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Lignin Deposition in Vascular Tissues of *Phaseolus vulgaris* Roots in Response to Salt Stress and Ca^{2+} Ions

By

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With 3 Figures

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Key words: *Phaseolus vulgaris*, lignification, root vascular tissues, salt stress, calcium ions.

Summary

CACHORRO P., ORTIZ A., ROS BARCELÓ A. & CERDÁ A. 1993. Lignin deposition in vascular tissues of *Phaseolus vulgaris* roots in response to salt stress and Ca^{2+} ions. – Phyton (Horn, Austria) 33 (1): 33–40, 3 figures. – English with German summary.

Lignin deposition in vascular tissues of bean *(Phaseolus vulgaris)* roots in response to salt stress and Ca^{2+} ions was studied through staining with toluidine blue O. The results showed that salt stress and Ca^{2+} ions exert a synergistic effect in the lignification of both protoxylem and metaxylem vessels and induce an earlier and stronger lignification of the secondary thickenings of the phloem fibre cell walls. These results are discussed in the light of lignin deposition as a factor which inhibits root growth and which contributes to the structural integrity of xylem vessels as an adaptation mechanism in resisting the stress imposed by salinity.

Zusammenfassung

CACHORRO P., ORTIZ A., ROS BARCELÓ A. & CERDÁ A. 1993. Lignineinlagerungen in Gefäßbündeln von Wurzeln bei *Phaseolus vulgaris* als Folge von Salz-Streß und Ca^{2+} -Ionen. – Phyton (Horn, Austria) 33 (1): 33–40, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Es wurden Lignineinlagerungen in Gefäßbündeln von Bohnenwurzeln *(Phaseolus vulgaris)* als Folge von Salzstreß und Ca^{2+} -Ionen untersucht, in denen Färbungen mit Toluidin blau 0 durchgeführt wurden. Die Ergebnisse zeigten, daß Salzstreß und Ca^{2+} -Ionen einen synergetischen Effekt in der Lignifizierung sowohl von Protoxylem- als auch Metaxylemgefäßen ausüben und eine frühere und stärkere Lignifizierung der Sekundärverdickungen der Fasern im Phloem induzieren. Diese Ergebnisse werden aus der Sicht der Ligningdeposition als ein Faktor diskutiert, welcher das Wurzelwachstum hemmt und als ein Anpassungsmechanismus zur Strukturerhaltung beiträgt, um dem Salzstreß zu begegnen.

Introduction

Salinity is known to affect many aspects of the metabolism of beans *(Phaseolus vulgaris)*, a glycophyte plant, and to induce changes in their anatomy, morphology and physiology (MEIRI & MAYBER 1967, MEIRI & al. 1971). These changes are often considered to be adaptations which increase the chances of beans to endure the stress imposed by salinity.

Salinity causes stunting of glycophytes and a marked reduction in growth. Calcium has long been known to have an ameliorating effect on the growth of plants under saline conditions (LAHAYE & EPSTEIN 1971). This effect has often been ascribed to Ca^{2+} preventing the uptake of the toxic Na^+ ions while allowing the continued uptake of K^+ (HADDAD & COUDRET 1991). LAHAYE & EPSTEIN (1971) demonstrated in beans that 1 mM Ca^{2+} ions restored the strong inhibition of growth found when bean plants were supplied with 50 mM NaCl.

Besides mineral imbalances and external osmotic pressures which cause a water deficit linked to a drop in water (ψ) and osmotic (π) potentials (HADDAD & COUDRET 1991), one of the most important factors that may limit plant cell growth is the plasticity of the cell wall, which is controlled by the phenolic cross-linking of cell wall polymers (BIGGS & FRY 1987). Since lignin deposition contributes to the loss of cell wall plasticity (BIGGS & FRY 1987), in this report we study the effect of Ca^{2+} ions on lignin deposition in the cell walls of the root vascular tissues of bean plants grown in saline conditions.

Materials and Methods

Beans *(Phaseolus vulgaris* cv. Contender) were germinated in filter paper saturated with 1 mM $CaSO_4$ in the dark at 28° C. After two days, 30 seedlings of a uniform size were transferred to hydroponic culture in buckets containing 10 L of aerated Hoagland solution (HOAGLAND & ARNON 1950). The pH of the solution was kept beween 5.5 and 6.0. The solutions were changed every three days. The plants were kept in an environment growth chamber with 70 % RH and subjected to a cycle of 16 h day with a flux density of 380–400 $\mu mol\ m^{-2}\ s^{-1}$ and 8 h night with temperatures of 25° C and 20° C, respectively.

The plants were allowed to accustom themselves to hydroponic culture for three days and then 80 mM NaCl (two additions of 40 mM on two consecutive days) was

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added to initiate saline treatment. Plants grown in the absence of NaCl were considered as control plants. Calcium treatments were 0.1, 0.5 and 5.0 mM Ca^{2+}, added from a $CaCl_2$ stock. Plants were harvested 15 days after treatment, and from these the roots were separated. Roots were washed and weighed. For lignin cytochemistry, 2 mm-thick sections were taken from the root 2 cm above the meristem.

For lignin cytochemistry, glutaraldehyde fixed root sections were dehydrated in a graded series of ethanol and propylene oxide and embedded in Epon. Thin sections were cut on a ultramicrotome and stained for 1 min with 0.5 % (w/v) toluidine blue 0 (CI 52040) (Ros Barceló & al. 1989). Lignified cell walls appeared more or less blue-green, while unlignified cell walls were clearly stained reddish purple (Feder & O'Brien et al. 1968).

Results and Discussion

The effect of salinity and calcium on the growth of roots of bean plants is shown in Figure 1. Plants grown in saline solutions (80 mM NaCl) were much smaller than the control plants. Calcium was a necessary requirement for root growth both in the presence and in the absence of salt (Fig. 1), although in its presence, 1–5 mM concentrations of calcium were required to obtain healthy plants as compared to 0.2–1.0 mM concentrations necessary for the control plants. Despite the healthy outward appearance of the leaves, the root growth of salt-stressed plants was always lower to that found in control plants (Fig. 1).

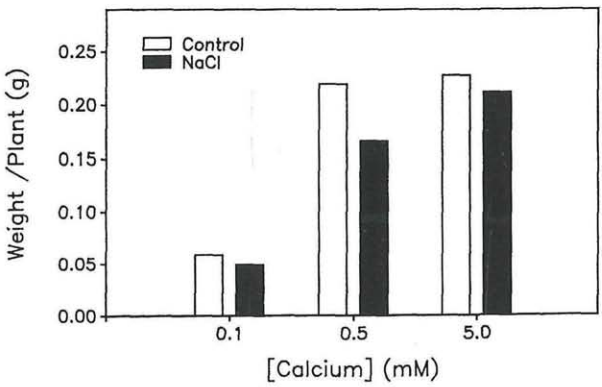

Fig. 1. Effect of calcium concentration on root growth of *Phaseolus vulgaris* plants in the presence and in the absence of 80 mM NaCl.

This encouraged us to investigate many factors which may be involved in the inhibition of root growth of salt stressed plants. For this, we investigated lignin deposition in the vascular cells of the roots as a function of calcium concentrations in both control and salt stressed plants. This was performed by staining with toluidine blue 0, a staining technique whose

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results are directly related to the level of lignins solubilized with acetylbromide and measured spectrophotometrically (Ros Barceló & al. 1989).

The results shown in Figure 2A illustrate that for infraoptimal Ca^{2+} concentrations (0.1 mM), lignification in control plants is almost totally restricted to the metaxylem and protoxylem vessels (arrowheads). This was also observed for salt stressed plants (Fig. 2B) although, in this case, metaxylem and protoxylem vessels were more heavily stained. However, the main differences were observed at the level of the phloem fibre walls which, in the case of salt-stressed plants, showed a weak lignification of the secondary thickenings (Fig. 2B, arrows).

Increases in Ca^{2+} concentration for salt-stressed plants from 0.5 mM to 5.0 mM invariably resulted in greater lignification of the protoxylem and metaxylem vessels (Figs. 3A, 3B, arrowheads) than occured in the control plants. This was accompanied by an earlier and stronger lignification of the secondary thickenings of phloem fibre walls (Figs. 3A, 3B, arrows). Lignification in control plants was almost totally restricted to the protoxylem and metaxylem vessels, which showed staining intensities similar to those found with calcium levels of 0.1 mM (Fig. 2A).

In all cases, no tissue injury or disorganization of the vascular tissues was observed either at the light (Figs. 2, 3) or electron microscope level (data not shown) following salt treatment for varying Ca^{2+} concentrations. In fact, beans grown in saline conditions largely maintain the tetrarch structure resulting from the secondary thickening of the roots (Figs. 2A, 2B).

The promotion of cell wall lignification by saline stress and Ca^{2+} concentrations in bean, a glycophytic plant, is in accordance with the induction of peroxidase activity, the key enzyme in lignin biosynthesis (Biggs & Fry 1987), by salt stress in C_3 (García & al. 1987b), and C_4 (García & al. 1987a) salt sensitive plants. These observations contrast with the adaptation mechanism shown by halophytic plants, which respond by lowering both peroxidase and lignin levels during salt tolerance (Hagege & al. 1988, Mittal & Dubey 1991).

From these results, it can be concluded that saline stress and calcium ions exert a synergic effect on the lignification of both protoxylem and metaxylem vessels, and induce an earlier and strong lignification of the secondary thickenings of phloem fibre cell walls (Figs. 2, 3). This induction of lignification, although related in part with root growth inhibition provoked by salt stress (Fig. 1), is not reversed when Ca^{2+} concentrations are increased (Figs. 2B, 3A, 3B). On the contrary, this leads to a strong promotion of root growth in salt stressed plants (Fig. 1). For this reason, a role for cell wall lignification as the limiting factor in root growth in salt-stressed beans is improbable. These results are in accordance with previous

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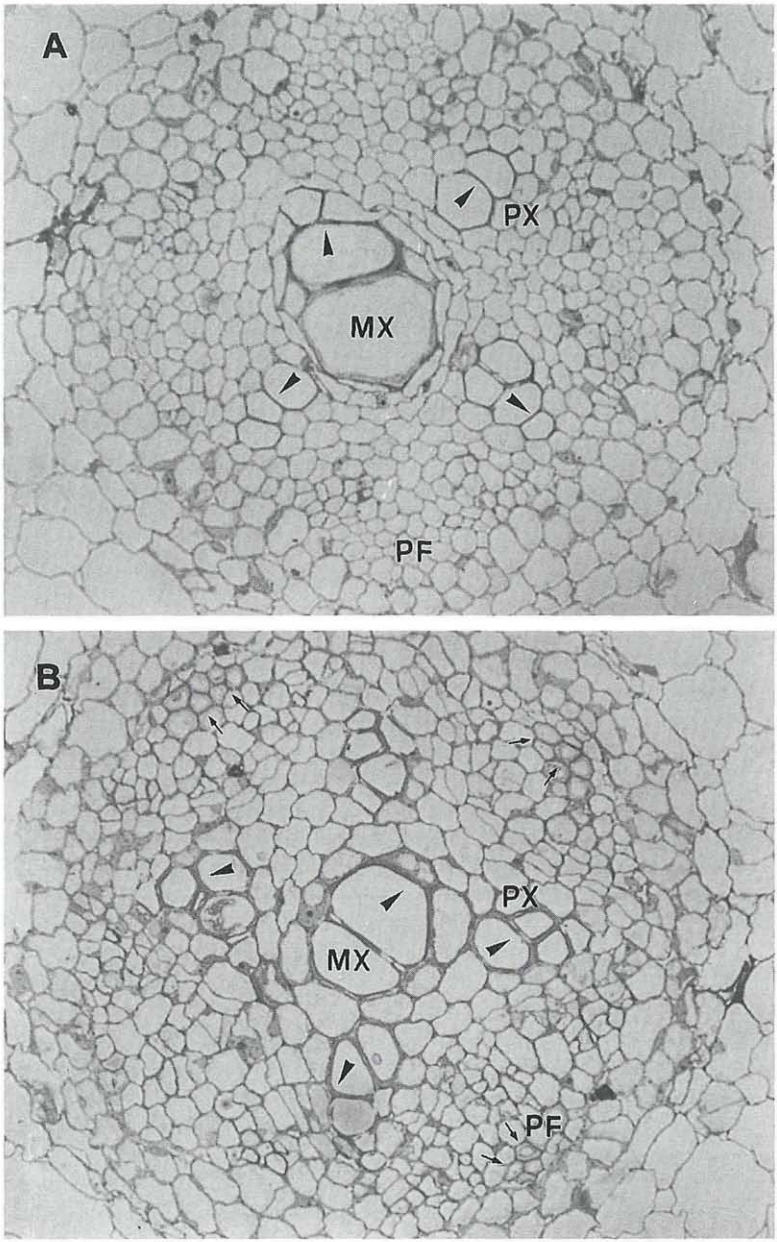

Fig. 2. Light microscopy of lignin deposition (arrows and arrowheads) in the root vascular cells of *Phaseolus vulgaris* plants grown in the absence (A) and the presence (B) of 80 mM NaCl for a Ca^{2+} ion concentration of 0.1 mM. MX = metaxylem, PF = phloem fibres, PX = protoxylem.

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reports (see ROS BARCELÓ & MUÑOZ 1992) in which it has been shown that cell wall lignification is not the limiting factor for plant cell and organ growth in wild glycophytic plants.

However, cell wall lignification of protoxylem and metaxylem vessels may contribute to the structural integrity of xylem vessels (SMART & AMRHEIM 1985) during the adaptation of bean plants to salt stress, through the formation of lignin-polysaccharide linkages (OHNISHI & al. 1992). This may be of great importance, since xylem vessels are subjected to the tensile and compressive forces of the transpiration stream, which may be severely affected in salt stressed glycophytic plants in order to maintain transpiration rates of similar magnitude to those found in control plants (see MEIRI & al. 1971).

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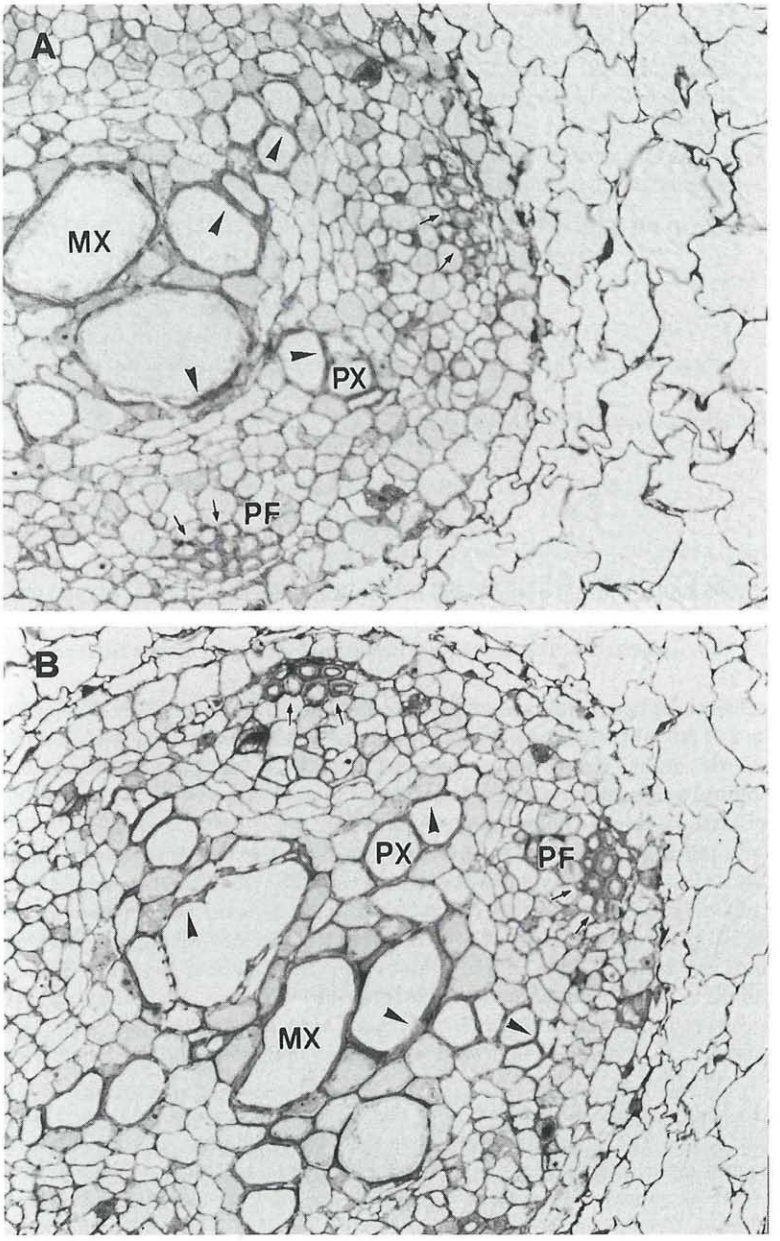

Fig. 3. Light microscopy of lignin deposition (arrows and arrowheads) in the root vascular cells of *Phaseolus vulgaris* plants grown in the presence of 80 mM NaCl for Ca^{2+} ion concentrations of 0.5 (A) and 5.0 (B) mM. MX = metaxylem, PF = phloem fibres, PX = protoxylem.

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Recensio

THE GARDEN. Journal of the Royal Horticultural Society. Vol. 144 (4) and (5), 1992. – Lex. 8°, p. I–XX, 151–191, LXIV–LXXXVI bzw. p. I–XXIV, 195–244, LXXV–XCXIV, zahlr. Abb.; geheftet. – The Royal Horticultural Society, Vincent Square, London, SW1P 2Pe.

Diese wichtige Gartenzeitschrift wurde zuletzt in Phyton 29 (2): 302 (1989) und 30 (2): 246 (1990) besprochen. In der Zwischenzeit (ab 1992) hat sich das Gesicht der Zeitschrift durch das größere Format wesentlich verändert, insbesondere die Farbbilder wirken jetzt viel besser als beim alten, kleinen Format. Zermürbend ist leider nach wie vor die komplizierte Paginierung.

An Beiträgen, die sicherlich auch das Interesse von Botanikern verdienen, enthält Heft 4 einen Aufsatz von Roy LANCASTER über *Helleborus thibetanus* (p. 165–159, mit drei Farbfotos), der erstmals durch Père Armand DAVID (*Davidia, Prunus davidiana* u. a.) in Baoxing in Sichuan gefunden und der westlichen Wissenschaft bekannt wurde. Tony SCHILLING berichtet auf p. 160–164 über Reisen in Bhutan [mit Farbbildern von *Pinus bhutanica, Rhododendron keysii, R. kesangiae, Primula deuteronana* mit blauen Blüten, *Bryocarpum himalaicum (Primulaceae), Osmunda claytoniana, O. cinnamomea, Daphne ludlowii* und *Magnolia campbellii* (Standortsübersicht)].

In Heft 5 sticht ein Artikel über das Leben von Robert FORTUNE (1812-1880) hervor (p. 214–217, 5 Abbildungen); zwischen 1843–1863 hat er viele Pflanzen aus China und Japan nach England eingeführt, darunter so bewährte wie *Mahonia bealei, Cryptomeria japonica, Anemone japonica, Dicentra spectabilis* und *Jasminum nudiflorum. Trachycarpus fortunei, Rhododendron fortunei, Fortunella* und *Fortunaea* tragen seinen Namen. Noch ein Aufsatz von Roy LANCASTER, über *Halesia diptera* var. *magnifica (Styracaceae)* mit Anmerkungen über die Gattung und über St. HALES (p. 226–227, 3 Abb.) sei erwähnt.

H. TEPPNER

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