

Short Communication

Calcium Distribution in the Abscission Zone of Bean Leaves

ELECTRON MICROPROBE X-RAY ANALYSIS¹

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ABSTRACT

The calcium content and distribution across the abscission zones of (2-chloroethyl)phosphonic acid-treated bean (*Phaseolus vulgaris* L. var. Contender) leaves were lower and not uniformly distributed as compared to the control. Calcium chloride-treated bean leaves had a higher calcium content, with more calcium localized in the potential abscission layer. Ethephon treatment promoted abscission in both debladed and nondebladed plants; there was a corresponding decrease in calcium in the abscission zone just prior to separation. Deblading of bean leaves under a calcium solution increased the calcium level in the abscission zone and delayed abscission.

a 1.0 cm petiole. In other experiments, deblading was done in 0.034 to 0.068 M CaCl₂ solution and 500 to 1000 µg/ml (2-chloroethyl)phosphonic acid (Ethephon) following the procedure of Poovaiah and Rasmussen (2). Leaf blades of nondebladed plants were immersed in CaCl₂ and Ethephon solutions until the leaf was completely wetted. Electron microprobe analysis for calcium was done 24 to 48 hr after treatment. Fresh, free-hand longitudinal sections from the abscission zone were affixed to polished carbon discs using a calcium-free, double adhesive tape and air dried. Sections were coated with a conducting layer of carbon prior to examination with an electron microprobe (Applied Research Laboratories Model EMX-SM). The operating conditions were 20 kv accelerating potential and 0.01 µamp sample current (5, 7).

There was a higher concentration of calcium in the abscission zone of nondebladed bean leaves than in the petiole and stem tissue (Fig. 1A). When the primary leaf was debladed under calcium solution, the calcium level in the abscission zone increased (Fig. 1B). Why the abscission zone has the capacity to accumulate more calcium than other areas may be related to activation of bound pectin methylesterase due to a decrease in the auxin supply to the abscission zone. This evidence supports the findings of Stösser *et al.* (9), that there is a differential capacity to bind calcium in the abscission zone with development of the abscission layer. The increased level of calcium delayed abscission by at least 2 days in these plants, which normally abscise in about 5 days. When plants were treated with an Ethephon solution, the calcium content in the abscission layer decreased with a concomitant accumulation of calcium immediately adjacent to the separation layer on the stem side (Fig. 1C). In Ethephon-treated plants, separation was complete in 2 to 3 days. After separation, the calcium level on both sides of the separation layer decreased (Fig. 1D). Foliar application of 1000 µg/ml Ethephon and leaves debladed under a 500 to 1000 µl/l Ethephon solution showed similar changes in calcium distribution, but the debladed petioles abscised earlier.

Calcium was most effective in delaying abscission; Mg²⁺ was not as effective as Ca²⁺ with K⁺ and Na⁺ having no effect. A sequential analysis of abscission layer formation and Ca²⁺ localization in the abscission zone suggests that the calcium is lost prior to separation (Fig. 1C) as reported by Rasmussen (3) and Rasmussen and Bukovac (6).

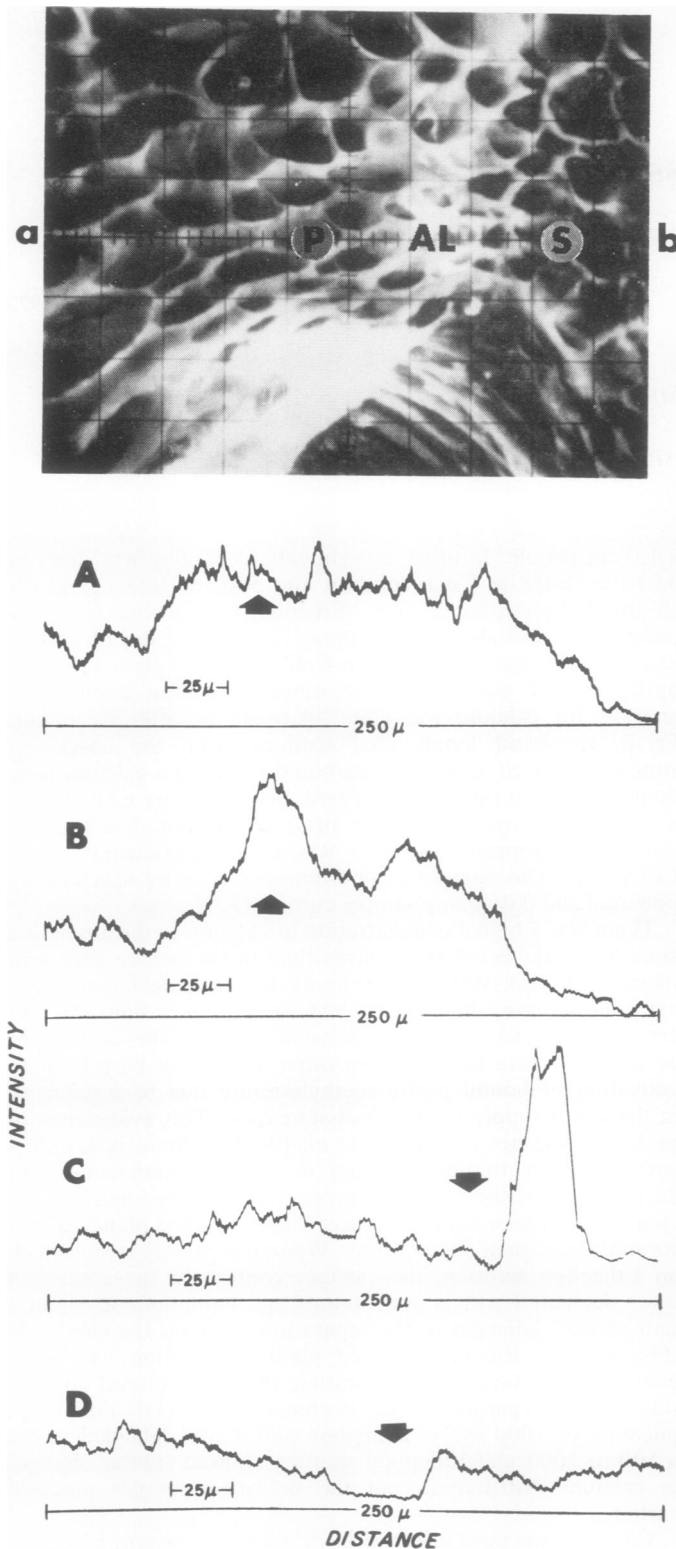
The movement of calcium from the abscission layer could be due to an increase or release of pectin methylesterase from a bound form in the abscission layer as well as the petiole. This

Calcium influences the abscission process in its maintenance of cellular integrity of both the cell walls and membranes. The influence of calcium on leaf abscission is largely understood in terms of its function in calcium pectate, the cementing substance between cells (1, 8). Calcium forms linkages between galacturonic acid chains, as well as between the galacturonates and the carboxyl groups of other components such as protein. Calcium has been shown to increase the break strength of leaves (4) and abscission layers (2). Evidence indicates that the cell walls in the leaf abscission layers (6) and fruit abscission layer (9) have lower affinity for calcium with progressive development of the layer. The loss of binding sites in the abscission zone due to degradation of the cell wall has been suggested.

This study was undertaken to determine the distribution of calcium under conditions of delaying the onset of abscission (CaCl₂) and a corresponding acceleration (Ethephon). The use of the microprobe allows *in situ* analysis of both the soluble and bound forms, whereas other techniques are primarily restricted to the bound form.

Bean seeds (*Phaseolus vulgaris* L. var. Contender) were germinated in vermiculite at 21 C, 1000 ft-c of light for 16 hr/day. About 8 days after germination, abscission of the lower pulvinus of the primary leaf was induced by deblading to

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action, coupled with the action of polygalacturonase, may form an organic complex and calcium would thus move from the abscission layer during senescence. Rasmussen (3) has discussed the role of these enzymes and the relationship of auxin in delaying abscission through involvement of these enzymes. The degree of mineral redistribution in plants is related to its mobility. Calcium is generally regarded as an immobile element. Whether or not a different mobility pattern exists during leaf abscission remains to be documented.

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FIG. 1. Electron microprobe x-ray analysis of the abscission zone of bean leaves. Secondary electron micrograph of the tissue used for line C. Line profile analysis of 250 μ across the abscission region showing distribution of calcium. In all figures, left side represents part of pulvinus with stem tissue to the right. Scan proceeded from left to right (a to b). Arrow identifies the abscission layer. A: nondebladed (control) plant. B: Debladed under 0.034 M calcium chloride solution. Sample was prepared for microprobe analysis 2 days after the treatment. C: Foliar application of 1000 μ g/ml Ethephon. Sample was prepared for microprobe analysis 2 days after the treatment. Large peak in the stem tissue is due to a calcium crystal. D: Folar application of 1000 μ g/ml Ethephon. Sample was prepared for microprobe analysis 3 days after the treatment. P: petiole; AL: abscission layer; S: stem.