Available online at: www.basra-science-journal.org

ISSN –1817 –2695

Effect of Sorbitol on Callus Induction and Somatic Embryos Regeneration in Two Local Wheat (*Triticum aestivum* L.) Cultivars

Hussein K. Al-Kaaby; Luma H. Abdul-Qadir and** Marwa E. Kareem

*Department of Biology ,College of Education for Pure Science – University of Basrah.

**Mesentery of Education.

kabi.zair7@gmail.com

Received 5-5-2013 , Accepted 19-8-2013

Abstract

A series of experiments were conducted in Plant Tissue Culture Laboratory, Department of Biology - College of Education for Pure Science – University of Basrah.

Two local wheat varieties cultivars named Dor29 and Fateh were evaluated to determine the impact of 0, 10, 20 and 30 $g.l^{-1}$ sorbitol for callus induction and somatic embryos regeneration. Mature seeds of the tested cultivars were selected as explants for callus induction and regeneration on MS medium with Gambrog vitamins containing 30 $g.l^{-1}$ sucrose, 5 $g.l^{-1}$ agar, 2 $mg.l^{-1}$ BA and 1 $mg.l^{-1}$ 2,4-D.

Results were showed that sorbitol at 20 $g.l^{-1}$ had a significant effect on callus fresh weight; mean globular somatic embryos number with no significant difference between the two cultivars under investigation. The obtained results from our study suggested that the optimal callus induction medium was MS + Gambrog vitamins + 30 $g.l^{-1}$ sucrose + 20 $g.l^{-1}$ sorbitol + 2 $mg.l^{-1}$ BA + 1 mg^{-}2,4-D + 5 $g.l^{-1}$ agar ,while maximum number of somatic embryos formed at the same previous medium with the amition of BA and 2,4-D.

Key words: Wheat, sorbitol, callus, somatic embryos.

Abbreviations : BA Benzyladenine , 2,4-D 2,4-Dichlorophenoxyacetic acid , MS Murashige and Skoog medium (1962)

1. Introduction

Carbohydrates are necessary as a source of energy and a carbon substrate for biosynthesis Continuous supply of carbohydrates to plants cultured *in vitro* is essential, since photosynthetic activity of *in vitro* grown tissues is usually reduced[1].

A number of carbohydrate, support growth but their requirement and suitability can vary according to plant species [2]. In general, sucrose is the carbohydrate of choice as carbon source for *In vitro* plant culture, probably because it is the most common carbohydrate in the phloem sap of many plants[3 ,4].

However, invertases that are released by the explants into the medium, split sucrose into glucose and fructose[5]. Thus, explants are usually exposed to a mixture of sucrose, glucose and fructose.

Sorbitol which also known as glucitol, is a sugar alcohol, is thought not usually to be metabolized by plant tissues and therefore unavailable as carbon sources[6]. In contrast, sorbitol is readily taken up and metabolized in some species. It has been found to support the growth of apple callus [7] and that of other rosaceous plants [8], occasionally giving rise to more vigorous growth than can be obtained on sucrose. The ability of Rosaceae to use sorbitol as a carbon source is reported to be variety dependent [9].

According to the literature, adding sorbitol into the tissue culture media may promote both callus induction and regeneration rates significantly in apples [10], loquats[11], maize[12] and rice[13].

However, the response of local wheat cultivars to varied concentrations of sorbitol is not yet studied. Therefore, this experiment was conducted in order to discover the impact of sorbitol concentrations on the tissue culture of two local wheat cultivars.

2. Materials and Methods

The mature seeds of two cultivars of local wheat (*Triticum aestivum* L.) known as Dor29 and Fateh were used as explants for callus induction. Seeds were surface sterilized by 70% ethanol for 1min followed by (% 5) commercial solution of sodium hypochlorite for 20 min with mild shaking, then rinsed three times with sterile distilled water. seeds then are placed on the media and kept in the dark at 25±2°C for 4 weeks.

Both the MS medium [14], with Gambrog vitamins [15], were used as the culturing medium in all experiments. This medium was fortified with 2 mg.l BA and 1 $mg.l^{-1}$ 2,4-D for callus induction, culture vessels used, were Pyrex test tubes(110x10 mm). The medium was supplemented with 30 $g\ l^{-1}$ of sucrose and 5 $g.l^{-1}$ agar as the solidifying agent.

In order to investigate the effect of sorbitol, various concentrations of sorbitol (0, 10, 20 and 30 $g.l^{-1}$ were added to the above medium. To discover the impact of sorbitol, subculture was performed every 30 days after inoculation and obtained callus were transferred to the same callus induction medium. For the regeneration experiment, embryogenic callus produced on the callus induction medium were transferred to the hormones free regeneration medium.

Concentration of sucrose, agar and sorbitol in the regeneration medium was the same as that for the callus induction.

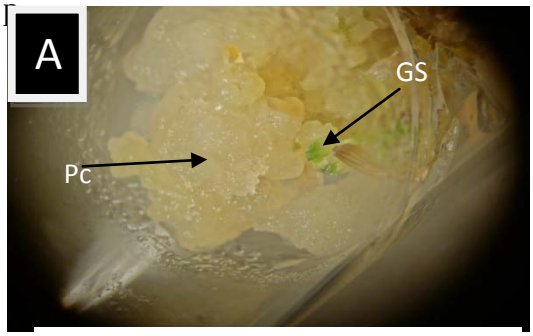
Two parameters including mean callus fresh weight and mean number of globular somatic embryos were measured.

The analysis of variance and revised least significant difference (rLSD) at 0.05 level of significance for a completely randomized factorial experiment with five replications were done , using GENSAT statistical program[16].

3. Results and Discussion

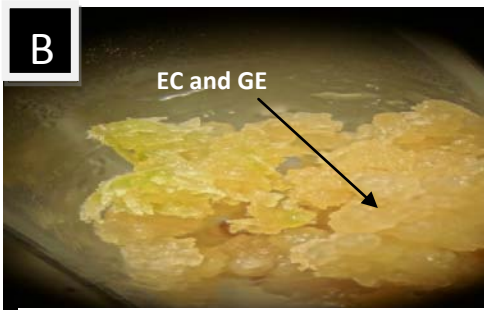
Callus from the tested media obtained after (14-18) days after culturing. Moreover, two types of callus; embryogenic and primary were detected. Callus that are nodular and compact, white to yellow in color are defined as embryogenic. By contrast, the primary callus was completely white and friable and was much greater in size than the embryogenic callus.

In the first subculture after 30 days about 250mg of the embryogenic callus with the proembryoid cells were transferred to the hormones-free medium for embryo formation. After (10-14) days from sub culturing the somatic embryos started to appear as clusters and was globular in shape when examined under dissecting microscope. Their numbers were measured when green spots appeared on the regeneration media after (12-16) days.



Pic. A – Primary callus

PC= primary callus, GS= green spots



Pic. B – Embryogenic callus and globular embryos

EC= embryogeneic callus

GE= globular embryos

Pic. C - Regenerated callus

Different concentrations of sorbitol incorporated into media were tested on callus induction and the regeneration. Remarkable differences were found in sorbitol effects. A significant differences were also observed in the interaction between varieties and sorbitol treatments

without a significant difference between the two local varieties in their response to increasing sorbitol concentrations in the medium.

The results of statistical analysis in Table (1) showed that adding different concentrations of sorbitol to the nutrient media of results no significant effect on callus fresh weight compared with control, in addition significant differences were observed in the interaction between varieties and sorbitol concentrations, while there were no significant differences between varieties .In terms of respect to

interaction, Dor29 showed the highest regeneration of 1.212 gm of mean callus fresh weight at 20 g.l of sorbitol and Fatah the lowest with 0.859 gm of callus at 30 g.l sorbitol.

The results of the effects of different sorbitol concentrations on mean number of globular somatic embryos shown in table (2). Based on this table, adding of sorbitol at 20 g.l to the medium had significant effect on the mean number of globular somatic embryos, which increased by (%24.3) compared with the control. Table (2) indicated that the interaction between Fateh and 20 g.l Sorbitol showed the highest mean number of somatic embryos (68 embryos), and the lowest (52.67) was in the interaction between Fateh and a control.

The results of this study focused on the positive effect of sorbitol on callus production and somatic embryos formation. The proper levels of sorbitol 20 g.l which enhanced the callus production, morphological observation also indicated that the adding of sorbitol caused the production of more embryogenic callus compared to the sorbitol-free medium.

The role of sorbitol in tissue culture was based on two functions: firstly, as a primary carbon source to enhance regeneration frequency of embryogenic callus; and secondly as an osmotic regulator which can have a positive impact on callus and regeneration ability [12 ,13].

Some studies mentioned that sorbitol can be used as an osmotic agent and for this reason callus cannot metabolize it [17]. Others reported that sorbitol can also contribute as a carbon source [18].

Geng *et al*. [13]stated that due to the fact sorbitol functions in the cell as a complex mechanism, this complexity results in a high level of interaction in different culture stages. Sorbitol may act as an osmotic agent at an early stage of culture, and becomes a source of carbon later.

Pua & Chong [19] stated that poor response of sucrose in shoot proliferation of peach rootstock might be due to the slow break up of sucrose into glucose and fructose. The availability of invertase enzyme required for the efficient conversion of sucrose into glucose and fructose is less in sorbitol.

Table (1) Effect of Sorbitol on callus fresh weight (gm) of two Wheat varieties

Sorbitol g.l / Cultivar	0	10	20	30	**Mean**
Dor29	**0.939**	**0.862**	**1.212**	**0.919**	**0.983**
Fateh	**0.895**	**0.819**	**1.033**	**0.859**	**0.901**
Mean	**0.917**	**0.841**	**1.123**	**0.889**	
rLSD	cultivars N.S	concentrations N.S	interaction 0.313		

Table (2) Effect of Sorbitol on globular somatic embryos numbers of two Wheat varieties

Sorbitol g.l^{-1} / Cultivar	0	10	20	30	Mean
Dor29	**55.33**	**55.67**	**66.33**	**59.67**	**59.25**
Fateh	**52.67**	**53.67**	**68.00**	**60.33**	**58.67**
Mean	**54.00**	**54.67**	**67.17**	**60.00**	
rLSD	cultivars N.S	concentrations 0.661	interaction 0.935		

References

1. Amiri, S and S.K. Kazmitbar.2011. Enhancement of callus induction and regeneration efficiency from embryo culture of *Datura stramonium*. Afr.J.Biotech.10 (50):10101-10107.
2. Karhu, ST (1997). Sugar use in relation to shoot induction by sorbitol and cytokinin in apple. J. Am. Soc. Hort. Sci., 122: 476–480.
3. Lemos, E.E.P. and D.A. Baker. 1998. Shoot regeneration in response to carbon source on internodal explants of *Annona muricata* L. Plant Growth Regul., 25: 105-112.
4. Fuentes, S.R.L., M.B.P. Calheiros, J. Manetti-Filho and L.G.E. Vieira. 2000. The effect of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffea canephora* Plant Cell Tiss. Org. Cult., 60: 5-13.
5. De Klerk, G.J.M. and A. Calamar. 2002. Effect of sucrose on adventitious root regeneration in Apple. Plant Cell Tiss. Org. Cult., 70: 207-212
6. George EF, Hall MA, Klerk GJD. 2008. Plant Propagation by Tissue Culture. 3rd Edition. Springer.
7. Chong, C and C.D. Taper.1971.Daily variation of sorbitol and related carbohydrates in Malus leaves. Can.J.Hort.Sci.49:173-177.
8. Coffin, R., Taper, C.D. & Chong, C. 1976 Sorbitol and sucrose as carbon source for callus culture of some species of the *Rosaceae*. Can. J. Bot. 54, 547-551.
9. Albrecht, C. 1986. Optimization of tissue culture media. Comb. Proc. Int. Plant Prop. Soc. 1985, 35, 196-199.
10. Lin, S. and Z. Chen, 1997. Effect of sorbitol on separation and culture of protoplast in loquat. J. Fujian. Agric. Univ., 26: 400–406
11. Ma, F. and F. Wang, 1996. Effect of sorbitol serving as carbohydrate on apple microproduction. J. Xibei. Agric. Univ., 24: 102–104
12. Swedlund, B. and R.D. Locy, 1993. Sorbitol as the primary carbon source for the growth of embryogenic callus of maize. *Plant Physiol.*, 103:1339–1346.
13. Geng, P.P., H.G. La, H.Q. Wang and E.J.C. Stevens, 2008. Effect of

sorbitol concentration on regeneration of embryogeneic calli in upland rice varieties *(Oryza sativa* L.). Plant Cell Tiss. Org. Cult., 92: 303–313.

14. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

15. Gambrog O.L., Miller R.A. & Ojma K. 1968 Nutrient requirements of suspension cultures of soybean root cells. Exp.Cell Res. 50, 151-158

16. Compton, M.E. 1994. Statistical methods suitable for the analysis of plant tissue culture data. Plant Cell Tiss. Org. Cult., 37: 217–242.

17. Wang, H.L., P.D. Lee, L.F. Liu and J.C. Su, 1999. Effect of sorbitol induced osmotic stress on the changes of carbohydrate and free amino acid pools in sweet potato cell suspension cultures. Bot. Bull. Acad. Sin., 40: 219–225.

18. Kumria, R., B. Waie and M.V. Rajam, 2001. Plant regeneration from transformed embryogeneic callus of an elite indica rice via Agrobacterium. Plant Cell Tiss. Org. Cult.
67: 63-71.

19. -Pua, E.-C. and C. Chong. 1984. Requirement for sorbitol (D-glucitol) as carbon source for *in vitro* propagation of *Malus robusta*. Can. J. Bot., 62: 1545-1549.

-1

دور السوربيتول في استحثاث الكالس وتكوين الاجنة الجسمية في صنفين من الحنطة المحلية *Triticum aestivum* L.

حسين خلف الكعبي و لمى حسين عبد القادر و مروى عماد كريم

فزل عهلطكحيئب / قكي بطكةنلي بلككعهلطك شدنغب / جئلعبطكا شنب

kabi.zair7@gmail.com luma_bio2004@yahoo.com maemad71@gmail.com

الخلاصة

اجريت مجموعة من التجارب في مختبر الزراعة النسيجية لقسم علوم ألحياة ،كلية التربية للعلوم الصرفة - جامعة ألبصرة استعمل فيها صنفين من الحنطة المحلية هما دور29 وفتح وذلك لغرض تحديد دور السكر الكحولي السوربيتول بعد اضافته الى الوسط الغذائي المعد للإكثار الدقيق لهما.

تم اختيار البذور الناضجة للصنفين المذكورين كأجزاء نباتية لغرض استحثاث الكالس وتكوين الاجنة ألجسمية اذ تم زراعة هذه البذور في وسط MS المغذي و المزود بفيتامينات Gambrog والذي اضيف اليه 30غم/لتر سكروز، 5غم/لتر اكار، 2ملغم /لتر BA، 1ملغم/لتر2,4-D. ولغرض تحديد دور السوربيتول تمت اضافته الى الوسط الغذائي بالتراكيز 0، 10، 20، 30 غم/لتر.

اظهرت نتائج التجارب ان اضافة السوربيتول بالتركيز 20ملغم/لتر كان له اثر معنوي في زيادة معدل الوزن الطري للكالس وفي زيادة معدل اعداد الاجنة الجسمية الكروية للصنفين دون ان تكون هناك فروق معنوية بينهما.

يمكن الاستنتاج من الدراسة ان الوسط الغذائي الملائم لاستحثاث الكالس في الصنفين المذكورين هو الذي يتكون من MS+ فيتامينات Gambrog+ 30 غم/لتر سكروز+2 ملغم/لترBA+ 1ملغم/لتر 2,4-D +5ملغم/لتر اكار. بينما كان الوسط الملائم لاستحثاث الاجنة الجسمية هو ذات الوسط بعد استبعاد منظمي النمو BA و 2,4-D.

الكلمات المفتاحية : الحنطة، السوربيتول، الكالس، ألأجنة الجسمية.