Effect of water stress on callus induction from shoot tips of date palm (*Phoenix dactylifera L.*) cv. Bream cultured *in vitro*

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Summary

The experiment carried out at plant cells and tissue culture laboratory, College of Education University of Basra, in 2010; on shoot tips quarters of date palm (*Phoenix dactylifera L.*) cv. Bream.

Shoot tips quarters were cultured on MS nutrient medium with addition of sucrose, sodium di-hydrogen orthophosphate, thiamine, Myo-inositol, activated charcoal, agar and using auxin 2,4-D and cytokinin BA as growth regulators. Water stress was induced by using Polyethylene glycol (PEG 3000) in concentrations of (0, 10 and 20) %.

The results indicate that using PEG 3000 with previous concentrations caused significant increase in callus fresh weight when compared with nutrient medium free of PEG 3000 with no significant difference between the two concentrations; while there was a significant increase in somatic embryos produced by nutrient medium with 10% PEG 3000 compared with that having 20% PEG or control treatment free of PEG.

Key words: Date palm, water stress, PEG 3000.

Introduction

Water availability is one of the principal limitations of crop production, particularly in the arid and semi arid regions where date palm is predominantly grown (Al-Khayri and AL-Bahrany, 2004).

The use of *in vitro* cultures to study stress response is based on the fact that those cultured cells behave similarly to the cells of intact plants subjected to water deficit and salinity stress conditions (Attree *et al*., 1991).

It has been estimated that 10% of the arable land can be classified under non stress category, which implies that crops grown under 90% of the arable land experience different types of environmental stresses, like water stress .The prediction is that the water deficits will continue the major single a biotic factor likely to affect crop yield globally (Sharma and Lavanya, 2002).

Iraq is considered as one of the most affected countries in Asia (Batanony, 1996), most of its lands suffer from salinity and drought. Date palm plantations in Iraq were destroyed due to wars, drought, salinity and diseases. For those reasons there is a great need for the establishment of new programs for rapid propagation of date palm along with producing a salt and drought tolerant cultivars (Al-Ka'aby, 2004)

Polyethylene glycol (PEG) widely used to induce water stress, is a nontoxic, nonionic polymer which is not expected to penetrate into plant cells (Djibril *et al*., 2005). It also lowers the water potential of the medium and has been used to stimulate drought stress in plants.

The aim of this study is producing *in vitro* of date palm callus and somatic embryos highly tolerate to drought by using (PEG) as water stress agent.

Materials and Methods

The study was carried out at plant cells and tissue cultures laboratory, college of education, university of Basra, Basra /Iraq in 2010.

Explant preparation

Shoot tips were separated from 2-4 years old offshoots of date palm (*Phoenix dactylifera* L.) cv. Bream, cut into four quarters (Mater, 1986), then put it in antioxidant solution (ascorbic acid 100mg/l + citric acid 150mg/l).

Surface sterilization

Shoot tips quarters were surface sterilized with commercial bleach containing 20% sodium hypochlorite with a few drops of tween-20, for 15 minutes, then rinsed with sterile distilled water for three times.

Preparation of nutrient medium

The basal nutrient medium consisted of Murashige and Skoog (1962) inorganic salts (MS) supplemented with (mg/l) sodium dihydrogen orthophosphate (170), Myo-inositol (100), sucrose (30000), adenine sulphate (40), thiamine-HCl (5), activated charcoal (3000), agar (7000) (Tisserat, 1981). Nutrient medium supplemented with 2,4Dichlorophenoxyacetic acid (2, 4-D) (10) and Benzyl aminopurine (BA) (3).

Water stress was induced by polyethylene glycol (PEG 3000) at concentrations (0, 10 and 20) %. Media were adjusted to pH 5.8 with 0.5 N. KOH. The media were then autoclaved at 121 °c and 1.2 Kg/cm^2 for 20 minutes.

Cultures were incubated in the growth room in darkness at 27±2 °c for sixteen weeks during which they were transferred at four weeks intervals.

After primary callus formation, it was transferred to callus development medium with the same previous constituents and maintain at 27±2 °c and 16h photoperiod at growth room, till embryogenic callus formation after twelve weeks (subcultures was made at four weeks intervals), one hundred milligram of the embryogenic callus forms were transferred to hormones free nutrient medium in order to initiate somatic embryos formation.

To determine the callus and somatic embryos responses to PEG induced water stress, the following parameters were calculated:

1-callus fresh weight (g). 2-time elapsed for somatic embryos formation (day). 3-number of somatic embryos. 4-time elapsed for germination of somatic embryos (day). 5- Germination percentage.

Statistical analysis

The experiment was set up as a completely randomized factorial experiment (1cultivar x 3 treatments) with 10 replications. The data were subjected to the analysis of variance (ANOVA) using SPSS statistical program. Revised least significant difference (RLSD) test was used to verify the differences between means at 0.05 significance level (Snedecor and Cochran, 1982).

Results and discussion

The results in Fig. (1) Indicate that PEG at 10% caused a significant increase in embryogenic callus fresh weight compared with control treatment, with no significant difference between the two PEG concentrations. This result may due to the ability of shoot tips and primary callus cells to accumulate small molecule compounds known as compatible solutes in their cells as a way of tolerating stresses such as drought, high salt concentration ,and so on (Chen and Murata, 2002).The compatible solutes so far reported in plants includes amino acids (proline), onium compounds (glycine betaine) mono sacchrides (fructose), sugar alcohol (Mannitol) and di- and oligosaccharides (sucrose).

The accumulation of compatible solutes protect cellular components from stresses by acting as osmoregulators, since their high solubility in water acts as a substitute for water molecules released from leaves (Akash *et al*., 2001) or by increasing cellular osmotic pressure (Delaunery and Verma, 1993). In date palm it was noticed that PEG caused a significant increase in the content of endogenous free proline (Al-Khayri and Al-Bahrany, 2004).

In Fig. (2) and Fig. (3) it is clear that PEG at 10% concentration enhanced all growth parameters, it caused a significant decrease in time elapsed for somatic embryos formation and for starting embryos germination. It also caused a significant increase in a number of somatic embryos formed Fig. (4) and on their germination percentage (Fig. 5) compared with 20% PEG treatment.

It is well documented that plant cells under water stress conditions try to adjust their water and osmotic potential by the accumulation of compatible solutes as mentioned above, but this adjustment stays for a limited period of time after which cells under high concentrations of PEG need to spent energy for their osmotic adjustment which may affect metabolic processes leading to decrease cells growth and development (Bolarin *et al*., 1995). Cell water deficits cause an increase in the concentration of ions that destabilize macromolecules or lead to toxicity (Flowers and You, 1988; Osaki *et al*., 1991; Yokota *et al*., 2006).

Regarding our experiment it is clear that PEG at 10% concentration is ideal to produce tolerant cell lines and tissues and could be used in future for date palm plantlets hardening in order to produce water stress tolerant plants.

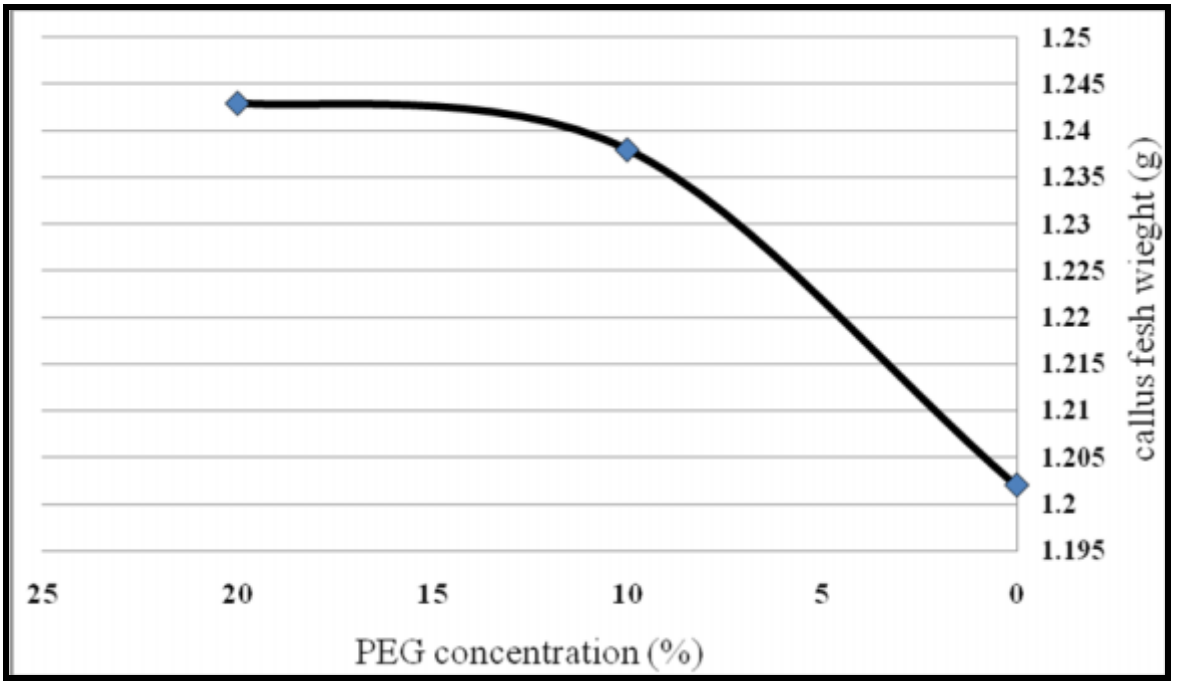


Fig.(1) Callus fresh weight as affected by PEG

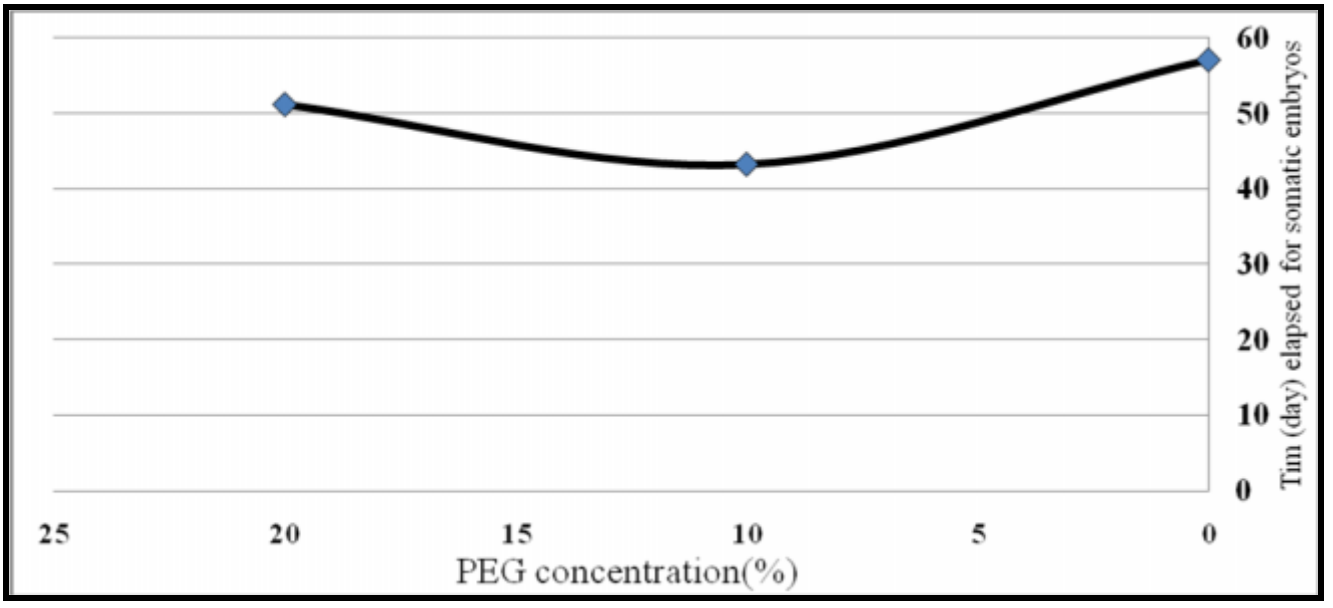


Fig. (2) Effect of PEG time elapsed for somatic embryos formation

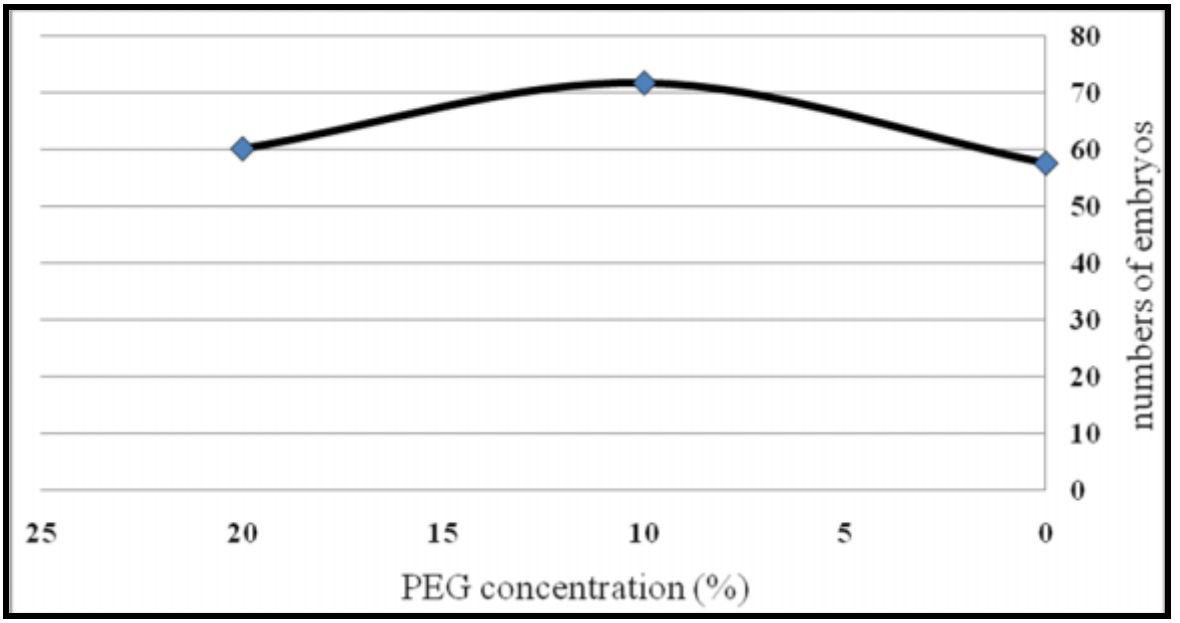


Fig. (3) Effect of PEG on numbers of somatic embryos formed

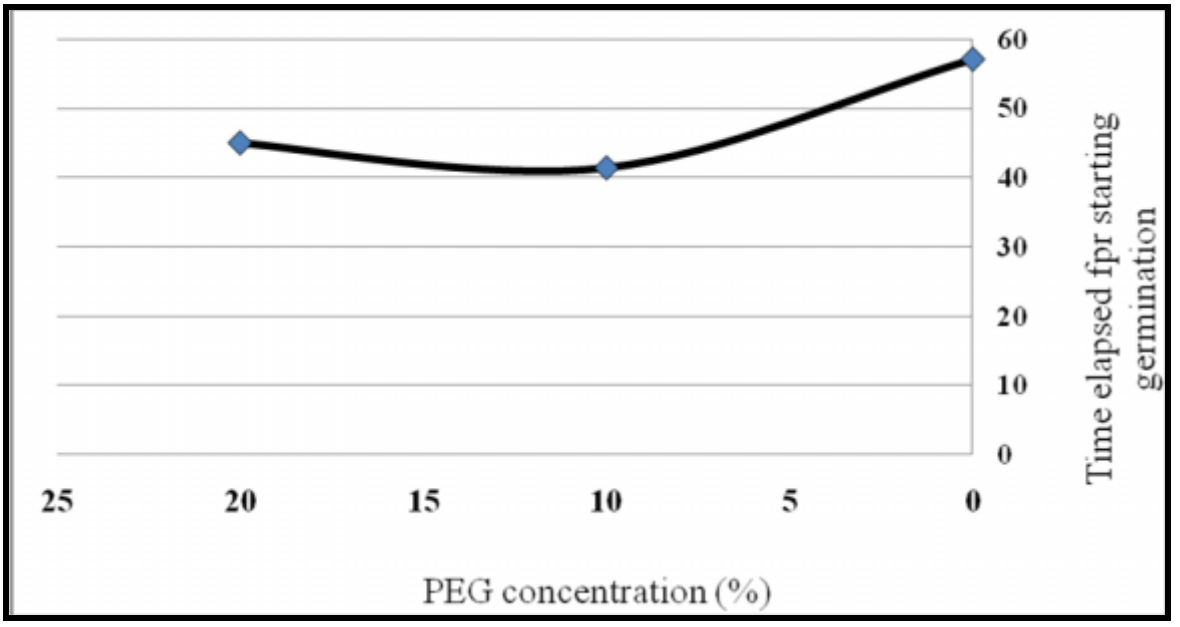


Fig. (4) Effect of PEG on elapsed time (day) for starting germination

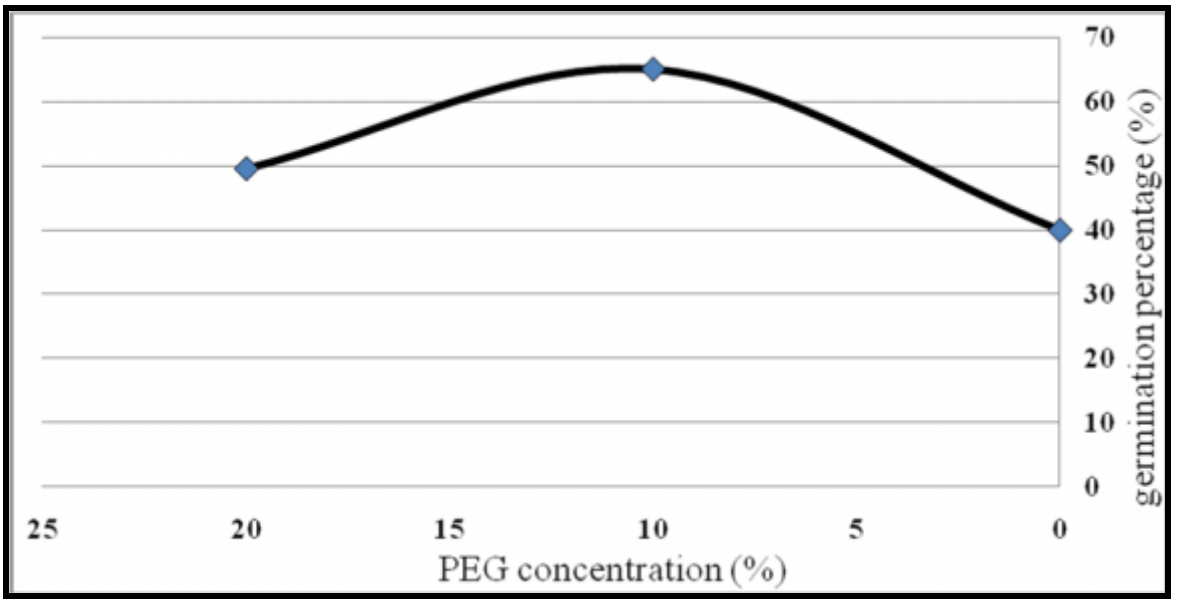
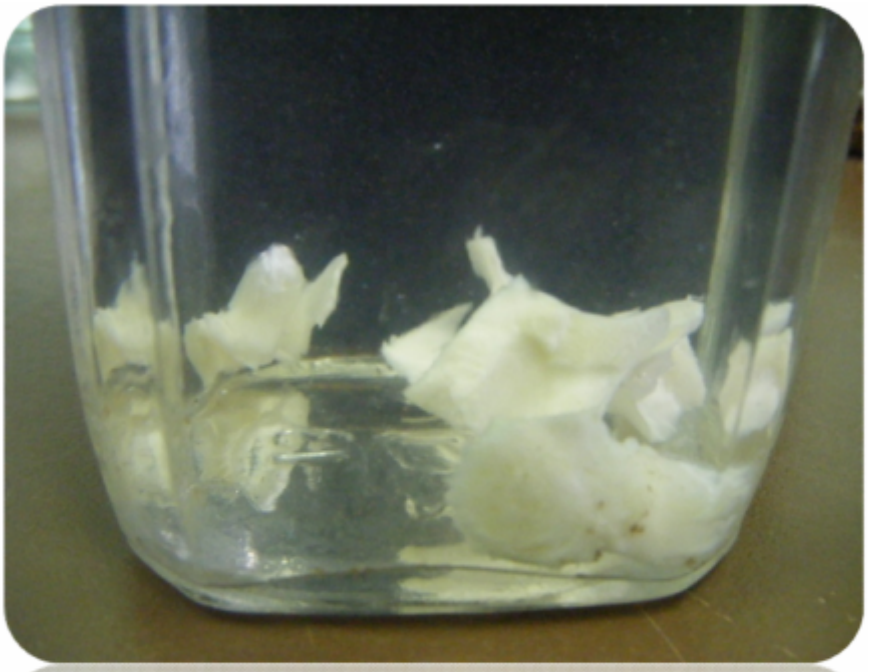
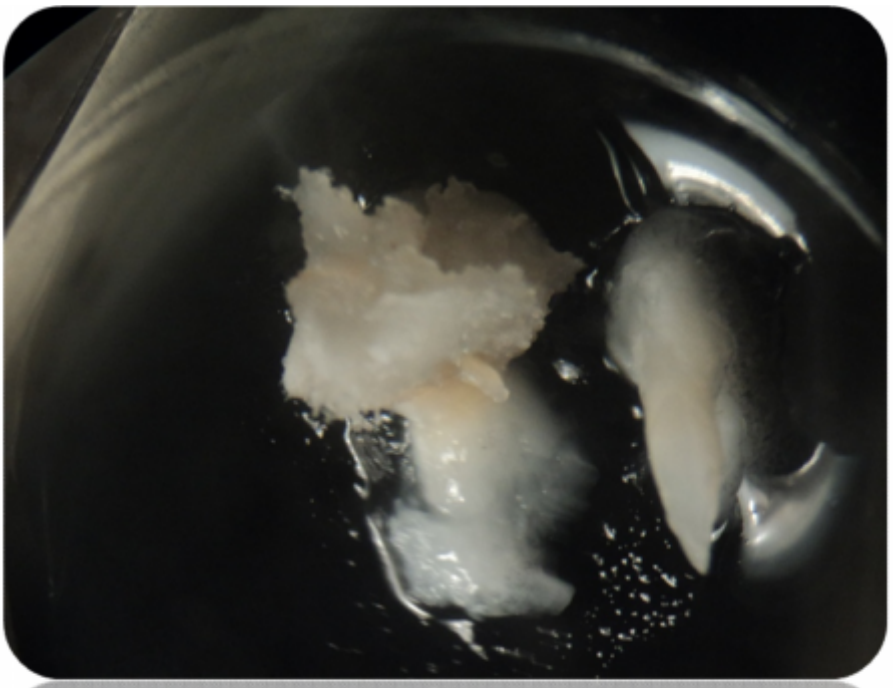


Fig.(5) Effect of PEG on germination percentage of somatic embryos

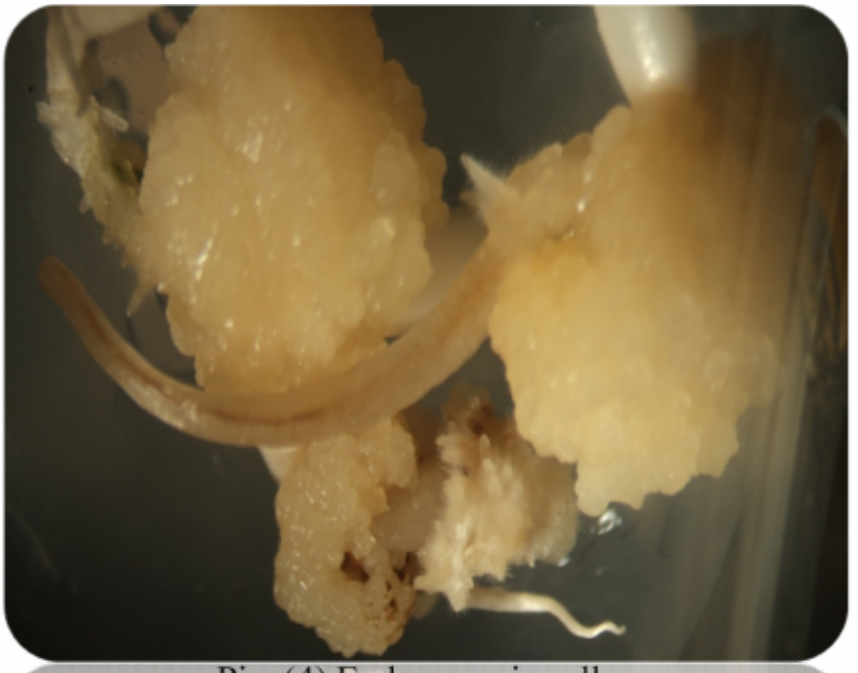
Pic. (1) Shoot tip



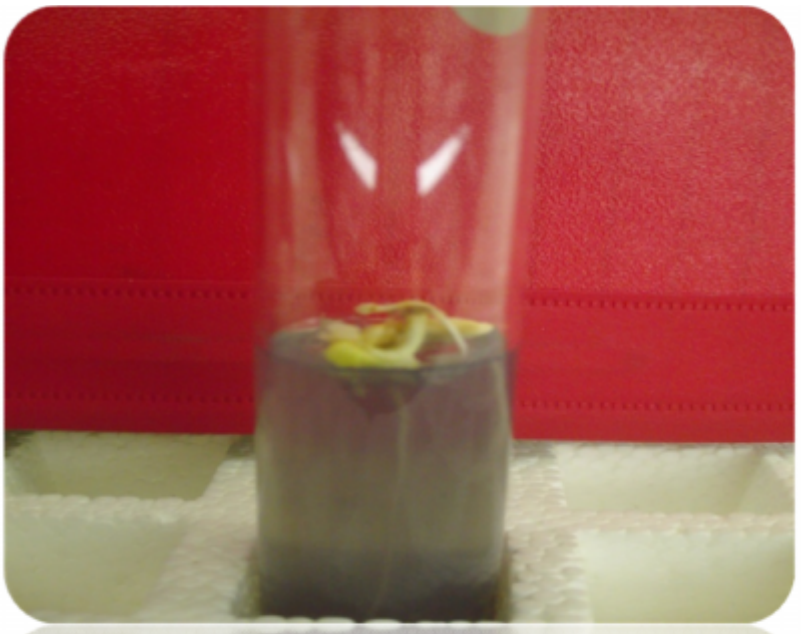
Pic. (2) Explants in antioxidant solution



Pic. (3) Primary callus



Pic. (4) Embryogenic callus



Pic.(5) germinated embryo

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تأثير الشد المائي في استحثاث تكوين الكالس من البراعم الطرفية لنخيل التمر صنف (بريم)عند الإكثار الدقيق

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الخلاصة

أجريت التجربة في مختبر الزراعة النسيجية التابع إلى قسم علوم الحياة – كلية التربية- جامعة البصرة في العام 2010 وقد استخدمت فيها أرباع البراعم الطرفية لنخيل التمر صنف بريم .

زرعت أرباع البراعم الطرفية على وسط غذائي يتكون من أملاح MS أللاعضوية مع عدد من المواد الإضافية وهي (السكروز، فوسفات الصوديوم الثنائية الهيدروجين، الثيامين، الاينووسيتول، الفحم المنشط والاكار) وباستخدام الاوكسين NAA بتركيز(30 mg/l) والسايتوكاينين 2ip بتركيز (3 mg/l) وتم استحثاث الشد المائي بواسطة(Polyethylene glycol (PEG 3000 بالتركيزين (10و20)%.

أوضحت النتائج المستحصلة من هذة التجربة ان استخدام PEG 3000 بالتركيزين المذكورين أدى إلى زيادة معنوية في كمية الكالس الناتجة من البراعم الطرفية بالمقارنة مع الوسط الغذائي الخالي منه دون ان تكون هنالك فروق معنوية بين التركيزين بينما سبب التركيز 10% زيادة معنوية في عدد الأجنة الجسمية الناتجة مقارنة مع التركيز 20% ومعاملة السيطرة بالوسط الغذائي الخالي من PEG.

الكلمات المفتاحية: نخلة التمر، الشد المائي ،PEG 3000،الإكثار الدقيق.