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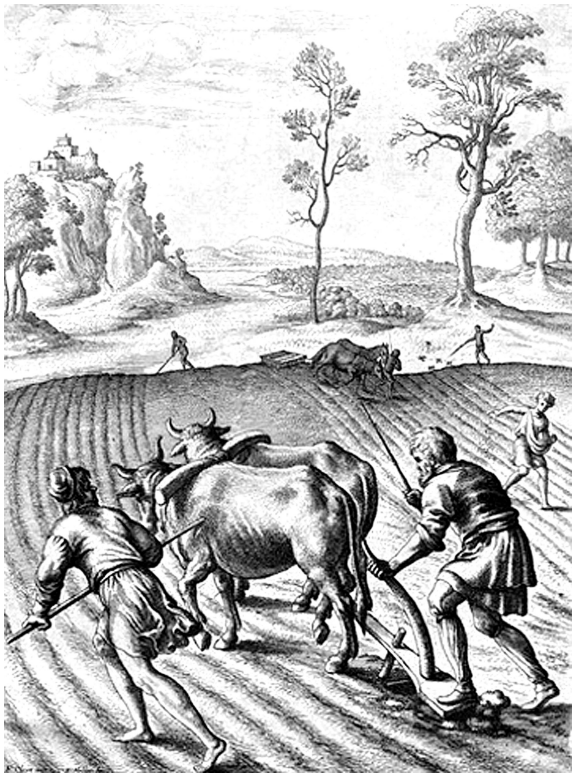

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Abscisic Acid Effects on Maize (*Zea mays* L.) Embryo Culture

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Abstract: Immature embryos of maize (*Zea mays* L.) were aseptically excised and cultured on Murashige and Skoog (MS) media containing four different Abscisic Acid (ABA) concentrations (Control 0.0, 0.1, 1.0 and 10.0 mg L^{-1}). The ABA effects on the germination of cultured embryos, number of leaves per plant, plant height, percentage of dry matter and protein expression for plants regenerated from ABA treated embryos were studied. Effects of ABA on germinating embryos were found to be associated with time; it reduced the germination during the first week. ABA affected the percentage of dry matter significantly.

Key words: Abscisic acid, maize embryo culture, *Zea mays* L.

INTRODUCTION

Embryo culture is the sterile isolation and growth of immature or mature embryos *in vitro* in order to obtain viable plants. This branch became one of important tissue culture practices with multiple goals such as, shortening the breeding cycle, overcoming seed dormancy and sterility, *in vitro* propagation and rescuing of incompatible hybrids. The use of maize (*Zea mays* L.) embryos in embryo culture experiments is due to its economic importance, its relative ease isolation and culture and due to the simple maintenance of the regenerated plants afterward.

The plant hormone Abscisic Acid (ABA) has been discovered in the middle twentieth century (Chwartz *et al*., 2003) and thought to be an auxin in the early beginnings of its use in plant tissue culture. It has been found to have important effects on the embryo culture by inhibiting the occurrence of precocious germination in embryo (White and Rivin, 2000; Pavia and de Oliveira, 1995), playing primary regulatory roles in the initiation and maintenance of seed dormancy (Molina *et al*., 2001). ABA has also some effects on genetic expression of the tissue cultured embryos (Thomas, 1993; Wilen *et al*., 1993). Moreover, ABA effects on tissue-cultured embryos could provide useful information about interactions between plant hormones (Ross and O'Neill, 2001; Hays *et al*., 1999) and the developmental processes of plant embryos (Dong *et al*., 1997). ABA plays important roles in many aspects of plant development, in the regulation of stomatal aperture and in the initiation of adaptive responses to various environmental conditions (Shinozaki *et al*., 2003; Nambara *et al*., 2002).

ABA is a sesquiterpenoid synthesized from xanthophylls (Taylor *et al*., 2000; Seo and Koshiba, 2002) and appears to influence several physiological and developmental events (Mauch-Mani and Mauch, 2005; Hsu and Kao, 2004; Borovskii *et al*., 2002; Kende and Zeevaart, 1997; Phillips *et al*., 1997; Tan, 1997; Zhang *et al*., 1997; Kapik *et al*., 1995; Xu *et al*., 1995). ABA content has been correlated with increased tolerance to chilling (Lee *et al*., 1993) and cadmium toxicity (Hsu and Kao, 2003). Also, under environmental stress conditions, cytokinin oxidized gene induction by abscisic acid results in aberrant degradation of cytokinins therefore impairing normal development (Brugière *et al*., 2003).

ABA is found in two isomers {(+)-S-ABA} and {(-)-R-ABA}. Some suggested that the effects of each form should be study independently from the other since they could have different effects and they are degraded to different compounds (Balsevich *et al*., 1994; Cramer *et al*., 1998). However, the hormone used in this experiment was a rasemic mixture of both ABA isomers, which is the usually used hormone in tissue culture experiment.

The objectives of this study, which conducted at Al-Balqa Applied University in Jordan, were to investigate the effects of ABA concentrations on the precious germination of immature maize embryos and to determine the effects of ABA concentrations on the growth and proteins expression of plants developed from these embryos.

MATERIALS AND METHODS

Media preparation: To prepare one liter of medium for each ABA concentration, 4.3 g of a commercial Murashige

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and Skoog (MS) medium was added to 200 mL distilled water. One mL of Thiamine stock (40 mg/100 mL) and 40 g of sucrose were added and the volume was brought to 800 mL using the required amount of distilled water. ABA was then added as follow: Control (0.0), 0.1, 1.0 and 10.0 mg L^{-1}. The volume was completed to one liter by the addition of distilled water and the pH was adjusted to 5.7. Eight grams of agar was added to each medium and the media were autoclaved for 15 min at 121°C, 15 psi. The media were cooled to about 50°C at room temperature and dispensed into sterile Petri dishes; each received about 20-25 mL. Petri dishes were then kept in refrigerator until using.

Embryo culture: The silks and husks of fresh maize ears (two to three weeks after pollination) were peeled. The kernels were carefully removed and transferred to a beaker and washed with running tap water for 15 min. Under the laminar airflow, the kernels were surface sterilized with 20% hypochlorite solution for 15 min and then rinsed in three changes of sterile distilled water. Young embryos (1-2 mm length) were aseptically excised from the immature kernels and placed embryo axis in contact with the culture media. Five embryos were cultured in each petri dish with total of 50 embryos in 10 petri dishes for each treatment. Petri dishes were incubated at 28°C under a 16/8 h (light/dark) photoperiod. The embryos were observed continuously to determine the effects of ABA on precocious germination.

The developed seedlings were transferred to small plastic pots containing sterilized peat moss and kept in the green house. The height of plants was measured from the surface of the pot to the top of the plant and the number of leaves per plant was counted. Samples of fresh leaves were kept for total protein isolation. The whole plants were cut (about 70 days from culturing embryos on media) and dried in an oven for 48 h at 60°C to calculate the percentage of the dry matter.

Total protein isolation and gel preparation:

Protein isolation: Ten grams of the kept leaves were washed with distilled water and then grinded by a blender in 40 mL of 0.1 Tris-HCl buffer pH 6.8. The homogenate was filtrated in a beaker through two layers of cheesecloth. The collected homogenate was kept in 50 mL plastic tube and centrifuged at 5,000 rpm for 15 min at 4°C. The supernatant was transferred to a new tube and 65% saturation of ammonium sulfate was added slowly to supernatant in order to precipitate proteins. After 1.5 h of incubation at room temperature, the supernatant was centrifuged at 5000 rpm for 15 min at 4°C. The pellet was resuspended in 5 mL Tris-HCl buffer pH 6.8. The precipitated protein was dialyzed against 2 L of 0.1 M Tris-HCl buffer pH 6.8 for overnight. The content of the dialysis tube was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant (protein) was transferred to a new tube and stored at -20°C.

Preparation of SDS-polyacrylamide gels: The resolving and stacking gels were prepared according to Sambrook and Russell (2001).

Preparation of samples: To prepare the protein samples for loading on the gel, one part of 1X SDS gel-loading buffer {50 mM Tris-HCl (pH 6.8), 100 mM dithiothreitol, 2% (w/v) SDS, 0.1% bromophenol blue, 10% (v/v) glycerol} was mixed with four parts (v/v) of each protein sample in different eppendorf tubes. The tubes were placed in a boiling water bath for 3 min to denature the protein. Twenty microliter of each sample was loaded into the bottom the gel wells.

Staining of SDS-polyacrylamide gels: The protein was separated by electrophoresis and the gel was then stained with coomassie brilliant blue dye as described by Sambrook and Russell (2001).

Data analysis: Data were analyzed statistically according to completely random design and the significant differences between means were tested by Least Significant Differences (LSD) at $p<0.05$ (Steel and Torrie, 1960).

RESULTS

The averages of germinated embryos after 7 days in culture showed significant differences among treatments (Table 2), with control, 0.1, 1.0 and 10.0 mg ABA/L gave 5, 3.8, 1.7 and 0.3 germinated embryos, respectively (Table 1). On the other hand, the effects of ABA on the total number of germinated embryos showed no significant differences among treatments; the control revealed an average of 5 germinated embryos, while the averages were 4.8, 4.4 and 4.1 for treatments 0.1, 1.0 and 10.0 mg ABA/L, respectively.

The effect of ABA concentrations on the plant height is presented in (Table 1). The results ranged between 28.5 cm for 10.0 mg ABA/L to 33.2 cm for the control. The averages for 0.1 mg and 1.0 mg ABA/L were 30.1 and 28.7 cm, respectively. These differences were statistically significant (Table 2).

The averages of developed leaves per plant were very similar among treatments with the control having the highest average (7.7), while 0.1 mg ABA/L having the

Table 1: Averages of four characters tested with four concentrations of ABA

Treatments	Germination		Leaves numbers	Plant height (cm)	Dry matter (%)
	After one week	Total (after four weeks)			
Control	5	5	7.7	33.2	18.6
0.1 mg L^{-1} ABA	3.8	4.8	7.3	30.1	17.5
1.0 mg L^{-1} ABA	1.7	4.4	7.5	28.7	16.7
10 mg L^{-1} ABA	0.3	4.1	7.4	28.5	15.6
LSD	0.67	NS	NS	2.20	1.76

Table 2: Mean squares, source of variation (SOV) and degree of freedom (DF) from analysis of variance for the effect of four ABA concentrations on four characters

SOV	df	Germination		Leaves numbers	Plant height (cm)	Dry matter (%)
		After one week	Total (after four weeks)			
Treat.	3	50.80*	1.63	0.34	48.38*	17.24*
Error	36	0.55	0.94	0.19	5.99	3.78

* Significant at 0.05

lowest (7.3). The averages were 7.5 and 7.4 leaves for 1.0 and 10.0 mg ABA/L, respectively (Table 1). These differences were not significant statistically (Table 2).

For dry matter accumulation of the plants, the averages were 18.6, 17.5, 16.7 and 15.6 for the control, 0.1, 1.0 and 10.0 mg ABA/L, respectively (Table 1). These differences were statistically significant (Table 2).

The effects of ABA on the protein patterns showed no change in the expression of the major protein for all tested treatments (Figure is not presented).

DISCUSSION

The significant effects of ABA concentrations on germination of embryos after seven days in culture could be explained by the increased ABA concentrations used in this study; this emphasizes that ABA prevent precocious germination during seed development in maize. This agree with the results of (Barlow and Pilet, 1984) who mentioned that when ABA applied to maize roots it decreases the rates of cell growth and retard cell enlargement in the meristem, which may in turn affect the rate of cell division. Beaudoin *et al.* (2000), Pilet and Barlow (1987) also indicated that ABA is an inhibitory regulator for maize root growth. On the other side, the results showed no significant differences among ABA concentrations on the total germination after 4 weeks. This could be explained by the fact that ABA is degraded rapidly by light and other factors and after many weeks in culture it may loose its effect on germination entirely or partially.

Regarding plant growth, the slightly reduced in the accumulation of the dry matter of the plants grown from immature embryos was probably due to the enhancement of the germination and growth of control compared with immature embryos treated with ABA. The results of the germination and plants height may support this, which showed that ABA concentrations delayed germination in the first week and plants height increased as ABA concentrations decreased. The results of the dry matter accumulation could be also due to the effects of ABA on some biological pathways that in turn reduced the accumulation of macromolecules.

Related to protein expression, the size and intensity of all protein bands resulted from all ABA concentrations were the same. However, this is not totally true since minor proteins expression could be altered but was not detected by the procedure used in this study, or the patterns of protein expression may be altered in other stages of the plant development like embryonic stage (Tan *et al.*, 1997). So, more precise protein isolation procedure could be used for the isolation of certain protein groups like dehydrin (Borovskii *et al.*, 2002) and proline (Jose-Estanyol and Puigdomenech, 1998) to detect the effect of ABA concentrations on their level of expression.

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