Hypocotyl Growth and Peroxidases of *Bidens pilosus*¹

**EFFECT OF COTYLEDONARY PRICKINGS AND LITHIUM PRETREATMENT**

Received for publication August 11, 1980 and in revised form November 24, 1980

Marie-Odile Desbiez and Nicole Boyer  
Laboratoire de Phytomorphogénèse, 4, rue Ledru, 63000 Clermont-Ferrand, France  
Thomas Gaspar  
Laboratoire de Biologie végétale, 22, quai Van Beneden, 4020 Liège, Belgium

**ABSTRACT**

Pricking one cotyledon of young *Bidens pilosus* plants induces rapid inhibition of hypocotyl growth, essentially in its middle portion. Analysis of soluble peroxidases indicates rapid changes (increase of activity) in basic isoenzymes followed by more progressive enhancement of the acidic ones. Pretreatment of the plants with lithium prevents the inhibition of elongation due to pricking as well as the peroxidase changes. The phenomenon is similar to the previously described thigmomorphogenetic process in *Bryonia dioica*.

---

Pricking one cotyledon of decapitated *Bidens pilosus* plants has been shown to induce precedence (gaining of apical dominance) between cotyledonary buds (5, 6). The pricking effect was prevented by a lithium pretreatment of the plants (7). Lithium similarly prevented the thigmomorphogenetic response of *Bryonia dioica* and inhibited the peroxidase changes due to rubbing (1). We therefore wondered if the cotyledonal prickings affected the hypocotyl growth and peroxidase pattern of intact *Bidens* plantlets and investigated the effects of a lithium pretreatment of the same.

**MATERIALS AND METHODS**

Plant Material, Growing Conditions, and Treatment. Young plants of *B. pilosus* L. (var. *radiatus*) were raised from seed (achenes) in Petri dishes (darkness; 22 °C) on filter paper and dilute nutrient solution, as used previously (8). Plantlets with 15-mm-long hypocotyls were selected at day 4 and placed in lots of 40 in glass hemolysis tubes containing the same dilute solution for 24 h. From that time, they were kept in a controlled environment room (9-h light daily at 24 w m²; 22 °C; 60% humidity). At day 5, they were transferred either to deionized water or to a LiCl (50 μM) solution. There is no effect of lithium on seedling growth between days 5 and 6 (7). At day 6, the 30-mm-long hypocotyls were marked mm by mm with China ink in their upper part. Four prickings with a needle were given to one of the cotyledons (on the middle vascular bundle) 24 h or 12 h before growth measurements and enzyme analyses at day 7.

Peroxidase Activity and Isoperoxidase Patterns. Crude enzymic extracts were prepared from 500 mg fresh material in 0.5 ml 0.1 M Na-K phosphate buffer (pH 7.0, supplemented by 10 mM thioglycolate in order to avoid browning) at 2 °C, 12 and 24 h after the pricking treatment. The macerates were centrifuged at 3,300g for 20 min, and the supernatants were used for enzyme determination. Guaiacol-peroxidase activity and peroxidase isoenzyme patterns (by vertical starch gel electrophoresis) were determined as previously reported (4). The gel was developed with benzidine and O-dianisidine. Three to five replicates were used in each experiment.

**RESULTS**

Pricking Effects on Growth and Peroxidases. Whole hypocotyl growth response to cotyledonary pricking is shown in Figure 1. The comparative increases in length of the control and pricked plants exhibited a severe restriction (up to 80%) of growth in the latter in the first 2 days following the treatment. Elongation of the pricked plants after 13 days was approximately one-half that of the controls. A preliminary analysis (Fig. 2) localized the inhibiting effect in the top and middle 5-mm-long portions of the hypocotyl, while the bottom part appeared unaffected. More detailed measurements made on marked stems (Fig. 3) confirmed the preceding observations in the total but also indicated the special behavior of the first 2 mm of the top portion, which practically were unaffected instead of being inhibited during the first 48 h after the prickings.

---

¹Research supported by the French CNRS (LA 45, RCP 474, ATP 4108) and by the Belgian Fonds de la Recherche Fondamentale Collective (grant 2.9009 to T. G.).
A preliminary determination of total peroxidase activity along control and pricked *Bidens* plants (Table 1A) indicated an increasing gradient from top to base in both samples. Pricking induced an enhanced enzyme activity all along the hypocotyl. The greatest effect was seen in the middle portion. This peroxidase gradient corresponded to a parallel gradient of activity of the basic isoperoxidases separated by electrophoresis (Fig. 4). The gradient did not appear so clearly for the acidic isoenzymes. Figure 4 also shows that the pricking effect was already visible after 12 h but occurred in the basic peroxidases only at that time. This effect
continued to be accentuated later on, while the acidic isoperoxidases of the middle and top portions also commenced to be clearly accentuated.

**Modifications of the Pricking Effects by Lithium Pretreatment.** Lithium by itself did not apparently affect the hypocotyl length of the control plants, but a more detailed analysis (cf. Figs. 2 and 5) revealed a slight growth stimulation of the bottom and middle portions. Comparing again the same two figures, it can be seen that the pricking effect on growth was clearly lower when lithium-pretreated plantlets were concerned. It was calculated that the ameliorating effect of lithium was 67.6 and 76.7% in the top and middle portions, respectively. Figure 5 also shows that lithium pretreatment even allowed the bottom portions of pricked plants to continue their growth, although this was less than that of the unpricked control. The effect of lithium in counteracting the effect of prickings could also be seen from the peroxidase determinations (on a protein basis, compare A and B in Table 1) and thezymograms (on a fresh weight basis, compare Figs. 4 and 6). The increase in peroxidase activity due to pricking was severely reduced if not reversed (in the middle hypocotyl portion), and the clearest differences previously noted in the activity of isoperoxidases of the pricked plants were not observed in the same isozymes when pretreated by lithium.

**DISCUSSION**

Pricking one cotyledon of *B. pilosus* thus significantly reduced hypocotyl growth, just as rubbing young internodes of *B. dioica* plants reduced their elongation (1, 3). The highest inhibition was observed in the middle portion of the hypocotyl (Fig. 2) where, as observed in *Bryonia* internodes (3), a maximum number of cells were in their elongating phase (unpublished data). Also, as occurred in *Bryonia*, inhibition of growth has been shown to correlate first with a rapid increase in intensity of preexisting basic peroxidases followed by a more progressive enhancement of the acidic ones. Biochemical (11) and anatomical (3) data led us to interpret these mechanically induced changes of growth through the auxin-catabolizing and lignifying roles of basic and acidic peroxidases, respectively (10). An essential difference between the *Bidens* and *Bryonia* systems is that the mechanical stimulus and the responses occurred in two different organs (cotyledon, hypocotyl) in the former but in the same organ (internode) in the latter, which apparently implies the transmission of a signal from the cotyledon to the hypocotyl in *Bidens*. Electrophysiological and autoradiographical data (8) indicate a rapid transmission of such a signal. The *Bidens* system has also some resemblance to that of the spinach plant, where a red light signal given to one leaf induced immediate peroxidase changes in other leaves (15). In all three cases, pretreatment of the plants with lithium prevented the morphogenetic responses due to the physical stimuli and suppressed the characteristic peroxidase changes they normally induced. A model of a switching “on” and “off” pump-and-leak, as a relay and amplification-mechanism in the control of morphogenesis, has been proposed by Thellier and Desbiez (16) to explain the mechanically induced precedence among axillary buds, which also was prevented by lithium. Following this model, it has been supposed that isoperoxidases were electrostatically associated with membranes (14), and changes in the activity of membrane-bound enzymes as a result of mechanical perturbation have been demonstrated (9).

**LITERATURE CITED**

8. Desbiez MO, M Thellier 1977 Contrôle ionique de la manifestation d’un rythme nycthéméral de présence entre bourgeois axillaires. Physiol Vég 16: 785-798