The effect of water stress on callus and somatic embryos formation of rice (*Oryza sativa* L.) cv. Jasmine cultured *in vitro*.

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Summary

Water availability is one of the major limitations of crop production in Iraq. The use of *in vitro* cultures to study stress responses is based on the fact that those cultured cells behave similarly to those cells of intact plants subjected to water deficit and salinity conditions.

For that reason the response of rice (*Oryza sativa* L.) cv. Jasmine to water stress induced by polyethylene glycol (PEG3000) was studied *in vitro*. Seeds of jasmine rice were used as explants and cultured on MS nutrient medium supplemented with PEG 3000 at 0, 3, 6 and% 9.

Results revealed that PEG at high concentrations (6 & 9%) caused a significant decrease in callus fresh weight, somatic embryos development and a significant increase in proline accumulation in callus compared with control.

Results also showed that PEG at 3% either had no significant effect or it encouraged callus fresh growth and somatic embryos development, and such concentration is suitable for enhancing drought tolerance in rice cell lines cultured *in vitro*.

Key words: water stress, PEG, rice. Callus, somatic embryos.

Introduction

Biotechnological approaches are indispensable tools to achieve crop improvement, but the application of such approaches requires the availability of a regeneration system for the crop of interest. Regeneration of rice *in vitro* has been well demonstrated [1] but little is known about Jasmine rice which is recently cultivated in Iraq.

Water deficit is one of the most important environmental disturbances, which influence the distribution of many species from year to year. The tissue culture method is a novel approach to this problem; the main idea is that cultivated cells are used as the selection units rather than whole plants [2].

Cells and callus cultures provide controlled, uniform environment for studying physiological and biochemical processes in plants, particularly mechanisms operative at the cellular level such as water stress responses [3].The method is based on spontaneous induced mutant *in vitro* after which resistant cells influenced by a selective agent are selected and plants are subsequently regenerated from the surviving resistant cells [4].

Polyethylene glycol (PEG), a non penetrated and non toxic osmotic lowers the water potential of the medium and has been used to stimulate drought stress with no injurious or toxic effects on the plant [5], but inhibit growth by lowering water potential of the medium [6]

PEG-adapted cell lines of tomato have shown a high level of tolerance to salt stress as compared to non-adapted cells. Successful *in vitro*

selection for drought tolerance has been reported for wheat [7] maize [8] and sugarcane [9].

The aim of this study was to produce cell lines tolerated to drought using PEG as a stress agent.

Materials and methods

Seeds were introduced from the rice research station in Mishkhab at Al-Najaf governorate. The following experiments were:

1- Germination percentage:

Seeds germination percentages were done by separating (100) seeds equally in four Petri dishes lined with filter paper 9cm in diameter, adding 5ml of distilled water. Germination percentage was calculated after (72) hrs. using the following equation:-

$$\% \text{ germination} = \frac{\text{no. of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

2- Seeds surface sterilization:

Seeds were dehusked manually and surface sterilized for 3 min. in 70 % ethanol, followed by 30 min. shaking in 20% of commercial bleach contain sodium hypochlorite with a few drops of tween-20, then rinsed three times in sterilized distilled water.

3- Preparation of nutrient medium:

Seeds of jasmine rice were cultured in 25x100 mm test tubes using MS nutrient medium [10] supplemented with (mg/l) Sucrose 30000, Na_2HPO_4 170 ,Glycine 200, Thiamine-HCl 500, Myo-inositol 100, Adenine sulphate 40, Agar 7000 and 2,4dichlorophenoxyacetic acid (2-4,D) 5.

The pH of the medium was adjusted to 5.8 with 0.1 KOH. The media were autoclaved at 121°C and 1.05 kg/m^2 for 20 minute. Light conditions in the growth room were fixed by fluorescent lights at photoperiod of 16h light / 8h dark at 25±2°C.

After callus initiation PEG3000 was added at concentrations of 0, 3, 6 and 9% to the nutrient medium. Subcultures were made every 4 weeks till somatic embryos establishment.

The following parameters were evaluated:

1-Callus fresh weight (gm) and callus water content percentage.

2-Proline accumulation (µg/gm fresh weight) in callus [11].

3-Numbers of somatic embryos and time elapsed for their formation (day).

Statistical analysis

The experiment was designed as a factorial experiment (two factors, variety x PEG treatments) with a completely randomized distribution of the treatments with 10 replications. The data were subjected to the analysis of variance using SPSS programme. Revised LSD test were used to verify the differences between means at 0.05 significant level. [12].

Results and discussion

Figure (1), show that increasing water stress induced by PEG caused a significant decrease in embryogenic callus fresh weight and in callus water content (Fig.2) especially at high concentrations of PEG 6 and 9% compared with control and 3% PEG treatment.

Somatic embryos number and time elapsed for somatic embryos formation were affected in the same manner , a significant decrease in the number of somatic embryos(Fig. 5), and a significant increase in time elapsed for somatic embryos formation(Fig 4), caused by PEG high concentrations 6 and 9 % .

Regarding proline accumulation (Fig. 3), show a significant increase in callus proline along with increasing PEG concentrations. Proline content of jasmine rice callus increase gradually with the increased in PEG concentration. The highest content of proline is observed at 9%PEG.

Because plant growth is a result of cell division and enlargement, water stress directly reduces growth by reducing cell division and enlargement [1]. One of the major consequences of water stress is the loss of protoplasmic water leading to the concentration of ions such as Cl and NO_3 at high concentrations these ions inhibit metabolic functions [13]. Additionally, the concentration of protoplasmic constituents and the loss of water from the cell lead to the formation of what is termed a glassy state. In this state whatever liquid is left in the cell, it has a very high viscosity, increasing the chances of molecular interaction that can cause protein denaturation and membrane fusion [14].

A means of increasing drought tolerance is by decreasing osmotic potential by accumulation of solutes. Generally plants accumulate some kinds of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake [15]. Among these solutes, proline is the most widely studied [16]. The beneficial role of proline is in conferring osmotolerance that has been widely reported [17].

Increasing PEG concentration was also association with increase in proline accumulation in rice and date palm [18, 19]. The concentration of PEG in the medium showed a great effect on the growth in tomato cultured *in vitro* [4].

Conclusion:

Regarding this experiment it seems that adding PEG -3000 at 3% concentration either has no inhibitory effect or it encouraged callus growth and somatic embryos formation, and this concentration is suitable to enhance water stress tolerance in rice cells cultured *in vitro*.

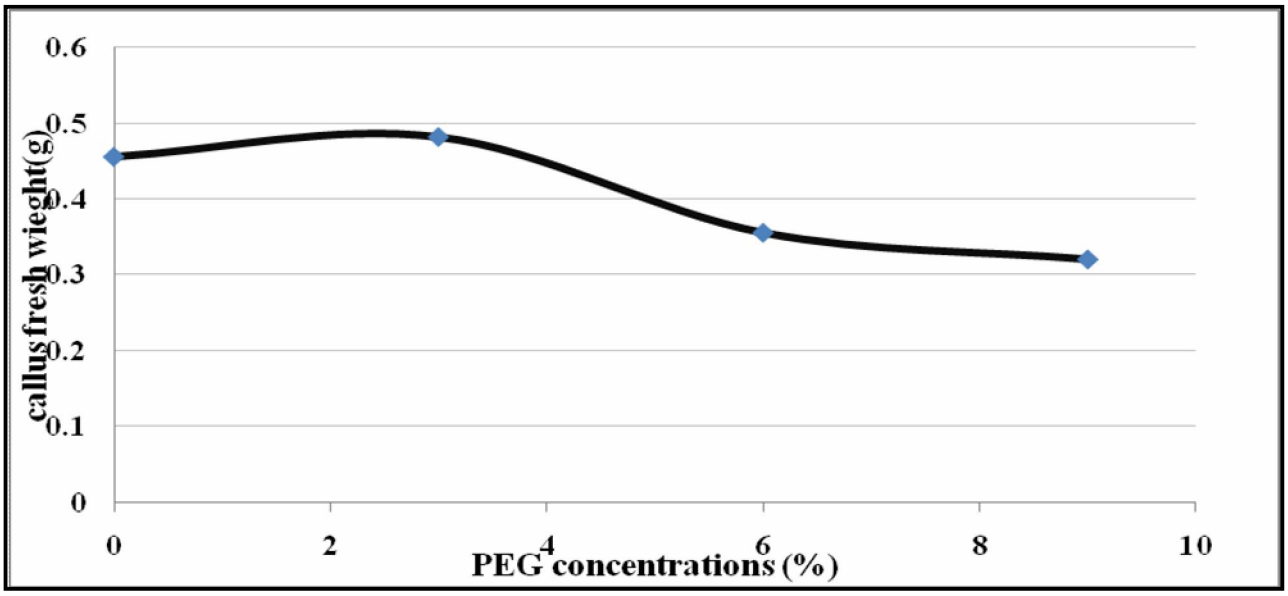

Fig. (1) Effect of PEG concentrations on callus fresh weight

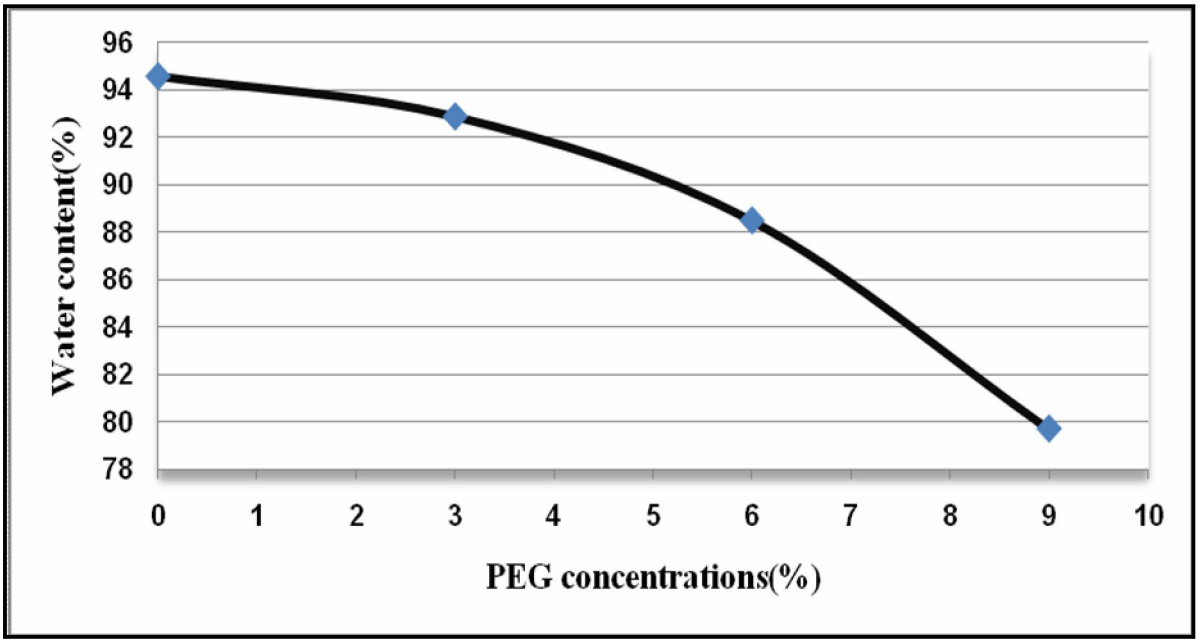

Fig.(2) Effect of PEG concentration on callus water content

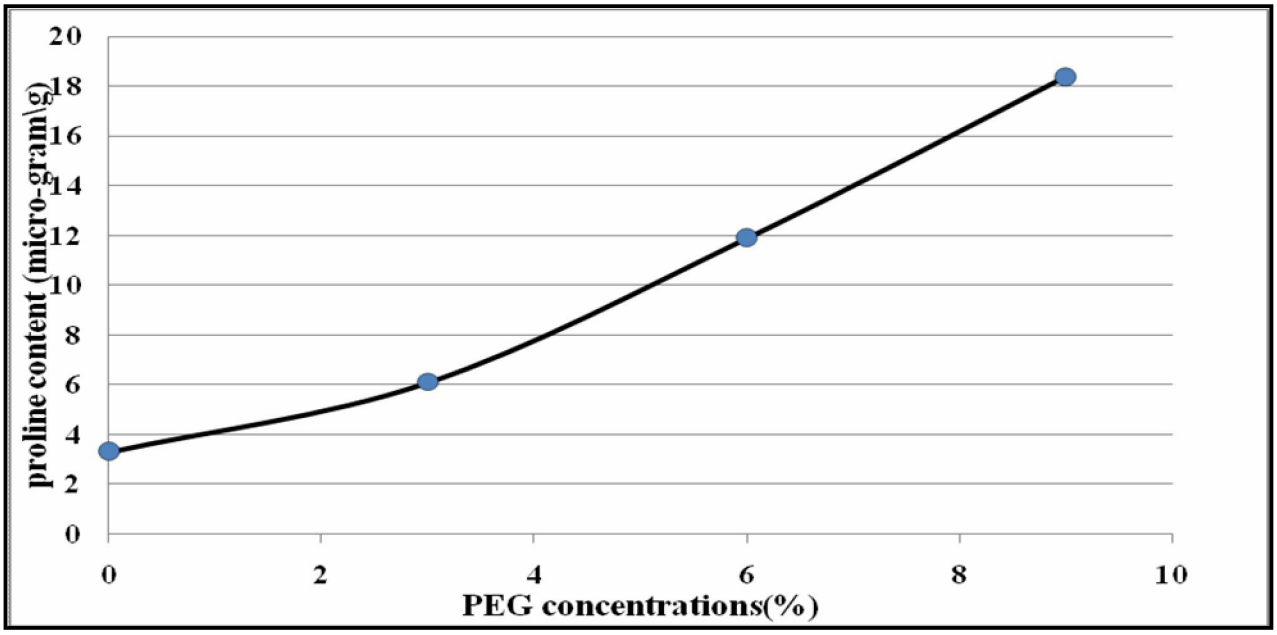

Fig. (4) Effect of PEG concentration on time elapsed for somatic embryos formation

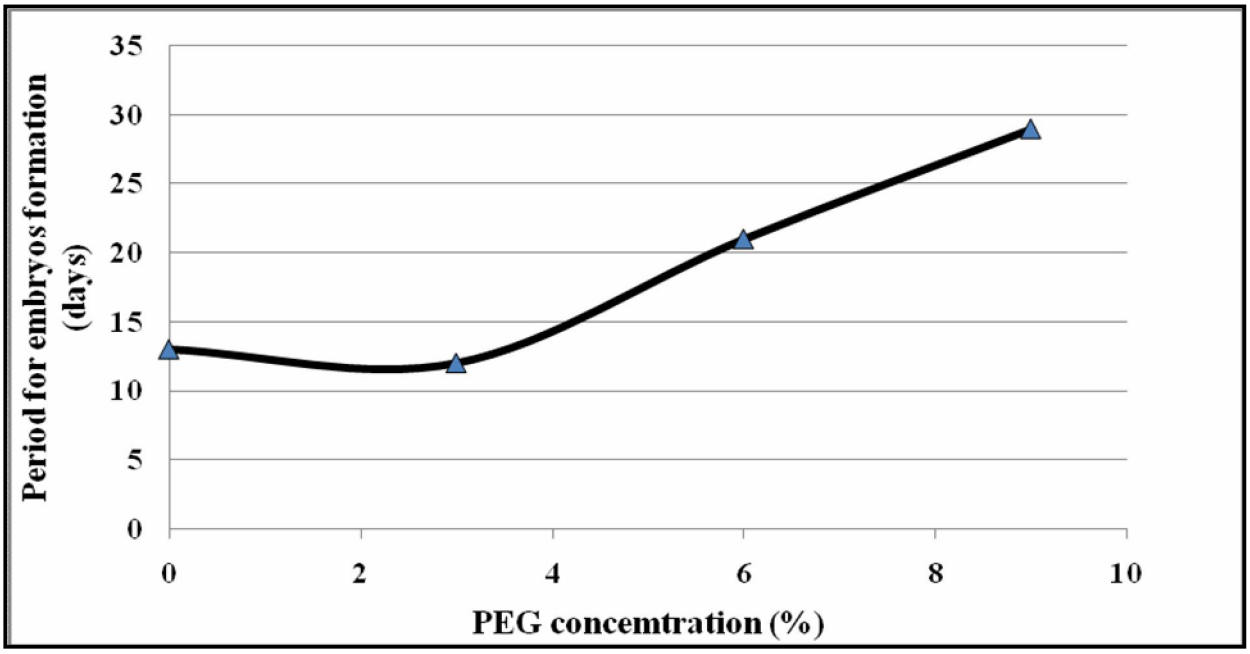

Fig. (5) Effect of PEG concentration on numbers of somatic embryos

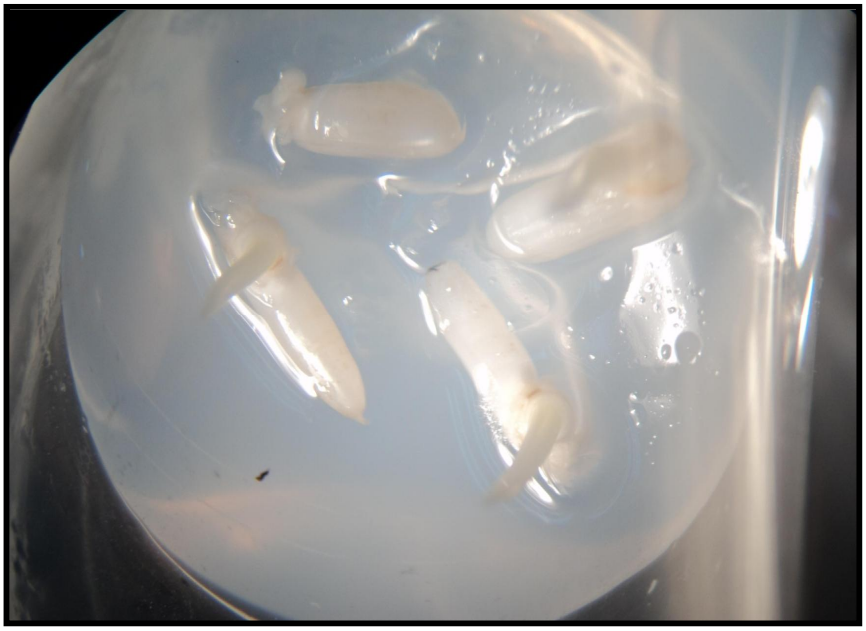

Fig. (1) Cultured seeds

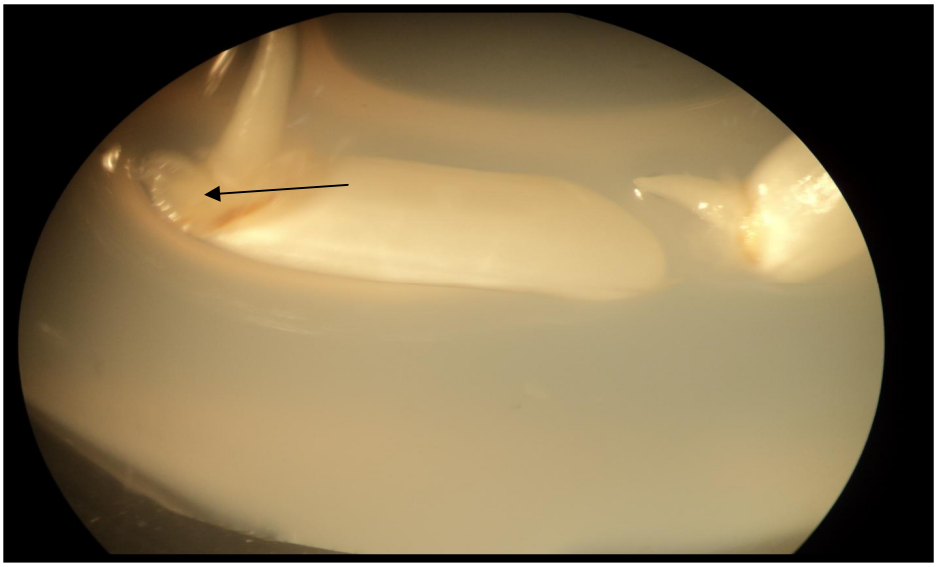

Fig. (2) Primary callus after 4- weeks

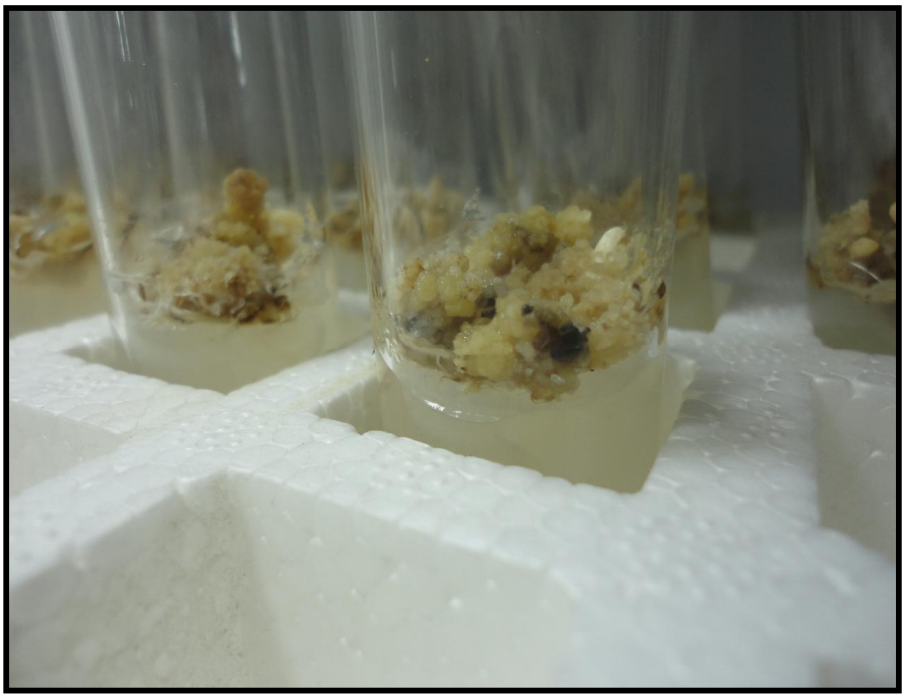

Fig.(3) **callus 8- weeks age**

Fig.(4) Embryogenic callus

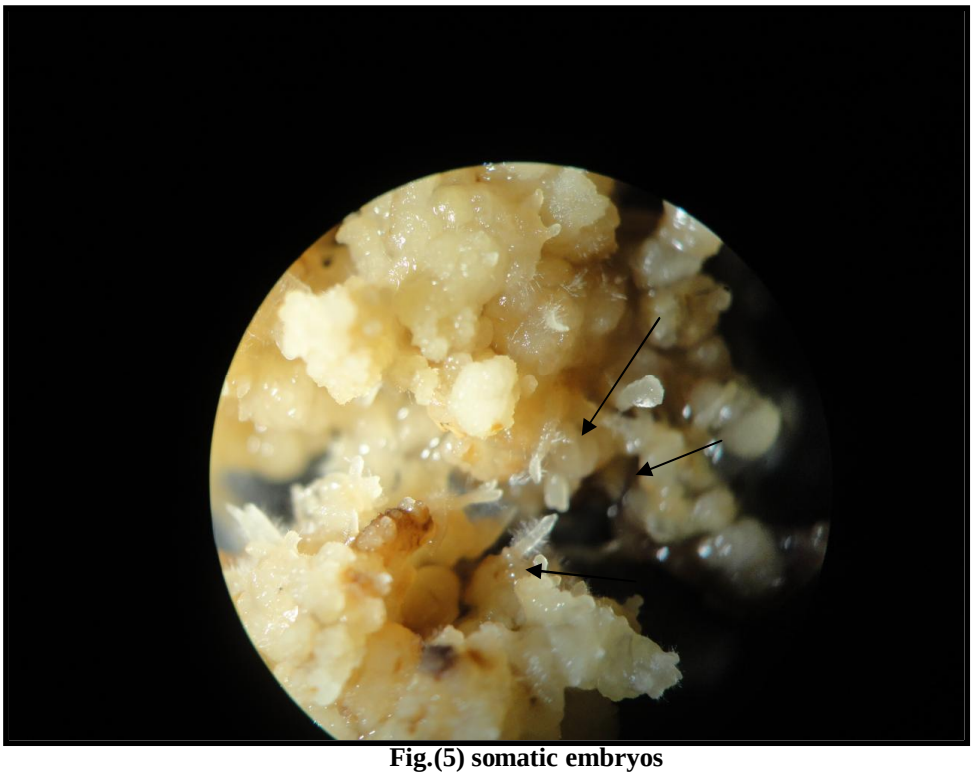

Fig.(5) somatic embryos

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تأثير الشد المائي في تكوين الكالس والأجنة الجسمية في نبات الرز (*Oryza sativa* L.) صنف ياسمين تحت ظروف الزراعة النسيجية

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الخلاصة

إن مشكلة نقص الإمدادات المائية تعد واحدة من أهم المشكلات التي تواجه الزراعة في العراق والذي يعتبر من أكثر البلدان تأثرا بالجفاف في قارة أسيا. كما إن تقانة زراعة الخلايا والأنسجة النباتية تعد من التقانات الحديثة التي ثبت إن استعمالها يؤدي إلى إنتاج نباتات مقاومة للشدود البيئية كالجفاف والملوحة إذ استنتجت البحوث إن استجابة الخلايا المزروعة نسيجيا لظروف الشد المائي تكون مماثلة لاستجابة خلايا النبات الكامل . وعلية فقد استهدفت الدراسة الحالية معرفة مدى استجابة نبات الرز صنف ياسمين إلى ظروف الشد المائي عند زراعته نسيجيا.

استخدمت في الدراسة البذور الكاملة للرز صنف ياسمين (الجنين مع السويداء) كأجزاء نباتية وقد تمت زراعتها على وسط MS والذي أضيف إليه مادة polyethylene glycol (PEG 3000) كمادة محفزة للشد المائي بالتراكيز (0 , 3 , 6, 9%).

أوضحت النتائج إن التراكيز العالية من PEG (6 , 9%) أدت إلى خفض معنوي في الوزن الطري للكالس وفي صفات الأجنة الجسمية كما أدت إلى زيادة معنوية في تجمع البرولين في الكالس بالمقارنة مع معاملة السيطرة. كما أوضحت النتائج إن التركيز 3% كان له أثر معنوي في زيادة الوزن الطري للكالس وفي صفات نمو الأجنة الجسمية . وعليه فأنا نوصي باستعمال هذا التركيز في الدراسات اللاحقة حول زيادة تحمل الشد المائي لنبات الرز تحت ظروف الزراعة النسيجية.