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EFFECTS OF AN IMPOSED 12-DAY PERIOD OF BORON DEFICIENCY ON REPRODUCTIVE DEVELOPMENT IN SUNFLOWER

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SUMMARY

The boron requirements for the sunflower oil-seed cultivar, Sunfola 68.3, and a hybrid, Suncross 150, were established by growing plants to maturity in sand culture with a wide range of boron supply levels. Reducing the boron concentration below 0.08 mg/l in the nutrient solution lowered seed yield per plant by reducing the size of the capitulum, seed number and weight per seed. Sensitivity to boron deficiency during flower initiation and floret production was examined by with-holding boron for 12 days either at an early stage of flowering, floral stage (FS) 0-2, or at a later stage (FS 4-6) immediately prior to and during floret production. At FS 0-2, boron deficiency induced rapid necrosis in the apical meristem and developing capitulum, resulting in death in 8-10 days. At the later stage, boron deficiency induced surface splits in the central region of the receptacle and growth was arrested in the floret bearing zone within 4-6 days. Following restoration of the boron supply, there was a rapid recovery in capitulum growth, but disc floret initiation did not resume. Staining of receptacle tissue with DNA specific fluorochromes showed that boron deficiency resulted in the arrest of cell division and depletion of DNA from cells in the disc floret generating zone of the receptacle, in both early and later stages of floret initiation. By contrast, the cells of the disc floret initials showed little cytological change during the boron stress period, providing an explanation for their tolerance of boron deficiency.

INTRODUCTION

Boron deficiency in crop plants is more widespread than deficiency of any other micronutrient (Gupta *et al.*, 1985). The commercial sunflower has a relatively high demand for boron and failure to appreciate this has resulted in boron deficiency reducing growth and seed yields in many regions of the world, including Bulgaria, Spain, South Africa and Thailand (Blamey *et al.*, 1987). The expansion of sunflower production in Australia and elsewhere since the 1970s has led to the crop being cultivated on a wide range of soil types, not all of which have adequate boron. Although boron deficiency has been reported for sunflower crops grown in the Central Tablelands of New South Wales, Australia (Haddad and Kaldor, 1984), the boron requirements of Australian sunflower cultivars have received little scientific attention, despite the evidence that genotypes show considerable variation in their response to boron supply (Blamey *et al.*, 1987). While boron deficiency is known to reduce yield by causing malformation of the capitulum and unfilled seeds (Blamey, 1976), the cytological and anatomical reasons for these effects have not been investigated. A 12-day period of imposed boron deficiency was used to examine the response of the capitulum to short term stress and its ability to recover. The boron stress was applied during two periods; (1) early, when the apex was in an early transitional stage, (2) prior to and during the disc floret production stage.

MATERIALS AND METHODS

Plants were grown in river sand/perlite (1/1) with daily applications of nutrient solution, made up in demineralised water containing (mg/l) Ca,160; K,195; Mg,48; N as nitrate, 168; P,31; S,64; iron and micronutrients (Hocking *et al.*, 1987). Boron was added as boric acid to provide 8 levels of boron ranging from deficient to supraoptimal.

The first experiment was carried out to establish the effects of boron supply on growth, boron content, and seed and oil yield components, for two commercial cultivars, Sunfola 68.3 (open-pollinated) and Suncross 150 (hybrid) maintained at 21/16°C air temperature in a naturally lit glasshouse at Canberra, Australia. Leaf area was estimated at the end of floret

initiation (FS 8 or star stage) by measuring the length and greatest width of laminae of attached leaves, and applying the empirical expression of Rawson *et al.* (1980). Mature heads were threshed and the number of filled seeds counted. The concentration of oil in the seeds was found by wide-band nuclear magnetic resonance. Boron concentration in tissue was determined by inductively coupled plasma emission spectrometry.

In a second experiment, the effects of a short period of boron deficiency on the anatomy and morphology of the young capitulum were examined for two stages of flowering, early transition at floral stage (FS) 0-2, and the period of floret production (FS 4-6) (Marc and Palmer, 1981). Using the same cultivation procedures, Suncross 150 was grown in a controlled environment cabinet at 26°C with an 18 hour photoperiod (420μE/m^2/sec, from incandescent lamps and Philips TL ME 125W fluorescent tubes). The plants were supplied with a maintenance level of 0.04 mg/l boron in the nutrient solution, for either 14 or 28 days after sowing (DAS), when boron was omitted from the nutrient solution for 12 days. At the end of this period, the ability of the plants to recover was tested by resupplying boron at a high level of 0.4 mg/l.

For anatomical investigation, apices were fixed for 3 days in ice-cold 8% glutaraldehyde in 0.25 M phosphate buffer at pH 6.8, dehydrated, embedded in LR white acrylic resin (The London Resin Co.) and serially sectioned at 2μm thickness. For cytological study, sections were stained with the DNA specific fluorochromes, acridine orange or Bizbenzimide (33258 Hoechst). Acridine orange was used as a 1% aqueous solution and Bizbenzimide as a 0.1% buffered solution in 5mM Tris/1mM EDTA at pH 7.4. Sections were stained for 2 min, washed, dried, and mounted in immersion oil for observation with incident UV light in a Leitz microscope equipped with a Ploemopak 2.1 fluorescence epi-illuminator. Colour micrographs were obtained using Kodacolor ASA100 film.

RESULTS

A. Effects of boron supply on vegetative growth and yield.
Since the responses of both cultivars to boron stress were similar, only results for Suncross 150 are presented. Plants which received the lowest supplies of boron showed severe symptoms of deficiency (Table 1). Although leaf number per plant was not affected by boron supply (data not shown), leaf area and plant dry weights were markedly reduced by boron deficiency (Table 1). Leaf area and dry matter production plateaued at a boron supply of greater than 0.08 mg/l. Boron concentrations in apical tissue increased markedly in response to the first increments of applied boron and, like dry matter yield, plateaued above the 0.08 mg/l level. The growth of the capitulum was severely curtailed by an inadequate supply of boron.

Table 1. Effects of boron supply on growth characters and boron concentration in the developing capitulum of sunflower cv. Suncross 150. Values ± SD.

Boron supply (mg/l)	Leaf# symptom rating	Leaf* area (cm^2)	Dry matter of tops at maturity (g/plant)	Capitulum diameter (mm)	Boron* concn in capitulum (mg/kg)
0.004	4.7	340 ± 23	8.9 ± 2.0	-	13 ± 2
0.01	4.1	404 ± 13	12.2 ± 4.5	58 ± 12	21 ± 4
0.04	2.9	452 ± 83	22.4 ± 6.1	77 ± 23	40 ± 6
0.08	1.7	629 ± 89	31.6 ± 10.5	NA	49 ± 0
0.13	1.0	615 ± 127	32.4 ± 9.1	NA	57 ± 14
0.40	1.0	584 ± 109	34.6 ± 10.6	118 ± 3	54 ± 3
1.25	1.0	632 ± 121	35.6 ± 10.2	NA	54 ± 1
3.95	1.0	514 ± 33	36.7 ± 9.5	NA	57 ± 5

Visual ranking of leaf symptoms at anthesis: 1, no symptoms: 5, very severe symptoms.
*Measured at the end of floret initiation (FS 8). NA, no data.

Seed and oil yields were reduced by boron deficiency, primarily because of a decrease in seed number per plant and lower single seed dry weights (Table 2). Seed number per plant was affected to a greater extent by boron deficiency than single seed weight. Plants which received the lowest boron supply of 0.004 mg/l produced small, badly distorted capitula which failed to set any seed. Seed yields did not increase significantly in response to boron levels higher than 0.08 mg/l. There was no consistent effect of boron supply on seed oil concentration.

Table 2. Effects of boron supply on yield components of sunflower cv. Suncross 150. Values ± SD.

Boron supply (mg/l)	Seed no. /plant	Single seed wt (mg)	Seed yield (g/plant)	Seed oil concn (%)	Oil yield (g/plant)
0.004	0	-	-	-	-
0.01	19 ± 16	37.9 ± 4.7	0.7 ± 0.5	41.3 ± 2.5	0.29 ± 0.21
0.04	100 ± 19	48.9 ± 5.8	4.9 ± 0.9	45.5 ± 1.2	2.23 ± 0.41
0.08	159 ± 25	60.7 ± 2.1	9.7 ± 2.4	42.7 ± 1.8	4.14 ± 1.02
0.13	158 ± 31	66.3 ± 8.6	10.4 ± 2.4	45.5 ± 2.0	4.73 ± 1.09
0.40	156 ± 17	70.0 ± 5.4	10.9 ± 2.0	42.4 ± 2.1	4.62 ± 0.85
1.25	161 ± 14	69.3 ± 4.9	11.2 ± 2.3	42.6 ± 3.1	4.77 ± 0.98
3.95	165 ± 20	68.8 ± 7.7	11.3 ± 3.0	42.7 ± 2.6	4.83 ± 1.28

B. Growth and developmental responses.

(i). Boron stress - FS 0-2 (DAS 14-26). Within 3 days of stopping the boron supply, the stem and leaf petioles ceased elongation growth, and the expansion of young leaves was arrested. The stem continued to increase in diameter suggesting that, as with other tissues, boron deficiency did not arrest cell expansion in the transitional apex (Halbrooks and Peterson, 1986). In the apical meristem, growth and the transition to flowering were arrested after 4 days. The meristem appeared to survive in a dormant state for a further 4 or 5 days before disintegration and death occurred. Use of the fluorochromes, acridine orange and Bizbenzimide showed that there was a rapid erosion of DNA from cells in the apex during the first 4 days of boron deficiency. Characteristically the surface of the meristem became brown in colour and transparent to transmitted light, possibly because of increased polyphenol production and pectin secretion into the wall (Dugger, 1983). The subapical meristem showed no evident pathology during the period of boron stress, and resumed normal growth following restoration of the boron supply. The high sensitivity of the cells in the apical meristem to a deficient boron supply to the plant may reflect their location at the end of the boron transport path-way, if boron is utilised preferentially by tissues closer to the root system. Restoration of boron at the end of the 12-day period resulted in axillary bud growth replacing the dead apical meristem.

(ii). Boron stress - FS 4-6 (DAS 28-40). Within 4 days of stopping the boron supply, deficiency symptoms appeared in young leaves in the apical bud and leaf expansion was arrested. The majority of young leaves tolerated boron deprivation for up to 12 days without permanent damage. In the developing head, the first formed involucral bracts died within 8 days. In contrast the younger, last formed involucral bracts were able to tolerate boron stress for the full 12-day period. Within 6 - 8, days the central uncommitted region of the receptacle turned brown in colour and became transparent to surface light. These symptoms were often accompanied by the appearance of crescent shaped splits in the receptacle surface indicating the cessation of cell division in the generative area of the receptacle, while cell division and/or cell expansion continued in the sub-apical region (Palmer and Marc, 1982).

The production of new disc floret initials stopped within 4-6 days, although existing initials were relatively unaffected. Growth of the floret bracts was also suspended. Staining with acridine orange and Bizbenzimide showed that while nuclear content was maintained in the

cells of the disc floret initials, there was severe erosion of DNA in the nuclei of surface cells of the receptacle and in the cells of the floret bracts, leading to the conclusion that either the cells in the disc floret initials are able to compete successfully for available boron or they have a low boron requirement.

When the boron supply was restored after 12 days, the disc floret initials resumed cell division to eventually produce mature fertile florets. However, the development of the whole capitulum was abnormal because the surface splits functioned as additional floret generating sites (Blamey, 1976; Palmer and Marc, 1982). In addition, the natural floret generation process was not restored resulting in heads with no disc florets at the centre, and consequent reduced head size. Despite these abnormal effects the capitulum survived to produce some normal seeds.

DISCUSSION AND CONCLUSIONS

This study has shown that not all regions of the developing capitulum are equally inhibited by boron deficiency. In particular, the subapical meristem in the transitional apex appears to be comparatively tolerant of boron stress. Fluorescent staining of capitulum sections has shown that nuclei of cells of the disc florets and disc floret initials are better able to survive boron stress than nuclei of neighbouring uncommitted cells of the receptacle surface. Although the reason for this has not been investigated, it may be that the active metabolism of the cells in these tissues provides temporary protection against damage by enhancing an ability to acquire and retain boron. While boron concentrations in the whole capitulum decline with increasing boron stress (Table 1), the magnitude and direction of change may not be uniform throughout the organ. Future work to detect such differential changes will require micro-analytical techniques to measure boron at low concentration in the small volumes of tissue.

The existence of the differential sensitivity to boron stress reported here emphasises the need to selectively investigate the metabolism and development of tissues within the capitulum in order to understand the way in which environmental factors such as nutrient stress affect seed yield. The failure of the capitulum to resume floret production when the boron supply was restored suggests that boron stress permanently removes the ability of the cells in the generative area of the receptacle to divide in the periclinal plane; this being the essential first step in floret production (Palmer and Steer, 1985). The irreversible damage to the developing capitulum caused by boron stress that has been shown in this study, emphasises the importance of preventing boron deficiency in sunflower crops at the sensitive early stages of flowering.

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