

## ABSCISIC ACID MODIFIES BORON STRESS IN CULTURED MAIZE KERNELS

NIRMALA NAUTIYAL\* AND RUBY SRIVASTAVA

Botany Department, Lucknow University, Lucknow-226007

Received on 23 Feb., 2005

### SUMMARY

Fertilized ovules of maize (*Zea mays* L.) were cultured at 14 days after anthesis in MS medium devoid of growth regulators. The developing kernels were supplied boron at three levels 0.01mM (low), 0.1mM (normal) and 0.5mM (high), each without or with 1mg l<sup>-1</sup> abscisic acid (ABA). Growth of kernels, after 8 days in culture, was observed as increase in length, breadth, fresh and dry weight with concomitant decrease in concentration of sugars and increase in that of nitrogen, protein and starch. Maize kernels cultured in absence of ABA showed growth enhancement at low B and poor growth at high B from those at normal B. In kernels at low B the concentration of sugars and phenols increased and that of nitrogen, proteins and starch decreased, while at high B protein nitrogen and starch contents increased. A supply of ABA increased the level of seed reserves and almost reversed the effects of boron stress in maize kernels cultured *in vitro*.

**Key words:** Abscisic acid, boron stress, *in vitro* culture, maize kernels, seed reserves.

### INTRODUCTION

Deficiency as well as toxicity of boron decline the seed yield more than the vegetative yield in crop plants (Mengel and Kirkby 1987). The response to low boron on seed development varies in different plant species. Boron deficiency decreases the seed yield, pod number; number of seeds in each pod, but in some species increases the seed size (Dear and Lipsett 1987, Rerkasem and Loneragan 1994). Seeds produced in plants grown in low B soils, even when they do not show reduction in weight, are low in quality as they are poor in viability and produce high percentage of abnormal seedlings (Bell *et al.* 1989). The intricate relationship between the boron supply and seed development needs to be explored. Culture of developing seed or ovule culture is one of the methods used for investigating the role of micronutrients in seed development. Isolated fertilized ovules containing proembryo can be grown successfully up to the formation

of new plants in absence of growth supplements (Matthys Rochon *et al.* 1998). Fertilized ovules of cotton cultured *in vitro* responded to hormones. Their growth was stimulated significantly by GA<sub>3</sub> and less significantly by IAA addition (Beasley *et al.* 1974). ABA addition to the medium reduced the amount of fiber induced by IAA whereas kinetin partially overcame the inhibition caused by ABA. Deletion of boron in culture medium prevented the fiber elongation and promoted callus formation in fertilized cotton ovules cultured *in vitro* (Beasley *et al.* 1974).

ABA level in seeds increases during late stages of embryogenesis and declines during maturation of drying (King 1982, Quatrane 1987, Kermod 1990). Exogenous application of ABA to developing embryo in culture induces desiccation tolerance, prolongs developmental processes and prevents precocious germination (Kermod 1990). In seeds abscisic acid increases the desiccation

\* Corresponding author

tolerance or osmotic stress and induces expression of specific (stress) genes (Skriver and Mundy 1991) and represses synthesis of proteins normally present (Marschner 1995).

This paper describes the growth and development of fertilized maize ovules or kernels 14 days after anthesis in tissue culture at three boron levels in presence or absence of ABA. The paper also reports the concentration of carbohydrate and nitrogen fractions in cultured seeds as influenced by boron and its interaction with exogenous ABA.

### MATERIALS AND METHODS

Maize (*Zea mays* L.) was grown in soil under natural conditions. Ears were harvested 14 days after anthesis (DAA) and fertilized ovules from middle part of the ear were taken as explants. The culture medium used in the study was Murashige and Skoog (MS) medium (1962), without IAA and kinetin and without or with (1 mg l<sup>-1</sup>) ABA. The macro and micronutrient salts used for preparation of the media were taken as AR salts and except for EDTA and KI were recrystallised to minimize the B content. Boron was supplied as H<sub>3</sub>BO<sub>3</sub> at three levels, low (0.01mM), normal (0.1mM) and high (0.5mM). All glasswares and the culture media were sterilized in an autoclave at 103 kPa at 120°C for 30 – 45 minutes. Each culture flask contained 50ml culture medium.

The surface sterilization of the explants was carried out before excision from the ear with 0.1% mercuric chloride for 5 min. The mercuric chloride from the surface was removed by repeated washings with sterile distilled water. Aseptic conditions were maintained during the sterilization and transfer of the explant to the media.

The flasks containing developing seeds were placed in a BOD incubator at 25±1°C provided with lights of 70 Wm<sup>-2</sup> for 16 h photoperiod. Three flasks were taken for each treatment and five ovules were cultured in each flask. Growth parameters were studied on the change in size, shape and color of kernels after 8 days in culture.

The fresh and dry weights were determined before and after drying the kernels in an electric oven at 80°C

for 48 h. The material for determination of carbohydrate and nitrogen fractions was fixed in 80% (v/v) boiling ethanol (1:10) and ground at room temperature. Sugars, phenols and non-protein nitrogen were determined in the alcohol soluble fraction, while the alcohol insoluble fraction was used for the estimation of starch and protein nitrogen content. Sugars and phenols from the alcohol soluble fraction were transferred into aqueous phase and the extract was cleared using lead acetate and precipitation of excess lead with dibasic sodium phosphate. Reducing and total sugars in the filtrate were estimated before and after hydrolysis of the extract with 1% invertase (Nelson 1944). The non-reducing sugar content was calculated by subtracting reducing sugar from the total sugar contents. Total phenols in the extract were determined using Folin-phenol reagent (Swain and Hillis 1959). Starch from the alcohol insoluble fraction was extracted in perchloric acid and estimated colorimetrically according to the procedure of Montgomery (1957).

The nitrogen content in both alcohol soluble and insoluble fractions was determined by semi-micro Kjeldahl method. Both fractions were digested separately in 5% salicylic acid in concentrated H<sub>2</sub>SO<sub>4</sub> (Chibnall *et al.* 1943). The nitrogen content in the digest was estimated by distillation as ammonia in Markam's apparatus and collected in borate buffer pH 4.8 and titrated against N/140 H<sub>2</sub>SO<sub>4</sub>.

### RESULTS AND DISCUSSION

*In-vitro* growth of maize kernels was observed after 8 days in culture in MS medium (1962) without growth regulators as increase in size, fresh and dry weight from that observed before culture (Table 1). The increment in kernel size was more at low (0.01mM) boron than normal (0.1mM) and high (0.5mM) boron. The term normal boron is applied in relation to the boron supply in the widely used MS medium and low and high boron levels are based on this. The increase in the size of kernels in response to low boron has been reported earlier in wheat (Rerkasem and Loneragan 1994) and subterranean clover (Dear and Lipsett 1987). The reduction in starch concentration was associated with sugar accumulation at low boron might be due to non-utilization of sugars and stimulation in starch

**Table 1.** Growth parameters of maize kernels at variable concentrations of boron and ABA.

Boron supply (mM)	Before culture	After culture	
		- ABA	+ ABA
<b>Length (mm)</b>			
0.01		8.3±0.3	7.3±0.3
0.1	6.3±0.2	8.0±0.0	8.0±0.0
0.5		7.7±0.3	8.0±0.3
<b>Breadth (mm)</b>			
0.01		6.3±0.3	5.8±0.2
0.1	5.7±0.3	7.2±0.2	6.3±0.3
0.5		6.2±0.2	5.3±0.3
<b>Fresh weight (mg)</b>			
0.01		235±3.7	226±5.8
0.1	178±8.6	215±2.9	217±1.5
0.5		208±6.7	204±6.0
<b>Dry weight (mg)</b>			
0.01		65±1.3	66±1.0
0.1	37±2.1	61±4.4	62±1.2
0.5		40±0.9	50±2.6

± SEm

phosphorylase activity as has been reported by Chatterjee *et al.* (1987) in maize grown in sand culture. The increase in length, fresh and dry weight of kernels at low B in absence of ABA was reversed by ABA (1mg l<sup>-1</sup>) addition to the medium. ABA might have lowered the sink activity and caused premature ripening of grains at low boron thus as a consequence the size and weight of maize kernels was decreased. At the same time concentrations of reducing sugars, starch, phenols, protein and non-protein nitrogen increased and that of non-reducing sugars decreased in boron deficient kernels in presence of ABA (Tables 2 and 3; Fig.1). The decline in sink activity in boron deficient kernels in presence of ABA is similar to the reports of Haeder and Beringer (1981) in K deficient wheat. The increase in the level of both nitrogen fractions in B deficient maize kernels might be the result of increase in permeability of the membrane as has been reported earlier (Glinka and Reinhold 1971) in presence of ABA.

**Table 2.** Sugar concentration (mg g<sup>-1</sup> fw) in maize kernels at variable concentrations of boron and ABA.

Boron supply (mM)	Before culture	After culture	
		- ABA	+ ABA
<b>Reducing sugars</b>			
0.01		3.50±0.25	4.24±0.07
0.1	5.55±0.21	2.85±0.28	2.70±0.13
0.5		4.92±0.35	2.55±0.03
<b>Non- reducing Sugars</b>			
0.01		6.23±0.17	1.29±0.23
0.1	13.31±1.21	4.12±0.02	8.23±0.38
0.5		5.61±0.03	7.58±0.33
<b>Total sugars</b>			
0.01		9.73±0.82	5.53±0.30
0.1	18.85±1.00	6.97±0.47	10.93±0.50
0.5		10.53±0.10	10.13±0.30

± SEm

**Table 3.** Nitrogen concentration (% fw) in maize kernels at variable concentrations of boron and ABA.

Boron supply (mM)	Before culture	After culture	
		- ABA	+ ABA
<b>Non -protein nitrogen</b>			
0.01		0.13±0.02	0.22±0.03
0.1	0.06±0.02	0.25±0.01	0.11±0.0
0.5		0.16±0.03	0.12±0.0
<b>Protein nitrogen</b>			
0.01		2.37±0.04	3.59±0.11
0.1	1.16±0.02	2.27±0.05	2.49±0.05
0.5		3.86±0.31	2.77±0.04
<b>Total nitrogen</b>			
0.01		2.50±0.05	3.81±0.09
0.1	1.22±0.06	2.52±0.04	2.60±0.05
0.5		4.02±0.34	2.89±0.04

± SEm

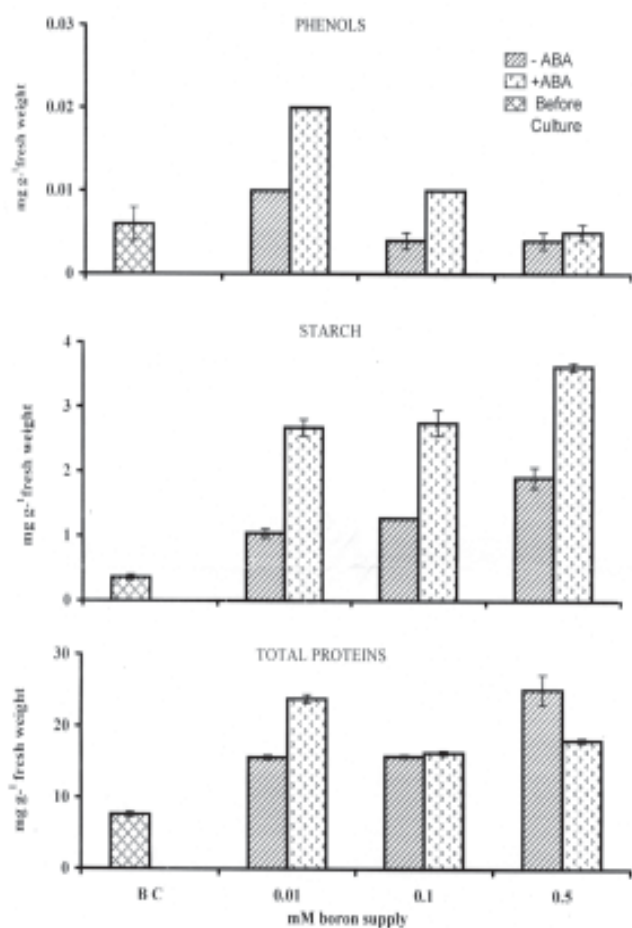


Fig. 1. Concentration of phenols, starch and total proteins ( $\pm$  SEM) in maize kernels before (BC) and after culture (AC) at variable concentrations of boron and ABA

The concentration of phenols in maize kernels increased at low B from that of other two B levels. ABA addition enhanced the level of phenols most significantly in the boron deficient kernels (Fig.1). This could be due to the inhibitory effect of ABA on utilization of sugars at low boron resulting in accumulation of reducing sugars as well as total phenols. The results support that phenol biosynthesis is stimulated in boron deficiency due to increase in the polyphenol oxidase activity and  $K^+$  efflux (Marschner 1995).

Excess supply of boron (0.5mM) was inhibitory for the *in vitro* growth of maize kernels in the presence of ABA. In boron excess size and weight of kernels were reduced and the concentration of sugars, starch and

nitrogen increased from that of normal boron. The results show that excess boron might have enhanced the levels of proteins by reducing the proteinase activity and that of starch by decreasing the starch phosphorylase activity. High sugar content at excess boron in absence of ABA might be due to their complexation with boron inhibiting the growth and decreasing the grain weight. These results further substantiate the involvement of boron in maintaining the carbohydrate flux between glycolysis and Pentose Phosphate Pathway (Marshner 1995). ABA addition modified the response to excess B by increasing the length and dry weight of kernels and decreasing the concentration of reducing sugars, protein and non-protein nitrogen, which were otherwise accumulated in maize kernels at excess B.

Boron deficiency often increases the IAA concentration from the normal level (Coke and Whittington 1968) and the boron deficiency symptoms resemble with that of higher auxin levels (Robertson and Loughman 1974). The results obtained here show that the increased seed weight in boron deficiency might be due to higher IAA levels in kernels in absence of ABA (Birnbaum *et al.* 1973). ABA addition counteracted the response to low boron resulting in decreased kernel weight and increased starch, sugar and protein contents. The inhibitory effect of ABA in modifying the response to low boron might be due to lowering the effect of IAA (Dunlap and Robacker 1990) by reducing the activity of membrane bound  $H^+$  pumping ATPase resulting in enhanced  $H^+$  extrusion. Boron deficiency has been reported to favour  $H^+$  excretion induced by IAA in tobacco cell suspension cultures (Goldbach *et al.* 1990).

## REFERENCES

- Beasley, C.A., Ting, I.P., Linkins, A.E. and Birnbaum, E.H. (1974). Cotton ovule culture: A review of progress and a preview of potential. In: H.E. Street (ed.), Tissue Culture and Plant Science, pp 169-192. Academic Press, London and New York.
- Bell, R.W., Malay, L., Plaskett, D., Dell, B. and Loneragan, J.F. (1989). Germination and vigor of black gram (*Vigna mungo* L. Hepper) seeds from plants grown with and without boron. *Aust. J. Agric. Res.* **40**: 273-279.

## BORON STRESS IN MAIZE KERNELS

- Chatterjee, C., Sinha, P., Nautiyal, N., Agarwala, S.C. and Sharma, C.P. (1987). Metabolic changes associated with boron-calcium interaction in maize. *Soil. Sci. Plant Nutr.* **33**: 607-617.
- Chibnall, A.C., Rees, M.W. and Williams, E.F.(1943). The total nitrogen content of egg albumin and other proteins. *Biochem. J.* **37**: 354-359.
- Coke, L. and Whittington, W.J. (1968). The role of boron in plant growth IV. Interrelationships between boron and indole-3-acetic acid in the metabolism of bean radicles. *J. Exp. Bot.* **19**: 295-308.
- Dear, B.S. and Lipsett, J. (1987). The effect of boron supply on the growth and seed production of subterranean clover (*Trifolium subterraneum* L.). *Aust.J.Agric.Res.* **38**: 537-546.
- Dunlap, J.R. and Robacker, K.M.(1990). Abscisic acid alters the metabolism of indole-3- acetic acid in senescing flowers of *Cucumis melo* L. *Plant Physiol.* **94**: 870-874.
- Glinka, Z and Reinhold, L.(1971). Abscisic acid raises the permeability of plant cells to water. *Plant Physiol.* **48**:103-105.
- Goldbach, H.E., Hartmann, D. and Roetzer, T. (1990). Boron is required for stimulation of the ferricyanide induced proton release by auxins in suspension- cultured *Daucus carota* and *Lycopersicon esculentum* *Physiol. Plant.* **80**: 114-118.
- Haeder, H.E. and Beringer, H. (1981). Influence of potassium nutrition and water stress on the abscisic acid content in grain and flag leaves during grain development. *J. Sci. Food Agri.* **32**: 552-556.
- Kermode, A.R. (1990). Regulatory mechanisms involved in the transition from seed development to germination. *Critical Rev. Plant Sci.* **9**:155-195.
- King, R.W. (1982). Abscisic acid and seed development. In: A.A. Khan (ed.), *The Physiology and Biochemistry of Seed Development, Dormancy and Germination*, pp. 157-181. Elsevier Biomedical Press, Amsterdam.
- Marschner, H. (1995). *Mineral Nutrition of Higher Plants* (2<sup>nd</sup> Ed.) Academic Press, New York.
- Mengel, K. and Kirkby, E.A. (1987). *Principles of Plant Nutrition*. International Potash Institute, Switzerland.
- Matthys- Rochon, E., Piola, F., Deunff, E. Le., Mol, R. and Dumas, C.(1998). *In vitro* development of maize immature embryos: a tool for embryogenesis analysis. *J. Exp. Bot.* **49**: 839-845.
- Montgomery, R. (1957). Determination of glycogen. *Arch. Biochem. Biophys.* **67**: 378-386.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-497.
- Nelson, N. (1944). Photometric adaptation of Somogyi method for determination of glucose. *J. Biol. Chem.* 153-375.
- Rerkasem, B. and Loneragan, J.F. (1994). Boron deficiency in two wheat genotypes in a warm, subtropical region. *Agron. J.* **86**: 887-890.
- Robertson, G.A. and Loughman, B.C. (1974). Response to boron deficiency: A comparison with responses produced by chemical methods of retarding root elongation. *New Phytol.* **73**: 821-832.
- Shelp, B.J. (1988). Boron mobility and nutrition in broccoli (*Brassica oleracea* var. Italica). *Ann. Bot.* **61**: 83-91.
- Skriver, K. and Mundy, J. (1990). Gene expression in response to abscisic acid and osmotic stress. *Plant Cell* **2**: 503-512.
- Swain, T. and Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. *J. Sci. Food Agri.* **10**: 65-82.