

**INITIATION AND DIFFERENTIATION OF FLORET  
PRIMORDIA IN NATURALLY WOUNDED CAPITULA  
OF BORON DEFICIENT SUNFLOWER  
(*Helianthus annuus* L.) PLANTS**

---

L. F. Hernández\*

---

*Departamento de Agronomía, Universidad Nacional del Sur,  
CIC de la Provincia de Buenos Aires, 8000, Bahía Blanca, Argentina*

*Received: April 08, 2002*

*Accepted: November 23, 2002*

SUMMARY

The effect of boron (B) deficiency in sunflower (*Helianthus annuus* L.) plants on early capitulum development was studied. Plants were grown under artificial conditions on an 18 h long-day photoperiod in B-deficient soil mixture (<0.1 ppm B) irrigated with B-deficient or complete Hoagland's solution (0.27 ppm B). B-deficient plants were transferred 25 days after seedling emergence (DAE) to a B-rich soil mixture (2.0 ppm B) and irrigated with complete B Hoagland's solution.

Compared with controls, plants grown in B-deficient soil mixture had 18% less leaf area, 25% less receptacle area, and 33% less shoot dry weight; all differences were statistically significant. In both treatments the generation of new floret primordia started at the capitulum rim at 35 DAE and continued towards its center. Each floret primordium differentiated gradually and centripetally over space and time, first as a slight dome that became a two-part structure, the bract and the floret corolla. Capitulum damage was observed in B-deficient plants early during floret differentiation, resulting in the onset of surface splits on the receptacle at floral stage (FS) 4 (28-30 DAE). The edges of these splits acted as centres of floret primordium differentiation which developed centrifugally and resulted in aberrant capitula and the development of ray flowers and involucre bracts in abnormal positions on the inflorescence. This paper provides a detailed description of the beginning of floret primordium differentiation during the floral morphogenetic process in sunflower capitula where damage was observed in plants grown under boron stress.

**Key words:** boron, capitulum, *Helianthus*, morphogenesis, sunflower

---

\* Corresponding author: Fax: 54 0291 459 5127, e-mail: lhernan@criba.edu.ar

## INTRODUCTION

The cultivated sunflower is highly sensitive to boron deficiency (Schuster and Stephenson, 1940; Blamey *et al.*, 1979, 1987). Boron deficiency in field-grown sunflower has been more frequently reported in soils of low organic matter content from the sandy west of Argentina (Diaz-Zorita and Duarte, 1998). It has been recognized that this area can have a potential problem of economic significance (Diggs *et al.*, 1988; Ratto de Miguez and Diggs, 1990).

Boron deficiency can also affect crop development in new sunflower growing areas of seed production with high soil pH and coarse soil texture (Paoloni and Hernández, personal communication, 2000). In these areas, large grain yields after fertilization with N and optimum management may also enhance the deficiency symptoms (Blamey, 1976). Boron deficiency in sunflower produces different symptoms ranging from severe cases of seedling emergence failures or seedling death (Blamey, 1976), to capitulum malformation (Palmer and Marc, 1982; Blamey, 1976; Palmer *et al.*, 1988; Palmer, 1998). Sunflower genotypes have also been found to differ in sensitivity to B deficiency (Blamey *et al.*, 1979), and the magnitude of damage could vary with genotype sensitivity (Ungaro *et al.*, 1988).

Boron deficiency during floret initiation in sunflower generally results in damage to the capitulum meristem and consequently causes the appearance of involucre bracts and ray florets in different positions at the capitulum center (Blamey, 1976; Palmer and Marc, 1982).

Palmer and Marc (1982) also reported this effect after pin-prick wounding of the undifferentiated receptacle surface in floral stage (FS) 4, 5 or 6, i.e., 30, 32 or 35 days after seedling emergence, respectively (Marc and Palmer, 1981). They concluded that in capitula from B-deficient plant, the rims of the wounds could provide first sites for organ initials (Palmer and Marc, 1982). Hernández and Palmer (1988) speculated further and found a response before floret differentiation commenced and after a circular wound of the receptacle surface was made. Hernández and Green (1993) and Hernández (1997) proposed that the wound rims could act as regenerating centers where the organogenetic process could begin. Palmer and Marc (1982) also observed malformed inflorescences under B deficiency, but they did not follow the morphogenetic process under B stress over time. Here, a sequential description is reported of the differentiation of newly arising florets in abnormal positions in capitula from plants under B stress. The implications of these findings for organogenesis of the sunflower capitulum have been extensively discussed (Hernández and Palmer, 1988; Hernández and Green, 1993; Hernández, 1997).

## MATERIALS AND METHODS

### Plant culture

Sunflower plants (*Helianthus annuus* L.) cv. Cargill 208A were grown under controlled environmental conditions with a light/dark regime of 18/6 h, air temperature of 25°C, photon flux of about 580  $\mu\text{mol s}^{-1} \text{m}^{-2}$  PPFD and a relative humidity of about 80% in 2 l plastic pots that contained a B-deficient (<0.1 ppm) soil mixture made of sand and perlite (1:1 w/w). Plants were watered daily, and 15 ml of incomplete (no B) Hoagland's solution (Hoagland and Arnon, 1950) were added every third days to maintain an optimal level of nutrients, except for B. Control plants were supplied the same regime with complete Hoagland's solution (0.27 ppm of B). The pH of the nutrient solution applied in both treatments ranged from pH 6.8 to 7.0. All the plants were repotted after 25 days to 4 l plastic pots containing a B-rich (2.0 ppm) soil mixture. The plants were maintained under automatic irrigation and supplemented with complete Hoagland's solution until sampling. To avoid contamination with B, all water used for preparing nutrient solutions and reagents for analysis were double distilled in a quartz apparatus and all chemicals were of analytical grade.

### Morphological observations

Starting at 23-25 DAE when the capitula reached FS 3 (visual scale of early inflorescence development after Marc and Palmer, 1981), the last-formed apical leaves and involucral bracts were removed and the incipient floral meristem (receptacle) was exposed. Sequential molds (Green and Linstead, 1990) of the receptacle surface were obtained (Hernández and Green, 1993). To obtain a primary mold, the exposed meristem was covered with a polyvinyl impression material, SDS Kerr's extrude wash (Sybron Dental Specialties, West Collins Orange, CA). The primary mold was removed after 10 min with tweezers. This method of producing the molds was applied twice a day to ten plants for each treatment for a period of 18 days. The mold was inverted, affixed to a slide and a cast was made. The molds were completely filled with Spurr's resin (hard mixture) and placed in an oven at 70°C for 72 h. After hardening, the casts were mounted on scanning electron microscope (SEM) stubs, and sputter-coated with gold. To protect the apex from desiccation, the receptacle meristem was immediately covered with a cap made from wet tissue paper and aluminium foil.

The sputter-coated replicas were examined in a Philips 505 scanning microscope at 10 kV and photographed. Details of its morphology, floret differentiation and pattern formation in capitula were then followed over time (Hernández, 1997).

Plants were harvested at 65 DAE when the capitula reached FS 8 (Marc and Palmer, 1981) and measurements made of the receptacle area, leaf area and dry weights of leaves and stems. Plant leaf area was measured using a LI-COR LI-

3000A area meter. Mean leaf and stem dry weights were obtained by drying the samples in an oven at 60°C for 48 h.

## RESULTS AND DISCUSSION

Control plants did not show any vegetative or reproductive morphological abnormalities. At 15 DAE all the plants in the B-deficient soils exhibited recognizable symptoms of boron deficiency, whereas none of the plants of controls showed symptoms of injury. By 22 DAE, 82 plants in the B-deficient soil showed typical B deficiency injury of limited aerial growth, most likely because of poor root system development (Hirsch and Torrey, 1980; Hirsch *et al.*, 1982) (Table 1). The effect of B-deficiency on the reduction of the total leaf area was significant (Table 1). The leaves were darker and broader in B-deficient plants than in controls. The total area of the receptacle was also significantly reduced in B-deficient plants (Table 1).

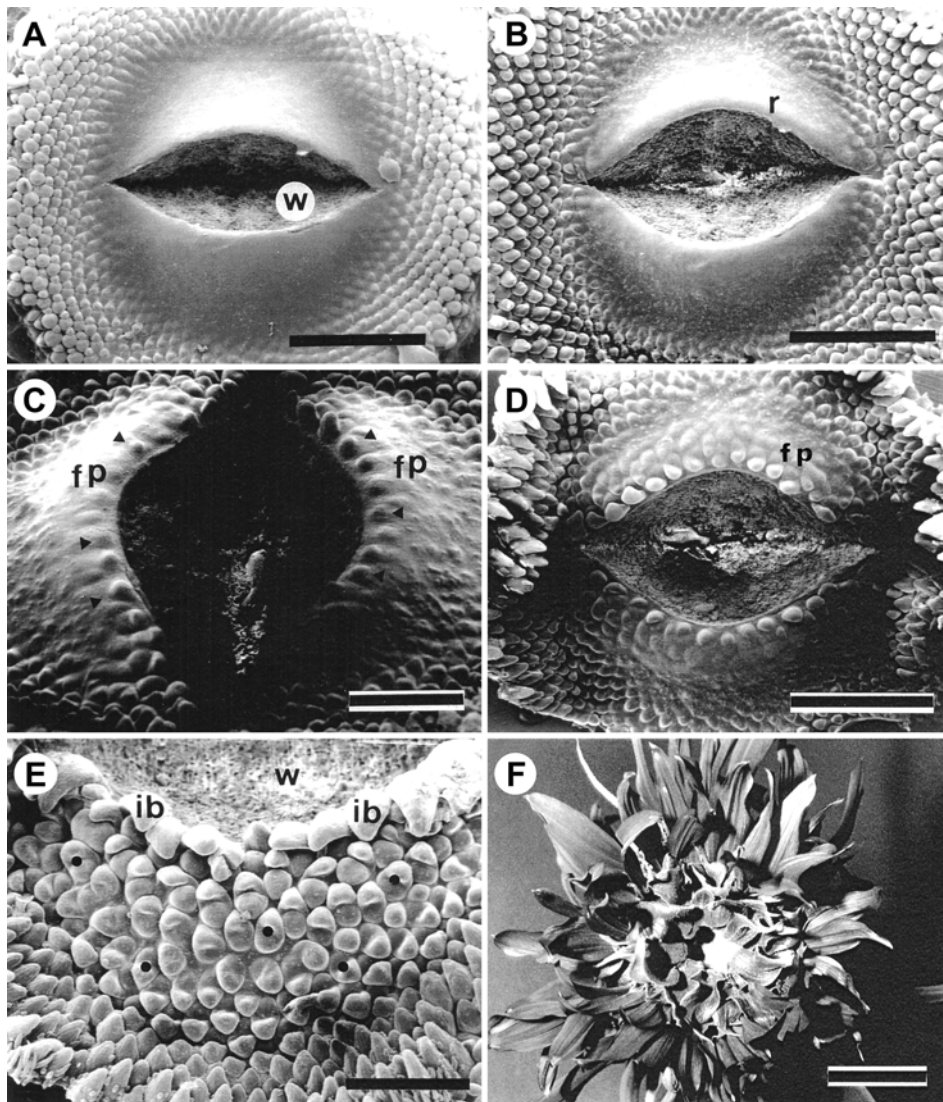
Table 1: Vegetative and reproductive development of control and B-deficient sunflower plants 65 days after seedling emergence. Numbers in parentheses are standard errors.

Treatment	Shoot dry weight (g)	Leaf area (cm <sup>2</sup> )	Root dry weight (g)	Receptacle area (mm <sup>2</sup> )
Control	6.3 (0.7)	1391.2 (234.3)	1.7 (0.5)	8139.1 (891.3)
B-deficiency	5.2 (0.9)	929.0 (91.4)	0.7 (0.2)	6101.2 (731.2)
LSD (p<0.05)	1.1	384.1	0.3	1232.4

Plants that were transferred to normal B media at 25 DAE showed partial recovery. At 34 DAE, 70% of the B-deficient plants developed some sort of damage at the surface of the meristematic receptacle. Floral development in one of these plants, presenting an almost symmetrical split on its receptacle surface at FS 4, is shown in Figure 1, A-D.

The sequential imaging of a single region of the wound zone permitted a detailed description of the floret initiation process in this particular case. At 37 DAE the wound developed into a gap 600  $\mu$ m wide (Figure 1A). At 39 DAE cell division activity in and beneath the epidermis adjacent to the wound edges (Hernández and Palmer, 1988) created an overhanging rim (Figure 1B). At 42 DAE irregularly spaced mounds appeared on the flank of the wound rims (Figure 1C). Primordia appeared first as small protuberances at the wound edges (Figure 1C) and rapidly developed into involucre bracts. Those florets formed later gave rise to ray or disc florets (Figure 1D-E).

Three days after the beginning of floret differentiation, each floret became a two-part structure: an abaxial bract and an adaxial flower (Figure 1E). All subsequent initials developed into disc florets (Figure 1E) until the receptacle surface was completely covered with flowers. At maturity, ray and disc florets differentiated at both sides of the wound rims were normal in appearance and developed into functional structures (Figure 1F).



**Figure 1:** Development of florets in a *B*-deficient naturally wounded sunflower capitulum. **A.** Top view of a capitulum showing the wound (*w*) at 37 DAE. **B.** The same capitulum at 39 DAE. A rounded rim (*r*) developed at each side of the wound. **C.** Floret primordia (*fp*) developing at the wound rim (42 DAE). Some irregularly spaced mounds developing on the flank of the wound rims are noted by darts. **D.** At 46 DAE the rim became covered with floret primordia (*fp*). **E.** Floret primordia at the edge of the wound rim become involucre bracts (*ib*). Other primordia (some noted by dots) become disc florets. **F.** A mature capitulum at first anthesis. Note the empty centre partially covered with outwardly grown involucre bracts. All subsequent initials developed into ray or disc florets. The last produced normal fruits. Bars: **A** and **B** = 1 mm; **C**, **D** and **E** = 500  $\mu$ m; **F** = 4 cm.

The developmental description of floret primordia differentiation presented here shows in detail how, at the level of the receptacle meristem, the slits induced by B-deficiency distort the normal sequence of floret organogenesis. The total growth of the sunflower plants was reduced when plants were grown in B-deficient media. The most characteristic effect, however, when the injuries occurred at the reproductive level, mainly reflects an alteration of the reproductive meristem of the young capitulum.

### ACKNOWLEDGMENTS

*This research was supported by the Secretaría de Ciencia y Tecnología - UNS and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC, La Plata, Argentina). L.F. Hernandez is member of the Scientific Career of CIC, (La Plata, Argentina). The valuable comments on the manuscript of Dr. M.H. Ebinger, Los Alamos National Laboratory, are deeply appreciated.*

### REFERENCES

- Blamey, F.P.C., 1976. Boron nutrition of sunflowers (*Helianthus annuus* L.) on Avalon medium sandy loam. *Agrochemophysica* 8: 5-10.
- Blamey, F.P.C., D.G. Edwards and C.J. Asher, 1987. Nutritional disorders of sunflower. Dept. of Agricult. Univ. of Queensland, St. Lucia, pp. 72.
- Blamey, F.P.C., D. Mould and J. Chapman, 1979. Critical boron concentration in plant tissues of two sunflower cultivars. *Agronomy J.* 71: 243-247.
- Diaz-Zorita, M. and G.A. Duarte, 1998. Aplicaciones foliares de boro en girasol en el Noroeste Bonaerense. XX Reunión Argentina de Oleaginosos, Actas, Bahía Blanca, pp. 123-124.
- Diggs, C.A., M.S. Ratto de Miguez and V.M. Shorrocks, 1988. Deficiencias de boro en girasol. Estudios en cultivos a campo en la pradera pampeana. 12<sup>th</sup> Internat. Sunflower Conf. Procs. Novi Sad, pp. 307-312.
- Green, P.B., and P. Linstead, 1990. A procedure for SEM of complex structures applied to the inflorescence of snapdragon (*Antirrhinum*). *Protoplasma* 158, 33-38.
- Hernández, L.F., 1997. Floret differentiation in the capitulum of sunflower. *Helia* 20: 63-68.
- Hernández, L.F. and P.B. Green, 1993. On the transductions for expression of pattern: Biophysical analysis in sunflower (*Helianthus annuus* L.). *The Plant Cell* 5: 1725-1738.
- Hernández, L.F. and J.H. Palmer, 1988. Regeneration of the sunflower capitulum after cylindrical wounding of the receptacle. *Am. J. Bot.* 75: 1253-1261.
- Hirsch, A.M. and Torrey, J.G., 1980. Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. *Can. J. Bot.* 58, 856-866.
- Hirsch, A.M., Pengelly, W.L., and Torrey, J.G., 1982. Endogenous IAA levels in boron-deficient and control root tips of sunflower. *Bot. Gaz.* 143, 15-19.
- Hoagland, D.R. and D.I. Arnon, 1950. The Water-Culture Method for Growing Plants without Soil. *Calif. Agric. Exp. Sta. Circ.* 347.
- Marc, J. and J.H. Palmer, 1981. Photoperiodic sensitivity of inflorescence initiation and development in sunflower. *Field Crops Res.* 4: 155-164.
- Palmer, J.H. and J. Marc, 1982. Wound-induced initiation of involucre bracts and florets in the developing sunflower inflorescence. *Plant and Cell Physiol.* 23: 1401-1409.
- Palmer, J.H., P.J. Hocking and P.J. Randall, 1988. Effects of an imposed 12-day period of boron deficiency on reproductive development in sunflower. 12<sup>th</sup> Internat. Sunf. Conf. Procs., Novi Sad, Yugoslavia, pp. 296-300.
- Palmer, J.H., 1998. The physiological basis of pattern generation in the sunflower. In: *Symmetry in Plants*, Jean, R.V. and D. Barabé (Eds.) World Scientific, Singapore, pp. 145-169.

- Ratto de Miguez, S. and C.A. Diggs, 1990. Niveles de boro en suelos de la pradera pampeana. Aplicación al cultivo de girasol. Ciencia del Suelo 8: 93-100.
- Schuster, C.E. and R.E. Stephenson, 1940. Sunflower as an indicator plant of boron deficiency in soils. J. Am. Soc. Agron. 32: 607-621.
- Ungaro, M.R. G., A.M.C. Furlani and J.A. Quaggio, 1988. Efficient boron uptake and use by sunflower genotypes grown in nutrient solution and soil. 12<sup>th</sup> Internat. Sunflower Conf. Procs. Novi Sad, Yugoslavia, pp. 301-306.

### **INICIACIÓN Y DIFERENCIACIÓN DE LOS PRIMORDIOS FLORALES EN CAPÍTULOS DE PLANTAS DE GIRASOL (*Helianthus annuus* L.) DEFICIENTES EN BORO**

#### RESUMEN

Se estudió el efecto de la deficiencia de boro (B) sobre el desarrollo temprano del capítulo en plantas de girasol (*Helianthus annuus* L.). Las plantas fueron crecidas bajo condiciones controladas con un fotoperíodo de 18 horas en una mezcla de suelo deficiente en B (<0.1 ppm B) y regadas con solución de Hoagland, completa (0.27 ppm B, control) o deficiente en B. Veinticinco días después de la emergencia de las plántulas (DAE) las plantas con deficiencias de B fueron transferidas a una mezcla de suelo rico en B (2.0 ppm B) y regadas con solución Hoagland completa.

Comparadas con el control, las plantas crecidas con deficiencia de B tuvieron un área foliar 18% menor, una superficie del receptáculo 25% menor y el peso seco del tallo se redujo 33%. En ambos tratamientos, la generación de nuevos primordios florales se inició al borde del capítulo a los 35 DAE y continuó hacia su centro. Cada primordio floral comenzó a diferenciarse apareciendo como una suave protuberancia para luego convertirse en una estructura bipartita, la bráctea y la corola de la flor.

Durante la diferenciación temprana de las flores se observaron en las plantas deficientes en B, fisuras en la superficie del capítulo. Ello se produjo el estado floral (FS) 4 (entre 28 y 30 DAE). Desde los bordes de estas fisuras se comenzaron a diferenciar primordios florales en sentido centrífugo, dando como resultado capítulos deformados y el desarrollo de brácteas involucrales y flores liguladas en posiciones anormales dentro de la inflorescencia.

Este trabajo describe en detalle el inicio de la diferenciación de los primordios florales durante el proceso morfogénico reproductivo temprano en capítulos de girasol de plantas crecidas con deficiencias severas de boro.

### **INITIATION ET DIFFÉRENTIATION DES PRIMODIA DANS LES CAPITULES DES PLANTES DE TOURNESOL (*Helianthus annuus* L.) DÉFICIENTS EN BORE**

#### RÉSUMÉ

On a étudié l'effect de la déficience de bore (B) sur le développement tôt du capitule dans les plantes de tournesol (*Helianthus annuus* L.).

Les plantes ont grandi sous des conditions contrôlées avec un photopériode de 18 heures dans un mélange du sol déficient en B (<0.1 ppm B) et arrosées avec une solution de Hoagland, complète (0,27 ppm B, Contrôle) ou déficient en B. Vingt-cinq jours après l'apparition des plantules (DAE), les plantes avec déficience de B, ont été transférées à un mélange d'un sol riche en B (2.0 ppm B) et arrosées avec solution Hoagland complète.

Comparées avec le contrôle, les plantes grandies avec déficience de B ont en une are foliée 18,0% mineure, une surface avec du réceptacle 25,0% mineure et le poids sec du tronc s'est réduit 33,0%.

Dans les deux traitements, la génération de nouveaux primordia floraux s'est initiée au bord du capitule après les 35 DAE et a continué vers son centre. Chaque primordium floral a commencé à se différencier en apparaissant comme une saute protubérance pour donner après, dans une structure bipartite, la bractée et la corolle de la fleur.

Pendant la différenciation des fleurs on a observé dans les plantes déficientes en B, des fissures dans la surface du capitule. La s'est produit à l'état floral (FS) 4 (entre 28 et 30 DAE). À partir des bords de ces fissures, on a commencé à différencier des primordia floreaux en sens centrifuge, en donnant comme résultat des capitules déformés et le développement des pousses des bractées involuquées et des fleurs ligulées en position anormale dans l'inflorescence.

Ce travail décrit en détail le début de la différenciation des primordia floreaux pendant le processus morphogénétique reproduit dans les capitules de tournesol dans les plantes grandies avec des déficiences sévères de bore.