BORON CONCENTRATION GRADIENT IN THE ENDOSPERM AND EMBRYO DURING OVULE DEVELOPMENT IN *CLIVIA MINIATA*

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ABSTRACT

Research on boron concentration gradient in the endosperm and embryo of *Clivia miniata* Regel ovules is a continuation of investigations on polar distribution of several compounds in the developing ovule. The polar distribution of chemical compounds in the ovule might constitute one of the factors determining embryogenesis.

It was found that: a) there occured a chalaza-micropyle gradient of boron concentration in the endosperm during the exponential phase of embryo growth (age of ovules – 50 to 120 days); boron concentration in the endosperm increased from 12.13 to 23.71 μg g^{-1} fr.wt. (micropylar part) and from 13.58 to 28.42 μg g^{-1} fr.wt. (chalazal part); the embryo elongated towards the chalazal part of the ovule, i.e. towards higher boron concentration in the endosperm; b) in the embryo a reversed gradient of boron was detected: a higher concentration in the micropylar part (decrease from 127.29 to 61.78 μg g^{-1} fr.wt.; ovules 86-123 days old), a lower one in the chalazal part (decrease from 99.47 to 53.16 μg g^{-1} fr.wt.; the exponential phase of embryo growth).

KEY WORDS: *Clivia miniata* Regel, gradient of boron concentration, endosperm, embryo.

INTRODUCTION

In developing ovule of higher plants the occurrence of gradients of certain factors along the chalaza – micropyle axis is postulated to induce polarity and thus influence morphogenesis of the zygote (Wardlaw 1955, Rodkiewicz 1973). In the endosperm and embryo of monocotyledonous plants Ryczkowski has found the existence of gradients of osmotic value, respiration rate, concentration of reducing sugars, amino acids and macro- and microelements (K, Ca, Mg, Fe, Mn, Zn, Cu) (Ryczkowski 1967, 1978, 1980, 1981, Ryczkowski and Reczynski 1988). All mentioned factors showed higher values in the chalazal part of the endosperm than in the micropylar part. In the embryo some of the gradients had the same direction as in the endosperm, while others had a reversed direction (Ryczkowski 1978, 1980).

Among micronutrients vital for plant growth there are no quantitative data concerning the existence of boron concentration gradient in the developing ovule. Boron plays a very important physiological role influencing: pollen tube growth, carbohydrates transport and metabolism, cell wall synthesis and structure, lignification, RNA metabolism, respiration, IAA metabolism ect. (Dugger 1983, Polster and Schwenk 1992, Ferrol and Donaire 1992, Loomis and Durst 1992, Barr et.al. 1993).

This paper contains the results of investigations on boron concentration gradient along the micropyle – chalaza axis in endosperm and embryo during the exponential phase of embryo growth.

MATERIAL AND METHODS

The ovules of *Clivia miniata* Regel were used as experimental material. The ovule and embryo dimensions and ovule age, counted from the day the perianth dropped till the day of sampling, were the chosen developmental criteria. Determination of boron concentration in the endosperm was made in ovules aged 50 through 123 days, i.e. during the second part of inhibitional, and exponential phases of embryo growth. Quantitative determination of boron in the embryo was performed during the exponential phase of its growth (ovules 87-123 days old) (Fig. 1).

The technique of endosperm and embryo preparation and their division into chalazal and micropylar parts was described earlier (Ryczkowski 1967). The obtained parts of endosperm and embryo were weighed (fresh weight), dried to a constant weight (dry weight) in a vacuum dryer at 60^{0} C, powdered in an agate mortar and stored in a dessicator. Boron concentration was determined by the method of Garcia et.al. (1985). Absorbance of the anionic complex of boric acid with 2,6 – dihydroxybenzoic acid, associated with cationic dye (crystal violet), was measured by means of Spectromom 195 (Hungary) spectrophotometer at 600 nm against the reagent blank.

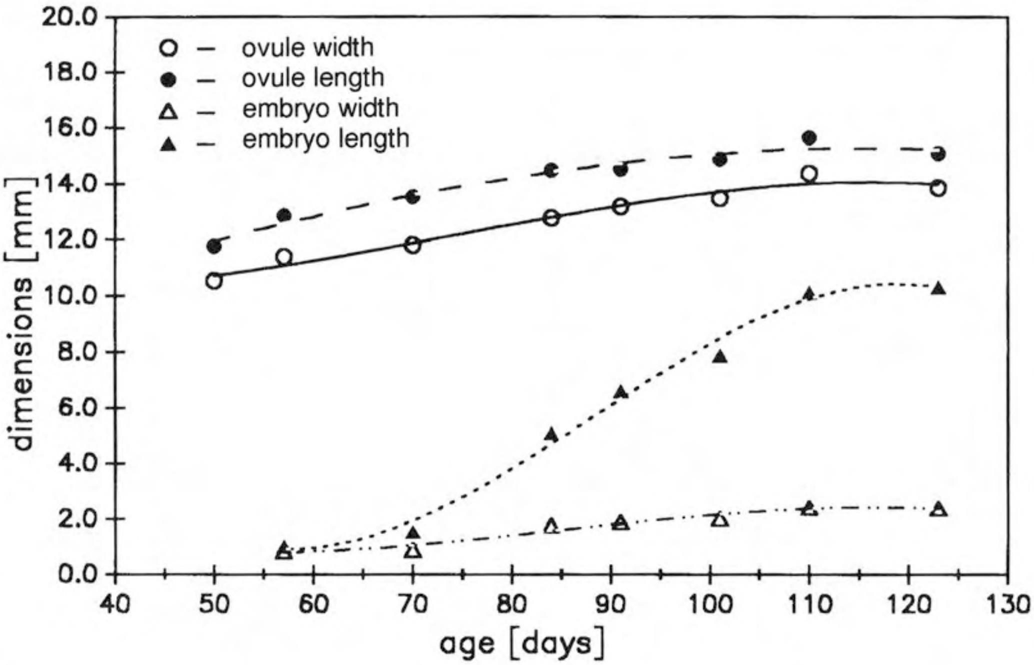

Fig. 1. *C. miniata.* Ovule and embryo dimensions.

Each value of boron concentration is the mean of at least two separate determinations. Standard deviation in Fig. 2 A and 2 B is represented by the vertical bars.

RESULTS

Endosperm

From the 50-th day after perianth dropped until the end of analysis (123-rd day of embryo exponential growth) boron concentration in the endosperm increased: in the micropylar part from 12.13 to 23.71 μg g^{-1} fr.wt. while in the chalazal part from 13.58 to 28.42 μg g^{-1} fr.wt. Boron concentration was higher in the chalazal than in micropylar part (Fig. 2 A).

Embryo

Concentration of boron in the micropylar part of the embryo (Mpe) decreased from 127.29 μg g^{-1} fr.wt. (84 days old ovules) to 61.78 μg g^{-1} fr.wt. (123 days old ovules). Boron concentration in the chalazal part of the embryo (Chpe) decreased from 99.47 μg g^{-1} fr.wt. (84 days old ovules) to 40.07 μg g^{-1} fr.wt. (110 days old ovules) and slightly increased to 53.16 μg g^{-1} fr.wt. in 123 days old ovules (Fig. 2 B).

In comparison to the endosperm, in the embryo the reversed gradient of boron concentration was found. During the whole examined period of embryogenesis a higher concentration of boron was in the micropylar part than in the chalazal part of the embryo.

DISCUSSION

The evident chalaza – micropyle gradient of boron concentration in endosperm was probably connected with the mechanism of transport of nutrients to the ovule and, on the other hand, with the function of endosperm in relation to the developing embryo. Nutrients are delivered from the vegetative parts of the plant to the chalazal part of the ovule (Thorne 1985) and are further distributed to the micropylar part of the endosperm. Thus, higher boron concentration in the chalazal part of endosperm might result from a better supply of the element from mother plant. On the other hand, endosperm cells neighboring the embryo are intensively digested (Rodkiewicz 1973, Schel et al. 1984, Szkukalek et al. 1989). In other areas of the endosperm an intensive formation of cell walls and synthesis of reserve substances takes place. As boron influences the above mentioned processes through regulation of carbohydrates polymerization, metabolism of peptides, nucleic acids and hormones (Dugger 1983, Loomis and Durst 1992) a higher boron concentration may be expected in the chalazal part of endosperm.

The last, but not least, factor, which might cause lower concentration of boron in the micropylar part of endosperm, was the vicinity of the embryo. During exponential growth the embryo has high demands for nutrients, among them boron, and absorbs these nutrients from the surrounding micropylar part of endosperm. The high embryo demand for boron is manifested by many times higher concentration of this element in the embryo than in endosperm.

The general increase in boron concentration in endosperm during embryo exponential growth was connected with development and accumulation of nutrients in endosperm. In the latter part of the embryo exponential growth (ovules 110-123 days old) a more intensive increase of boron concentration in the endosperm was detected. It was connected with gradual dehydration and slowing down of endosperm development. The slower embryo development favored also boron accumulation in the endosperm.

The direction of boron concentration gradient in the endosperm agreed with the gradients of osmotic value, respiration rate and concentration gradients of free amino acids, macro-

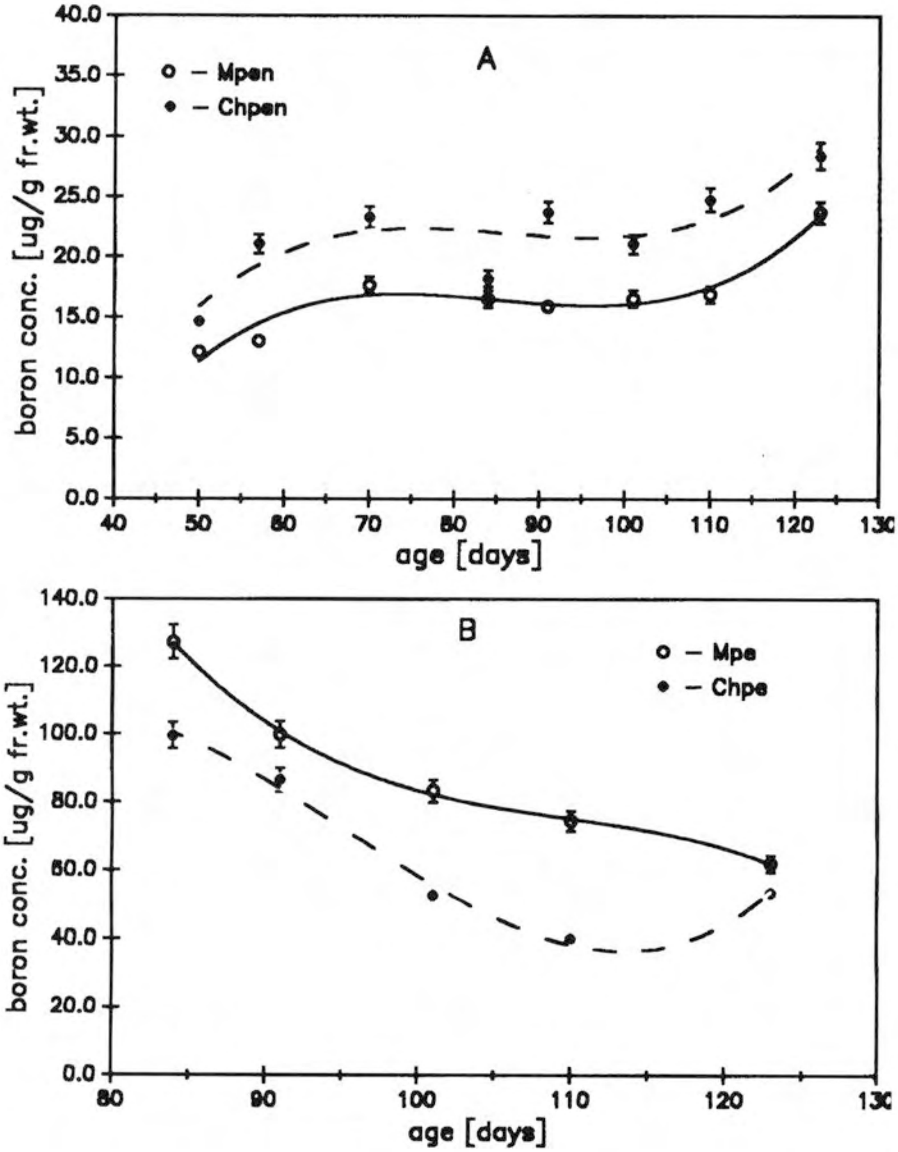

Fig. 2 A-B. Boron concentration in the micropylar (Mpen) and chalazal (Chpen) parts of the endospem (inhibitional and exponential phase of embryo growth) and in the micropylar (Mpe) and chalazal (Chpe) parts of the embryo (exponential growth phase).

and micronutrients (Ryczkowki 1967, 1978, 1980, 1981, Ryczkowski and Reczyński 1988).

Boron concentration in the embryo was several times higher than in the endosperm and decreased gradually during the exponential phase of embryo growth. This decrease was coupled with intense growth of the embryo and with the synthesis of structural and reserve compounds. In that phase of embryo development, boron delivery to the embryo was not sufficient in relation to embryo requirements. Boron is considered as an immobile element in plants, hence, its deficiency is observed mainly in young, differentiating organs (Dugger 1983). During the first part of the stationary phase of embryo growth a substantial increase of boron concentration was found (Reczyński and Ryczkowski 1994), probably as a result of slower embryo growth.

In the *C. miniata* embryo the direction of boron concentration gradient was opposite to that found in the endosperm. Nevertheless, it was consistent with the gradient of respiration rate (Ryczkowski 1981) and Ca, Mg, Mn and Cu concentration gradients (Ryczkowski and Reczynski – unpublished data). Such direction of boron and other gradients in the embryo (higher concentration in the micropylar than in the chalazal part) results most probably from the fact, that the micropylar part of the embryo comprises vital parts of the future plant, i.e. shoot tip, leaves primordia and root cap. The chalazal part of the embryo embodies only a major part of the cotyledon, in which processes of differentiation gradually decline (Erdelska and Ryczkowski 1972).

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GRADIENT STĘŻENIA BORU W BIELMIE I ZARODKU PODCZAS ROZWOJU ZALĄŻKA *CLIVIA MINIATA*

STRESZCZENIE

Badania nad gradientem stężenia boru w bielmie i zarodku zalążków *Clivia miniata* Regel są kontynuacją badań nad polarnym rozmieszczeniem szeregu związków w rozwijającym się zalążku. Polarne rozmieszczenie związków chemicznych w zalążku może stanowić jeden z czynników wpływających na embriogenezę.

Stwierdzono: a) istnienie gradientu chalaza-mikropyle stężenia boru w bielmie podczas wykładniczej fazy wzrostu zarodka (wiek zalążków – 50-120 dni); stężenie boru wzrosło w badanym okresie embryogenezy w części mikropylarnej bielma z 12.13 do 23.71 $\mu g\ g^{-1}$ fr.wt., a w części chalazalnej z 13.58 do 28.42 $\mu g\ g^{-1}$ fr.wt.; zarodek wydłużał się w kierunku chalazalnym t.j. w kierunku wyższego stężenia boru w bielmie; b) w zarodku stwierdzono odwrotny gradient stężenia boru: wyższe stężenie w części mikropylarnej (spadek z 127.29 do 61.78 $\mu g\ g^{-1}$ fr.wt., wiek zalążków – 86 do 123 dni), niższe w części chalazalnej (spadek z 99.47 do 53.16 $\mu g\ g^{-1}$ fr.wt., ten sam okres wykładniczej fazy wzrostu).

SŁOWA KLUCZOWE: *Clivia miniata* Regel, gradient stężenia boru, bielmo, zarodek.