., 2011). Also Al-Taha and Al-Mazini (2016)reported that TDZ active at low concentration (0.5 – 1 mg $.L^{-1}$)wich gave the high rate of shoot number from shoot tips , hoever ,white brownish callus raised around the nodes grown *in vitro* . The positive effect of TDZ on date palm growth was in a good agreement with studies which revealed that the TDZ at the appropriate concentrations enhanced the plant growth and development of many plants (Jones *et al*., 2007, Zambre *et al*., (2001), Casanova *et al*., (2004), Nhut *et al*., (2006), Jheng *et al.,* (2006), and Ružić and

Vujović, (2008). As indicated by Naif (2019), the medium provided with cytokinin was superior to the control treatment in the weight of callus. The number of buds, as well as between culture with Bioreactor system, which superior on solid medium, the reason may be attributed to the benefit of the nutrient medium being limited in the solid system, as the part in contact with the medium is the one that benefits from the components of the nutrient medium without the other parts of the tissue. The immersion of a part tissue in a solid medium leads to a decrease in gas exchange, which affects the growth and development of the cultivated plant tissue.

The superiority in the bioreactor may be due to the abundance of nutrients and their increased readiness, which causes ease of absorption in the moving liquid medium compared to the solid medium, and the movement of the parts implanted in the liquid medium works to exchange gases and overcome the phenomenon of deficiency of some elements that may appear on the tissue parts as a result of their assembly. (Ducos *et al*., 2003) As for cultivation in solid media, it impeded the absorption of nutrients by the implanted tissue, which negatively affected the growth and multiplication of crops compared to the liquid medium (Al Khateeb and Alturki, 2014).

The emergence of adventitious buds from tissues and organs cultivated *in vitro* has been observed in many plants; that the emergence of adventitious buds is from cells exposed to the nutrient medium that is in contact with it, as these cells lose their differentiation and return to the meristematic state and then re-differentiate them by the effect of the composition of the medium and

conditions surrounding environment into meristematic regions that grow and develop into shoots with the same phenotypic composition as the buds found in the axils of leaves (Torrey, 1967). These results are consistent with what was found by Huetteman and Preece (1993) and Husain *et al*. (2007), and (Pradhan *et al*., (1998)

PG effect in stimulating the tissue to form a greater number of buds in the nutrient medium may be due to its role in promoting growth and lateral shoots and adventitious roots in some woody plants (James, 1979; Jonse and Hatfield, 1976). PG reduces the peroxidase activity within the cultivated plant part and thus protects endogenous auxin from oxidative stress (De Klerk *et al.* 1999; Sarkar and Naik, 2000). and Agud*, et al*. 2010) The results of the research are consistent with those found by Jani *et al*. 2015, Salekjalali (2012), Rustaei *et al*., (2009), Londe*, et al*., (2017) and Wang, *et al*., (2011) and (Kumar *et al*. (2005)Index fig(4).

4-1-6- The percentage of Browning

It is noticed from the results in Table (14) that there is no significant difference between the liquid medium and the solid medium in the percentage of browning, as its percentage in the liquid medium reached 7.8 %, while in the solid medium is reached (5.6)%

The same table also shows the superiority of the treatment TDZ at 1 mg.L^{-1} and the control treatment over treatment 0.5 mg .L^{-1} of TDZ where it reached 7.2 %.While the lowest percentage at 0.5 mg. L^{-1} TDZ, reached 5.6%. The control treatment was superior to the two PG treatments in the two concentrations (50 and 25) mg. L^{-1}

gave it the highest percentage 7.8%, while the lowest percentage for tanning was recorded at 50 mg. L^{-1} reached (5.6%).

Results are in agreement with Fayek *et al*., (2017) and Kim *et al*., (2007), As for the interaction between the medium and PG. Table (14) shows that there is a significant superiority for the control treatment with the liquid medium and the treatment with 25 mg L^{-1} PG with the liquid medium on the other of the treatments, it gave the highest value of browning 8.9%, where the treatment 25 mg.L^{-1} PG with the solid medium the lowest value in the browning ratio 4.4%, the interaction between the medium and the TDZ, the treatment was superior to 0.5 mg. L^{-1} TDZ with the liquid medium in the percentage of browning reached 8.9% , while the lowest percentage was 0.5 mg. L^{-1} TDZ with the solid medium 2.2%. These results are agreement with those found by Ali and Abdulzhara, (2018).

The interactions between PG and TDZ, Table (14) showed the superiority of the control treatment overall concentrations by giving it the highest browning percentage 10% , while the lowest percentage was recorded when treatment was 0.5 mg. L^{-1} TDZ with 25 or 50 mg.L^{-1} PG (5%).

Table (14) also showed the effect of triple interactions. The control treatment in both solid and liquid media superiority to all treatments by giving it the highest browning ratio 10%, while the treatment of 0.5 mg. L^{-1} TDZ with 25 mg.L^{-1} PG with the solid medium did not appear any browning.

Table (14) The effect of medium type, TDZ, and PG and their interactions on the percentage of browning percent (%)

Medium type	PG(mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	10.0	10.0	6.7	8.9
	25	6.7	10.0	10.0	8.9
	50	3.3	6.7	6.7	5.6
solid medium	0	10	3.3	6.7	6.7
	25	6.7	0.0	6.7	4.4
	50	6.7	3.3	6.7	5.6
					Mean of medium
TDZ and medium	Liquid	6.7	8.9	7.8	7.8
	solid	7.8	2.2	6.7	5.6
					Mean of PG
TDZ and PG	0	10.0	6.7	6.7	7.8
	25	6.7	5.0	8.3	6.7
	50	5.5	5.5	6.7	5.6
Mean of TDZ		7.2	5.6	7.2	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
4	5.2	5.2	7.4	7.4	9.2	1.2

4-2-Effect medium type, TDZ, and PG on endogenous hormones content *in vitro* grown tissues

4-2-1- Indole acetic acid (IAA) content in callus tissue.

It is noted from the results in Table (15) that the liquid medium showed significantly superior on the solid medium in the callus content of auxin, which was 27.097 µg.Kg^{-1}, while in the solid medium, reached (26.462 µg. Kg^{-1}).

The same table showed that the optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 27.847 µg. Kg^{-1}, which showed significant superiority in increasing IAA callus compared to the two concentrations (0 and 1) mg. L^{-1} , while the lowest value was at the control treatment, which reached (25.638 µg. Kg^{-1}).

The data in table 15 showed that the treatment of PG at a concentration of 50 mg. L^{-1} was a significant superior compared to the two concentrations (0 and 25) mg. L^{-1} and the auxin value was 27.817 µg. Kg^{-1}, while the lowest value at control treatment reached. (25.660 µg. Kg^{-1})

According to the results obtained, the PG treatment at 50 mg .L^{-1} with the liquid medium was significantly superior to other treatments, except for the treatment 50 mg.L^{-1} PG with the solid medium, which recorded the highest rate of auxin content 28.066 µg. Kg^{-1}, while the lowest value was recorded at the control treatment with the solid medium (24.975 µg.Kg^{-1}).

Table (15) Effect of medium type, TDZ , PG and their interactions on auxin content in callus (µg. Kg^{-1})

Medium type	PG (mg .L^{1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	24.981	27.835	26.219	26.345
	25	25.325	28.127	27.194	26.882
	50	27.414	29.258	27.525	28.066
solid medium	0	23.915	25.256	25.755	24.975
	25	25.170	28.091	27.267	26.843
	50	27.025	28.513	27.164	27.567
					Mean of medium
TDZ and medium	Liquid	25.907	28.406	26.979	27.097
	Solid	25.370	27.287	26.729	26.462
					Mean of PG
TDZ and PG	0	24.448	26.545	25.987	25.660
	25	25.248	28.109	27.231	26.862
	50	27.219	28.886	27.345	27.817
Mean of TDZ		25.638	27.847	26.854	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.3786	0.4637	0.4637	0.6558	0.6558	0.8032	1.1359

Also, the interaction between the type of medium and TDZ had a significant effect. The treatment of 0.5 mg. L^{-1} TDZ with the liquid medium showed a significant superior quality on all treatments, which recorded the highest value reached 28.406 µg. Kg^{-1},while the lowest value was recorded at the control treatment with the solid medium (25.970 µg. Kg^{-1}).

Table (15) showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1}PG in the content of auxins compared to the other interaction treatments except for treatment 0.5 TDZ with 25 PG, where there are no significant difference between them, ther were it reached (28.886 and 28.108 µg. Kg^{-1}) for each of them respectively, after 12 weeks of culture. Whereas the lowest auxin content at the control treatment and reached (24.448 µg.Kg^{-1}).

Table (15) indicated the significant superiority of as for the triple interaction. the treatment 50 mg L^{-1} of PG and 0.5 mg. L^{-1} of TDZ in the liquid medium recrded the highest valuinthe the content of auxins compared to the other of the treatments except for the treatment (0.5 mg. L^{-1} TDZ with 25 mg. L^{-1} PG in the liquid medium and the treatment 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG in the solid medium), where the highest auxin content was recorded at 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG in the liquid medium reached 29.258 µg.Kg^{-1}. While the control treatment with the solid medium recorded the lowest content of the auxin (23.91 µg. Kg^{-1}).

4-2-2- Indole acetic acid (IAA) content in buds.

It is noted from the results in Table 16 that the liquid medium showed significantly superior on the solid medium in the buds content of auxin, which was 28.36 µg. Kg^{-1}, while in the solid medium, reached (27.76µg. Kg^{-1}).

The same table showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ 29.30 µg. Kg^{-1}, which showed significant superiority in increasing the content of the of IAA in buds compared to the two concentrations (0 and 1) mg. L^{-1}, while the lowest value was at the control treatment, which reached 27.02 µg. Kg^{-1}, additionally, the treatment 1mg.L^{-1} TDZ showed a significant superiority compared to the control treatment.

The data in table 16 showed that the treatment of PG at a concentration of 50 mg. L^{-1} was significantly superior compared to the two concentrations (0 and 25) mg. L^{-1}, which recorded the highest value 29.18 µg. Kg^{-1}, while the lowest value at control treatment reached (26.93 µg. Kg^{-1}).

Table (16) also indicated the significant superiority of the interaction treatment between 50 mg. L^{-1} PG with the liquid medium recorded the highest value in auxins content 29.41 µg.K g^{-1} compared to the other treatments except for the treatment 50 mg L^{-1} PG with the solid medium, the differences between them were not significant. Simultaneously, the lowest value was recorded at the control treatment with the solid medium(26.51 µg. Kg^{-1}).

Also, the interaction between the type of medium and TDZ had a significant effect. The treatment of 0.5 mg L^{-1} TDZ with the liquid medium showed a significant superior compared to thetreatment, except for the treatment of 0.5 mg. L^{-1} TDZ with a solid medium, which recorded the highest value reached 29.68 µg. Kg^{-1}. While the lowest value recorded at the control treatment with the solid medium (26.58 µg. Kg^{-1}).

Table (16) showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in auxins content compared to the other interaction treatments recorded the highest value reached 31.11 µg.Kg^{-1} after 12 weeks of culture, while the lowest value recorded at the control treatment (25.93 µg. Kg^{-1}).

As for the triple interaction, Table (16) indicated the significant superiority of the treatment 50 mg. L^{-1} of PG and 0.5 mg. L^{-1} of TDZ in the liquid medium in the content of auxins compared to the other treatments and reached 31.24 µg.Kg^{-1}, except for the treatment (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG in the solid media), that the differences between them were not significant, while the lowest auxin content was recorded at the control treatment in both media types (25.93 µg.Kg^{-1}).

Table (16) The effect of medium type, TDZ , PG and their interactions on buds content of auxin (µg. Kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	25.93	28.69	27.44	27.35
	25	28.14	29.11	27.70	28.32
	50	28.29	31.24	28.71	29.41
solid medium	0	25.93	27.06	26.55	26.51
	25	26.65	28.70	28.14	27.83
	50	27.16	30.98	28.71	28.95
					Mean of medium
TDZ and medium	Liquid	27.45	29.68	27.95	28.36
	Solid	26.58	28.92	27.80	27.76
					Mean of PG
TDZ and PG	0	25.93	27.88	26.99	26.93
	25	27.40	28.91	27.92	28.07
	50	27.73	31.11	28.71	29.18
Mean of TDZ		27.02	29.30	27.88	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.579	0.709	0.709	1.003	1.003	1.228	1.737

4-2-3- Indole acetic acid (IAA) content in shoots.

It is observed from the results in Table (17) the superiority of the solid medium on the liquid medium in the content of the shoot of

auxin; it was reached 28.98 µg. Kg^{-1}, while in the liquid medium, it reached (28.87 µg. Kg^{-1}).

The same table showed that the optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 30.33 µg. Kg^{-1}, which showed significant superiority in increasing the content of IAA's shoots compared with the two concentrations (0 and 1) mg. L^{-1}, while the lowest value was at the control treatment, which reached (27.28 µg. Kg^{-1}).

PG also significantly affected the shoot content of auxin, where table (17) shows that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority compared with other concentrations (0 and 25) mg. L^{-1} after 24 weeks of culture, which recorded the highest value 29.96 µg. Kg^{-1}, while the lowest value at control treatment, reached (27.90 µg. Kg^{-1}).

As for the interaction between the type of medium and PG, Table (17) indicated that the PG treatment at 50 mg L^{-1} with the liquid and solid medium significantly superior to other treatments except for the treatment 25 mg. L^{-1} PG with the solid medium, where they recorded the highest value 29.96 µg. Kg^{-1} for each of them, while the lowest value was recorded at the control treatment with both media type (27.90 µg. Kg^{-1}).

The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg. L^{-1} TDZ with the solid medium showed a significantly superior treatment except for the

treatment at 0.5 mg. L^{-1}TDZ with the liquid medium, which recorded the highest value reached 30.41 µg. Kg^{-1}. While the lowest value recorded at the control treatment with both media types (27.82 µg. Kg^{-1})

As for the interactions between the TDZ and PG, table 17 showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in auxins content compared to the other interaction treatments recorded the highest value reached 32.34 µg. Kg^{-1} after 12 weeks of culture, while the lowest value recorded at the control treatment (26.51 µg. Kg^{-1})

As for the triple interaction, Table (17) indicated the significant superiority of the two treatments (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid and solid media), recording the highest value 32.34 µg.Kg^{-1} compared to the other interaction treatments; the lowest auxin content was recorded at the control treatment in both media type (26.51 µg. Kg^{-1})

Table (17) Effect of medium type, TDZ, PG and their interactions on shoots content of auxin (µg. Kg^{-1})

Medium type	PG (mg .L^{1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	26.51	28.97	28.21	27.90
	25	28.63	29.42	28.19	28.74
	50	28.31	32.34	29.24	29.96
solid medium	0	26.51	28.97	28.21	27.90
	25	28.63	29.93	28.64	29.07
	50	28.31	32.34	29.24	29.96
					Mean of medium
TDZ and medium	Liquid	27.82	30.24	28.55	28.87
	Solid	27.82	30.41	28.70	28.98
					Mean of PG
TDZ and PG	0	26.51	28.97	28.21	27.90
	25	28.63	29.67	28.42	28.91
	50	28.31	32.34	29.24	29.96
Mean of TDZ		27.82	30.33	28.62	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.595	0.728	0.728	1.030	1.030	1.262	1.784

4-2-4- Cytokines content in callus tissue

It is observed from the results in Table (18) the significant superiority of the liquid medium on the solid medium in callus content

of cytokinins it was reached 2.00 µg. Kg^{-1}, while in the solid medium, which was (1.97 µg. Kg^{-1}).

The study results indicated in the same table that there was a significant effect of TDZ on the content of callus tissue from cytokines. Optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 2.05 µg.Kg^{-1} , which showed significant superiority in increasing the content of the callus tissues of cytokines compared to the two concentrations (0 and 1) mg. L^{-1}, while the lowest value was at the control treatment, which reached (1.88 µg. Kg^{-1}).

PG also had a significant effect on cytokinins callus content. Table (18) showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed a significant superiority compared to the other concentrations (0 and 25) mg. L^{-1} , which recorded the highest value 2.06 µg. Kg^{-1}, while the lowest value at control treatment reached (1.90 µg. Kg^{-1}).

The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg. L^{-1} TDZ with the liquid medium showed a significantly superior treatment except for the treatment at 0.5 mg. L^{-1} TDZ with the solid medium, which recorded the highest value reached 2.06 µg. Kg^{-1}. While the lowest value recorded at the control treatment with the solid medium (1.85 µg . Kg^{-1}).

As for the interaction between the type of medium and PG, Table (18) indicated that the PG treatment at 50 mg L^{-1} with the

liquid medium was significantly superior to the other treatments, which recorded the highest value 2.08 µg. Kg^{-1}, while the lowest value was recorded at the control treatment with the solid medium (1.88 µg. Kg^{-1})

Table (18) the effect of medium type, TDZ PG and their interactions on callus content of cytokinins (µg. Kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	1.7677	2.0299	2.0093	1.9356
	25	1.9802	2.0261	2.0114	2.0059
	50	2.0031	2.1340	2.1125	2.0832
solid medium	0	1.6247	2.0223	2.0031	1.8834
	25	1.9573	2.0123	1.9940	1.9879
	50	1.9798	2.0934	2.0605	2.0446
					Mean of medium
TDZ and medium	Liquid	1.9170	2.0633	2.0444	2.0082
	Solid	1.8539	2.0427	2.0192	1.9719
					Mean of PG
TDZ and PG	0	1.6962	2.0261	2.0062	1.9095
	25	1.9687	2.0192	2.0027	1.9969
	50	1.9915	2.1137	2.0865	2.0639
Mean of TDZ		1.8855	2.0530	2.0318	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.01591	0.01949	0.01949	0.02756	0.02756	0.03376	0.04774

As for the interactions between the TDZ and PG, table 18 showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1}PG in cytokines' content compared to other interaction treatments recorded the highest value reached 2.11 µg. Kg^{-1} except (50 mg. L^{-1} PG and 1 mg. L^{-1}TDZ)where the differences between them were not significant, while the lowest value recorded at the control treatment (1.69 µg. Kg^{-1}).

As for the triple interaction, Table (18) indicated the significant superiority of the treatment(50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ with the liquid media), recording the highest value 2.13 µg. Kg^{-1} compared to other interaction treatments; the lowest cytokinin content was recorded at the control treatment with the solid medium, which reached (1.62 µg. Kg^{-1}).

4-2-5- Cytokinin content in buds .

It is observed from the results in Table (19) the significant superiority of the solid medium on the liquid medium in buds content of cytokinins; it was reached 2.29 µg. Kg^{-1}, while in the solid medium, is reached (2.16 µg. Kg^{-1}).

The study results indicated in the same table that there was a significant effect of TDZ on the content of buds from cytokinins. Optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 2.37 µg. Kg^{-1} showed a significant superiority in increasing the buds' content of cytokinins compared with the two concentrations (0 and 1) mg. L^{-1} , while the lowest value was at the control treatment, which reached (2.07 µg. Kg^{-1}).

PG also had a significant effect on the buds content of cytokinins. Table (19) showed that the optimal treatment was observed in the medium containing 50 mg.L^{-1} of PG, 2.27 µg. Kg^{-1} showed significant superiority compared to the control treatment, which recorded the lowest value 2.14 µg. Kg^{-1}, while the differences were not significant compared to the PG treatment at a concentration of 25 mg.L^{-1}

The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg. L^{-1} TDZ with the liquid medium showed significantly superior performance on other treatments, which recorded the highest value reached 2.46 µg. Kg^{-1}. While the lowest value recorded at the control treatment with the solid medium (2.00 µg. Kg^{-1})

As for the interaction between the type of medium and PG, Table (19) indicated that the PG treatment at 25 mg L^{-1} with the liquid medium was significantly superior to the other treatments, which recorded the highest value 2.33 µg. Kg^{-1}, but statistical analyzes did not show significant differences between the two treatments 25 and 50 mg. L^{-1} PG with the liquid medium.

As for the interactions between the TDZ and PG, table (19) shows the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in the buds content of cytokinins compared to the other interaction treatments recorded the highest value reached 2.51 µg. Kg^{-1}, while the lowest value was recorded at the control treatment (1.93 µg. Kg^{-1}).

Table (19) the effect of medium type, TDZ , PG, and their interactions on buds content of cytokinins (µg. Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	1.989	2.364	2.290	2.214
	25	2.215	2.453	2.348	2.339
	50	2.209	2.579	2.171	2.320
solid medium	0	1.883	2.200	2.135	2.073
	25	2.018	2.233	2.297	2.183
	50	2.114	2.446	2.157	2.239
					Mean of medium
Effect TDZ and medium	Liquid	2.138	2.466	2.269	2.291
	Solid	2.005	2.293	2.196	2.165
					Mean of PG
TDZ and PG	0	1.936	2.282	2.212	2.144
	25	2.117	2.343	2.323	2.261
	50	2.161	2.512	2.164	2.279
Mean of TDZ		2.071	2.379	2.233	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0519	0.0635	0.0635	0.0899	0.0899	0.1101	0.1557

As for the triple interaction, Table (19) indicated the significant superiority of the treatment (50) mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ with the liquid media), recording the highest value 2.57 µg. Kg^{-1} compared to the other interaction treatments; the lowest

cytokinin content was recorded at the control treatment with the solid medium, which reached (1.88 µg. Kg^{-1}).

4-2-6-Cytokinin content in shoots.

It is noted from the results in Table (20) that the liquid medium was significantly superior to the solid medium in shoot content of cytokinin, where the cytokinin in the liquid medium was 2.64 µg. Kg^{-1}, while in the solid medium, which was (2.39 µg. Kg^{-1}).

The results indicated in the same table that there is a significant effect of TDZ on the content of shoots of cytokinin. Optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 2.74 µg. Kg^{-1} showed significant superiority in increasing the content of cytokinins shoots compared to the two concentrations (0 and 1) mg. L^{-1} , while the lowest value was at the control treatment, which reached 2.27 µg. Kg^{-1}, PG also had a significant effect on the content of cytokinins' shoot, where table (20) shows that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority compared to the other concentrations (0 and 25) mg. L^{-1}, which recorded the highest value 2.67 µg. Kg^{-1}, while the lowest value at control treatment reached (2.39 µg. Kg^{-1}).

The interaction between the type of medium and PG, Table (20) indicates that the PG treatment at 50 mg L^{-1} with the liquid medium is significantly superior to the other treatments, recording the highest value 2.80 µg. Kg^{-1}, while the lowest value was recorded at the control treatment with the solid medium 2.28 µg. Kg^{-1}. As well

as, the PG treatment at 25 mg L^{-1} with the liquid medium showed significantly superior compared to other interaction treatments.

The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg. L^{-1} TDZ with the liquid medium showed significantly superior performance on other treatments, which recorded the highest value reached 2.82 µg. Kg-1. While the lowest value recorded at the control treatment with the solid medium 2.12 µg. Kg^{-1}. As for the interactions between the TDZ and PG, table (20) shows the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in cytokinin content compared to the other interaction treatments recorded the highest value 3.08 µg. Kg^{-1}, while the lowest value recorded at the control treatment, reached (2.12 µg. Kg^{-1}).

As for the triple interaction, Table (20) indicated the significant superiority of the treatment(50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ with both liquid and solid media), recording the highest value 3.17 and 2.99 µg. Kg^{-1} for each, respectively. Compared to the other interaction treatments, while the lowest cytokinin content was recorded at the control treatment with the solid medium, which reached (1.95 µg. Kg^{-1}).

Table (20) The effect of medium type, TDZ , PG and their interactions on shoots content of cytokines (µg. Kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	2.289	2.595	2.645	2.510
	25	2.508	2.693	2.677	2.626
	50	2.480	3.175	2.768	2.808
solid medium	0	1.954	2.521	2.391	2.289
	25	2.146	2.513	2.383	2.347
	50	2.275	2.992	2.369	2.545
					Mean of medium
TDZ and medium	Liquid	2.426	2.821	2.697	2.648
	Solid	2.125	2.675	2.381	2.394
					Mean of PG
TDZ and PG	0	2.122	2.558	2.518	2.399
	25	2.327	2.603	2.530	2.487
	50	2.378	3.084	2.568	2.676
Mean of TDZ		2.275	2.748	2.539	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0661	0.0810	0.0810	0.1145	0.1145	0.1403	0.1984

4-2-7- Gibberellins content in callus tissue .

The results in Table (20) It was observed from the significant superiority of the liquid medium on the solid medium in callus content of gibberellins it was reached 43.10 µg. Kg^{-1}, while in the solid medium, which was (41.93 µg. Kg^{-1}).

As for the effect of TDZ, it is clear from the same table that the optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 46.96 µg. Kg^{-1}, which showed significant superiority in increasing the content of the callus of gibberellins compared with the two concentrations (0 and 1) mg.L^{-1} ,while the lowest value was at the control treatment, which reached 36.42 µg. Kg^{-1}. As the treatment 1 mg.L^{-1} TDZ showed significant superiority on the control treatment.

Gibberellins' callus content on PG also had a significant effect. Table (17) showed that the optimal treatment was observed in the medium containing 50 mg.L^{-1} of PG, recording the highest value 47.67 µg. Kg^{-1}, which showed significant superiority compared with other concentrations (0 and 25) mg. L^{-1}, while the lowest value at control treatment reached (37.50 µg. Kg^{-1}).

As for the interaction between the medium and PG, Table (21) indicated the two treatments' superiority 50 mg. L^{-1} PG with the liquid medium on other treatments, recording the highest value 48.41 µg. Kg^{-1}, while the lowest value recorded at the control treatment with the solid medium was (36.72 µg. Kg^{-1}).

The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg. L^{-1} TDZ with the liquid medium showed significantly superior performance on other treatments 47.86 µg. Kg^{-1}.While the lowest value recorded at the control treatment with the liquid medium 36.39 µg. Kg^{-1}. In contrast, the statistical analyzes did not show significant differences between

the treatment of 1TDZ at 0.5 mg. L^{-1} in both media (liquid and solid) in the content of callus tissue from Gibberellins.

Table (21) The effect of medium type, TDZ, PG and their interactions on callus content of Gibberellins (µg. Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	30.25	42.54	42.03	38.27
	25	38.17	45.49	44.14	42.60
	50	40.74	55.54	48.94	48.41
solid medium	0	30.15	41.22	38.78	36.72
	25	38.17	44.45	43.76	42.13
	50	41.04	52.54	47.21	46.93
					Mean of medium
TDZ and medium	Liquid	36.39	47.86	45.04	43.10
	Solid	36.45	46.07	43.25	41.93
					Mean of PG
TDZ and PG	0	30.20	41.88	40.41	37.50
	25	38.17	44.97	43.95	42.36
	50	40.89	54.04	48.08	47.67
Mean of TDZ		36.42	46.96	44.15	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
1.534	1.878	1.878	2.656	2.656	3.253	4.601

As for the interactions between the TDZ and PG, table 21 showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in the content of gibberellins, which recorded

the highest value reached 54.04 µg. Kg^{-1} compared to the other interaction treatments; the lowest value was recorded at the control treatment 30.20 µg. Kg^{-1}. Table 21 shows the treatment (50 PG with 1 TDZ $mg.L^{-1}$) on other interaction treatments.

As for the triple interaction, Table (21) indicated the significant superiority of the treatment (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid medium), recording the highest value 55.54 µg. Kg^{-1} compared to the other interaction treatments, except for treatment of 0.5 mg. L^{-1} TDZ with 50 $mg.L^{-1}$ PG with the solid medium, while the lowest gibberellins content was recorded at the control treatment in the solid medium was (30.15µg. Kg^{-1}).

4-2-8- Buds contents from gibberellins .

The results in Table (22) indicated no significant differences of the liquid medium and the solid medium in the buds content of gibberellins; it was in the liquid medium 53.74 µg. Kg^{-1}, while in the solid medium was (51.20 µg. Kg^{-1}).

The study results indicated in the same table that there was a significant effect of TDZ on the content of buds from gibberellins. Optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 56.26 µg. Kg^{-1} showed a significant superiority in increasing the buds' content of gibberellins compared to the two concentrations (0 and 1) mg. L^{-1}, while the lowest value was at the control treatment, which reached 47.30 µg. Kg^{-1}. As the same table shows the significant superiority of the treatment 1 mg. L^{-1} TDZ on the control treatment.

PG also had a significant effect on the content of buds of gibberellins. Table (22) showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority which recorded the highest value reached 58.27 µg. Kg^{-1}, compared to the other concentrations (0 and 25) mg. while the lowest value at control treatment, which was (48.40 µg. Kg^{-1}).

As for the interactions between the TDZ and PG, table 22 showed the significant superiority of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG in the buds content of gibberellins which recorded the highest value reached 65.24 µg. Kg^{-1} compared to the other interaction treatments, while the lowest value was recorded at the 1.0 TDZ with 0 PG mg. L^{-1}, reached (44.52 µg. Kg^{-1})

Also, the interaction between the type of medium and TDZ had a significant effect on the buds content of gibberellins, the treatment 0.5 mg. L^{-1} TDZ with the liquid medium showed significantly superior performance on other treatments, which recorded the highest value reached 56.41 µg. Kg^{-1}. While the lowest value recorded at the control treatment with the solid medium (45.59 µg. Kg^{-1}).

Additionally, it was noticed that no significant differences were recorded between the treatment of 0.5 mg. L^{-1} in the liquid medium and the treatment of 0.5 mg. L^{-1} TDZ in the solid medium

Regarding the effect of the interaction between PG and the type of medium, Table (22) showed the significant superiority of the

treatment PG with concentration 50 mg. $L^{-1,}$ with the liquid medium in the gibberellins' content, recorded the highest value reached 60.26 µg. Kg^{-1} compared to other interaction treatments, the lowest value was recorded at (0 mg. L^{-1} PG with the solid medium), which was (47.45 µg. Kg^{-1}).

Table (22) Effect of medium type TDZ, PG and their interactions on buds content of Gibberellins (µg. Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	47.21	50.57	50.26	49.35
	25	49.96	53.01	51.84	51.60
	50	49.84	65.64	65.30	60.26
solid medium	0	41.83	50.97	49.55	47.45
	25	46.30	52.51	50.77	49.86
	50	48.64	64.84	55.34	56.28
					Mean of medium
Effect TDZ and medium	Liquid	49.01	56.41	55.80	53.74
	Solid	45.59	56.11	51.89	51.20
					Mean of PG
TDZ and PG	0	49.91	50.77	44.52	48.40
	25	51.31	52.76	48.13	50.73
	50	60.32	65.24	49.24	58.27
Mean of TDZ		47.30	56.26	53.85	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
2.694	3.300	3.300	4.667	4.667	5.715	8.083

As for the triple interaction, Table (22) indicated the significant superiority of the treatment (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid medium), recording the highest value 65.64 µg. Kg^{-1} compared to the other interaction treatments, except for the two interaction treatments (50 mg.L^{-1} PG and 0.5 mg.L^{-1} TDZ in the solid medium). Simultaneously,the lowest gibberellin content was recorded at the control treatment in the solid medium (41.83 µgKg^{-1}).

4-2-9- Gibberellins content *in vitro* grown shoot (µg. Kg^{-1}**).**

It is noticed from the results in Table (23) that there was no significant effect of the medium type on the content of the shoot of gibberellins, where the gibberellin in the solid medium reached 55.40 µg. Kg^{-1}, while in the solid medium, which was (54.93 µg. Kg^{-1}).

The results indicated in the same table that there is a significant effect of TDZ on the content of shoots of gibberellins. Optimal treatment was observed in the medium containing 0.5 mg L^{-1} of TDZ 58.88 µg. Kg^{-1},which showed significant superiority in increasing the content of gibberellin's shoots 58.88 µg. Kg^{-1} compared to the control treatment, which recorded the lowest value reached 50.49 µg. Kg^{-1}, while the statistical analyzes did not show significant differences between the two TDZ treatments 0.5 mg.L^{-1} and 1.0 mg, L^{-1}, in the content of gibberellins of shoots.

PG also had a significant effect on the content of gibberellins' shoot, where table (23) showed that the optimal treatment was observed in the medium containing 50 mg. L^{-1} of PG, which showed significant superiority which recorded the highest value 59.82 µg. Kg^{-}

^{1}, compared to the other concentrations (0 and 25) mg. L^{-1}, while the lowest value at control treatment reached (51.65 µg.Kg^{-1}).

The interaction between the medium type and PG, it is evident from table (23) the significant superiority of the interaction treatment 50 mg. L^{-1} PG with the solid medium on other treatments, except for the treatment 50 mg. L^{-1} PG with the liquid medium, which gave the highest value of 61.00 µg. Kg^{-1}, while the lowest value was recorded at the control treatment with the solid medium, which was (50.47 µg. Kg^{-1}).

The interaction between the medium and TDZ had a significant effect on the content of gibberellins shoots. The treatment of 0.5 mg.L^{-1} TDZ with the solid medium was significantly superior to other treatments, reaching 59.92 µg. Kg^{-1}, while the lowest value was recorded at the control treatment with the solid medium 49.94 µg. Kg^{-1}, while the statistical analyses did not show significant differences between the treatment of 0.5 mg. L^{-1} TDZ in the solid medium and the treatment 0.5 mg.L^{-1} TDZ in the liquid medium.

The interactions between the TDZ and PG, table (23) shows the superior of the treatment TDZ at 0.5 mg. L^{-1} with the 50 mg. L^{-1} PG which recorded the highest value 68.44 µg. Kg^{-1} in the gibberellins' content compared to the other interaction treatments, the lowest value recorded at the control treatment was 49.35 µg.Kg^{-1}.

The triple interaction Table(23) indicated the significant superiority of the treatment 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with

the solid medium, recording the highest value 71.34 µg. Kg^{-1} compared to the other interaction treatments; the lowest gibberellin content was recorded at the control treatment in the solid medium (46.61 µg. Kg^{-1}).

Table (23) Effect of medium type TDZ, PG and their interactions on shoots content of gibberellins (µg. Kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	52.09	53.51	52.90	52.84
	25	50.26	54.43	55.24	53.31
	50	50.74	65.54	59.61	58.63
solid medium	0	46.61	52.29	52.50	50.47
	25	51.38	56.13	56.66	54.72
	50	51.84	71.34	59.81	61.00
					Mean of medium
TDZ and medium	Liquid	51.03	57.83	55.92	54.93
	Solid	49.94	59.92	56.32	55.40
					Mean of PG
TDZ and PG	0	49.35	52.90	52.70	51.65
	25	50.82	55.28	55.95	54.02
	50	51.29	68.44	59.71	59.82
Mean of TDZ		50.49	58.88	56.12	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
2.327	2.849	2.849	4.030	4.030	4.935	6.980

4-2-10- Abscisic acid content in callus tissues .

The results in Table (24) It is observed from the significant superiority of the liquid medium on the solid medium in callus content of abscisic acid, and it wa s reached 10.34 µg. Kg^{-1}, while in the solid medium, which was (7.25µg. Kg^{-1}).

The results also showed (tablt 24) table that the control treatment was significantly superior to the two treatments (0.5 and 1 mg. L^{-1}TDZ) in the content of the callus tissue of abscisic, which reached 11.36 µg. Kg^{-1}. While the lowest value was recorded at the treatment of 0.5 mg. L^{-1} reached (7.28 µg. Kg^{-1}).

The control treatment was significant superior compared to the two concentrations (25 and 50) $mg.L^{-1}$ PG, where it gave the highest content of abscisic acid 11.03 µg. Kg^{-1}, while the lowest content at 50 $mg.L^{-1}$PG, which was 6.85 µg. Kg^{-1}, as well as the treatment of PG at a concentration of 25 mg. L^{-1} showed significant superiority compared to the treatment of 50 mg. L^{-1}.

The same table showed the effect of the interactions between TDZ and PG, where the control treatment showed a significant superiority over other treatments in the content of abscisic acid, where the highest value was recorded, which was 16.50 µg. Kg^{-1}, while the lowest value was recorded at the treatment 0.5. $Mg.L^{-1}$ TDZ with 50 $mg.L^{-1}$ PG, which was (6.16 µg. Kg^{-1}).

Table (24) The effect of medium type, TDZ , PG and their interactions on the callus content of abscisic acid (µg. Kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	16.786	10.184	10.521	12.497
	25	12.304	9.300	9.892	10.499
	50	10.176	6.856	7.019	8.017
solid medium	0	16.208	5.830	6.666	9.568
	25	6.859	6.100	6.505	6.488
	50	5.860	5.465	5.766	5.697
					Mean of medium
Effect TDZ and medium	Liquid	13.089	8.780	9.144	10.338
	Solid	9.642	5.799	6.312	7.251
					Mean of PG
TDZ and PG	0	16.497	8.007	8.593	11.033
	25	9.581	7.700	8.198	8.493
	50	8.018	6.161	6.393	6.857
Mean of TDZ		11.366	7.289	7.728	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.2816	0.3449	0.3449	0.4878	0.4878	0.5975	0.8449

Table (24) showed that the control treatment with a liquid medium significant superiority over the other treatments for TDZ, as it gave the highest value for abscisic acid, which reached 13.09 µg. Kg^{-1}, while the lowest value was recorded for treatment 0.5 mg $.L^{-1}$ in the solid medium which was 5.80 µg. Kg^{-1} , As for the interactions

between the medium and the PG, the table shows the superiority of the control treatment in the liquid medium significantly on the other treatments, where the highest value of abscisic acid was recorded, which reached 12.50 µg. Kg^{-1}, and the lowest value was recorded at the treatment 50 $mg.L^{-1}$ PG with the solid medium, which reached (5.70 µg. Kg^{-1}).

Table (24) showed the effect of triple interactions. It was noticed that there was a significant superiority of the control treatment with the liquid medium in the content of abscisic acid, which the record it the highest value of abscisic acid, which was 16.79 µg. Kg^{-1}, while the lowest value was recorded at the treatment 50 $mg.L^{-1}$ PG and 0.5 $mg.L^{-1}$ TDZ in a solid medium, which was (5.47 µg. Kg^{-1}).

4-2-11- Abscisic acid content in buds.

The results in Table (25) indicated the liquid medium's superiority, with a significant difference to the solid medium in the buds content of abscisic acid, as it reached 13.90 µg. Kg^{-1}, in the liquid medium, while the solid medium reached 12.91 µg. Kg^{-1}. Whereas, as shown in the same table, the control treatment significantly superiority the two concentrations (0.5 and 1) mg. L^{-1} TDZ in the buds content of abscisic acid, where ABA's content in the control treatment was (14.25 µg. Kg^{-1}).

Table (25) also showed that the control treatment was significantly superior, which recorded the highest value of abscisic acid 15.10 µg. Kg^{-1}, compared with the other two concentrations (25 and 50) mg. L^{-1} PG, while the lowest value at 50 $mg.L^{-1}$ PG 12.26

µg. Kg^{-1}, while the statistical analyses did not show significant differences between the treatment 25 mg. L^{-1}P G and the treatment 50 mg.L^{-1} PG

Table (25) also showed that the control treatment was significantly superior, recording the highest abscisic acid content, which was 18.07 µg. Kg^{-1} compared to other treatments, while the lowest value was for treatment 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG, which was reached (11.94 µg. Kg^{-1}).

The same table also indicated the significant superiority of the control treatment in the liquid medium in the buds contents of abscisic acid by giving it the highest value of 14.97 µg. Kg^{-1}, while the lowest value of abscisic acid, was recorded at the interaction treatment of 1 mg. L^{-1} TDZ with the solid medium, which was 12.57 µg. Kg^{-1}.

Also, Table (25) shows that the control treatment was significantly superior to all concentrations of PG, as the standard treatment with the liquid medium gave the highest value of abscisic acid, which reached 15.83 µg. Kg^{-1}. While the treatment recorded 50 mg. L^{-1} PG with the solid medium had the lowest acid value, which reached 11.43 µg. Kg^{-1}, and the control treatment with the solid medium showed a significant superiority over the other treatments.

As for the triple interactions, the liquid medium's control treatment gave the highest value, reaching 20.26)µg. Kg^{-1}, with a significant superiority on other treatments, while the treatment (50 mg.L^{-1} PG and 0.5 mg. L^{-1} TDZ) recorded in the solid medium the

lowest value for the content of abscisic acid. In the bud stage, which reached 10.59 µg. Kg^{-1}, the control treatment significantly superior to other treatments.

Table (25) The effect of medium type, TDZ, PG and their interactions on the buds content of abscisic acid (µg. Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	20.26	13.65	13.57	15.83
	25	12.42	13.48	12.48	12.79
	50	12.25	13.29	13.72	13.09
solid medium	0	15.89	13.65	13.57	14.37
	25	12.42	13.72	12.69	12.94
	50	12.25	10.59	11.45	11.43
					Mean of medium
TDZ and medium	Liquid	14.97	13.47	13.26	13.90
	Solid	13.52	12.65	12.57	12.91
					Mean of PG
TDZ and PG	0	18.07	13.65	13.57	15.10
	25	12.42	13.60	12.59	12.87
	50	12.25	11.94	12.59	12.26
Mean of TDZ		14.25	13.06	12.92	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.554	0.679	0.679	0.960	0.960	1.176	1.663

4-2-12- Abscisic acid content in shoots.

It is noted from the results in Table (26) the significant superiority of the solid medium was significantly superior to the liquid medium in the content of shoots of abscisic acid, as the rate of abscisic acid in the solid medium was 20.10 µg. Kg^{-1}, while in the liquid medium, the value of abscisic acid was reached.(19.24 µg. Kg^{-1}).

The same table also shows that the control treatment was significantly superior to (0.5 and 1) $mg.L^{-1}$ TDZ in the Abscisic acid shoot stage.The highest value for it was the control treatment 20.59 µg. Kg^{-1}, while the lowest value was at the treatment 0.5. $Mg.L^{-1}$ TDZ, which was 18.79 µg. Kg^{-1}.The control treatment also significantly superiority over the two treatments for PG (50 and 25) mg. L^{-1} by giving it the highest value of 21.46 µg. Kg^{-1}, while the lowest value was recorded when treatment was 50 mg. L^{-1}, reaching 18.22 µg. Kg^{-1} .The statistical analyzes also showed that PG's treatment at the concentration of 25 mg. L^{-1} was superior compared to the treatment 50 mg. L^{-1}

As for the interaction between the medium and PG, Table (26) showed the significant superiority of the control treatment with the solid medium on the other treatments. It gave the highest content of shoots of abscisic acid 21.99 µg. Kg^{-1}, whereas the treatment reached 17.92 µg. Kg^{-1} in the 50 mg .L^{-1} PG in the liquid medium. As for the interaction between the medium and the TDZ, the control treatment with the solid medium significantly affected the content of

Abscisic acid of shoots, which reached 21.71µg. Kg^{-1}, while the lowest value was when the treatment was 0.5 mg . L^{-1} TDZ in the liquid medium, which reached (18.51 µg. Kg^{-1}).

Table (26) The effect of medium type, TDZ and PG and their interactions on shoot content of abscisic acid (µg. Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	23.74	18.62	20.42	20.93
	25	19.02	19.18	18.43	18.88
	50	17.80	17.73	18.22	17.92
solid medium	0	25.61	19.42	20.93	21.99
	25	20.42	19.83	19.15	19.80
	50	19.11	17.94	18.52	18.52
					Mean of medium
TDZ and medium	Liquid	20.19	18.51	19.02	19.24
	solid	21.71	19.06	19.53	20.10
					Mean of PG
TDZ and PG	0	24.67	19.02	20.67	21.46
	25	19.72	19.51	18.79	19.34
	50	18.45	17.84	18.37	18.22
Mean of TDZ		20.95	18.79	19.28	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.412	0.505	0.505	0.714	0.714	0.874	1.236

As for the interactions between TDZ and PG, the table mentioned above shows that the control treatment is significantly

superior to other concentrations by giving it the highest value in content of Abscisic acid 24.67 µg. Kg^{-1}, while the lowest value was recorded when the treatment was 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1} PG reached 17.84 µg. Kg^{-1}. Table (26) indicates the effect of triple interactions. The control treatment in solid media is superior to other treatments. It gives it the highest content of abscisic acid shoot, which reached 25.61 µg.Kg^{-1}, and the lowest content of abscisic acid was recorded when the treatment was 0.5 mg.L^{-1} TDZ with 50 mg.L^{-1} PGL2 with the liquid medium (17.73 µg. Kg^{-1}).

Tables (15-26) illustrate the effect of TDZ on the content of developing tissues from plant hormones, as it increased the content of plant tissue from auxin, cytokinIN, and gibberellins s, and the addition of TDZ also led to a reduction in the percentage of abscisic acid, due to the presence of direct effects of TDZ on endogenous auxin and cytokinin. Its direct effect on Apical meristems during the initial developments in tissue culture, as the addition of 1.8 µm of TDZ had a direct effect on auxins and cytokinins compared to plants grown with TDZ-free medium (Ferreira *et al*., 2006). Naif (2019) also showed that adding cytokinins to the nutritional medium increased the vegetable tissue content of auxins, cytokinins, gibberellins, and decreased abscisic acid content. The results of the study agree with (Kou *et al*., 2016).

It was also noted from the previous tables that the liquid medium was superior in the temporary immersion technique in the content of the plant tissue of auxins, cytokinins, and gibberellins, while the solid medium was superior in the content of the plant tissue

of Abscisic acid. In the explant, as its content of auxin, cytokinins and gibberellins was higher in the bioreactor compared to the solid medium. At the same time, the percentage of abscisic acid was higher in the solid medium.

4-3-The effect of medium type, TDZ and PG on tissue mineral content

4-3-1- Nitrogen content in callus tissues (%).

The results in Table (27) indicate the superiority of the liquid medium over the solid medium in tissue content of nitrogen 0.42 %. In comparison, its percentage in the solid medium was (0.39 %).

The results of the study also show in the same table that there is a significant effect of the compound TDZ on the callus content of the nitrogen, as the TDZ treatment at a concentration of 0.5 mg $.L^{-1}$ significantly superior compared with the two concentrations (1 and 0) mg $.L^{-1}$ TDZ, which the highest value was recorded 0.44 %. While the lowest value at the control treatment, which reached 0.37 %, as shown in Table (27), the PG at 50 mg $.L^{-1}$ was significantly higher at concentrations (0 and 25) mg $.L^{-1}$, where the highest nitrogen percentage was recorded 0.44 %. In comparison, the lowest percentage was recorded at the control treatment reached 0.37 %, and table (27) shows the treatment was superior to 0.5 mg $.L^{-1}$ TDZ with 50 mg $.L^{-1}$ PG significant in the content of callus of nitrogen compaded to the other treatments, as it recorded its highest percentage, which reached 0.9 %, while it was the lowest percentage recorded in the control treatment (0.32 %).

The same table shows that the treatment TDZ and the type of medium had a significant effect on callus content from the nitrogen component. The treatment 0.5 mg $.L^{-1}$ with the liquid medium, recorded the highest percentage was 0.46 %. In contrast, the lowest percentage was recorded when the control treatment with a solid medium, which was 0.35 %. As shown in Table (27), the superiority of treatment with PG at a concentration of 50mg.$L^{-1}$and liquid medium significantly on the other treatment 0.46%, while the lowest percentage of nitrogen was recorded at control treatment in the solid medium was (0.36 %).

As for the triple interaction, the same table indicates the treatment at 50 mg $.L^{-1}$ PG and 0.5 mg.L^{-1} TDZ in the liquid medium superior significantly to the other treatments by giving it the highest percentage of nitrogen, which reached (0.51)%, while the lowest percentage of nitrogen was recorded when the control treatment with the liquid medium (0.31%).

Table (27) The effect of medium type, TDZ , PG and their interactions on callus content of nitrogen (%)

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.3133	0.4333	0.3900	0.3789
	25	0.3633	0.4200	0.4200	0.4011
	50	0.4467	0.5133	0.4333	0.4644
solid medium	0	0.3200	0.3967	0.3667	0.3611
	25	0.3367	0.3967	0.3833	0.3722
	50	0.4133	0.4567	0.3933	0.4211
					Mean of medium
TDZ and medium	Liquid	0.3744	0.4556	0.4144	0.4148
	Solid	0.3567	0.4167	0.3811	0.3848
					Mean of PG
TDZ and PG	0	0.3167	0.4150	0.3783	0.3700
	25	0.3500	0.4083	0.4017	0.3867
	50	0.4300	0.4850	0.4133	0.4428
Mean of TDZ		0.3656	0.4361	0.3978	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0243	0.0297	0.0297	0.0421	0.0421	0.0515	0.0729

4-3-2- Nitrogen content in grown buds (%).

The results in Table (28) indicated the significant superiority of the liquid medium over the solid medium in nitrogen content 0.56 % in the liquid medium. In comparison, its percentage in the solid medium was (0.47%).

The same table also showed a significant effect of the compound TDZ on the nitrogen content. The treatment is 0.5 mg.L^{-1}, significantly superior on the two concentrations (0 and 1) mg .L^{-1} TDZ, which recorded the highest percentage 0.56%. In contrast, the lowest percentage was in the control treatment, which reached 0.51 %. Likewise, between the table, the treatment PG at the concentration of 50 mg .L^{-1} was significant on two concentrations (0 and 25) mg .L^{-1} PG in nitrogen content, where the highest percentage was recorded 0.58%. In contrast, the lowest value was recorded at 25 mg .L^{-1} PG, reaching (0.50 %). The same table also shows the treatment's superiority of 0.5 mg-L^{-1} TDZ with 50 mg .L^{-1} PG significantly in nitrogen content on the other treatments. The highest percentage was recorded 0.64 %, while the lowest percentage was treatment (25 PG with 0 TDZ). mg .L^{-1}, which reached to 0.48 %, and the table also shows that the treatment TDZ in the liquid medium had a significant effect on the nitrogen content of the tissue, as the treatment 0.5 mg .L^{-1} TDZ with the liquid medium showed significant superior on the other treatments, which reached 0.62 %, while the lowest percentage of nitrogen was recorded when treatment 0 mg .L^{-1} TDZ in the solid medium, where it was (0.44 %).

The effect of PG was also significant in the liquid medium, as Table(28)showed the superiority of treatment PG with a concentration of 50 mg .L^{-1} with the liquid medium significantly on the other treatments in the buds content of nitrogen, where the highest percentage was recorded for it 0.63 %. In contrast, the lowest

percentage was for the treatment 0 mg .L^{-1} PG with the solid medium 0.43 % . The treatment at 50 mg .L^{-1} PG in the liquid medium was non-significant difference with the treatment at (0 mg .L^{-1} PGwith the liquid medium).

Table (28) The effect of medium type, TDZ, PG and their interactions on the buds content of nitrogen (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.637	0.607	0.583	0.609
	25	0.543	0.563	0.547	0.551
	50	0.563	0.700	0.627	0.630
solid medium	0	0.397	0.477	0.430	0.434
	25	0.417	0.467	0.443	0.442
	50	0.500	0.573	0.540	0.538
					Mean of medium
Effect TDZ and medium	Liquid	0.581	0.623	0.586	0.597
	Solid	0.438	0.506	0.471	0.471
					Mean of PG
TDZ and PG	0	0.517	0.542	0.507	0.522
	25	0.480	0.515	0.495	0.497
	50	0.532	0.637	0.583	0.584
Mean of TDZ		0.509	0.564	0.528	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0429	0.0525	0.0525	0.0743	0.0743	0.0909	0.1286

As for the triple interaction, Table (28) indicated the significant treatment superiority 50 mg .L^{-1} PG and 0.5 mg .L^{-1} TDZ with the liquid medium on the other treatments by giving it the highest percentage of nitrogen content, which reached 0.70 %, while the lowest percentage of nitrogen was recorded when the control treatment in the solid medium was(0.40%) .

4-3-3- Nitrogen content in shoots.

It is noticed from the results in Table (29) that the liquid medium was significantly superior to the solid medium in the content of the vegetative growth of the nitrogen component, as the percentage of nitrogen in the liquid medium reached 0.75 %, while in the solid medium is reached (0.69%).

It was also found that there was a significant effect of TDZ, as it was observed that the treatment was significantly superior to the control treatment by 0.5 mg .L^{-1} TDZ by recording the highest percentage of nitrogen amounting to 0.77 %, while the lowest percentage was in the control treatment, which amounted to 0.67%. Also, PG had a significant effect, as Table (29)showed that the treatment was superior to 50 mg .L^{-1} PG significantly on the two concentrations (0 and 25) mg.L^{-1} PG by giving it the highest percentage, which was 0.78 %. In contrast, the lowest percentage was recorded when the control treatment reached (0.66%).

The interactions between the medium and PG, the table above explained that there is a significant effect on the content in shoots of nitrogen, as the treatment surpassed 50 mg .L^{-1} PG with

the liquid medium, with a significant difference compared of the treatments by giving it the highest precentage of 0.81 %, while it was the lowest percentage recorded is when the standard treatment with the solid medium was 0.63 %. Simultaneously, the superiority was not significant for the treatment 50 mg $.L^{-1}$ PG with the liquid medium on 25 mg $.L^{-1}$ PG with the liquid medium, and the 50 mg $.L^{-1}$ PG in solid medium.

As for the bilateral interaction between the medium and the TDZ, the treatment of 0.5 mg $.L^{-1}$ TDZ with the liquid medium superiority all treatments, recording the highest rate of nitrogen reaching 0.78 %, while the lowest percentage of nitrogen was recorded at the control treatment with the solid medium was (0.64%).

As for the interactions between the two compounds TDZ and PG, the same table showed that the treatment 0.5 mg $.L^{-1}$ TDZ with 50 mg $.L^{-1}$ PG was superior to the other treatments, by giving it the highest percentage of nitrogen as it reached 0.84 %, while the lowest percentage was at the treatment was (25 PG with 1 TDZ) mg $.L^{-1}$, which recorded (0.64 %).

Table (29) showed the effect of triple interactions, where the two interaction treatments (1 mg$.L^{-1}$ TDZ with 50 mg $.L^{-1}$ PG with the liquid medium) and the treatment (0.5 mg $.L^{-1}$ TDZ with 50 mg $.L^{-1}$PG with the solid medium) showed a significant superiority compared to the other interactions, they recorded the highest percentage of nitrogen, which was 0.87 , 0.86% respectively.In comparison,the lowest percentage was recorded for nitrogen at (0 mg$.L^{-1}$ TDZ and 50 mg $.L^{-1}$ PG) in the solid medium, which reached (0.62 %).

Table (29) The effect of medium type, TDZ, PG and their interactions on the shoots content of nitrogen (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.690	0.707	0.687	0.694
	25	0.660	0.820	0.767	0.749
	50	0.733	0.823	0.867	0.808
solid medium	0	0.663	0.630	0.607	0.633
	25	0.627	0.770	0.663	0.687
	50	0.623	0.863	0.773	0.75
					Mean of medium
Effect TDZ and medium	Liquid	0.694	0.783	0.773	0.750
	solid	0.638	0.754	0.681	0.691
					Mean of PG
TDZ and PG	0	0.677	0.668	0.647	0.664
	25	0.643	0.795	0.715	0.718
	50	0.678	0.843	0.820	0.781
Mean of TDZ		0.666	0.769	0.727	
RLSD 0.05					
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.052	0.064	0.064	0.090	0.090	0.111	0.157

4-3-4- Phosphorous content in callus tissue %.

It is noticed through the results in Table (30) that the liquid medium was significantly superior to the solid medium in the content of phosphorus, where the percentage of phosphorus in the liquid medium was 0.29%, while in the solid medium is reached (0.27%.).

It was also found that there is a sigificant effect of TDZ, where it was observed that the treatment at 0.5 mg.L^{-1} was significant superion on the control treatment by giving it the highest percentage of phosphorous,which was 0.29 %.In contrast, the lowest percentage was recorded at the control treatment 0.26 %.The treatment 1 mg.L^{-1} TDZ was significantly superior to the control treatment.

The results from Table (30) also showed that the treatment 50 mg .L^{-1} -PG was significantly superior to the two concentrations (0 and 25) mg .L^{-1} PG by giving it the highest value, which was 0.29 %. At the same time, the lowest percentage in the control and 25 mg. PG L^{-1} treatment, which was (0.27%.)for each of them

On the effect of the interaction between the medium and PG on the callus content of phosphor, Table (30) indicated the superiority of the interaction treatment of 50 mg .L^{-1} PG with the liquid medium on all treatments by giving it the highest percentage 0.30 %, while the lowest percentage recorded is When treatment was 25 mg .L^{-1} PG with solid medium, which was 0.26 %. While the same table shows that the treatment 0.5 mg .L^{-1} TDZ with the liquid medium was significantly superior on other treatments, recording the highest percentage of phosphorous amounted to 0.30 %. The lowest percentage was recorded for solid medium control treatment, which was 0.25 %.No significant difference was observed between the treatment. 0.5 mg .L^{-1} TDZ in liquid medium and 0.5 mg .L^{-1} TDZ in solid medium in callus content of phosphorus and 1 mg .L^{-1} TDZ in liquid medium.

Table (30) The effect of medium type, TDZ, PG and their interactions on the callus tissue content of phosphor (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.2667	0.2967	0.2933	0.2856
	25	0.2633	0.3000	0.2767	0.2800
	50	0.2933	0.3000	0.3000	0.2978
solid medium	0	0.2467	0.2800	0.2600	0.2622
	25	0.2367	0.2700	0.2733	0.2600
	50	0.2700	0.2933	0.2933	0.2856
					Mean of medium
Effect TDZ and medium	Liquid	0.2744	0.2989	0.2900	0.2878
	solid	0.2511	0.2811	0.2756	0.2693
					Mean of PG
TDZ and PG	0	0.2567	0.2883	0.2767	0.2739
	25	0.2500	0.2850	0.2750	0.2700
	50	0.2817	0.2967	0.2967	0.2917
Mean of TDZ		0.2628	0.2900	0.2828	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0123	0.0151	0.0151	0.0214	0.0214	0.0262	0.0371

As for the interactions between the two compounds TDZ and PG, the table mentioned above showed the superiority of the treatment 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1} PG on other treatments, by giving it the highest percentage of phosphorous which was 0.30%,

while the lowest percentage for it was at (0 TDZ with 25 PG mg $.L^{-1}$) which recorded (0.25%)for each.

Table (30) showed the effect of triple interactions on phosphorus content, where the treatment was superior to 0.5 mg $.L^{-1}$ TDZ with 25 + 50 mg $.L^{-1}$ PG with liquid medium significantly on other treatments except for treatment (0.5 mg $.L^{-1}$ TDZ with 50 mg $.L^{-1}$). PG with the solid medium), which recorded the highest percentage of phosphorous, which was 0.30%, while the lowest percentage recorded of phosphorous at treatment (0 TDZ with 25 PG) mg $.L^{-1}$ in the solid medium, which was (0.24%).

4-3-5- Phosphorous content in buds (%).

The results in Table (31) indicated a significant superiority of the liquid medium over the solid medium in phosphorus content, as it reached in the liquid medium 0.34 %. In comparison, in the solid medium, it was (0.31%).

The same table also showed that there is a significant effect of TDZ on the phosphorous content, as the treatment 0.5 mg $.L^{-1}$ significantly superior on the control treatment, which it recorded the highest percentage 0.34 %, while the lowest percentage was at the control treatment 0.31%. According to the results obtained, the treatment of PG with a concentration of 50 mg $.L^{-1}$ was significantly superior to the other concentrations in the phosphorus content, where the highest percentage 0.35 %, while the lowest percentage was at the treatment 0 and 25 mg $.L^{-1}$, which reached 0.31 %. As for the interaction between the medium and PG, it is evident in Table

(31) that the treatment 50 mg .L^{-1} PG with the liquid medium was superior on all treatments by giving it the highest percentage of 0.37 %, while the lowest percentage was Phosphorous was recorded at the two treatments (0 and 25)mg.L^{-1} PG with the solid medium which was 0.30 % for both treatments.The bilateral interaction between the medium and TDZ had a significant effect on the buds content of phosphorus.The treatment significant superior 0.5 mg.L^{-1}TDZ with the liquid medium that recorded the highest percentage of phosphorus was 0.35 %. The lowest percentage was recorded when the control treatment in the solid medium is reached (0.30%) .

The same table showed that the treatment 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1}PG was superior significantly in the content of phosphorus buds comparted to the treatments, as it recorded the highest percentage of it, which amounted to 0.36 %, while the lowest percentage was at the treatments (0 TDZ with 25PG). mg .L^{-1} , (1TDZ with 0 and 25 PG) mg .L^{-1} and the control treatment was (0.31%).

As for the triple interaction, Table (31) indicated the superiority of the treatment (50 mg .L^{-1} PG and 0.5 mg .L^{-1} TDZ) in the liquid medium significantly on the other treatments, as the treatment at 50 mg .L^{-1} PG and 0.5 mg .L^{-1} TDZ in liquid medium gave recorded, the highest percentage of phosphorus content reached 0.38%, while the lowest rates for phosphorous were recorded at the control treatment in the solid medium and the treatment (0 TDZ with 25 PG) mg .L^{-1} with the solid medium and reached 0.29 % for both treatments.

Table (31) The effect of medium type, TDZ, PG and their interactions on the buds content of phosphor (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.3167	0.3467	0.3167	0.3267
	25	0.3167	0.3300	0.3133	0.3200
	50	0.3400	0.3833	0.3800	0.3678
solid medium	0	0.2933	0.3167	0.2967	0.3022
	25	0.2933	0.3100	0.2967	0.3000
	50	0.3200	0.3267	0.3367	0.3278
					Mean of medium
Effect TDZ and medium	Liquid	0.3244	0.3533	0.3367	0.3381
	solid	0.3022	0.3178	0.3100	0.3100
					Mean of PG
TDZ and PG	0	0.3050	0.3317	0.3067	0.3144
	25	0.3050	0.3200	0.3050	0.3100
	50	0.3300	0.3550	0.3583	0.3478
Mean of TDZ		0.3133	0.3356	0.3233	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.01721	0.0210	0.0210	0.0298	0.0298	0.0365	0.0516

4-3-6-Phosphorous content in shoots(%).

It is noted from the results in Table (32) the superiority of the liquid medium over the solid medium in the content of the shoot of phosphorus, as the percentage of phosphorus in the liquid medium

was 0.43 %. In comparison, in the solid medium, the percentage of phosphorus was (0.42%).

The same table also showed the effect of TDZ on shoot content of phosphorus where it noted that the treatment 0.5 TDZ was superior on the two concentrations (0 and 1) mg .L^{-1} TDZ by giving it the highest percentage of phosphorus 0.44 %, while the lowest percentage was at the control treatment reached 0.41%. The table also shows the significant superiority of treatment PG at a concentration of 50 mg. L^{-1}, on the control treatment, which recorded the highest percentage of phosphorous 0.44%, while the lowest percentage was at the control treatment reached (0.40 %).

The interaction between the medium and PG, the same table showed that the treatment 50 mg .L^{-1} PG with the liquid medium was superior to other treatments, by giving it the highest percentage of phosphorous 0.45%, while the lowest percentage of phosphorous was at the control treatment in the solid medium (0.40%). Also, the interaction between the medium and TDZ had an insignificant effect on the content of phosphorus's shoot, as the treatment recorded 0.5 mg.L^{-1} TDZ with the liquid medium. The highest percentage of phosphorus was 0.44 %. The lowest percentage was recorded at the control treatment in the solid medium was (0.40 %).

Table (10) showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG, which showed significant superiority on the control treatment in the phosphorus content, which recorded the highest value reached 0.45

%. In contrast, the control treatment recorded the lowest value reached (0.38 %).

Table (32) The effect of medium type, TDZ, PG and their interactions on the shoots content of phosphor (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.3867	0.4267	0.4133	0.4089
	25	0.4167	0.4333	0.4533	0.4344
	50	0.4500	0.4567	0.4333	0.4467
solid medium	0	0.3733	0.4067	0.4167	0.3989
	25	0.3867	0.4400	0.4367	0.4211
	50	0.4300	0.4467	0.4367	0.4378
					Mean of medium
TDZ and medium	Liquid	0.4178	0.4389	0.4333	0.4300
	Solid	0.3967	0.4311	0.4300	0.4193
					Mean of PG
TDZ and PG	0	0.3800	0.4167	0.4150	0.4039
	25	0.4017	0.4367	0.4450	0.4278
	50	0.4400	0.4517	0.4350	0.4422
Mean of TDZ		0.4072	0.4350	0.4317	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.02871	0.0351	0.0351	0.0497	0.0497	0.0609	0.0861

Table (32) indicated the effect of triple interaction on the shoot content of phosphorus. The treatment of 0.5 mg .L^{-1} TDZ with 50 mg

.L^{-1} PG with the liquid medium was superior with a significant difference compared to control treatments, including the control treatment in both liquid and solid mediums. It reached 0.46 % , while the lowest percentage was recorded at the control treatment with the solid medium, which was (0.37%).

4-3-7- Potassium content in callus tissues.

It is noticed from the results in Table (33) that there was no significant effect of the medium type in the callus content of potassium, as the results showed that the average percentage of potassium in both media which was (0.35%).

As for the effect of TDZ in the callus content of potassium, it was noted that the treatment 0.5 mg .L^{-1} significantly superior to the control treatment by giving it the highest percentage of potassium, which was 0.37 %. In contrast, the lowest percentage was recorded at the control treatment, which was 0.32 %. As well as the 1 mg .L^{-1} TDZ treatment was significantly superior compared with the control treatment.

PG showed a significant effect, as the treatment 50 mg .L^{-1} PG significantly superior on the two concentrations (0 and 25) mg .L^{-1}PG by giving it the highest percentage, which was 0.39 %. In contrast, the lowest percentage was recorded at the control treatment reached (0.32 %).

As for the interaction between the medium and PG, Table (33) showed a significant effect of the two treatments 50 mg .L^{-1} PG with both media (liquid and solid) on potassium's callus content,

which recorded the highest value at the treatment 50 mg. PG L^{-1} with the liquid medium was 0.39 %. While the lowest percentage recorded was at the control treatment with the liquid and solid media was 0.32 % .

Table (33) The effect of medium type, TDZ , PG and their interactions on the callus tissue content of Potassium (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.3233	0.3200	0.3167	0.3200
	25	0.2767	0.3500	0.3700	0.3322
	50	0.3633	0.4200	0.3967	0.3933
solid medium	0	0.3333	0.3300	0.3033	0.3222
	25	0.2900	0.3600	0.3433	0.3311
	50	0.3467	0.4400	0.3900	0.3922
					Mean of medium
Effect TDZ and medium	Liquid	0.3211	0.3633	0.3611	0.3485
	solid	0.3233	0.3767	0.3456	0.3485
					Mean of PG
TDZ and PG	0	0.3283	0.3250	0.3100	0.3211
	25	0.2833	0.3550	0.3567	0.3317
	50	0.3550	0.4300	0.3933	0.3928
Mean of TDZ		0.3222	0.3700	0.3533	
RLSD 0.05					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.02043	0.0250	0.0250	0.0353	0.0353	0.0433	0.0612

While the same table showed that the interaction between the medium and the TDZ, where the treatment 0.5 mg .L^{-1} TDZ with the solid medium was significantly superior to the control treatment in the callus content of potassium, which reached 0.38 %, and the lowest percentage was recorded at the control treatment in the liquid and solid media, which was (0.32%).

Table (33) showed that the optimal treatment was observed in the medium containing 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} of PG, which showed significant superiority compared to the other treatments in the percentage of potassium, which recorded 0.43%, while the lowest percentage was at treatment (0 TDZ) with 25 PG) mg .L^{-1}, which reached (0.28%).

Table (32) indicates the effect of triple interaction on the callus tissue content of Potassium, where the treatment 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1} PG with the solid medium showed a significantly superior compared to the control treatment by recording the highest percentage of 0.44 %. In comparison, the lowest percentage of potassium was recorded at the treatment (0)TDZ with 25 mg. L^{-1} with the liquid medium, which was (0.28 %).

4-3-8- Potassium content in buds.

The results in Table (34) indicated a significant superiority of the liquid medium on the solid medium in buds content of potassium, as it reached (0.48)% in the liquid medium. In comparison, it was 0.44 % in the solid medium.

The same table also showed that there is a significant effect of TDZ on the potassium content, as the treatment 0.5 mg $.L^{-1}$ TDZ significantly superior to the control treatment, as it recorded the highest percentage 0.49 %, while the lowest percentage was at the control treatment, reached 0.43%. As it was noted a significant superiority for the 1 mg $.L^{-1}$ TDZ treatment on the control treatment. The same table also showed that the treatment PG at the 50 mg $.L^{-1}$ was significantly superior on the two concentrations (0 and 25) mg $.L^{-1}$ in the potassium content. The highest percentage was recorded 0.50 %. In contrast, the lowest percentage was recorded at the control treatment, reaching (0.43 %).

Table (34) showed that the optimal treatment was observed in the medium containing 0.5 TDZ with 50 PG, which showed superiority compared to the other treatments in the buds content of potassium, where the highest value reached 0.57%, while the lowest percentage was recorded at the treatment (0 TDZ with0 or 25 PG) mg $.L^{-1}$ which was (0.42 %).

The same table also showed that the treatment TDZ in the liquid medium had a significant effect on the buds content of potassium, as the treatment 0.5 mg $.L^{-1}$ with the liquid medium showed a significant superiority over the control treatment, reaching 0.51%, while the lowest percentage was recorded at the control treatment in the solid medium, which reached (0.40 %).

The effect of PG was also significant in the liquid medium, as the table mentioned above showed the significant superiority of the treatment PG with a concentration of 50 mg $.L^{-1}$ in the liquid medium

on other treatments in buds content of the potassium, where the highest percentage recorded 0.53 %. In contrast, the lowest percentage was recorded at the control treatment in the solid medium, which reached (0.41 %).

Table (34) The effect of medium type, TDZ, PG and their interactions on the buds content of Potassium (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.4400	0.4733	0.4500	0.4544
	25	0.4367	0.4633	0.4867	0.4622
	50	0.4667	0.6033	0.5300	0.5333
solid medium	0	0.4000	0.4467	0.3900	0.4122
	25	0.4000	0.4367	0.4567	0.4311
	50	0.4100	0.5333	0.4833	0.4756
					Mean of medium
TDZ and medium	Liquid	0.4478	0.5133	0.4889	0.4833
	solid	0.4033	0.4722	0.4433	0.4396
					Mean of PG
TDZ and PG	0	0.4200	0.4600	0.4200	0.4333
	25	0.4183	0.4500	0.4717	0.4467
	50	0.4383	0.5683	0.5067	0.5044
Mean of TDZ		0.4256	0.4928	0.4661	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0309	0.0379	0.0379	0.0536	0.0536	0.0656	0.0928

Table (34) indicated the effect of triple interaction on the buds content of potassium, where the treatment 0.5 mg $.L^{-1}$ TDZ with 50 mg $.L^{-1}$ PG with the liquid medium showed a significantly superior compared with other treatments by recording the highest percentage of 0.60 %, except for treatment 50 mg $.L^{-1}$ PG with 0.5 mg $.L^{-1}$ TDZ in the solid medium and 50mg L^{-1} PG and 1mg L^{-1} TDZ in the liquid media , where the differences between them were not significant. In contrast, the lowest potassium percentage was recorded at the 0mg L^{-1} PG and 1mg L^{-1} TDZ in the solid media, which was (0.39%).

4-3-9- Potassium content in shoots.

It is noticed from the results in Table (35) that the liquid medium was significantly superior to the solid medium in the vegetable growth content of the potassium, as the percentage of potassium in the liquid medium reached 0.77%, while in the solid medium is reached (0.71%).

The same table also showed the effect of TDZ on the content of shoots of potassium, were observed that the treatment TDZ at 0.5 was significantly superior to the two concentrations (0 and 1) mg $.L^{-1}$TDZ, with the highest percentage of potassium 0.79 %, while the lowest percentage was at the control treatment, which reached 0.71%. As for the effect of PG, the same table showed that the treatment 50 mg $.L^{-1}$ PG was significantly superior to the two concentrations (0 and 25) mg $.L^{-1}$ PG by giving it the highest percentage of potassium 0.79%. Simultaneously, the lowest was recorded percentage at the control treatment, reaching (0.71%).

Table (35) The effect of medium type, TDZ and PG and their interactions on the shoots content of Potassium (%)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.7133	0.7833	0.7367	0.7444
	25	0.7500	0.7800	0.7300	0.7533
	50	0.7667	0.9033	0.7967	0.8222
solid medium	0	0.6500	0.7233	0.6800	0.6844
	25	0.6733	0.7200	0.6933	0.6956
	50	0.7133	0.8267	0.7600	0.7667
					Mean of medium
TDZ and medium	Liquid	0.7433	0.8222	0.7544	0.7733
	Solid	0.6789	0.7567	0.7111	0.7156
					Mean of PG
TDZ and PG	0	0.6817	0.7533	0.7083	0.7144
	25	0.7117	0.7500	0.7117	0.7244
	50	0.7400	0.8650	0.7783	0.7944
Mean of TDZ		0.7111	0.7894	0.7328	
RLSD 0.05					
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.03591	0.0439	0.0439	0.0621	0.0621	0.0761	0.1077

Table (35) showed the interaction between the medium and PG,as the treatment 50 mg.L^{-1} PG with the liquid medium significantly superior to other treatments,as it gave the highest percentage of 0.82%, while the lowest percentage of potassium was

when the control treatment in the solid medium was reached (0.68%).

The bilateral interaction between the medium and TDZ showed a significant effect on the content of potassium shoots, as the treatment at 0.5 mg .L^{-1} TDZ with the liquid medium significantly outperformed all treatments, which amounted to 0.82%. The lowest percentage was recorded when the control treatment in the solid medium was 0.68. As for the bilateral interaction between TDZ and PG, it is evident from the same table that the treatment 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1} PG was superior to all treatments, where the highest percentage of potassium was recorded at 0.87 %, while the lowest percentage was at the control treatment, which amounted to 0.68 %. At the same Table showed the effect of triple interactions on the content of potassium in shoots, where the treatment exceeded 0.5 mg .L^{-1} TDZ with 50 mg .L^{-1} PG with liquid medium significantly over the control treatment, by giving it the highest percentage of 0.90 %, while the lowest percentage recorded when the control treatment was in the solid medium, which was (0.65 %).

4-3-10- Magnesium content in callus tissues.

The results in Table (36) showed the superiority of the liquid medium over the solid medium by a significant difference in the content of magnesium, as its percentage in the liquid medium reached 72.87 mg. kg^{-1}. In contrast, the solid medium is reached (71.29 mg. kg^{-1}).

The results also showed in the same table the significant superiority of the treatment 0.5 mg. L^{-1} TDZ in the magnesium content of the treatment 1 mg. L^{-1} of it, with the highest percentage of 37.89 mg. kg^{-1}, while the statistical analyzes did not show significant differences between the control treatment and 0.5 mg. L^{-1} TDZ treatment.

Table (36) The effect of medium type, TDZ, PG and their interactions on the callus content of magnesium (mg.Kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	71.22	72.79	72.21	72.08
	25	70.81	73.25	70.53	71.53
	50	76.24	75.62	73.15	75.01
solid medium	0	68.17	70.48	68.83	69.16
	25	70.15	67.85	70.15	69.38
	50	71.47	83.34	71.14	75.32
					Mean of medium
TDZ and medium	Liquid	72.76	73.89	71.97	72.87
	solid	69.93	73.89	70.04	71.29
					Mean of PG
TDZ and PG	0	69.70	71.64	70.52	70.62
	25	70.48	70.55	70.34	70.46
	50	73.86	79.48	72.15	75.16
Mean of TDZ		71.35	73.89	71.00	

RLSD 0.05						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
2.113	2.588	2.588	3.659	3.659	4.482	6.338

The same table also showed the superiority of the treatment 50 mg. L^{-1} PG was significantly higher on the two concentrations (0 and 25) mg. L^{-1} PG in the content of magnesium callus, where the highest percentage of magnesium was recorded at 75.16 mg. kg^{-1}, while the lowest percentage was recorded when the treatment was 25 mg. kg^{-1} PG, which was 70.46 mg. kg^{-1}.The table mentioned above showed that the treatment exceeded 0.5 mg. L^{-1} TDZ with both media (liquid and solid) significantly over the control treatment, as the highest percentage of magnesium, was recorded in both of the two treatments, 73.89 $mg.Kg^{-1}$, the lowest percentage was recorder in control treatment in solid media wich was (69.93 mg. kg^{-1}) .

As the same table showed that the treatment was superior to 50 mg. L^{-1} PG with solid medium significant over the control treatment, as the highest percentage of magnesium content, was recorded at 75.32 mg. kg^{-1}, while the control treatment in the solid media was recorded recorded the lowest value 69.16 mg. kg^{-1}. As shown in Table (36), the effect of interaction between TDZ and PG was higher, where the treatment was superior to 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG significantly on all treatments, as it was recorded the highest content of the magnesium content, which amounted to 79.48 mg. kg^{-1}, while the control treatment recorded the lowest percentage of (69.70 mg. kg^{-1}).

Table (37) indicated the superiority of the treatment(50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the solid medium) significantly on the other treatments by giving it the highest content of magnesium was

83.34 mg. kg $^{-1}$. In comparison, the lowest content of magnesium was recorded in callus when treatment 25 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the solid medium where It reached (67.85 mg. kg $^{-1}$).

4-3-11- Magnesium content in buds.

Table (37) results indicated the liquid medium superiority over the solid medium in the content of magnesium element in buds, as its amount in the liquid medium was 74.50 mg. kg $^{-1}$. In contrast, the solid medium was (73.64 mg. kg $^{-1}$).

The results also showed in the same table that there is a significant effect of the compound TDZ on the magnesium content, as the treatment 0.5 mg. L^{-1} was a significant superior compared to (0 and1) mg. L^{-1}, which recorded the highest percentage 75.62 mg. kg $^{-1}$, while it was The lowest percentage when treatment 1 mg. L^{-1}, which was 73.05 mg. kg $^{-1}$, as shown in Table (37), PG treatment was superior to 50 mg. L^{-1} was significantly higher on the two concentrations (0 and 25) mg. L^{-1} in the buds content of the magnesium element, where the highest percentage was recorded, which was (76.14 mg. kg $^{-1}$).

The same table also showed the superiority of the treatment 0.5 m mg. L^{-1} TDZ in the liquid medium significantly on all treatments (except for the treatment of 0.5 mg. L^{-1} in the solid medium). The highest value was recorded 76.13 mg. kg $^{-1}$, while the lowest value was recorded at the treatment 1 mg. L^{-1} TDZ with the solid medium, which amounted to 72.14. The table also showed that PG treatment at 50 mg. L^{-1} in the liquid medium was significant superior compared

to the other concentrations except for the treatment (50 mg. L^{-1} PG with the solid medium) in the content of magnesium in buds, where the highest amounted to (76.63 mg. kg^{-1})

Table (37) The effect of medium type, TDZ and PG and their interactions on the buds content of magnesium (mg. kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	72.72	74.12	73.56	73.46
	25	73.31	73.80	73.11	73.41
	50	74.21	80.48	75.19	76.63
solid medium	0	71.81	73.79	71.15	72.25
	25	72.14	73.79	73.13	73.02
	50	77.08	77.74	72.14	75.65
					Mean of medium
Effect TDZ and medium	Liquid	73.41	76.13	73.95	74.50
	solid	73.68	75.11	72.14	73.64
					Mean of PG
TDZ and PG	0	72.26	73.95	72.35	72.86
	25	72.73	73.80	73.12	73.21
	50	75.65	79.11	73.66	76.14
Mean of TDZ		73.55	75.62	73.05	

RLSD 0.05						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.990	1.212	1.212	1.714	1.714	2.099	2.969

Table (37) also showed the superiority of the treatment at 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG significantly in the rate of magnesium on the other of treatments, as it recorded the highest percentage of it, which amounted to 79.11 mg. kg $^{-1}$, while the lowest percentage recorded when the control treatment was 72.26 mg. kg $^{-1}$. As for the triple interaction . Table (37) indicated the superiority of the treatment (50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the liquid medium) significantly on the other treatments except for the treatment (50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the solid medium) by giving it the highest content of magnesium was 80.48 mg. kg $^{-1}$. In comparison, the lowest content of magnesium was recorded in buds when treatment 0 mg. L^{-1} PG and 1 mg. L^{-1} TDZ in the solid medium where It reached (71.15 mg. kg $^{-1}$)

4-3-12- Magnesium content in shoots.

Table (38) showed the significant superiority of the liquid medium over the solid medium in the content of the shoots of magnesium, which reached in the liquid medium 75.41 mg. kg $^{-1}$, while the solid medium gave the lowest percentage of magnesium, which was (74.62 mg. kg $^{-1}$)

The results also showed in the same table that there is a significant effect of the compound TDZ on the magnesium accumulation in shoot of the date palm,the treatment is 0.5 mg. L^{-1} significantly superior on the control treatment by recording the highest percentage of 75.99 mg. kg $^{-1}$, while the lowest content was at the control treatment that recorded 74.42 mg. kg $^{-1}$. Moreover,

the treatment of 0.5 mg. L^{-1} TDZ showed a non-significant superiority on treatment 1 mg. L^{-1}.

Table (38) The effect of medium type, TDZ, PG and their interactions on the shoot content of magnesium (mg. kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	73.70	74.29	74.14	74.04
	25	75.07	75.28	75.82	75.39
	50	74.76	80.07	75.53	76.79
solid medium	0	72.79	75.10	73.12	73.67
	25	74.11	74.77	74.44	74.44
	50	76.09	76.41	74.77	75.76
					Mean of medium
TDZ and medium	Liquid	74.51	76.55	75.16	75.41
	Solid	74.33	75.43	74.11	74.62
					Mean of PG
TDZ and PG	0	73.63	74.69	73.63	73.85
	25	75.13	75.02	75.13	74.91
	50	75.15	78.24	75.15	76.27
Mean of TDZ		74.42	75.99	74.64	
RLSD 0.05					

Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.739	0.905	0.905	1.279	1.279	1.567	2.216

The table also showed that the treatment PG at a 50 mg.L^{-1} was significantly higher than the two concentrations (0 and 25) mgL^{-1}

in its magnesium content, where the highest content was 76.27 mg. kg $^{-1}$. Simultaneously, the lowest percentage was recorded at the control treatment, which was (73.85 mg. kg $^{-1}$).

As for the bilateral interactions, Table (38) showed the superiority of the treatment 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG significant in the vegetative growth content from magnesium element compared to the other treatments, where the highest percentage was recorded 78.24 mg. kg $^{-1}$. Simultaneously, the lowest value was at the control treatment was (73.63 mg. kg $^{-1}$).

The same table also showed that the TDZ treatment, 0.5 mg. L^{-1} TDZ with the liquid medium was a significant superio in the content of magnesium shoots than all treatments except for the treatment (1 mg. L^{-1} TDZ with the liquid medium) and the treatment (0.5 mg. L^{-1} TDZ with the solid medium), where the highest amount was recorded 76.55 mg. kg $^{-1}$, while the lowest amount was recorded at a solid medium with 1 mg. L^{-1} TDZ, which was 74.11 mg. kg $^{-1}$. The same table also shows a significant superior of the treatment PG at a 50 mg. L^{-1} with the liquid medium on the other treatments in the mean of magnesium, where the highest content was recorded, which was 76.79 mg. kg $^{-1}$, while the lowest content was recorded when the control treatment with the solid medium was 73.67 mg. kg $^{-1}$. As for the triple interference, Table (38) indicates that the treatment 50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in a liquid medium was a significant superior to other treatments by giving it the highest magnesium content of the shoots, which was (80.07 mg. kg $^{-1}$).

4-3-13- Manganese content in callus tissues.

The results in Table (39) indicated that the liquid medium was significantly superior to the solid medium in the content of manganese callus, as it reached 0.58 mg. Kg^{-1}, while in the solid medium, it was (0.46 mg. Kg^{-1}).

Table (39) The effect of medium type, TDZ, PG, and their interactions on the callus tissue content of manganese (mg. kg^{-1})

Medium type	PG (mg $.L^{-1}$)	TDZ (mg $.L^{-1}$)			PG and medium type
		0	0.5	1	
Liquid medium	0	0.268	0.653	0.505	0.475
	25	0.618	0.602	0.397	0.539
	50	0.651	0.807	0.721	0.727
solid medium	0	0.317	0.508	0.430	0.419
	25	0.324	0.512	0.430	0.422
	50	0.524	0.611	0.488	0.541
					Mean of medium
Effect TDZ and medium	Liquid	0.512	0.688	0.541	0.580
	Solid	0.388	0.544	0.449	0.460
					Mean of PG
TDZ and PG	0	0.293	0.580	0.468	0.447
	25	0.471	0.557	0.413	0.481
	50	0.588	0.709	0.604	0.634
Mean of TDZ		0.450	0.616	0.495	
RLSD 0.05					
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.082	0.1013	0.1013	0.1433	0.1433	0.1755	0.2483

The results also show in the same table that there is a significant effect of the compound TDZ on manganese callus content, as the treatment is 0.5 mg. L^{-1} significantly superior on (0 and 1) mg. kg $^{-1}$, of it, where the highest percentage was recorded 0.62 mg. kg $^{-1}$. When the lowest percentage was when the control treatment was 0.45 mg. kg $^{-1}$, The same table also showed that treatment PG with a 50 mg .L^{-1} concentration was significantly superior to the two concentrations (0 and 25) mg. L^{-1} PG, where the highest percentage of manganese was recorded, reached 0.63 mg. kg $^{-1}$, while the lowest percentage was at the control treatment reached 0.45 mg. kg $^{-1}$.

Table (39) also showed the superiority of treatment of 0.5 mg. L^{-1} TDZ with the liquid medium significantly on the other treatments, as its highest percentage reached 0.69 mg. Kg^{-1}, while the lowest percentage was recorded when the control treatment with the solid medium, which was 0.39 mg. kg $^{-1}$ About the effect of PG on the content of callus from manganese, the study showed that the treatment of PG at the concentration of 50 mg. L^{-1} with the liquid medium were significantly superior compared to the other other treatments, where the highest percentage was recorded reached 0.73 mg. Kg^{-1}, while the lowest percentage was in the control treatment in the solid medium, which reached (0.42 mg. kg $^{-1}$).As for the triple interaction, Table (39) showed. that the treatment 50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ in the liquid medium was significantly superior compared to the other treatments by giving it the highest manganese content 0.81mg Kg^{-1}, while the lowest manganese

content was recorded when the control treatment with the liquid medium was (0.27 mg. Kg^{-1})

4-3-14-Manganese content in buds.

The results in Table (40)indicated the significant superiority of the liquid medium over the solid medium in manganese buds content, as in the liquid medium, it reached 0.68 mg. Kg^{-1},while in the solid medium it was(0.61mg.Kg^{-1})

The same table also showed that the treatment 0.5 mg. L^{-1} TDZ significantly superiority on the two concentrations (0 and 1) mg. L^{-1} TDZ, where the highest percentage was recorded 0.73 mg. kg^{-1}. The treatment PG at the 50 mg. L^{-1} was significantly superior to the two concentrations (0 and 25) mg. L^{-1} of it in the manganese content, where the highest percentage was recorded 0.73 mg.Kg^{-1}, while the lowest percentage was recorded at the control treatment was (0.59 mg Kg^{-1}).

Table (40) also showed that the treatment of 0.5 mg. L^{-1} TDZ with the liquid medium was a significant superior comparted to the other treatments by recording the highest manganese content of 0.78 mg. Kg^{-1}, except the treatment (0.5 mg. L^{-1} with the solid medium) while it recorded the lowest value. At the control treatment in the solid medium, which was 0.54 mg. Kg^{-1}. The same table also showed that the treatment of PG was a significant superior at the concentration of 50 mg. L^{-1} in the liquid medium compared to the other treatments except for the treatment (50 mg. L^{-1} PG in the solid medium) in manganese buds content, where the highest value was

recorded 0.79 mg. Kg $^{-1}$, while the lowest value was at the control treatment with the solid medium., which was 0.55 mg. Kg^{-1}. As the same table showed, the treatment at 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG was a significant superior in manganese buds compared to the

Table (40) The effect of medium type, TDZ, PG and their interactions on the buds content of manganese (mg. kg $^{-1}$)

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.538	0.725	0.614	0.625
	25	0.687	0.657	0.570	0.638
	50	0.743	0.950	0.668	0.787
solid medium	0	0.400	0.646	0.588	0.545
	25	0.581	0.559	0.675	0.605
	50	0.639	0.812	0.588	0.680
					Mean of medium
Effect TDZ and medium	Liquid	0.656	0.777	0.617	0.683
	Solid	0.540	0.673	0.617	0.610
					Mean of PG
TDZ and PG	0	0.469	0.686	0.601	0.585
	25	0.634	0.608	0.623	0.622
	50	0.691	0.881	0.628	0.733
Mean of TDZ		0.598	0.725	0.617	

RLSD 0.05						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.074	0.091	0.091	0.129	0.129	0.158	0.223

other treatments, where the highest value was recorded at 0.88. mg. kg $^{-1}$, while the lowest value was when the control treatment was (0.47 mg. Mg^{-1}).

As for the triple interference, Table (40) showed that the treatment (50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ) with the liquid medium was a significant superior compared to the other treatments, except for the two interaction treatments (50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ) in the solid medium and the treatment (50 mg. L^{-1} PG and 0 mg. L^{-1} TDZ) in the liquid medium by giving it the highest value of the buds content of manganese was 0.95 mg. Kg^{-1}, while the lowest manganese content was recorded at the control treatment in the solid, medium (0.40 mg. Kg^{-1}).

4-3-15- Manganese content in shoots.

It is noticed from the results in Table (41) that the liquid medium was significantly superior to the solid medium in the content of shoot of the manganese element, where the rate of manganese in the liquid medium reached 0.86 mg. kg -1, while in the solid medium the value of manganese was(0.70 mg. kg $^{-1}$).

It is also noted from the same table that treatment 0.5 mg. L^{-1} TDZ significantly superior the control treatment by giving the highest value for the content of shoots of manganese, which amounted to 0.88 mg.Kg^{-1} , while the lowest value was for the control treatment, which reached 0.69 mg. kg $^{-1}$, PG also had a significant effect, as the table showed that the treatment at 50 mg. L^{-1} PG was significant superior compared to the two concentrations (0 and 25) mg. $L^{-1}$by

giving it the highest value of 0.96 mg. $Kg^{-1,}$ while the lowest value was recorded when the control treatment was (0.67 mg. Kg^{-1}).

As for the bilateral interaction between the medium and PG, the table showed that the treatment at 50 mg. L^{-1} PG treatment in the liquid medium was exceeded with a significant difference compared to the other treatments, gives it the highest value of 1.06mg. Kg^{-1}, while the lowest value was recorded when the control treatment with the solid medium, which was 0.60 mg.Kg^{-1}. The bilateral interaction between the medium and the TDZ had a significant effect on the content of manganese's shoot, where the treatment at 0.5 mg. L^{-1} TDZ with the liquid medium was a significant superior over the other treatments (except for the treatment 1 mg. L^{-1} with the liquid medium) and (0.5 mg. L^{-1} with the solid medium) and the control treatment (with the solid medium) reached 0.78 mg. Kg^{-1}, while the control treatment with the solid medium recorded the lowest value of manganese in the shoots 0.60. Kg^{-1}. As for the interactions between the two compounds TDZ and PG, it is evident from the same table that the treatment 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG superiority on all treatments, by giving it the highest value of manganese was 1.18 mg. Kg^{-1}, while the lowest value was when the control treatment, which was (0.58 mg. Kg^{-1}).

The table (41) the effect of triple interactions, where the treatment 0.5 mg.L^{-1} TDZ with 50 mg. L^{-1} PG with solid medium was superior to on the rest of the treatments except for treatment (0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid medium) And the treatment (0 and 1 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with the liquid

medium), where the highest value for manganese TDZ was recorded at 1.21mg.Kg^{-1}, while the lowest value was recorded for manganese when the control treatment in the solid medium (0.51 mg. Kg^{-1}).

Table (41) The effect of medium type, TDZ, PG and their interactions on the shoot content of manganese (mg. kg^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	0.646	0.823	0.711	0.727
	25	0.746	0.765	0.830	0.781
	50	0.950	1.155	1.069	1.058
solid medium	0	0.516	0.660	0.632	0.603
	25	0.624	0.639	0.588	0.617
	50	0.675	1.212	0.718	0.868
					Mean of medium
TDZ and medium	Liquid	0.781	0.915	0.870	0.855
	Solid	0.605	0.837	0.646	0.696
					Mean of PG
TDZ and PG	0	0.581	0.742	0.671	0.665
	25	0.685	0.702	0.709	0.699
	50	0.813	1.184	0.894	0.963
Mean of TDZ		0.693	0.876	0.758	

RLSD 0.05						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.1002	0.1227	0.1227	0.1735	0.1735	0.2125	0.3005

The cultivation system by bioreactors in liquid media has a positive effect on increasing the concentration of elements in the

tissue. It may be due to the ease of absorption of nutrients and increased availability from the liquid medium compared to the solid medium. The movement of cultured tissues in the liquid medium can also be an easy gas exchange, which helps to treat the phenomenon of deficiency of some elements in tissues due to their aggregation and non-proliferation. For these reasons, some researchers have resorted to using a moving liquid medium (Khierallah *et al*., 2011; El-Hammady *et al*., 1999; Hamid 2001).

The importance of the bioreactor is used air and liquid medium. The concentration of nutrients in the medium is influenced by the rate of absorption and cell activity effected by air and element availability(Archambault *et al*.,1995).The concentration of mineral elements in the plant tissue may change upon cultivation in the liquid medium, especially the two elements of phosphorus, potassium, and nitrates, elements which are rapidly depleted as Ca^{2} and Mg^{2} As a result of its use in physiological activities(Ilan *et al*., 1995).The use of growth regulators (especially cytokinins) affects the rate of absorption of basic elements in callus plant under laboratory conditions, as it directly affects the formation and differentiation of vessels and the regulation of enzymes involved in the metabolism of elements especially nitrogen(Aloni *et al,*.2004; Donato *et al* 2003). The increase in the potassium element leads to a decrease in the percentage of the sodium element in the plant tissue based on the competition between them, as the increase in the absorption of the potassium element from the cell membrane leads to preventing the absorption of sodium into the plant tissue as they enter the cell from

one Na / K pump to the presence of its positive charge (Munns and Tester, 2008), that the accumulation of nutrients in the plant tissue may be due to the effect of cytokines such as (2iP) which have a role in attracting and withdrawing nutrients from the source to the places of growth (Salman, 1988).

4-4- The effect of the medium type, TDZ, and PG on some physiological characteristics:

4-4-1- Carbohydrate content in buds.

The results in Table (42) showed the significant superiority of the liquid medium over the solid medium in the carbohydrate content of the buds, as the carbohydrate rate in the liquid medium reached 92.5 $mg.g^{-1}$, while in the solid medium is reached (77.9 $mg.g^{-1}$) .

There was also a significant effect of TDZ on carbohydrate content buds , as the treatment's at 0.5 mg. L^{-1} TDZ was a significant superior compared to the other treatments (0 and 1) mg. L^{-1} TDZ, where the percentage of carbohydrates was 96.3 $mg.g^{-1}$, while the lowest value was recorded 76.5 $mg.g^{-1}$ at the control treatment. PG treatment had a significant effect on carbohydrates content in buds, as Table (42) showed The treatment 50 mg. L^{-1}. The PG is a significant superior compared to the two treatments (0 and 25) mg. L^{-1} it's gave the highest value of 95.8 $mg.g^{-1}$, while the control treatment was recorded the lowest value 79.4 $mg.g^{-1}$. As for the bilateral interaction between the medium and PG, Table (42) showed the treatment at 50 mg. L^{-1}PG in the liquid medium was a significant superiority compared to the other treatments, as the

highest value was recorded in the content of buds of carbohydrates was 107.9 mg.g $^{-1}$, while the lowest value was recorded at the control treatment in solid medium amounted to (72.4 mg.g^{-1}).

Table (42) The effect of medium type, TDZ , PG and their interactions on the buds content of carbohydrates (mg.g^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	80.4	91.6	87.5	86.5
	25	74.9	102.3	72.2	83.1
	50	86.4	127.6	109.6	107.9
solid medium	0	66.0	79.0	72.1	72.4
	25	71.5	80.0	81.0	77.5
	50	80.0	97.2	74.0	83.7
					Mean of medium
TDZ and medium	Liquid	80.6	107.2	89.8	92.5
	Solid	72.5	85.4	75.7	77.9
					Mean of PG
TDZ and PG	0	73.2	85.3	79.8	79.4
	25	73.2	91.2	76.6	80.3
	50	83.2	112.4	91.8	95.8
Mean of TDZ		76.5	96.3	82.7	
RLSD 0.05					

Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
9.78	11.98	11.98	16.94	16.94	20.75	29.35

The same table also shows the significant effect of the bilateral interaction between the medium type and the TDZ. The treatment at 0.5 mg L^{-1} TDZ with in liquid medium type is a significant superior compared to the other to all treatments, as the carbohydrate content reached 107.2 $mg.g^{-1}$.But the controltrtment in solid medium was recorded the lowest value 72.5 $mg.g^{-1}$ .As for the bilateral interactions between the two compounds TDZ and PG, the table showed the treatment's superiority of 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG on all treatments except for the treatment(1.0 mg. L^{-1} TDZ with 50 mg. L^{-1} PG)by recording it the highest content. of carbohydrates 112.4 $mg.g^{-1}$, while the lowest content was in the control treatment and 0TDZ +25 PG mg l^{-1}, which recorded (73.2 $mg.g^{-1}$).

While the results mentioned in Table (42) show the effect of triple interactions, where the two treatments outperformed 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG with liquid medium significantly on all treatments except for the two treatments (0.5 mg. L^{-1} TDZ with 25 mg. L^{-1} PG) and 1.0 mg. L^{-1} TDZ with 50 mg. L^{-1} PG) with the liquid medium, where the highest value of the bud content of carbohydrates was recorded at 127.6 $mg.g^{-1}$, while the lowest value for carbohydrates was recorded when the control treatment in the solid medium was (66.0 $mg.g^{-1}$).

4-4-2-Carbohydrate content in shoots.

It is noticed from the results in Table (43) the significant superiority of the liquid medium over the solid medium in the content of shoots for carbohydrates, the carbohydrates in the liquid medium

reached 120.2 mg.g $^{-1}$, while in the solid medium reached (101.6 mg.g $^{-1}$).

There was also a significant effect of TDZ on the carbohydrate content for the shoots, where the significant superiority of the treatment was observed at 0.5 mg. L^{-1} TDZ on the control treatment, while there was no significant difference between it and the treatment 1.0 mg. L^{-1}TDZ, where the percentage of carbohydrates was 119.5 mg.g $^{-1}$, while the lowest value was recorded at the control treatment, which reached to 103.6 mg. g^{-1}. PG also had a significant effect on the carbohydrate content of shoots. Table (43) showed the treatment's superiority 50 mg. L^{-1} of PG significantly over the two treatments (0 and 25) mg. L^{-1} by giving it the highest value 124.6 mg .g^{-1}. Simultaneously, the lowest value was recorded when the control treatment was (102.6 mg.g^{-1}).

As for the bilateral interaction between the medium and PG, Table (43) shows the two treatments' superiority 50 mg. L^{-1} of PG with the liquid medium significantly over all other treatments, where the highest value was recorded 135.9 mg.g^{-1}, while the lowest value was recorded for the treatment of 25 mg. L^{-1} of PG with the solid medium 94.8 mg.g $^{-1}$, As it is evident from the same table the significant effect of the bilateral interaction between the medium and the TDZ, where the treatment 0.5 mg. L^{-1} TDZ with the liquid medium was significantly superior to all other treatments except for 1 mg. L^{-1} TDZ in the liquid medium, where the content of the shoots of carbohydrates in them reached 128.9 mg.g $^{-1}$, and the lowest value was recorded when the treatment was 1 mg. L^{-1} TDZ with the solid

medium 96.2 mg. g^{-1} , As for the bilateral interactions between the two compounds TDZ and PG, the same table showed that the treatment 0.5 mg. L^{-1} of TDZ with 50 mg. L^{-1} PG superior on all other treatments, by giving it the highest carbohydrate content 141.7 mg.g^{-1}, while it was the lowest content at the control treatment was recorded (95.5 mg.g^{-1}).

The results mentioned in Table (43) showed the effect of triple interactions, the treatments 0.5 mg. L^{-1} TDZ , 50 mg. L^{-1} PG with liquid medium significantly superior on all other treatments except for the two treatments (0.5 mg. L^{-1} TDZ, 50 mg. L^{-1} PG) With the solid medium and the treatment (1 mg. L^{-1} TDZ and 50 mg. L^{-1} PG) with the liquid medium, the highest value was 149.6 mg.g^{-1}, while the lowest value of the carbohydrate content of shoot was recorded at the control treatment in the solid medium was (90.0 mg.g^{-1}).

The reason for the superiority of the treatments in the liquid medium in total soluble sugars may be due to the role that the liquid media allows for gas exchange as well as the increased available of nutrients and sucrose which the tissue can used to synthetic sugars (Scragg , 1992; Borisjuk *et al*, 2004 and Naif, 2019). The other reason may also be due to the fact that cytokinins act as a sink for the nutrients and sugars present in the nutrient medium important in syntheses processes in tissues, which leads to the accumulation of sugars in them (Davies, 2004).

Table (43) The effect of medium type, TDZ and PG and their interactions on the shoot content of carbohydrates (mg.g^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	100.9	115.1	108.8	108.3
	25	106.1	122.1	120.7	116.3
	50	119.2	149.6	139.0	135.9
solid medium	0	90.0	104.6	95.9	96.8
	25	100.1	91.8	92.6	94.8
	50	105.6	133.9	100.2	113.2
					Mean of medium
TDZ and medium	Liquid	108.7	128.9	122.9	120.2
	Solid	98.6	110.1	96.2	101.6
					Mean of PG
TDZ and PG	0	95.5	109.8	102.3	102.6
	25	103.1	106.9	106.6	105.6
	50	112.4	141.7	119.6	124.6
Mean of TDZ		103.6	119.5	109.5	
RLSD 0.05					

Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
8.71	10.67	10.67	15.09	15.09	18.48	26.14

4-4-3- Chlorophyll (a, b and total) content of shoots (mg. 100g^{-1} fresh weight)

The results in Tables (44, 45, and 46) indicated the superiority of the liquid medium over the solid medium in the content

of chlorophyll (a, b and total), it reached in the liquid medium (101.2, 56.1 and 157.3), respectively, while it reached in the solid medium (88.8 , 51.8 and 140.6), respectively. The superiority was significant in chlorophyll a and total, while the superiority was not significant in the shoot content from chlorophyll b.

The same table also shows an effect of the TDZ compound on the chlorophyll content, where the treatment superiority of 0.5 mg. L^{-1} significantly in the chlorophyll content (a and total) which reached (99.3 and 156.2) respectively, while the results did not show a significant superiority in the chlorophyll content (b) which reached to 56.9 , the lowest value recorded for chlorophyll (a, b and total) at the control treatment was (90.6, 50.7 and 141.3). The PG at 50 mg. L^{-1} was a significant effect on the two concentrations (0 and 25) mg. L^{-1} in the chlorophyll content (a, b and total), which recorded (101.8, 59.1 and 160.9), respectively, while the lowest value was at the control treatment (93.2, 50.5 and 143. 8) for each chlorophyll type sequentially.

Tables (44,45 and 46) also showed an effect of the TDZ compound with the liquid medium on the chlorophyll content, where the treatment is 0.5 mg. L^{-1} exceeded on the liquid medium significantly in the chlorophyll content (a and total), which reached (106.1 and 164.0), respectively, while not noted a significant superiority in the chlorophyll b 57.9 mg.$100g^{-1}$. The lowest value was recorded for chlorophyll (a, b and total) at the standard treatment with solid medium(84.5, 48.8 and 133.3), respectively. The PG at50 mg. L^{-1} with the liquid medium has significant over the other

treatment in the chlorophyll (a, b and total), the highest value was recorded (108.1, 60.9 and 169.0) respectively, the lowest value was recordedof chlorophyll b with the control treatment in the solid medium reaching 46.9 while the lowest value of total chlorophyll and a was recorded with the25 mg. L^{-1} (82.4 , and 133.6) respectivly.

The same table showed the superiority of the treatment of 0.5 mg. L^{-1} TDZ with 50 mg. L^{-1} PG significantly in the content of chlorophyll (a and total) over the rest of the treatments except for the treatment (1 mg. L^{-1} TDZ with 50 mg. L^{-1} PG),which recorded the highest value (108.4and 171.4) respectively, while the lowest value at the treatment 0 mg. L^{-1} TDZ with 25 mg.L^{-1} PG, which was (80.8 and 132.4), respectively, the lowest value for chlorophyll (B) was reached at the control treatment 46.6 mg.$100g^{-1}$.

The triple interaction we are noted in table (44,45 and 46) which shows that the treatment 50 mg. L^{-1} PG and 0.5 mg. L^{-1} TDZ with the liquid medium was significantly higher over the control treatment in the solid medium by giving it the highest value for the plant tissue content of chlorophyll (a and b) and total as it reached (117.7, 65.5, and 183.1) respectively, while the lowest chlorophyll content (a and total) was recorded when treatment 0 mg. L^{-1} TDZ with 25 mg. L^{-1} PG in the solid medium reaching (67.7 and 121.8) respectively, while chlorophyll (b) recorded its lowest value when the control treatment in the solid medium (37.6).

Table (44) The effect of medium type, TDZ and PG and their interactions on the content of chlorophyll a in the shoot (mg.100g^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	95.9	102.0	96.4	98.1
	25	93.9	98.6	99.7	97.4
	50	100.2	117.7	106.4	108.1
solid medium	0	91.9	87.6	85.5	88.3
	25	67.7	90.5	88.9	82.4
	50	93.9	99.1	93.7	95.6
					Mean of medium
TDZ and medium	Liquid	96.7	106.1	100.8	101.2
	Solid	84.5	92.4	89.4	88.8
					Mean of PG
TDZ and PG	0	93.9	94.8	91.0	93.2
	25	80.8	94.6	94.3	89.9
	50	97.0	108.4	100.0	101.8
Mean of TDZ		90.6	99.3	95.1	

RLSD 0.05						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
6.01	7.36	7.36	10.41	10.41	12.74	18.02

Table (45) The effect of medium type, TDZ and PG and their interactions on chlorophyll b content in the shoot (mg.100g^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	55.6	51.5	55.3	54.1
	25	49.1	56.7	54.3	53.3
	50	53.1	65.5	64.2	60.9
solid medium	0	37.6	54.3	48.9	46.9
	25	54.1	52.8	46.8	51.2
	50	54.7	60.6	56.6	57.3
					Mean of medium
TDZ and medium	Liquid	52.6	57.9	57.9	56.1
	Solid	48.8	55.9	50.7	51.8
					Mean of PG
TDZ and PG	0	46.6	52.9	52.1	50.5
	25	51.6	54.7	50.5	52.3
	50	53.9	63.0	60.4	59.1
Mean of TDZ		50.7	56.9	54.3	
RLSD 0.05					

Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
5.29	6.48	6.48	9.16	9.16	11.22	15.87

Table (46) The effect of medium type, TDZ, PG and their interactions on content of total chlorophyll in the shoot (mg.100g^{-1})

Medium type	PG (mg .L^{-1})	TDZ (mg .L^{-1})			PG and medium type
		0	0.5	1	
Liquid medium	0	151.5	153.6	151.6	152.2
	25	142.9	155.3	154.0	150.8
	50	153.3	183.1	170.5	169.0
solid medium	0	129.5	142.0	134.4	135.3
	25	121.8	143.3	135.7	133.6
	50	148.6	159.7	150.3	152.8
					Mean of medium
TDZ and medium	Liquid	149.2	164.0	158.7	157.3
	solid	133.3	148.3	140.1	140.6
					Mean of PG
TDZ and PG	0	140.5	147.8	143.0	143.8
	25	132.4	149.3	144.8	142.2
	50	150.9	171.4	160.4	160.9
Mean of TDZ		141.3	156.2	149.4	

RLSD 0.05						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
6.20	7.60	7.60	10.74	10.74	13.16	18.61

There is much evidences proving that cytokinins activate the synthesis of proteins and plant pigments and differentiate membranes in chloroplasts (Goltsev *et al*. 2001; Kulaeva; *et al*. 2002). Studies also indicate that treatment with cytokinins contributed to prolonging the life of plastids (Reski *et al*., 1986), the

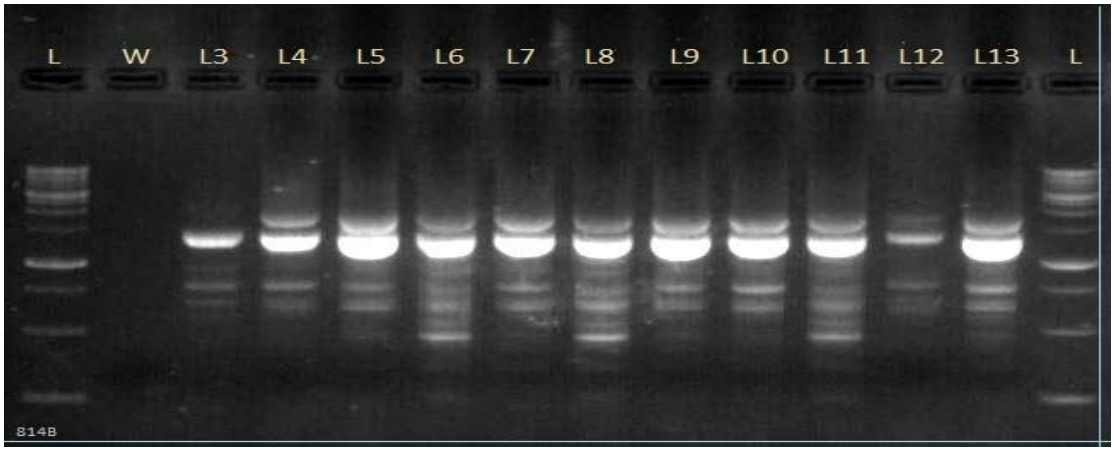
cytokinins react with a light then stimulating gene expression and increased formation of plastid proteins (Reski *et al*., 1991).The treatment with cytokinin increases the DNA of chloroplasts, that increases protoplast construction, maintains the level of pigments and changes the permeability of the membranes, as well as stimulates the replication of plastids and the formation of grana membrane (Vanstaden J. *et al*., 1988). High chlorophyll retention efficacy, especially in high concentrations (Sabovljevi A. *et al*., 2010).

4-5-: Molecular Genetics

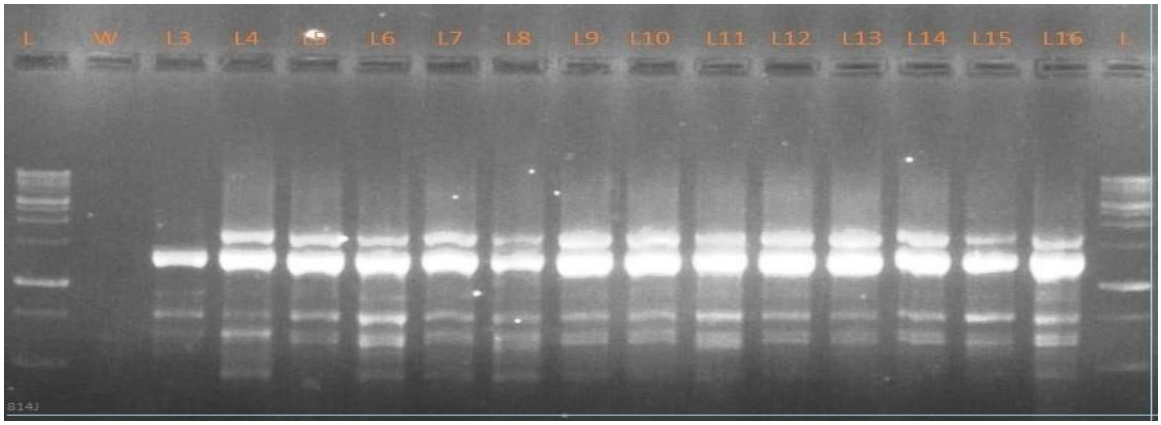
4-5-1: Simple Tandem Repeat (ISSR) technical Markers.

4-5-1-1: ISSR primer 814:

It is evident from the figure (12 and 13) that the Primers 844 had given a total number of (150) Bands in both mediums, where it reached (74) bundles in the liquid medium and reached (76) bands in the solid medium, the liquid medium was the highest number of bands that reached (7) bands where the amplification values ranged between (0.700-0.400) base pair, while the lowest number of bundles was recorded at the parameters (callus, standard, and 0.5 $mg.L^{-1}$ TDZ), which reached (6) bund, that the genetic match-rates of the parameters in the liquid and solid mediums did not show genetic variations at the genetic level described in the above marker.



Figure(12) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) mg. L^{-1} of PG in Bioreactors system using ISSR 814 marker (L:ladder, W:water, L3:callus, L4:control,L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50,L12:1+25,L13:1+50)

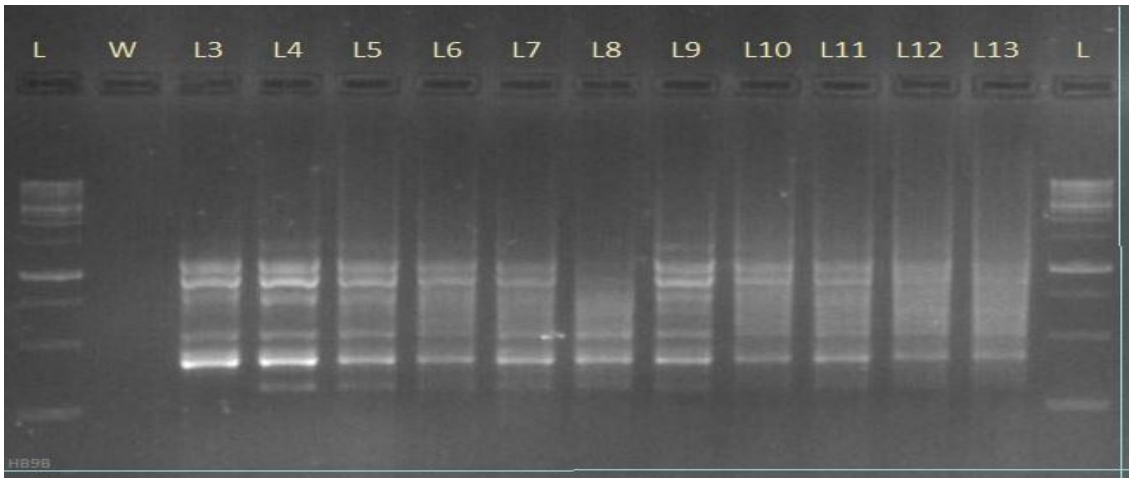


Figure(13) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) mg.L^{-1} of PG in Solid system using ISSR 814 marker (L:ladder, W: water, L3: Berhi leaf, L4: callus,L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, 16:1+100)

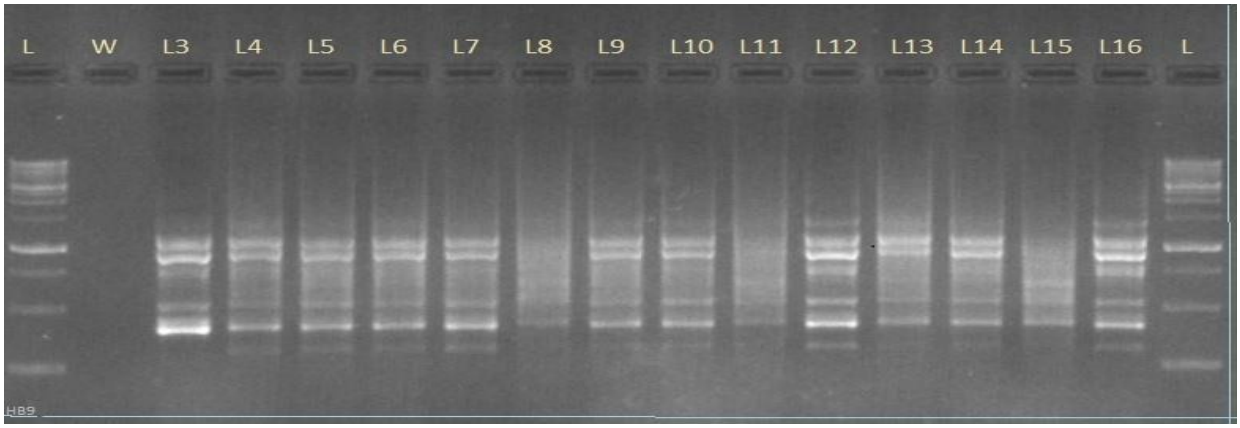
4-5-1-2: ISSR primer HB9

Figure (14 and 15) showed the results of amplifying the HB9 markers for samples of different parameters, as it gave the highest

number of multiplexed bands and the treatment was similar in the number of bands. The total of the formed bands in the liquid and the solid mediums were(169). The number of bands in the liquid medium was (70). Simultaneously, the number of solid media is (99), where the treatment in the liquid medium was equal in the number of the resulting bands, as the parameters recorded 6 BANDS with molecular weights ranging between (0.700-0.239). It is noticed from the figure that the genetic matching ratios of the transactions in the liquid and solid media did not show genetic variations at the genetic level.



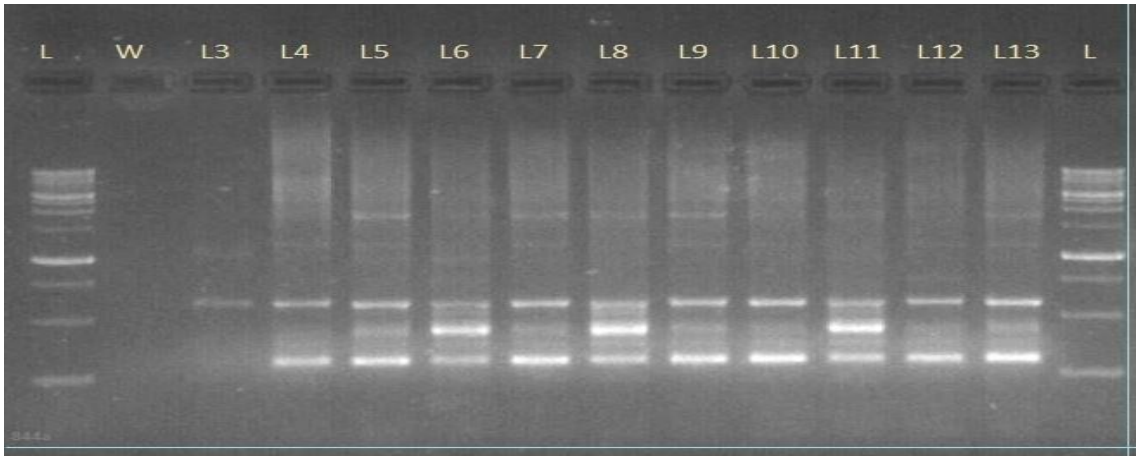
Figure(14) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1 of TDZ and 25, 50, and 100) mg. L^{-1} of PG in Bioreactors system using ISSR HB9 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)



Figure(15) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with (0.5 and 1) mg . L^{-1} concentrationsof TDZ and (25, 50 and 100) mg. L^{-1} of PG in Solid system using ISSR HB9 marker (L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-1-3: ISSR primer 844a:

Figure(16,17) showed the number of amplified bands that were produced by the marker 844 for the treatments in a liquid and solid medium, which reached (134) Bands as it gave amplified BANDS in the liquid medium (62) while it reached (72) in solid medium. The initiator ISSR-844 gave their molecular weights ranged between (0.900-0.235). Marker 844a showed the amplified bands number in the solid media and liquid medium were reached(6) bsnds. It is noticed that the genetic match-rates of the parameters in the solid and liquid medium did not show genetic variations at the genetic level.



Figure((16) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1 of TDZ and 25, 50 and 100) mg. L^{-1} of PG in Bioreactors system using ISSR 844a marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)

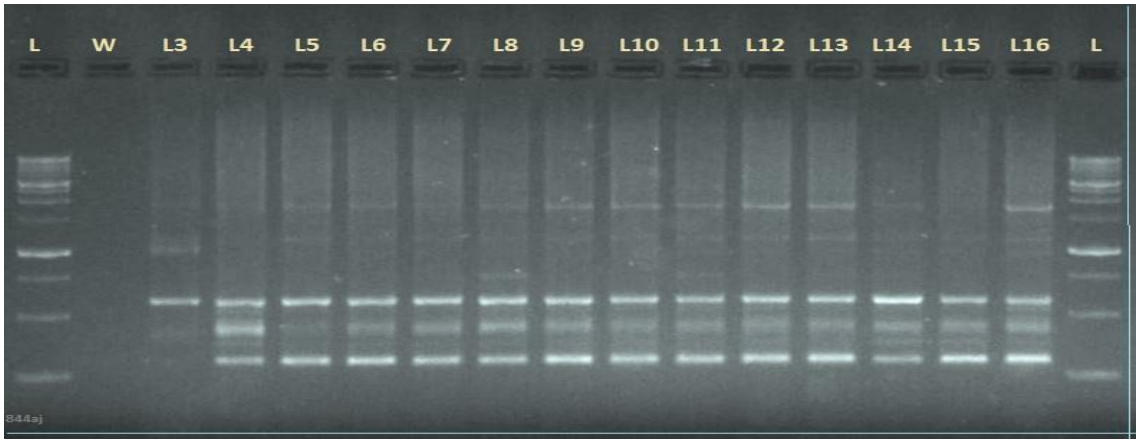


Figure (17) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) mg. L^{-1} of PG in Solid system using ISSR 844a marker (L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100).

4-5-1-4: ISSR primer HB10:

The figure (18 and 19) showed that the marker HB10 gave total number of multiplication band reached (134) bundles in both

media, where it reached (62) bundles in the liquid medium and reached (72) in the solid middle, as the treatments in the liquid and solid mediums were represented by packages of same molecular weights of (0.555-0.302) a base pair. The genetic match-rates of the liquid and solid medium parameters did not show genetic variations at the genetic level.

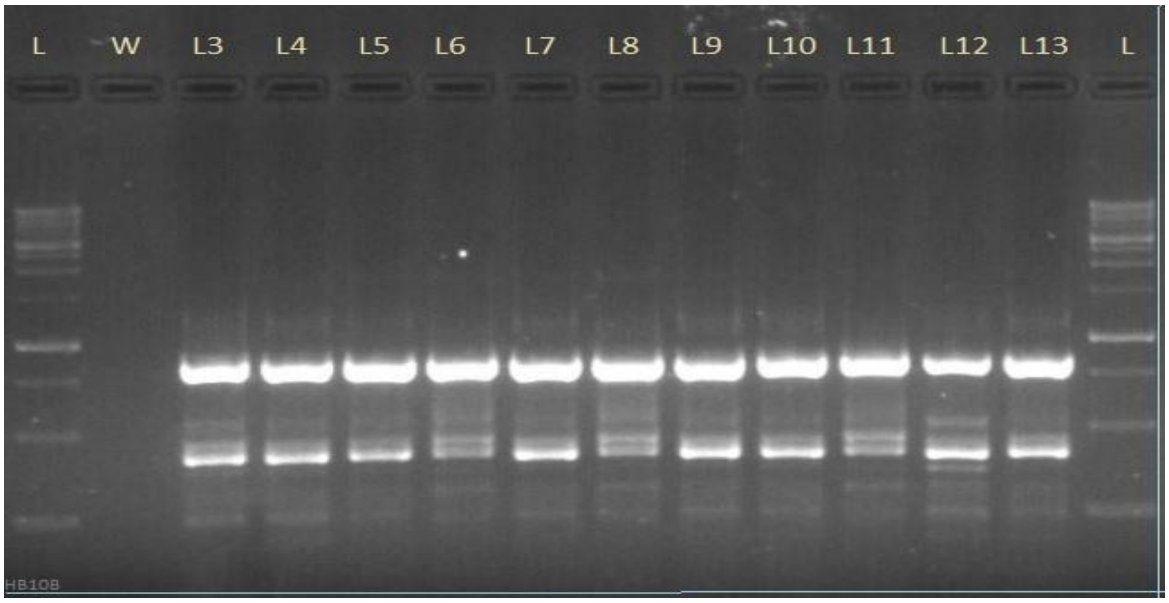


Figure (18) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1)of TDZ and (25, 50 and 100) mg. L^{-1} of PG in Bioreactors system using ISSR HB10 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)

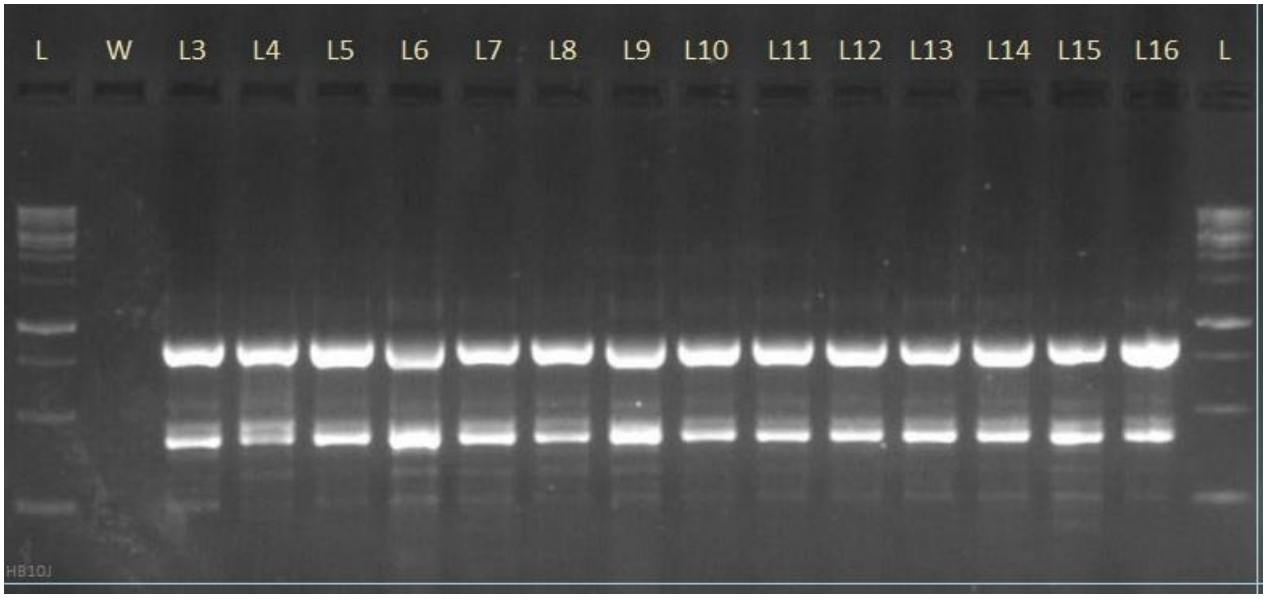
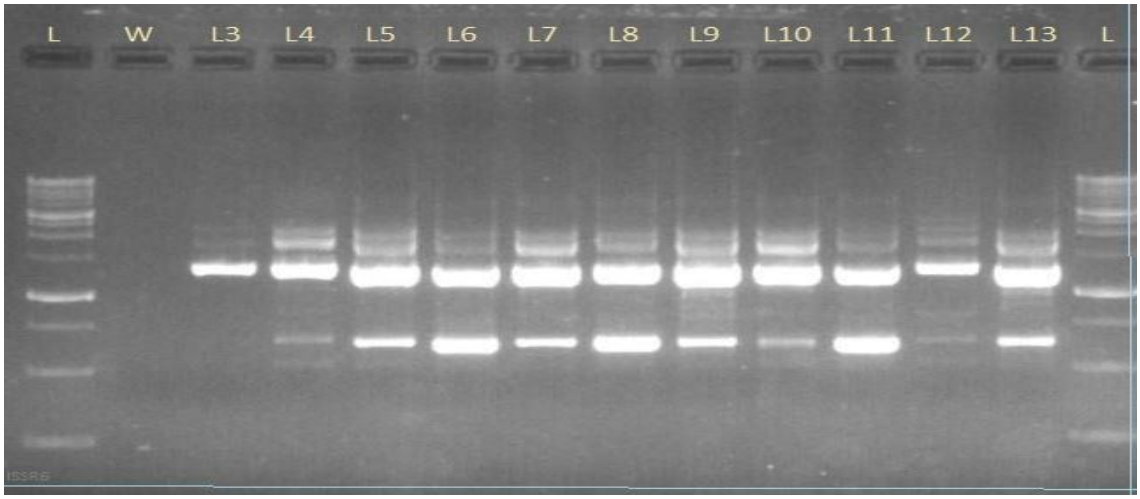


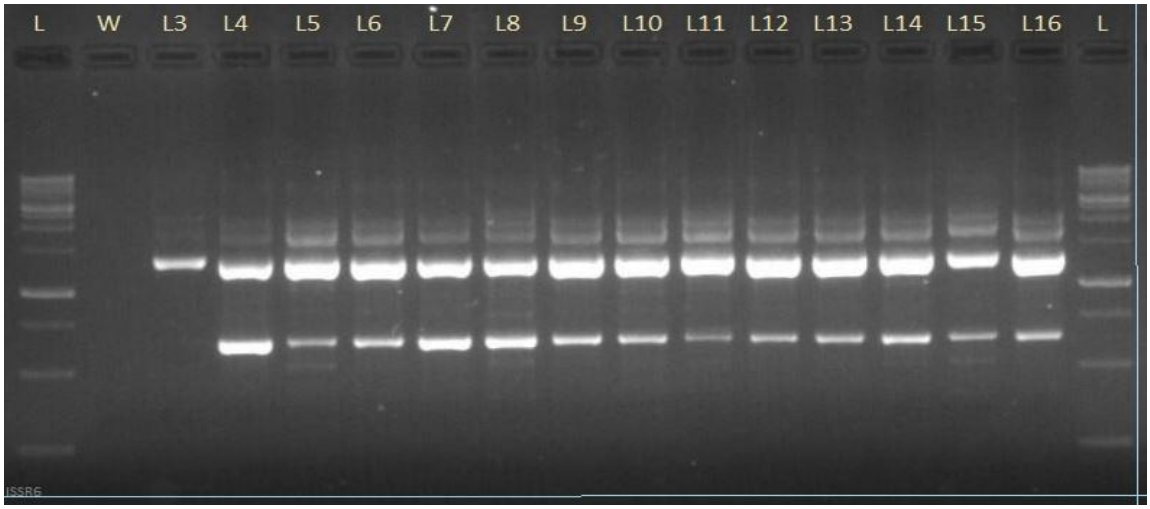
Figure (19) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} **of TDZ and (25, 50 and 100)** mg. L^{-1} **of PG in Solid system using ISSR 844a marker (L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100).**

4-5-1-5: ISSR primer ISSR6:

The figure (20 and 21) showed that the marker ISSR6 has given a total number of Bands (106) Bands in both mediums, where it reached (53) Bands in the liquid medium and reached in the solid medium (53) Bands, as all the treatment in the liquid and solid medium were the highest number of bundles which reached 5 bands for each of them where the amplification values ranged between (0.643-0.217) base pair, the genetic match ratios of the parameters in the liquid and solid medium did not show genetic variations at the genetic level described in the picture



Figure(20) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) of PG in Bioreactors system using ISSR6 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)



Figure(21) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} **of TDZ and (25, 50 and 100) mg. L^{-1} of PG in Solid system using ISSR 6 marker (L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100).**

The results showed a great genetic matching in all the markers used, which makes the use of ISSR markers a powerful tool in detecting the resulting genetic variance. Moreover, its accuracy in finding the genetic match. All markers have recorded a high genetic match. However, some bands were seemed unclear, causing some error in showing genetic matching, this may be due to the effect of the treatments used in increasing some chemical compounds within the plant tissue such as polysaccharides and phenols which it affected the process of DNA extraction in the plant tissue and weakens the process of DNA multiplication.(Khierakllah, H. and *et al*. (2014))

4-5-2- RAPD DNA (Random amplified polymorphic DNA) Technique

4-5-2-1-The primer OPA02

It is evident from the figure (22,23) that the primer Opa02 gave a total number of bands (136) Bands in both mediums (solid and liquid), where it reached (66) bands in the liquid medium and reached (70) bundles in the medium. There was not noted in the picture any morphism bands. The amplification values ranged between (0.588-0.287) base pairs. It was noted from the picture(18) that the genetic match of the liquid medium's parameters bands did not include variations with the solid medium.

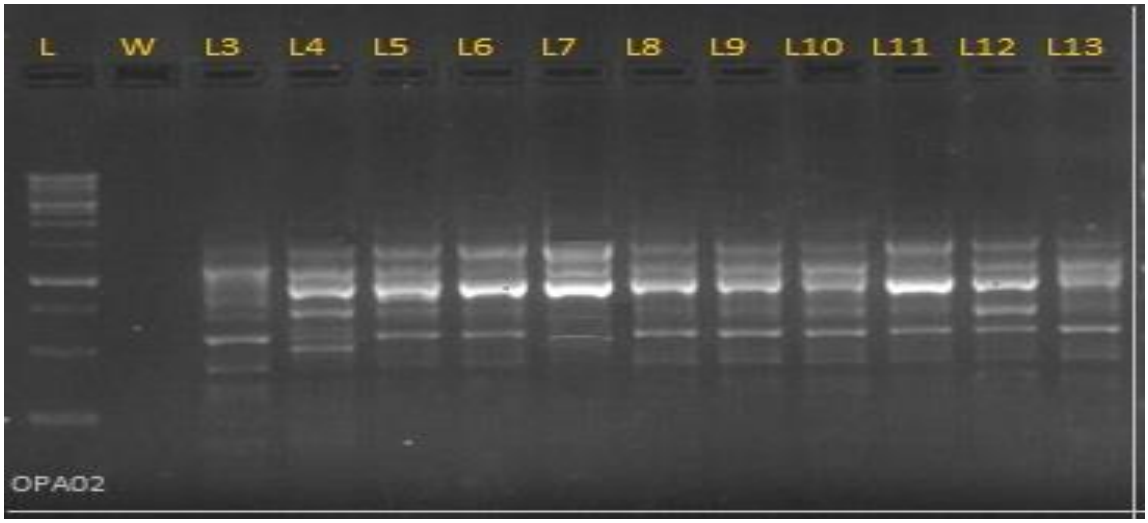


Figure (22) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) of PG in Bioreactors system using RAPD OPA02 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50

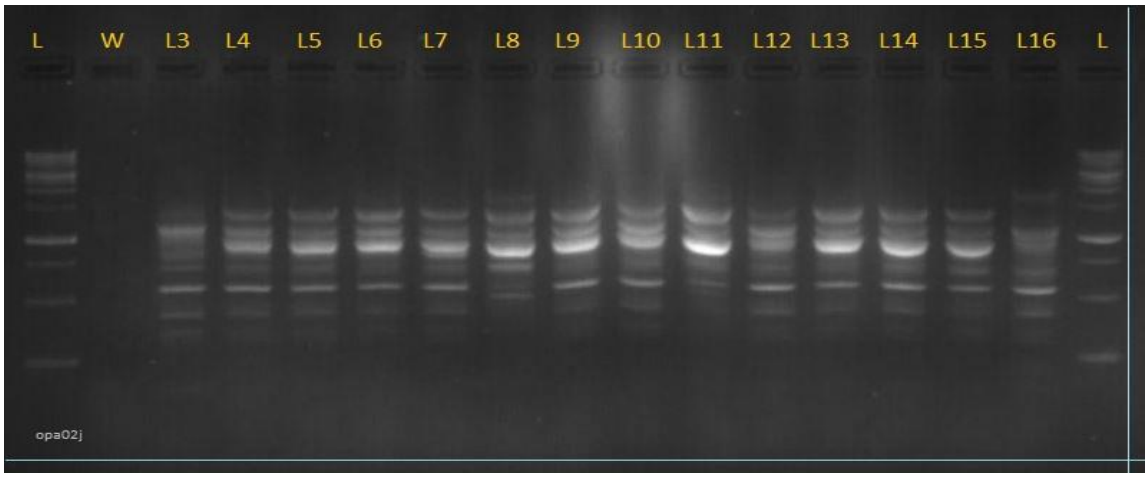


Figure (23) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) of PG in Solid system using RAPD OPA02(L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-2-2- The primer OPA12

The figure (24,25) showed that the primer Opa12 gave a total number of bands (166) bands in both mediums (liquid and solid), when it reached (85) bundles in the liquid medium and reached (81) bundles in the solid medium, all treatment give the same number of bands, the bands represented in the liquid medium (exception of the callus treatment) with the highest number of bundles, which reached 8 bands for each of treatments, when the amplification values ranged between (0.546-0.141) a base pair, while the lowest number of Bands was recorded when the callus treatment reached (7) bundles with an amplification force of (0.472-0.143) as a base pair. It is noticed from figure(20, 21) that the liquid medium genetic match did not show genetic variations with the solid medium.

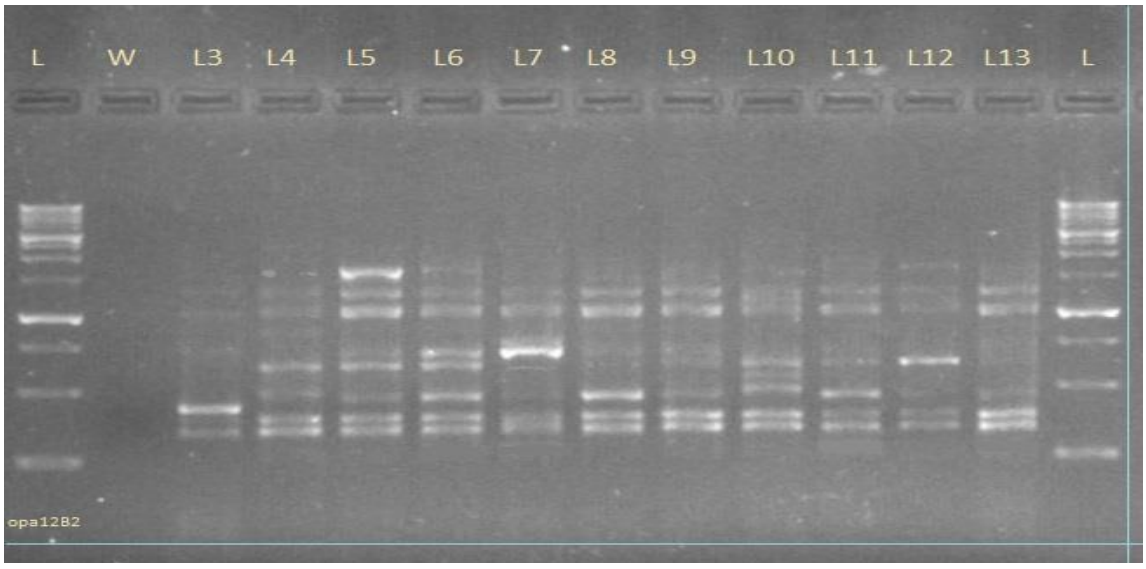


Figure (24) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L-1 of TDZ and (25, 50 and 100) of PG in Bioreactors system using RAPD OPA12 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50

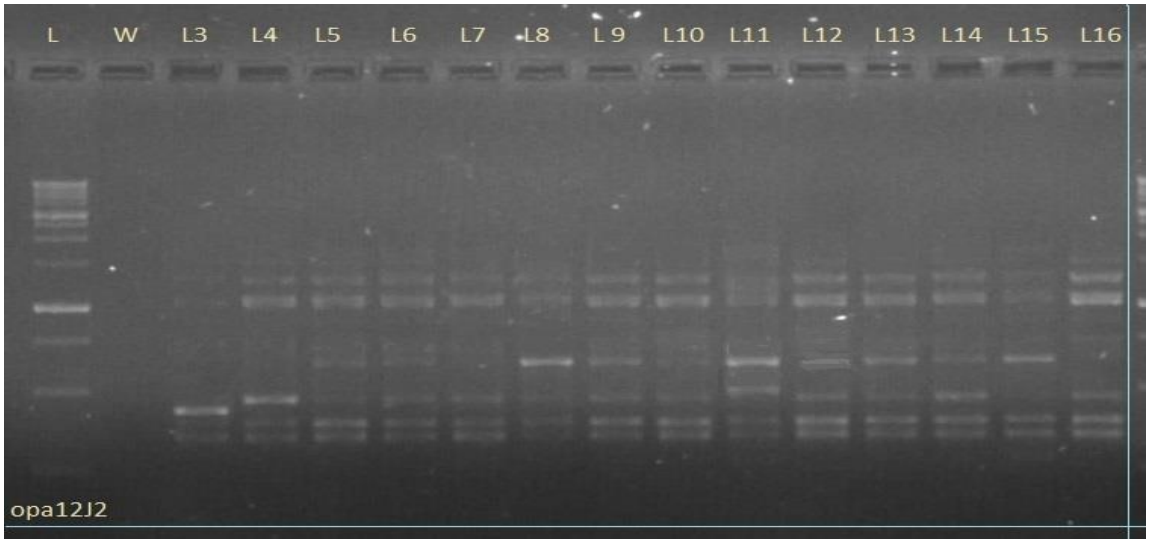


Figure (25) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L^{-1} of TDZ and (25, 50 and 100) of PG in Solid system using RAPD OPA12(L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-2-3- The primer opb07

The results from the figure (26, 27) showed that the primer Opb07 gave the number of clear Bands reached (161) Bands in both mediums (solid and liquid), the number of bands in the liquid medium was (77) while the number of bands in the solid medium was (84) bands, this primer did not record any polymorphism of the liquid medium (bioreactors), all the bands were identical, which amounted to 7 bands values ranged between (0.732-0.328) base pair, it was observed from figure (22, 23) show a positively the genetic match between the treatments.

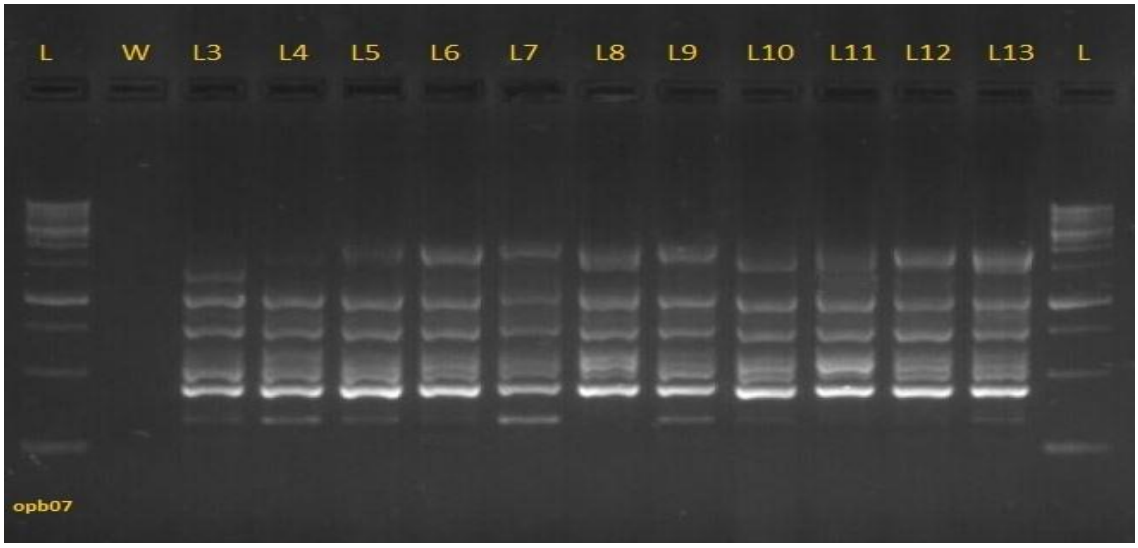


Figure (26) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L-1 of TDZ and (25, 50 and 100) of PG in Bioreactors system using RAPD OPA07 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)

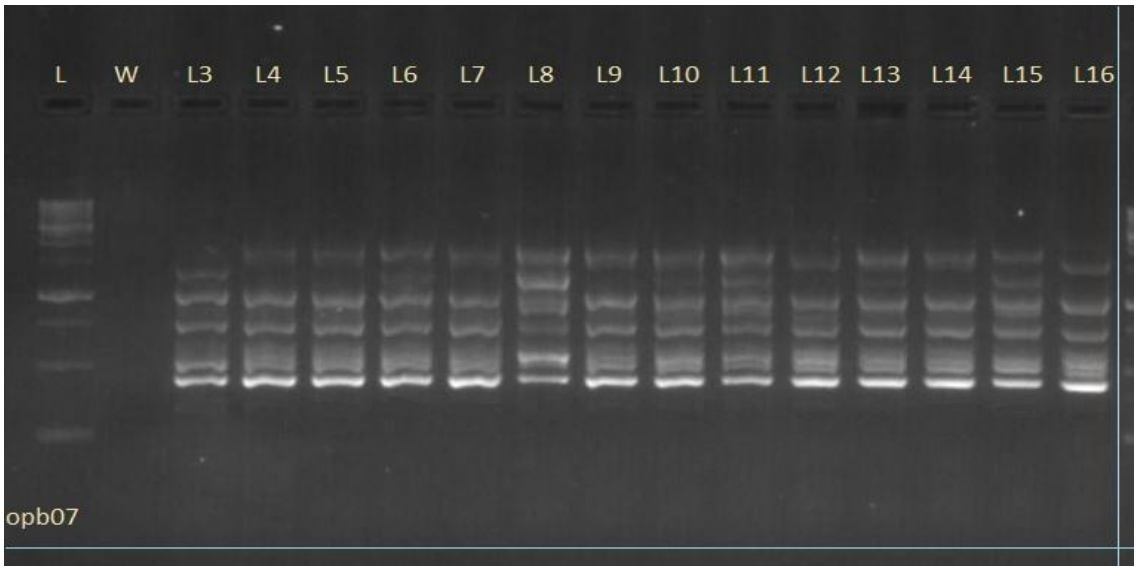


Figure (27) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L-1 of TDZ and (25, 50 and 100) of PG in Solid system using RAPD OPA07(L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-2-4- The primer opd10

The Figure (28, 29) showed that the primer Opd10 recorded the lowest number of bands than the other primers. It gave(124) Bands in both mediums (solid and liquid). The coefficients bands in the liquid and solid medium did not give a polymorphisms bands, All the bands were given monomorphism characteristics, and the amplification values ranged between (0.656-0.250) base pair; it was observed from the picture(24, 25) that the genetic marching was a highest between all treatments

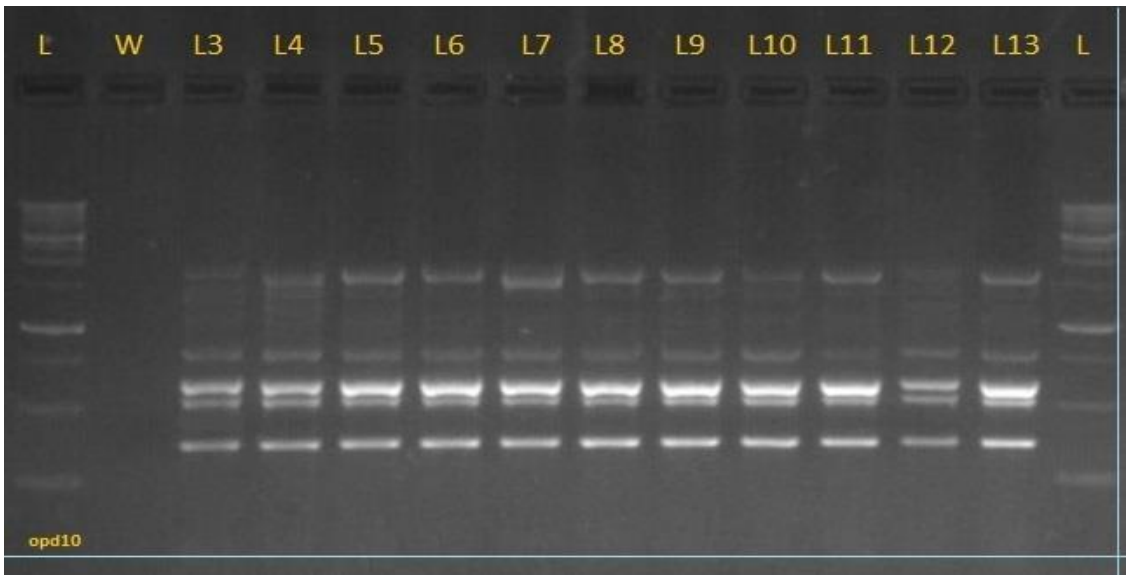


Figure (28) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with concentrations of (0.5 and 1) mg. L-1 of TDZ and (25, 50 and 100) of PG in Bioreactors system using RAPD OPD10 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50).

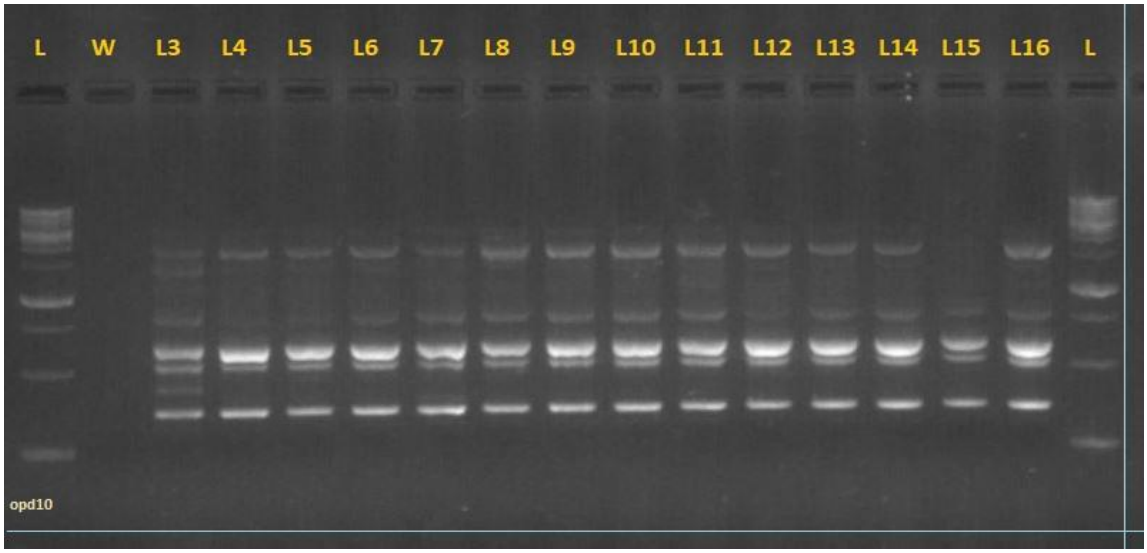


Figure (29) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with (0.5 and 1) mg concentrations. L-1 of TDZ and (25, 50 and 100) of PG in Solid system using RAPD OPD10(L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-2-5 Primer OpO07

The figure (30 , 31) showed there were a (186) bands protected from this primers in liquid and solid medium at all treatment, the primer in the liquid medium product (88) bands put the primer in a solid medium gave more than liquid medium reached (98) bands. The molecular weight of these bands ranges between (0.276-0.778), there were no polymorphisms bands were noted from these treatments. Moreover, there were noted genetically marching in all treatment in both media.

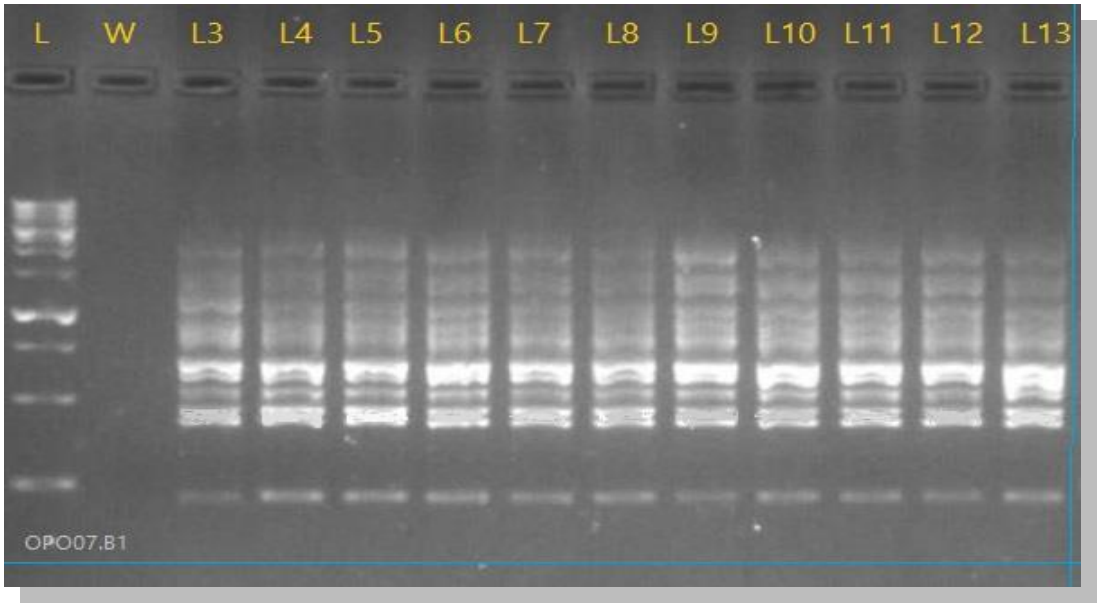


Figure (30) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with (0.5 and 1) mg concentrations. L^{-1} of TDZ and (25, 50 and 100) of PG in Bioreactors system using RAPD OPO07 marker (L: ladder, W:water, L3:callus, L4:control, L5:0.5, L6:1, L7:25, L8:50, L9:100, L10:0.5+25, L11:0.5+50, L12:1+25, L13:1+50)

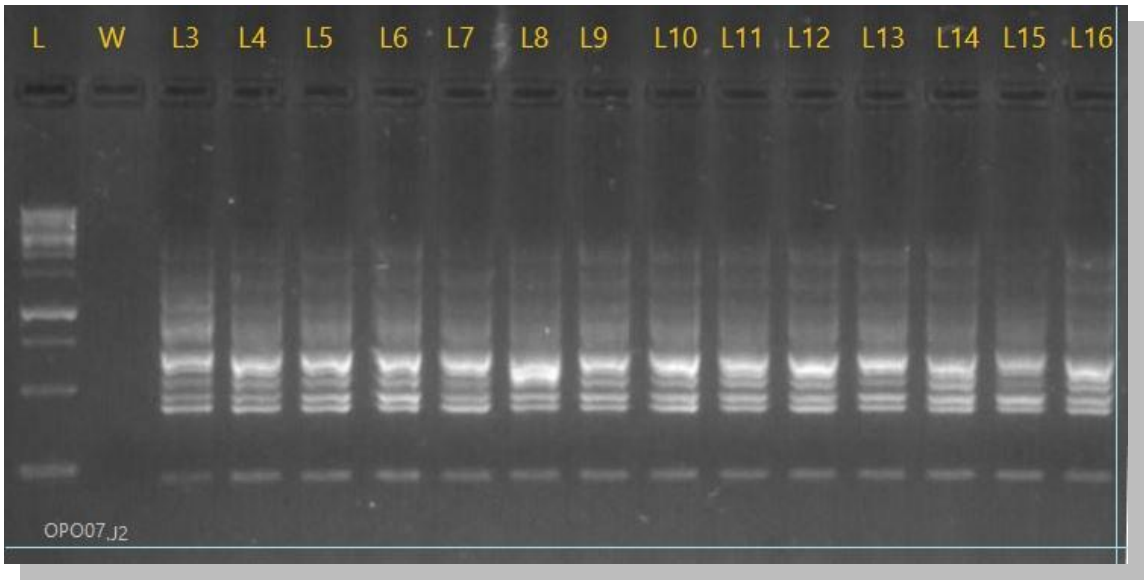


Figure (31) Genetic matching of DNA extracted from tissue culture samples of Barhi date palm treated with (0.5 and 1) mg concentrations. L^{-1} of TDZ and (25, 50 and 100) of PG in Solid system using RAPD OPO07(L: ladder, W: water, L3: Berhi leaf, L4: callus, L5:con., L6:0.5, L7:1, L8:25, L9:50, L10:100, L11:0.5+25, L12:0.5+50, L13:0.5+100, L14:1+25, L15:1+50, L16:1+100)

4-5-3- Transfer of a *SeNHX1* salt tolerance gene to a Date palm callus.

The *SeNHX1* gene was transferred from Saliconia (*Salicornia europaea*) Figure (32) and Index figure(5,6), Salicornia is a genus of succulent, halophyte (salt tolerant) flowering plants in the family Amaranthaceae that grow in salt marshes, it is a genus of saline succulents that tolerate high salinity to own it salt-resistant genes, the *SeNHX1* gene is one of the plant's most important defenses against salt stresses by ion exchange Na + / H +, and by overexpressing Na + / H + exchangers, as it was transferred to the date palm tissue by using a type of agrobacteria via the plasmid pCAMBIA3301 to achieve an increase in the plant's response to salinity resistance, and then study the effect of the gene expression process on the genetic traits and the plant's tolerance to high salt concentrations.

The gene, *SeNHX1*, was transferred to the date palm callus of the Barhi cultivar. Its presence was verified by amplifying the gene with its primer. The *SeNHX1* gene was amplified by using PCR with a template for the plasmid containing the gene where the bands appeared equal to the template. The Figure (33) shows the success of the gene transfer in samples a, b and e.

After making sure that the gene was transferred to the callus tissue, the callus containing the gene was transferred to a medium containing NaCl at a 200 mM concentration. The success of the transfer process was monitored using the two techniques, the first

technique, visual monitoring of the growth of the callus and its non-death, there was weak growth or weight loss and compared with the control samples (Figure 36), the second technique is to monitor the gene expression of the *SeNHX1*, after performing a dnase-DNA removal process (Figure 34) this step to ensure there wasn't interaction error with cDNA steps. The RNA was extracted then creating cDNA. After the cDNA process, the products were transferred to agarose gel where the band expressing the gene appeared in the Figure (35) shows tolerance to high salinity levels compared to plants that have not been treated, this is the process to proofing to gene expression success. These results agreed with (Yang X *et. al.,* 2011; Liu *et al.,*2011; Wu *et al.,* 2015).

Salicornia europaea

Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Order:	Caryophyllales
Family:	Amaranthaceae
Subfamily:	Salicornioideae
Genus:	***Salicornia***

Figure (32) Saliconia (*Salicornia europaea*) is a genus of succulent, halophyte (salt tolerant) flowering plants in the family Amaranthaceae

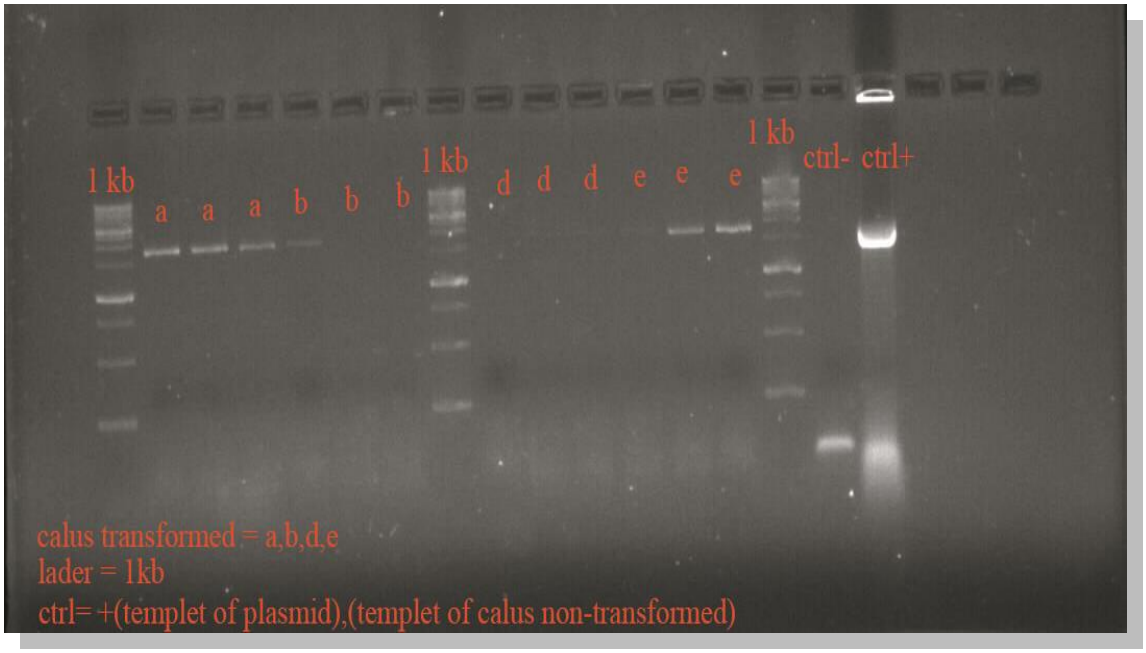


Figure (33) Transfer of a *SeNHX1* salt tolerance gene to a Date palm callus. the success of the gene transfer in samples a, b and e, L: lader, + Cat

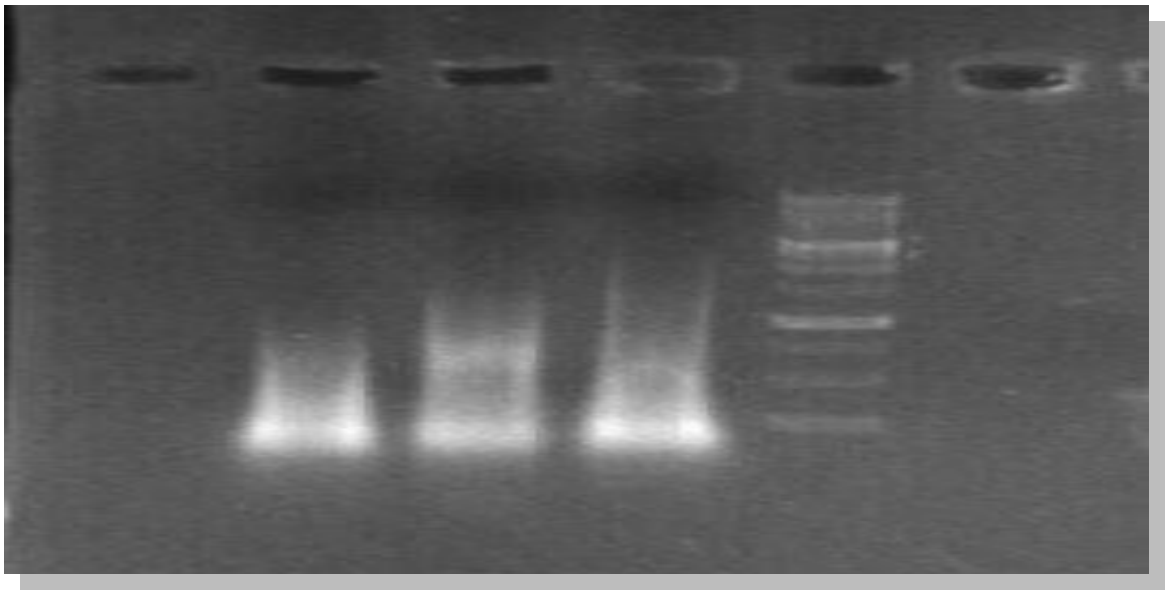


Figure (34) The appearance test for the *SeNHX1* gene after removing the DNA with DNase

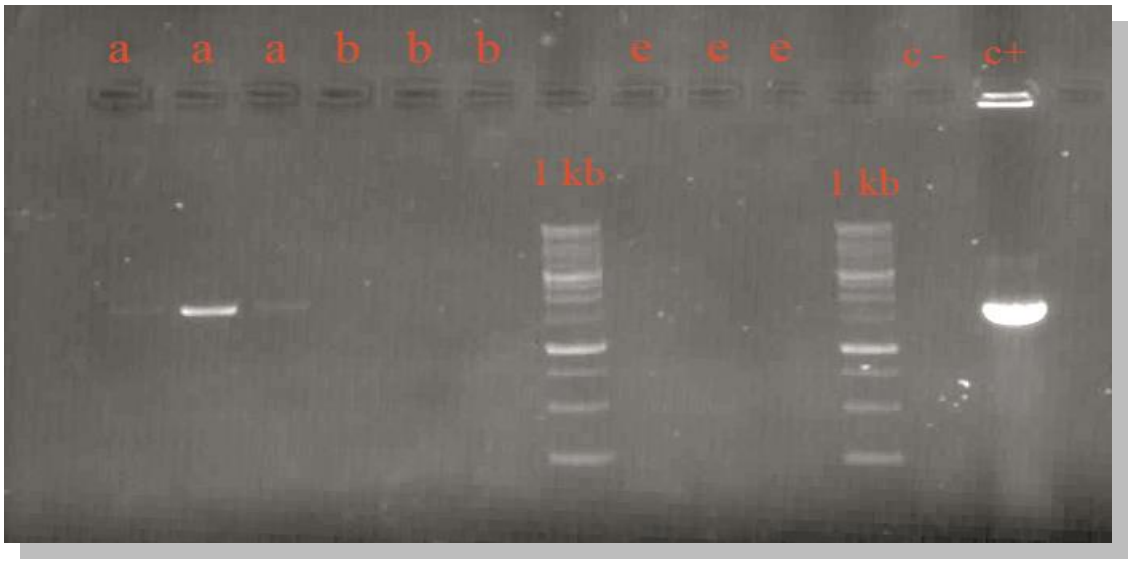


Figure (35) Gene expression of *SeNHX1* salinity stress gene by cDNA technique, a, b and c: sample; -c sample negative; +c: sample positive; L: lader

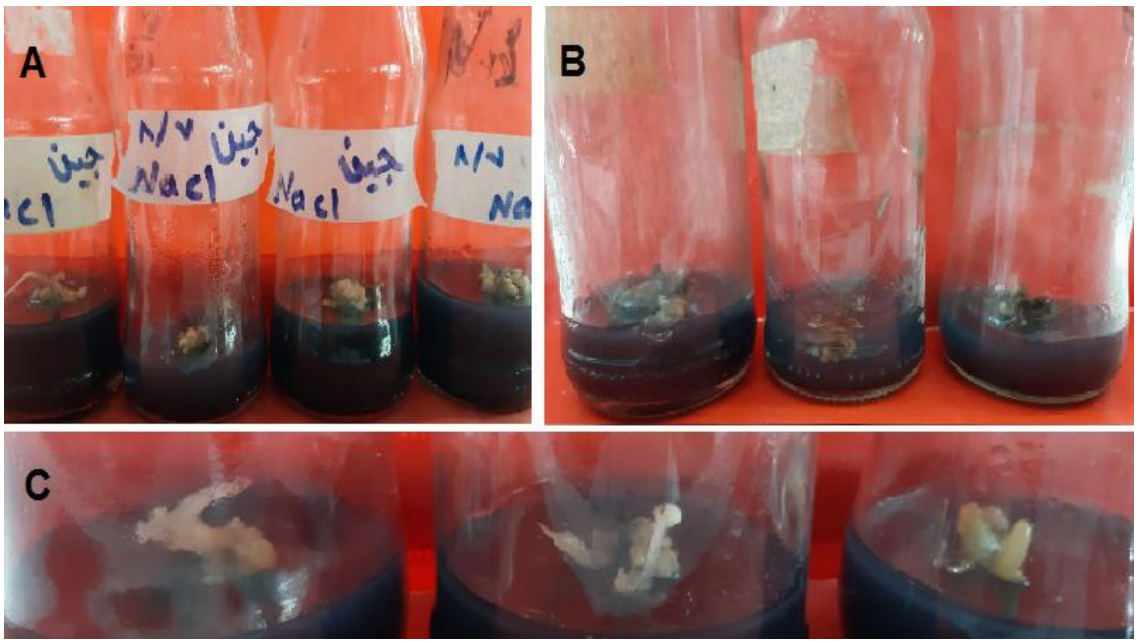


Figure **(36) transgenic callus of date palm by *SeNHX1* salinity stress gene, growing on solid medium with 200mM NaCl. A and C: Callus success for the gene transfer process, B: control**

5- Conclusion and Recommendation

5-1: Conclusions:

1- The explant grown in the liquid medium using the Bioreactors system was superior in the physical, chemical, physiological, and endogenous hormone content of the plant tissue. It gave positive indicators better than the explant grown in the solid medium.

2- The TDZ was a significant effect in most of the physical and chemical characteristics by increasing the concentration, and the concentration at 50 mg. L^{-1} was recorded best values .

3-The PG was a significant effect in most physiological and chemical traits, and the best concentrations were 0.5 mg. L^{-1} .

4- There is a variance between the concentrations of (PG, TDZ) in all the growth stages at the physical, chemical, and physiological characteristics, as the treatment gave 50 mg. L^{-1} PG with 0.5 mg. L^{-1} TDZ in both medium, the best indicator for the studied characteristics.

5- The triple interactions gave the highest mean when the treatment was 50 mg-l-1 of PG with 0.5 mg-l1of TDZ in the bioreactor system.

6- Molecular study using ISSR technology using the primers(814, 844a, HB9, HB10, ISSR6) showed no genetic variation between the treatments.

7- The molecular study using RAPD technology and using the primers(814, 844a, HB9, HB10, ISSR6) showed no genetic variation between the treatments.

8- Successful gene transfer of the gene (*SeHNXI*) using the plasmid (pCAMBIA3301)

9- Growth of the plant tissue (callus) to which the salt-tolerant gene was transferred when cultivated in a nutrient medium added to 200 mM NaCl

5-2- Recommendations:

1- Using the bioreactor to obtain a large number of multiple shoots.

2- Using other concentrations of PG and TDZ to obtain the best result

3- Using new compounds or growth regulators with bioreactors or any other system to obtain the using explant date palm production

4- Using other techniques to detect genetic match or difference.

5- Since the transfer genes experience into date palm tissue is a recent experiment, we recommend that you delve into this study and conduct a special and detailed study on the gene transfer to callus tissue of date palm and other economi plant

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Index

Figure (1) Bioreactor with a control system

Figure(2) Mass propagation in a bioreactor system

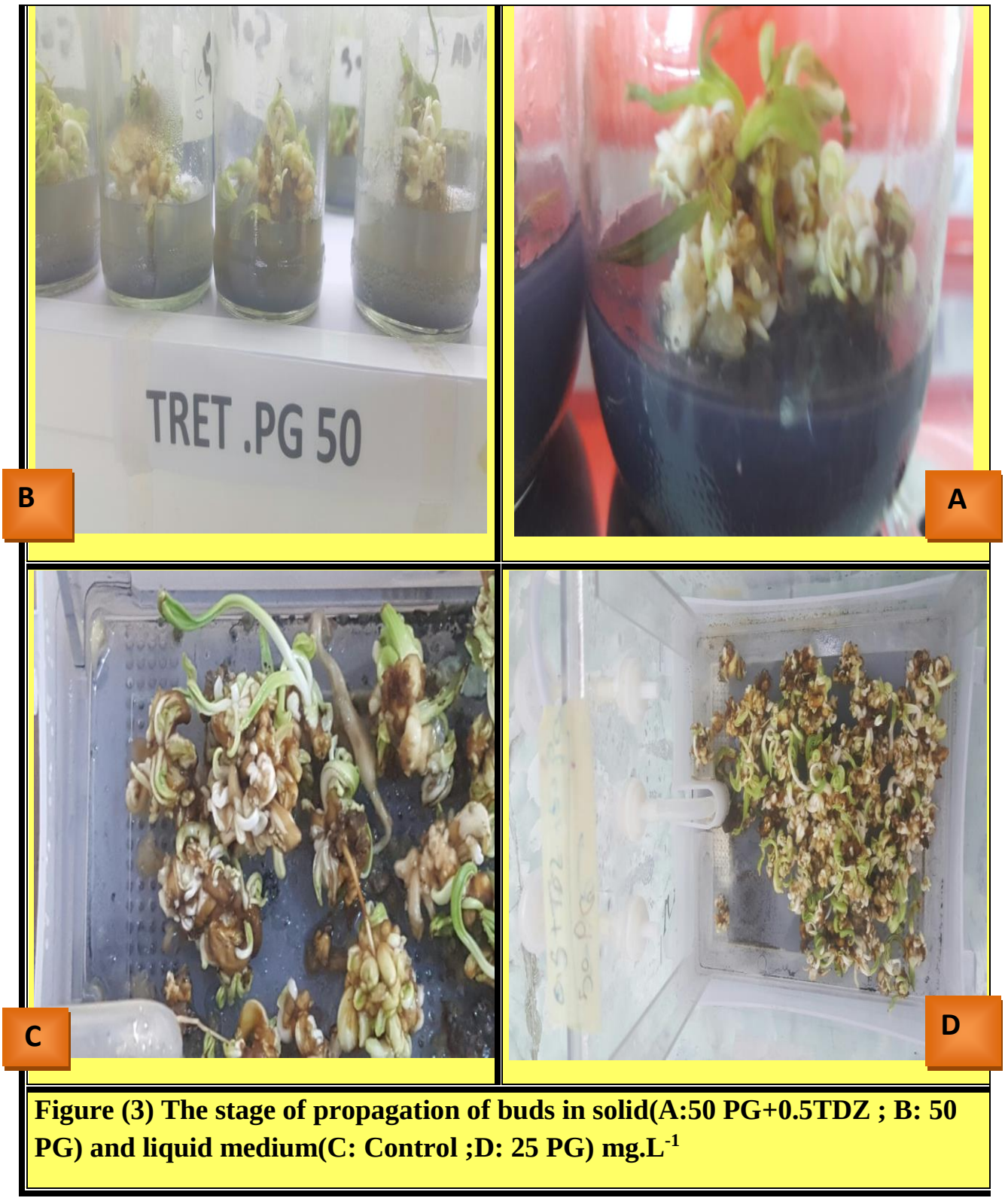


Figure (3) The stage of propagation of buds in solid(A:50 PG+0.5TDZ ; B: 50 PG) and liquid medium(C: Control ;D: 25 PG) mg.L^{-1}

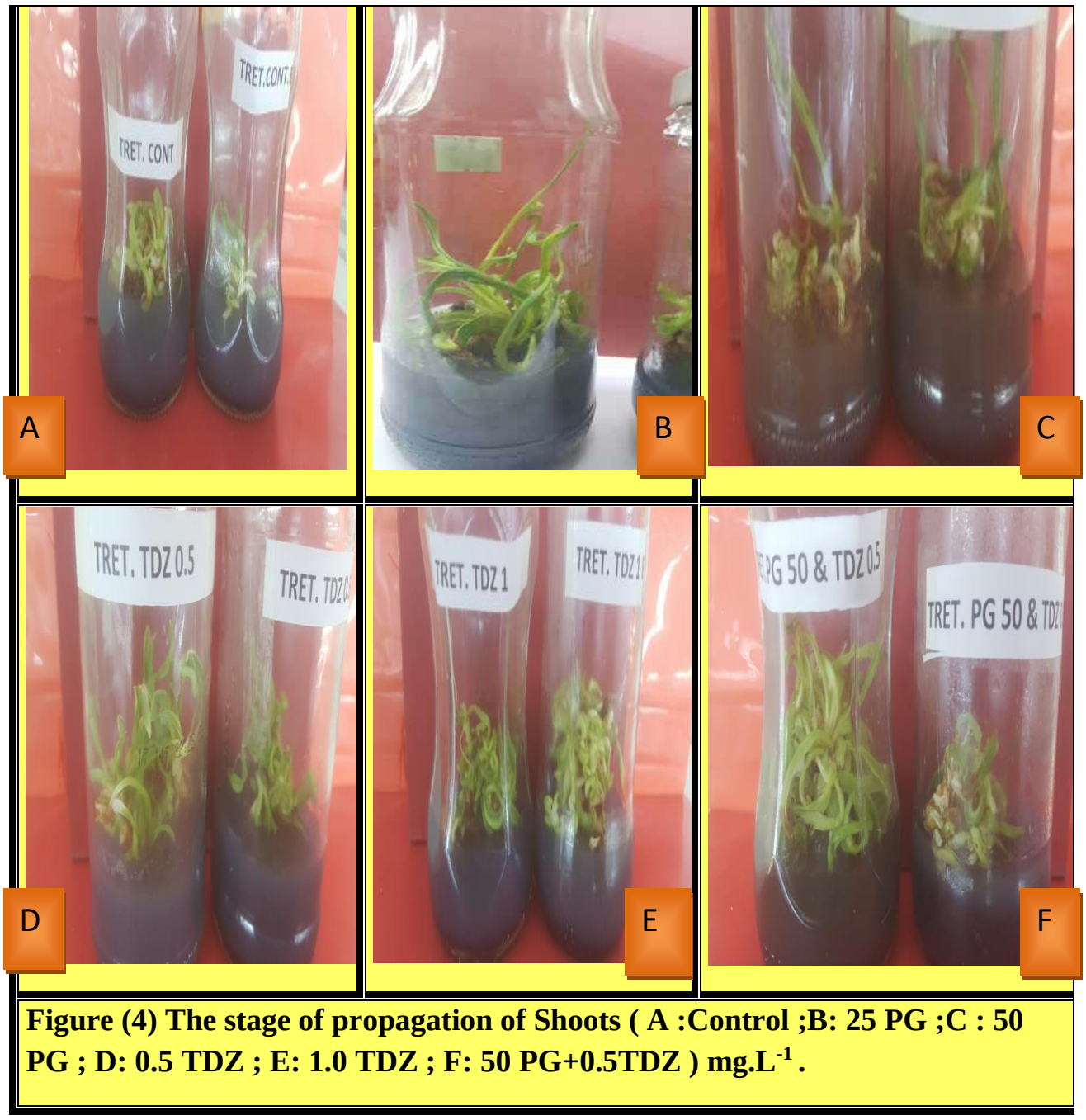
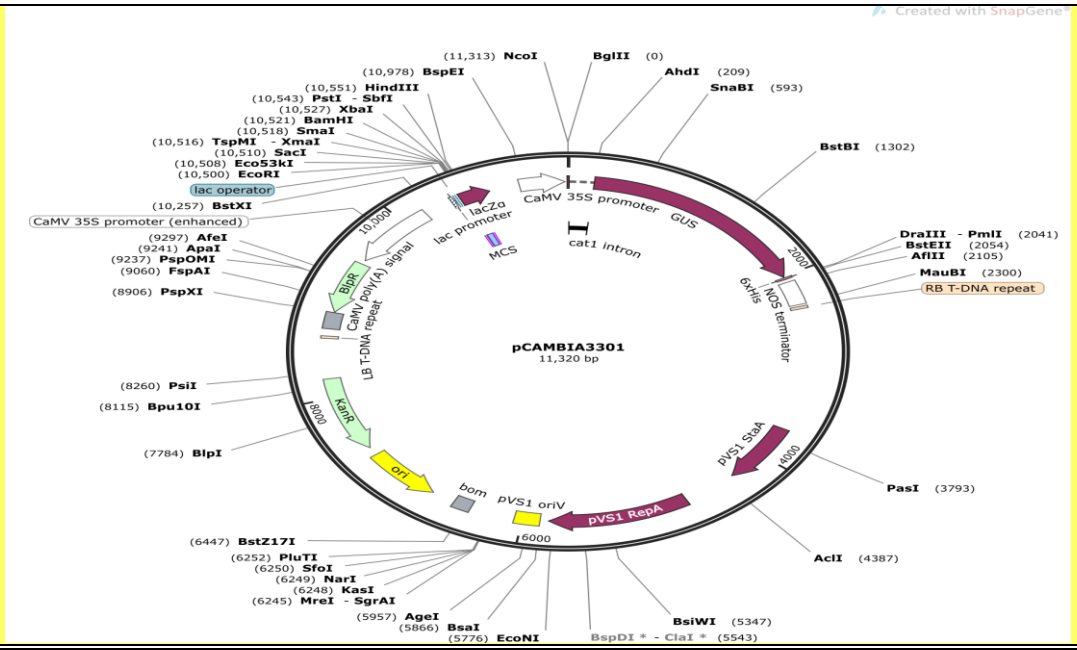


Figure (4) The stage of propagation of Shoots (A :Control ;B: 25 PG ;C : 50 PG ; D: 0.5 TDZ ; E: 1.0 TDZ ; F: 50 PG+0.5TDZ) $mg.L^{-1}$.

Figure(5) Shoot Elongation on sold medium ((A)Control (B)0.5 TDZ , (C)50PG ,(D)50+O.5)mg.L^{-1}



Saccharomyces cerevisiae S288C chromosome IV, complete sequence

NCBI Reference Sequence: NC_001136.10

GenBank Graphics

>NC_001136.10:1367485-1369386 Saccharomyces cerevisiae S288C chromosome IV, complete sequence

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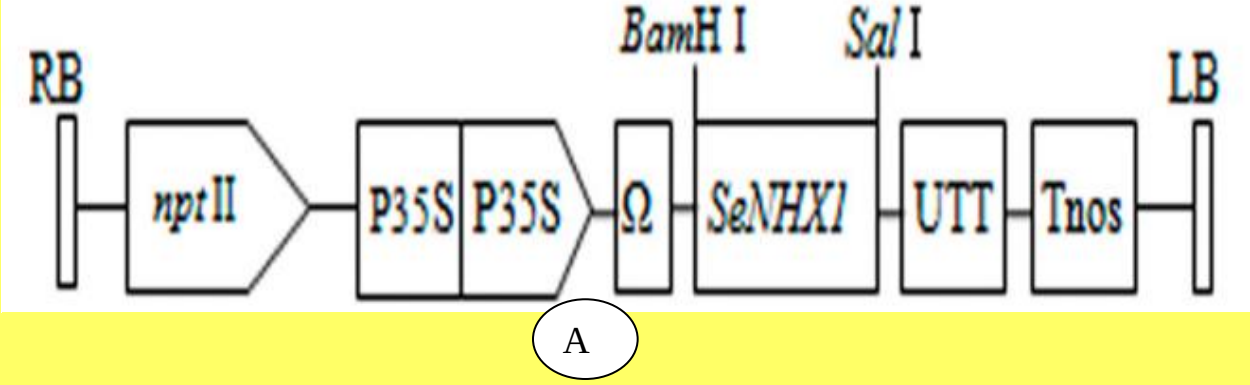
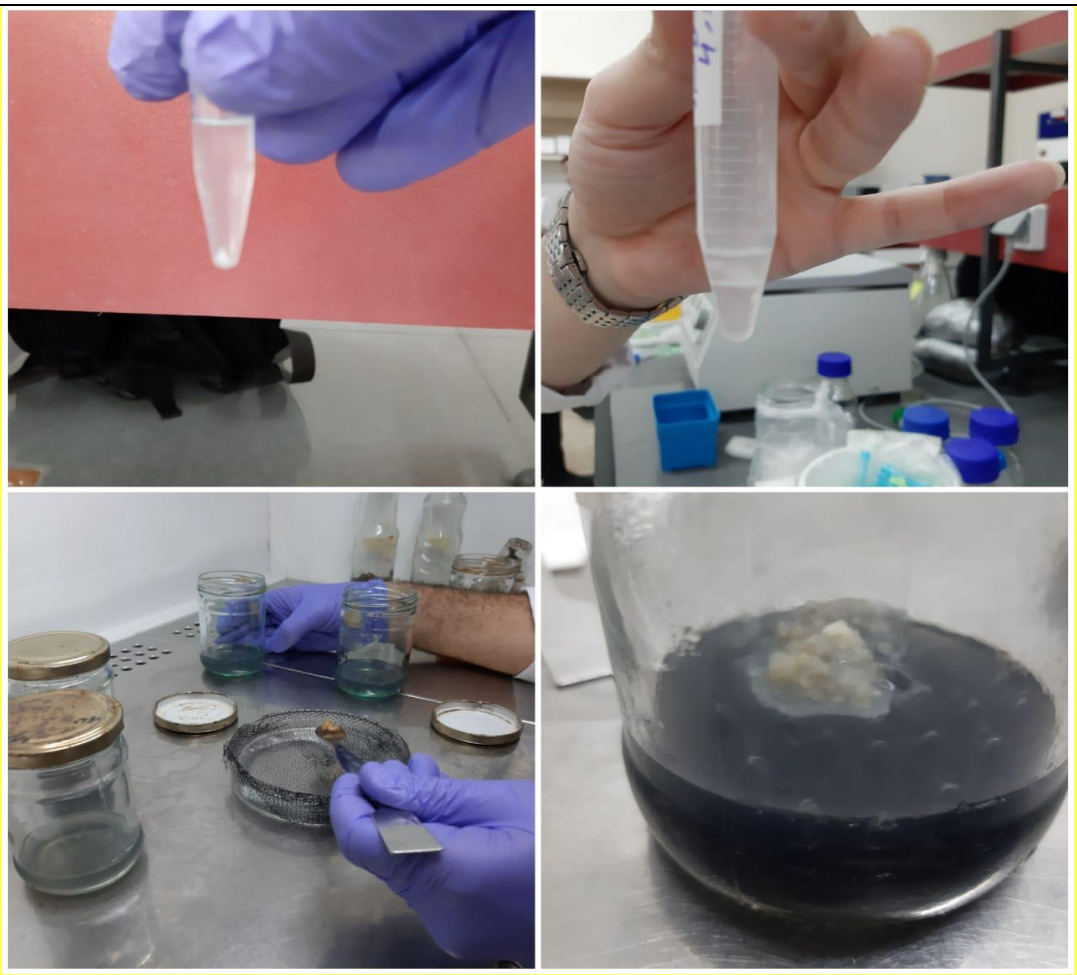
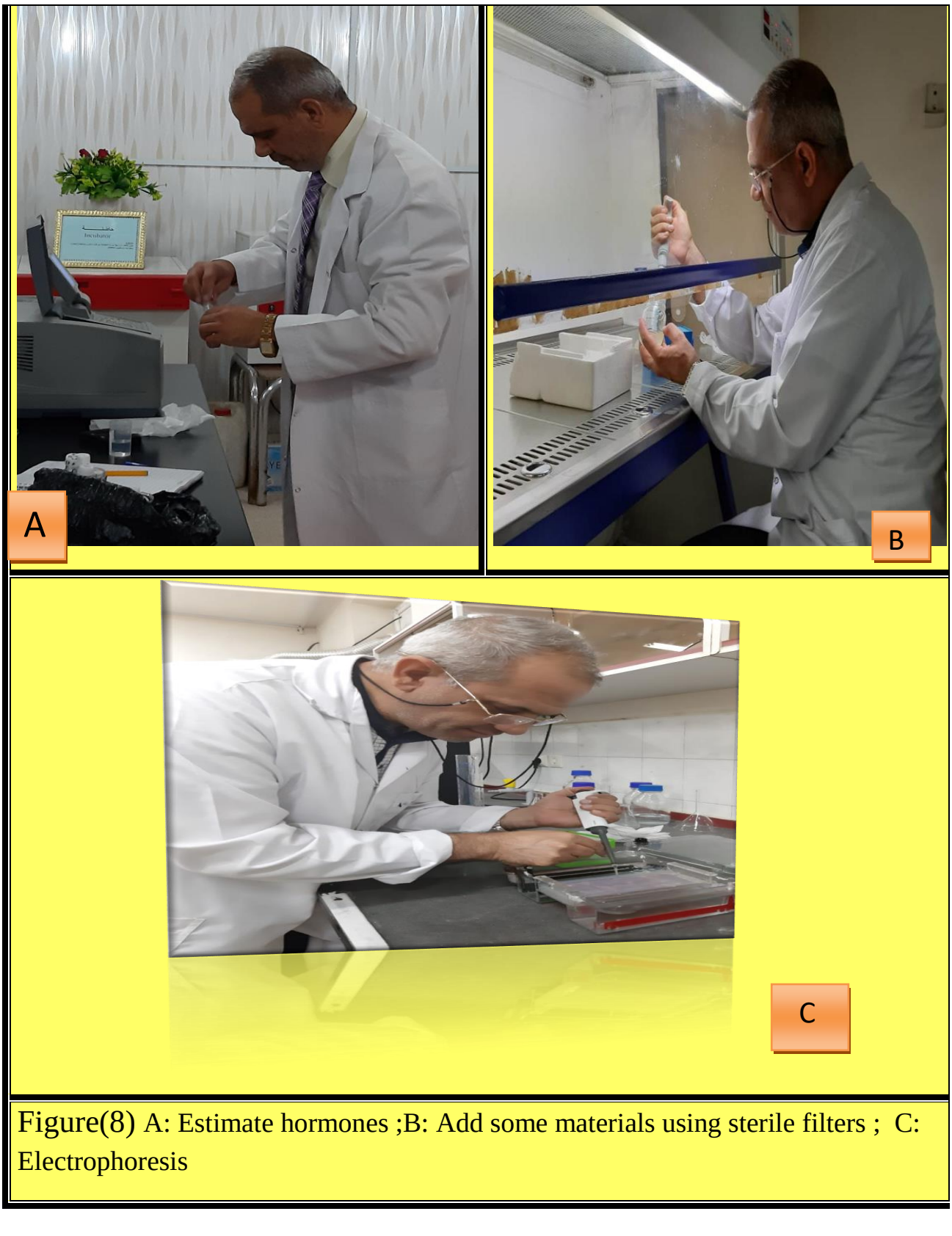
Figure(6) A: Plasmid pCAMBIA3301 B:Sequance SeHNXI genes Mass propagation in a bioreactor system



A



Figure(7) A: Schematic diagram of the vector region of SeNHX1, P35S, cauliflower mosaic virus 35S promoter B: steps of gene transfer



Figure(8) A: Estimate hormones ;B: Add some materials using sterile filters ; C: Electrophoresis

الخلاصة :

نفذت التجربة في مركز ابحاث النخيل التابع الى جامعة البصرة خلال المدة من كانون الاول 2017 الى اب 2019 م .حيث ان نجاح زراعة أنسجة نخيل التمر ذات التطابق الجيني العالي يمثل تحديًا يسعى إليه الباحثون. لذلك ، هدفت التجربة إلى التحقق من فعالية TDZ بتركيز (0 و0.5 و1.0)ملغم .لتر$^{-1}$ وPG بتركيز (0 و25 و 50) ملغم .لتر$^{-1}$ والحالة الفيزيائية للوسط في التكاثر الدقيق لنخيل التمر *Phoenix dactylifera* L. في نظام المفاعل الحيوي ، وإثبات نجاحها في الكشف الجيني والتقنية المعدلة وراثيًا عن طريق نقل الجين المقاوم للملح *SeNHX1* إلى الكالس ، وظهرت نتائج الدراسة ما يلي:

1- تفوق نظام الوسط السائل (المفاعل الحيوي) في وزن الكالس الطازج والبراعم والنمو الخضري ، ومتوسط عدد البراعم والنموات الخضرية ، ومحتوى الكالس والبراعم من الأوكسينات ، و الجبريلينات ، و السايتوكاينين ، وكذلك المحتوى والنسبة المئوية. من النيتروجين والفوسفور والمغنيسيوم والمنغنيز والكربوهيدرات والبوتاسيوم في معظم مراحل النمو في حين تفوق الوسط الصلب في محتوى النسيج النباتي من حامض الابسيسك .

2- اثرت اضافة TDZ وPG للوسط الغذائي كل على حده في معظم المؤشرات الفيزيائية والكيميائية والفسيولوجية اذ ادت اضافة TDZ بتركيز 0.5 ملغم .لتر$^{-1}$ والمعاملة 50 ملغم .لتر$^{-1}$ PG الى زيادة معدل وزن الكالس والبراعم والنموات الخضرية وعدد البراعم والنموات الخضرية وكذلك تفوقت في محتوى الكالس والبراعم والنموات الخضرية من الأوكسينات والسايتوكاينينات والجبرلينات كما

تفوقت في نسبة النتروجين والفسفور والبوتاسيوم والمغنسيوم والمنغنيز وكذلك في محتوى البراعم والنموات الخضرية من الكربوهيدرات. في حين تفوقت المعاملة القياسية على جميع تراكيز الـ TDZ وPG في محتوى الكالس والبراعم والنموات الخضرية من حامض الأبسسيك كما تفوقت في نسبة الاسمرار في مرحلة الكالس .

3- كما كان لتداخل الوسط مع الـ TDZ تأثير معنويا اذ اعطت المعاملة TDZ بتركيز 0.5 ملغم .لتر$^{-1}$ مع الوسط السائل زيادة معنوية في اغلب الصفات قيد الدراسة. ومن ناحية اخرى كان تفوقت المعاملة 50 ملغم .لتر$^{-1}$ PG مع الوسط السائل على باقي التراكيز في اغلب الصفات المدروسة ، في حين تفوقت المعاملة القياسية مع الوسط الصلب في محتوى الكالس والبراعم والنموات الخضرية من حامض الأبسسيك كما تفوقت في نسبة الاسمرار في مرحلة الكالس .

4- اوضحت الدراسة ان التداخل بين (TDZ وPG) له تأثير في الصفات الفيزيائية والكيميائية والفسيولوجية اذ اعطت المعاملة 0.5 ملغم .لتر$^{-1}$ TDZ مع 50 ملغم .لتر$^{-1}$ PG زيادة معنوية في معدل الصفات المدروسة مقارنة ببقية التراكيز ،في حين تفوقت المعاملة القياسية في محتوى الكالس والبراعم والنموات الخضرية من حامض الأبسسيك كما تفوقت في نسبة الاسمرار في مرحلة الكالس .

5- اما بالنسبة للتداخل الثلاثي فقد تفوقت المعاملة 0.5 ملغم .لتر$^{-1}$ TDZ مع 50 ملغم .لتر$^{-1}$ PG مع الوسط السائل على باقي المعاملات اذ ادت الى زيادة في معدل وزن الكالس والبراعم والنموات الخضرية وعدد البراعم والنموات الخضرية كما تفوقت في نسبة النتروجين والفسفور البوتاسيوم

والمغنسيوم والكربوهيدرات والمنغنيز وكذلك تفوقت في محتوى الكالس والبراعم من الاوكسينات والسايتوكاينينات والجبرين في حين تفوقت المعاملتان(0.5 ملغم .لتر$^{-1}$ TDZ مع 50 ملغم .لتر$^{-1}$ PG في الوسط السائل والصلب) في محتوى النموات الخضرية من الاوكسينات والسايتوكاينينات والجبرلين في حين تفوقت المعاملة 0.5 ملغم .لتر$^{-1}$ TDZ مع 50 ملغم .لتر$^{-1}$ PG مع الوسط السائل في محتوى البراعم والنموات الخضرية من في حين اعطت المعاملة 0.5 ملغم .لتر$^{-1}$ TDZ مع 50 ملغم .لتر$^{-1}$ PG مع الوسط الصلب اعلى محتوى للكالس من عنصري البوتاسيوم والمغنيسيوم كما سجلت اعلى محتوى للنموات لخضرية من المنغنيز ، في حين تفوقت المعاملة القياسية مع الوسط السائل في محتوى الكالس والبراعم من حامض الأبسسيك بينما سجلت المعاملة القياسية مع الوسط الصلب اعلى محتوى للنموات الخضرية من حامض الابسيسك كما تفوقت المعاملة القياسية مع الوسط الصلب والمعاملة 1.0 ملغم .لتر$^{-1}$ TDZ مع 0 ملغم .لتر$^{-1}$ PG مع الوسط الصلب في محتواها من عنصر الصوديوم في حين سجلت المعاملة القياسية في الوسطين السائل والصلب اعلى نسبة اسمرار لنسيج الكالس .

6- اشارت نتائج دراسة التطابق الوراثي بتقنيتيISSR(ISSR6 ,HB10 ,HB9 ,844a ,814) و RABD(opo07 ,opd10 ,opb07 ,opa12 ,opa02) الى ان المعاملات لم تؤثر في الصفات الوراثية للنسيج النباتي ، وقد لوحظ وجود تطابق وراثي عالي بين المعاملات

7- بينت الدراسة نجاح انتقال الجين *SeNHX1* المسؤول عن مقاومة النبات للملوحة الى نسيج الكالس.

تأثير طريقة الاستزراع وبعض المعاملات وتقنية النقل الجيني على نمو وتطور كالس نخيل التمر *Phoenix dactylifera* L. صنف البرحي

أطروحة مقدمة إلى

مجلس كلية الزراعة – جامعة البصرة

وهي جزء من متطلبات نيل شهادة دكتوراه فلسفة

في العلوم الزراعية–البستنة وهندسة الحدائق

(زراعة انسجة النبات)

من قبل الطالب

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