Plant and Soil **240:** 191–199, 2002.

Abscisic acid concentration, root pH and anatomy do not explain growth differences of chickpea (*Cicer arietinum* L.) and lupin (*Lupinus angustifolius* L.) on acid and alkaline soils

Wolfram Hartung[1,2,7], Laurent Leport[1,3], R. George Ratcliffe[4], Angela Sauter[2], Regina Duda[5,6] & Neil C. Turner[1,5]

[1]*Centre for Legumes in Mediterranean Agriculture, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.* [2]*Lehrstuhl Botanik 1, Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, Julius-von-Sachs-Platz 2, D97082, Würzburg, Germany.* [3]*UMR 6026, Université de Rennes 1, F35042, Rennes Cedex, France.* [4]*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, U.K.* [5]*CSIRO Plant Industry, Private Bag No. 5, Wembley, WA 6913, Australia.* [6]*Institute of Plant Nutrition, Am Hochanger 2, D85354 Freising, Germany.* [7]*Corresponding author**

Received 20 August 2001. Accepted in revised form 12 February 2002

Key words: apoplastic barriers, duplex soils, pH regulation, root morphology

Abstract

The ABA concentrations of leaves, roots, soils and transport fluids of chickpea and lupin plants growing in acid (pH=4.8) and alkaline (pH=8.0) soils and an acid soil with an alkaline subsoil and an alkaline soil with an acid subsoil were measured with the aim of explaining the poor growth of narrow-leafed lupins in alkaline soil. The ABA concentration in the leaves was higher in lupin than chickpea, but did not differ when the plants were grown in alkaline compared to acid soil. The ABA concentration of the roots and xylem sap of lupin did not differ significantly when grown in acid or alkaline soil. Chickpea roots and xylem sap had, however, lower ABA concentrations in acid soil. The ABA concentration in the soil solution was higher in the acid than in the alkaline soil. Roots of lupin and chickpea showed no suberization of the hypodermis or exodermis whether grown aeroponically or hydroponically and the pH of the cytoplasm did not change significantly when root cells of lupin and chickpea were exposed to external pHs of 4.8 or 8.0. The chickpea roots had greater suberization of the endodermal cells adjacent to radial xylem rays and maintained a slightly higher vacuolar pH than lupin in both acid and alkaline external media, but these small differences are insufficient to explain the reductions in lupin growth in alkaline soil.

Introduction

In the south-west of Western Australia, approximately 20% of the soils are deep coarse-textured acid sands, 20% are fine-textured, neutral-to-alkaline, clay loams and 60% are classified as duplex soils in which coarse-textured acid sands overlie fine-textured, alkaline clays (Tennant et al., 1992). Narrow-leafed lupin (*Lupinus angustifolius* L.) is an important pulse crop that grows on low-pH sandy-surfaced soils, but it does not grow well on neutral-to-alkaline, fine-textured soils (Tang et al., 1992, 1995). On these soils, pulses such as chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medik.) are being increasingly grown as they are better adapted to the particular soil conditions (Jayasundara et al., 1998; Siddique et al., 1999). Root growth and water uptake of narrow-leafed lupin have been shown to decrease in response to increasing alkalinity (Atwell, 1991; Tang et al., 1992, 1993a,b), leading to poor water uptake, stomatal closure and poor shoot growth (Tang et al., 1992, 1993, 1996; Tang and Turner, 1999). Tang et al. (1995) showed that soil pH and not texture was the main constraint to lupin

* E-mail: hartung@botanik.uni-wuerzburg.de

root growth. Comparisons with field pea by Tang et al. (1992, 1996) showed that root growth and function of field pea were not inhibited to the same degree by alkalinity as the roots of narrow-leafed lupin. However, no comparisons have been made with chickpea. The present study was initiated to determine whether the growth of lupin and chickpea on the two soil types was related to the sensitivity of the roots in the two species to the external pH.

Abscisic acid (ABA) is widely recognised as a stress hormone induced by unfavourable conditions in the edaphic and aerial environment. Studies have shown that it is produced in the roots and leaves of plants and is transferred from the roots to the leaves in the xylem and from the leaves to the roots in the phloem (Wolf et al., 1990). ABA inhibits shoot growth, but increases root growth especially under stress conditions, thereby maintaining root growth in drying soils (Saab et al., 1990). Simulations by Daeter et al. (1993) and Slovik et al. (1995) suggested that in alkaline soils ABA may leak from the roots to the soil rather than being released into the xylem, thereby inhibiting root growth when water shortage occurs. Abscisic acid is known to redistribute into alkaline compartments according to the anion trap concept (Slovik et al., 1995). Thus an alkaline substrate may cause ABA release from the roots to the surrounding medium. Studies with lupin in the field showed that the concentration of ABA in the acid soil was higher than observed with other species such as wheat (Hartung et al., 1996). This could indicate greater leakage from lupin than wheat roots, as has been observed when faba bean was compared with maize (*Zea mays* L.) under alkaline conditions (Degenhardt et al., 2000). Alternatively, it could indicate a slow breakdown of ABA under the acidic conditions of the field soil (Hartung et al., 1996) or the production of ABA by soil microorganisms (Cutler and Kronchko, 1999). Differences in root ABA concentration and ABA leakage between lupin and chickpea may account for differences in performance of these two species on alkaline soil, but these comparisons have not been made.

The aim of the present study was to determine whether ABA production and exudation cause the growth responses to soil pH in lupin and chickpea. Narrow-leafed lupin and chickpea were each grown in an acid coarse-textured soil traditionally used for lupin, an alkaline fine-textured soil traditionally used for chickpea, and duplex soils of sand over clay and clay over sand. Three sets of measurements were made in order to try to understand the growth of the two species on the different soils. First, the ABA content of the leaves, roots and adjacent soil was measured to determine whether the soil conditions induced ABA production by the plant or its exudation into the soil. Second, the ABA concentration of roots was measured when the two species were grown aeroponically and hydroponically and the possibility that chickpea and lupin differed in the development of apoplastic barriers was investigated. Finally, cytoplasmic and vacuolar pH values were measured using nuclear magnetic resonance to determine whether chickpea and lupin roots differed in their sensitivity to changes in the external pH.

Materials and methods

Culture of chickpea and lupin

Chickpea (*Cicer arietinum* L. accession T1587) and narrow-leafed lupin (*Lupinus angustifolius* L. cv. Merrit) were grown in a controlled-temperature glasshouse, set at 22/15 °C day/night temperatures, at CSIRO, Floreat Park, Western Australia. The plants were grown in polyvinyl chloride pots, 150 mm in diameter and 420 mm high, filled with (i) 12.35 kg of sieved, fine-textured loam (Calcic Haploxeralf, pH 8.0 in 1 m*M* $CaCl_2$) from the top 10 cm of the field in Merredin, Western Australia (Thomson et al., 1997) mixed with 1.35 kg of coarse sand (hereafter referred to as the alkaline soil), (ii) 12.35 kg of coarse-textured soil (Typic Natrixeralf, pH 4.8 in 1 m*M* $CaCl_2$) from the top 10 cm of the field of Beverley, Western Australia (hereafter referred to as the acid soil), and (iii) one of two different layers of the two types of soil. In one, lupin was grown in 10 cm of acid coarse-textured sand on top of the alkaline fine-textured soil (hereafter referred to as acid/alkaline soil) while in the second chickpea was grown in 10 cm of the alkaline fine-textured soil on the top of the acid coarse sand (herafter referred to as alkaline/acid soil). To ensure that the coarse-textured acid soil below the fine-textured alkaline soil was maintained near field capacity, a pipe was placed within the alkaline soil to allow the acid soil to be watered directly. For both soils 1.0 g of a commercial microelement preparation (Richgrow), 7.51 g of potassium nitrate, 7.13 g of ammonium nitrate, 10.67 g of calcium nitrate and 7.61 g of triple superphosphate, corresponding to 1.4 g of N, 0.6 g of P and 0.8 g of K per pot, was mixed with each 50 kg of soil.

Six seeds per pot were sown at a depth of 5 cm on 4 November after the pots had been watered to field capacity. Immediately before sowing, all seeds were inoculated with a commercial *Bradyrhizobium*. Each treatment had four replicates and the total of 24 pots were randomised on benches in the glasshouse and moved weekly. At 33 days after sowing (DAS), plant height was measured and three plants per pot were harvested. At 43 DAS, 3 days after flowering started, the remaining plants were harvested. All pots were irrigated commencing 3 DAS with 200 ml of water per pot every second day. Previous work had shown that this provided sufficient water to maintain the maximum rate of transpiration without waterlogging the soil.

Leaves, roots and the soil adjacent to the roots were collected at both harvests. At the first harvest, xylem sap leaking out of the freshly-cut stems was collected, before the roots were carefully pulled from the soil. Adhering soil was removed from the roots by gentle shaking and brushing and taken as the soil sample. At the second harvest, phloem sap was collected in lupin from the petiole of freshly cut flowers, while for both species, xylem sap was collected from roots placed in a pressure chamber and pressurised to 1 MPa. Samples of leaf, root and soil were then frozen in liquid nitrogen and freeze-dried. The dry weight of the freeze-dried samples was measured and the samples stored dry and in the dark until extraction for ABA measurement. The xylem and phloem sap samples were weighed, freeze dried and then stored with the plant samples until ABA measurement.

Root anatomy

To determine the influence of the external environment on the ABA content and on the apoplastic barriers to water and ABA movement in the root, chickpea and narrow-leafed lupin were cultivated under hydroponic and aeroponic (mist) culture as described by Freundl et al. (2000) and the roots were sampled 28 DAS for ABA analysis and anatomical studies.

Freehand cross sections of fresh primary roots of 21-day-old lupins and chickpea plants were made 10 cm behind the root tip. Sections were either stained for 1 h with 0.1% berberine hemisulfate and subsequently for 75 min with 0.5% toluidine blue O (w/v) (Brundrett et al., 1988), or with Sudan III. The berberine hemisulfate stained sections were viewed under an epifluorescence microscope using a violet filter set (exciter filter 365 nm, dichroitic mirror FT 395, barrier filter LP 397). Microscope pictures were retained using a video camera connected to a computer to produce colour images (Intas Colour LC 100C low light camera; Göttingen, Germany).

Measurement of cytoplasmic and vacuolar pH

Lupin and chickpea seeds were germinated in the dark at 25 °C between sheets of absorbent paper soaked in 0.1 m*M* $CaSO_4$. After germination for 2 days (lupin) or 3 days (chickpea), 80 (lupin) or 120 (chickpea) 5 mm root tips were cut into a continuously aerated medium containing either 50 m*M* glucose, 10 m*M* citrate, 0.5 m*M* $CaSO_4$, pH 4.8 (pH 4.8 adjusted with KOH) or 50 m*M* glucose, 10 m*M* Hepes, 0.5 m*M* $CaSO_4$, pH 8.0 (pH 8.0, adjusted with HCl). After cutting, the tissue was transferred to a 10-mm nuclear magnetic resonance (NMR) tube and oxygenated buffer was circulated through the tube at 0.13 ml s^{-1} (Lee and Ratcliffe, 1983).

In vivo ^{31}P NMR spectra were recorded at 121.49 MHz on a Bruker (Bruker GmbH, Karlsruhe, Germany) CXP300 spectrometer equipped with an Oxford Instruments (Oxford, U.K.) 7.05 T superconducting magnet and a 10-mm dia. double-tuned $^{13}C/^{31}P$ probe. ^{1}H-decoupled ^{31}P NMR spectra were accumulated with a 45° pulse angle, a recycle time of 0.5 s and a total acquisition time of either 0.5 or 1 h. Spectra were recorded for periods of up to 16 h from the same sample, and in some cases the buffer was switched from pH 4.8 to pH 8.0, or from pH 8.0 to pH 4.8, during the course of the experiment. Chemical shift values were measured relative to the signal from a capillary containing a 2% (v/v) aqueous solution of the tetraethyl ester of methylenediphosphonic acid and are quoted on the scale that puts the signal from 85% H_3PO_4 at 0 ppm. Estimates of cytoplasmic pH (pH_{cyt}) and vacuolar pH (pH_{vac}) were obtained from the chemical shifts of the cytoplasmic and vacuolar inorganic phosphate (P_i) signals using the calibration curves described by Spickett et al. (1993).

ABA analyses

The dried leaves and roots were washed and homogenised in liquid nitrogen and extracted in 2 ml of 80% methanol for 24–48 h at −20 °C. After centrifugation, sediments were resuspended in 1 ml of 80% methanol for 16 h. The combined extracts were purified by passing samples through C18-Sep-Pak® cartridges (Waters GmbH, Eschborn, Germany). Afterwards the organic solvent of the effluents was removed in vacuo (Bachofer Vacuum Concentrator,

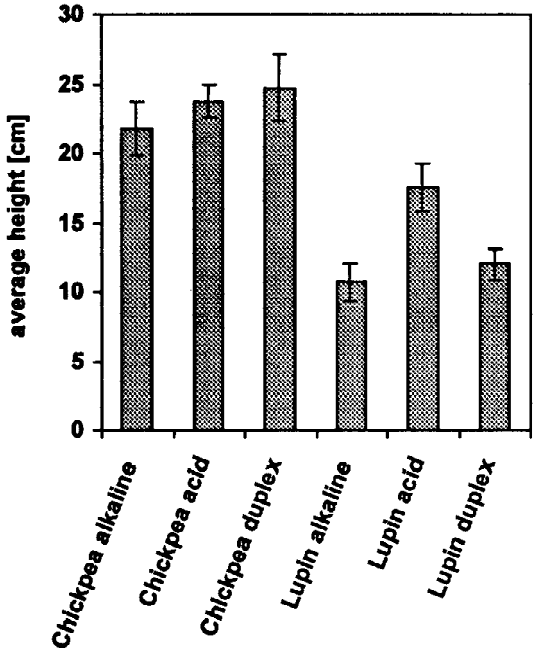

Figure 1. The height of chickpea and lupin plants grown in an acid soil, an alkaline soil, and a duplex soil for 33 days. Values are means of three plants±one standard error (n=4).

Bachofer, Reutlingen, Germany). Residues were resuspended in 1 ml of water and adjusted with a few drops of 1 *M* HCl to a pH of 2–3. The samples were partitioned three times against 1 ml ethyl acetate. The combined ethyl acetate fractions were reduced to dryness and finally taken up in 0.3 ml of TBS buffer (tris-buffered saline; 50 m*M* Tris, pH 7.8, 1 m*M* $MgCl_2$, 10 m*M* NaCl; pH adjusted with HCl). To guarantee complete solubilisation an ultrasonic treatment was applied (Sonorex Super RK 255, Bandelin, Germany). Dried transport fluids (xylem and phloem sap) were taken up in TBS buffer and analysed for ABA without further purification.

Soil samples were extracted in 3-fold excess of 1 m*M* $CaCl_2$ for 1 h. The extract was purified by partitioning against ethyl acetate as described above. ABA of the soil extract was expressed on the soil water basis at the time of harvest, assuming, that all the ABA was dissolved in the soil water (Hartung et al., 1996).

ABA was determined immunologically using ELISA (enzyme-linked immunosorbent assay) with monoclonal ABA antibodies as described by Weiler (1986). The validity of the immunoassay has been previously checked for tissues of maize, a range of other plant species and soil solutions (Weiler, 1986; Hartung et al., 1994; Hartung et al., 1996).

Results

ABA in leaves and roots of chickpea and lupin grown in soil

At 33 DAS, the height of chickpea was unaffected by the soil pH, but the height of lupin was greatest in the acid soil and it was reduced by 40–50% in the alkaline and in the alkaline/acid soil (Figure 1).

The ABA concentration of lupin leaves was twice as high as of chickpea leaves in both the acid and alkaline soils (Table 1). The ABA concentration of the roots was substantially lower than that in the leaves, and the chickpea roots showed a significantly increased ABA content when grown in the alkaline soil compared to chickpea in acid soil. Both species had similar ABA concentrations in the xylem sap (10–20 n*M*). The ABA concentration in the soil solution around the lupin roots was similar to that reported earlier for field grown lupins, around 1 n*M* (Hartung et al., 1996), whereas the ABA in the soil solution around the chickpea roots was surprisingly high relative to lupins, particularly in the acid soil. When the chickpeas were grown in the alkaline/acid soil, ABA increased in the leaves, roots and xylem sap but not the soil (Table 1). When lupin was grown in the acid/alkaline soil, low ABA concentrations were measured in the roots, xylem sap and soil solution but not leaves (Table 1). The phloem sap was always at least 6-fold higher in ABA than the xylem, and was lower in lupin plants growing in alkaline, and particularly acid/alkaline soil, compared to acid soil throughout the root zone (Table 1).

ABA and anatomy of roots in aeroponic and hydroponic culture

Roots of chickpea and lupin had substantially higher ABA concentrations when grown aeroponically than when grown hydroponically (Table 2), especially in lupin. The aeroponically grown lupin roots had remarkably similar concentrations of ABA to those of lupin roots grown on moist filter paper in an earlier study (Hartung and Turner, 1997). When grown hydroponically, the lupin roots had only 4% of the ABA of lupin roots grown aeroponically, whereas in chickpea the ABA concentration of the roots was reduced to half that in the aeroponically-grown roots.

No Casparian bands could be detected in the hypodermis of either chickpea or lupin roots, whether the plants were grown hydroponically (data not shown) or

Table 1. ABA concentration of leaves and roots of chickpea and lupin cultivated in an alkaline and acid soil. The ABA concentration of the xylem sap, the phloem sap and the soil solution surrounding chickpea and lupin roots is also given[†]

	Chickpea			Lupin		
	Acid soil	Alkaline soil	Alkaline/acid soil	Acid soil	Alkaline soil	Acid/alkaline soil
pH of solution	4.8	8.0	8.0/4.8	4.8	8.0	4.8/8.0
ABA concentration in tissues (nmol g^{-1} DW)						
Leaf	2.38±0.08	2.27±0.38	7.17±0.72	4.70±0.30	4.76±0.71	4.25±0.96
Root	0.04±0.00	0.29±0.05		0.26±0.10	0.17±0.08	
Top			0.54±0.08			0.09±0.02
Bottom			0.70±0.05			0.03±0.00
ABA concentration in soil solution and transport fluids (n*M*)						
Soil	5.11±1.22	2.13±0.54		1.58±0.23	0.75±0.44	
Top			5.64±0.72			0.57[‡]
Bottom			1.70±0.61			0.79[‡]
Xylem sap	14.8±3.4	19.8[‡]	30.7±9.6	10.9±0.25	8.85±0.20	2.90±0.45
Phloem sap	N.d.	N.d.	179[‡]	1031±184	493[‡]	19.4[‡]

[†]Values are means±one standard error (*n*=4).
[‡]Combined sample from the four replicate plants or soils.
N.d. not determined.

Table 2. Abscisic acid concentration (nmol g^{-1} FW) of roots from 21-day-old chickpea and lupin seedlings grown under hydroponic and aeroponic conditions[†]

Culture	Chickpea	Lupin
Aeroponic	0.98±0.20 (100%)	10.1±0.70 (100%)
Hydroponic	0.52±0.03 (53%)	0.42±0.03 (4.2%)

[†]Values are means±one standard error (*n*=4).

aeroponically (Figure 2B and D). However, the endodermis of both roots was in the primary stage with clearly-developed Casparian bands (Figure 2A, C and D). The chickpea roots also developed single cells in the endodermis with complete suberin lamellae which could be stained with Sudan III (Figure 2E). These cells were in a position close to the radial xylem ray where passage cells are usually found (Figure 2C and E).

Measurements of cytoplasmic and vacuolar pH

The *in vivo* ^{31}P NMR spectra recorded from excised root tips of lupin and chickpea showed the expected resonances (Ratcliffe, 1994), including well defined signals from the cytoplasmic and vacuolar inorganic phosphate (P_i) pools (Figure 3). At both pH 4.8 and 8.0 the chemical shifts of the P_i signals were stable over periods of at least 16 h for both species and the corresponding values of cytoplasmic and vacuolar pH are summarised in Table 3. The values of cytoplasmic pH were similar for the two species, both at a pH of 4.8 and 8.0. Lupin root tips only acidified slightly at a pH of 4.8. The vacuolar values were 0.3 pH units higher in chickpea roots than lupin roots at values of pH of both 4.8 and 8.0 and were slightly more dependent on the external pH in both species.

Discussion

Narrow-leafed lupins are well adapted to the acid sandy-surfaced soils of south-west Western Australia, but in fine-textured alkaline soils their performance is very poor (Tang et al., 1995). In the present study the growth of the lupin was reduced in the alkaline and alkaline/sandy soils, whereas chickpea growth was not reduced in the acid sand. Tang et al. (1992, 1993b, 1996) found that high pH markedly reduced lupin root growth, induced physical damage to the root

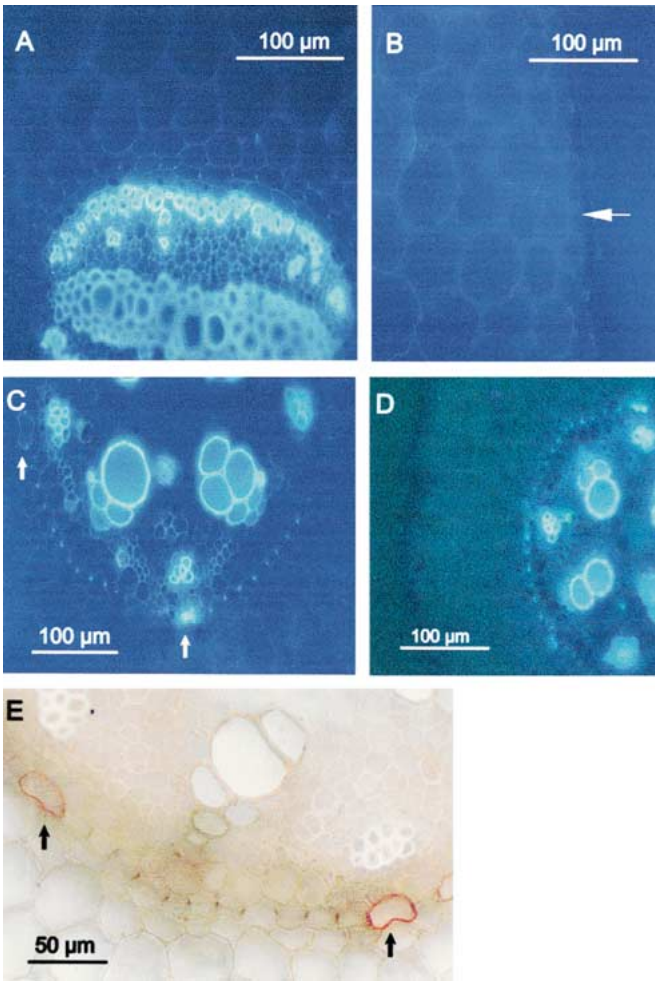

Figure 2. Cross sections of roots of 21-day-old lupin aeroponically-grown lupin (A–B) and chickpea (C–D) seedlings, 10 cm behind the tip, stained with berberine hemisulfate and toluidine blue (A–D) or Sudan III (E). (A) shows the region of the central cylinder with an endodermis in a primary state; (B) shows part of the cortex and the rhizodermis; (C) and (D) show the same parts of the root tissue in chickpea. Note that there is no hypodermis with Casparian bands (exodermis) in either species, but the endodermis of both species contains Casparian bands. Some of the endodermal cells of chickpea are in the secondary state (arrows in C and E).

Table 3. Chemical shift values for the cytoplasmic and vacuolar inorganic phosphate (P_i) signals observed in the ^{31}P nuclear magnetic resonance spectra of lupin and chickpea root tips, and the corresponding values of cytoplasmic (pH_{cyt}) and vacuolar (pH_{vac}) pH[†]

Species	External pH	Cytoplasmic P_i (ppm)	pH_{cyt}	Vacuolar P_i (ppm)	pH_{vac}
Lupin	4.8	2.85±0.01	7.43±0.01	0.84±0.01	5.20±0.02
	8.0	2.93±0.00	7.53±0.00	0.90±0.00	5.38±0.01
Chickpea	4.8	2.86±0.01	7.44±0.01	0.97±0.00	5.53±0.01
	8.0	2.89±0.01	7.48±0.01	1.05±0.01	5.67±0.01

[†]Values are means±one standard error (n=6). Chemical shifts are quoted on the scale that puts the signal from 85% H_3PO_4 at 0 ppm.

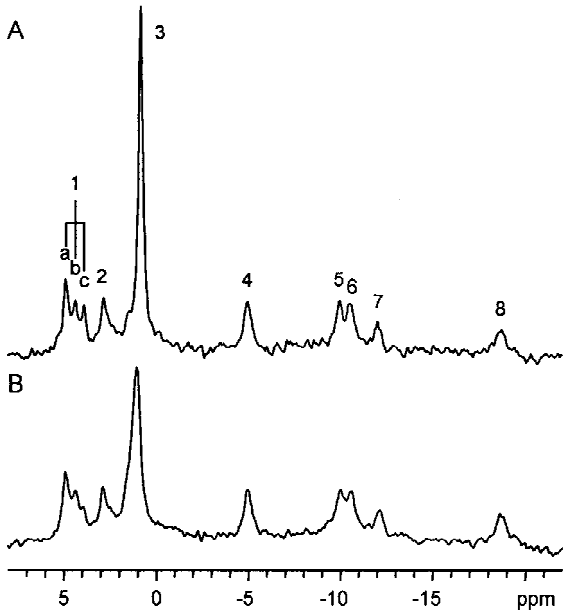

Figure 3. In vivo ^{31}P nuclear magnetic resonance spectra of (A) lupin root tips suspended in pH 4.8 buffer and (B) chickpea root tips suspended in pH 8.0 buffer. The numbered resonances can be assigned to: 1, phosphomonesters, including glucose 6-phosphate (1a) and phosphocholine (1c); 2, cytoplasmic P_i; 3, vacuolar P_i, 4, 5, 8, the γ- and α- and β-phosphates, respectively, of nucleoside triphosphate; 6, UDP-glucose and NAD(P)(H); 7, UDP-glucose. Each spectrum was recorded over a period of 1 h.

surface (Tang et al., 1993a), but did not affect the cortical cell membranes (Dracup et al., 1998). Also, the water and nutrient uptake by lupin roots was impaired in alkaline conditions, resulting in water deficits and stomatal closure (Tang and Turner, 1999). Very poor growth of legumes in an alkaline, but otherwise well-fertilised and well-watered, substrate was also observed by Degenhardt et al. (2000), whereas, maize plants grew well in this substrate. The good growth of maize was associated with the development of Casparian bands in the hypodermis of their roots and the development of a complete exodermis that appeared to provide protection to the root and prevent solute and ABA loss in well-aerated soils or under aeroponic conditions (Freundl et al., 2000). The protective Casparian bands in the hypodermis which developed under aeroponic culture in maize significantly slowed down ABA loss and resulted in ABA accumulation (Freundl et al., 2000). As shown above, ABA is required to maintain root growth under stress conditions (Saab et al., 1990). While the aeroponically-grown lupin and chickpea roots accumulated substantially higher amounts of ABA than roots that were cultivated in well-aerated hydroponic culture, no Casparian bands could be detected in the hypodermis when both the lupin and chickpea were grown aeroponically. While neither chickpeas nor lupin, as in other legumes (Perumalla et al., 1990), developed a Casparian band in the root hypodermis, the chickpea roots did develop suberised endodermal cells adjacent to the radial xylem rays of the stele where passage cells are normally located, and these may slow down the symplastic exchange of water and solutes between stele and cortex.

The small anatomical differences, however, do not appear to explain the differences in levels of ABA in the roots of aeroponically-grown chickpea and lupin. If the suberization of the cells located adjacent to the xylem rays were reducing ABA loss from roots, the ABA content of the chickpea roots should have been higher than that of lupin, while the reverse was true (Table 2). Likewise, the ABA concentrations of the roots were similar in chickpea and lupin in alkaline soil and only lower in chickpea in acid soil (Table 1). Thus, we conclude that the differences in growth of lupin in acid and alkaline soil, as far as they are related to ABA, do not arise from differences in root anatomy, as was observed previously in maize.

Surprisingly, the ABA in the soil surrounding the chickpea roots was high, especially in the acid soil. This is in contrast to the calculations of Daeter et al. (1993) and Slovik et al. (1995) who suggested that ABA may leak from roots in alkaline soils rather than

be released to the xylem. The concentration of ABA in the xylem sap of lupin in alkaline soil was not significantly reduced compared to that in acid soil, and if anything, was higher in the sap of chickpea on alkaline soil, especially in the alkaline/acid soil (Table 1). Likewise, the ABA concentration in the alkaline soil tended to be similar or higher than in acid soil (Table 1). This high amount may be explained by poor ABA degradation under acid conditions (Hartung et al., 1996). Alternatively, the high ABA concentration in the soil surrounding the chickpea roots may be the result of ABA-producing micro-organisms in the vicinity of chickpea roots or of a high number of root hair and root cap cells released from the root surface to the soil.

In principle, the poor performance of lupins on alkaline soil might also arise from the effect of external pH on the intracellular pH values of the roots. However, *in vivo* ^{31}P NMR measurements showed that pH_{cyt} was very similar to the values that have been reported for other root tissues (Ratcliffe, 1994), and there was no evidence that the lupin roots were unusually sensitive to alkaline pH values or that the chickpea roots were unusually sensitive to acidic pH values. The very weak dependence of the cytoplasmic pH on the external pH between pH 4.8 and 8.0 in the roots is consistent with measurements on plant cell suspension cultures where it has been shown that the cytoplasmic pH is effectively independent of the external pH over the same pH range (Fox and Ratcliffe, 1990; Gout et al., 1992). The external pH also had little effect on the vacuolar pH values, and again this observation was in agreement with results obtained for plant cell suspensions. Comparison of the vacuolar pH showed that the vacuoles in the chickpea roots were 0.3 pH units more alkaline than in the lupin roots at both 4.8 and 8.0. This small difference in the pH of the principal subcellular compartment could protect the chickpea roots from loss of ABA to the soil solution, since the partitioning of ABA is strongly dependent on pH and the higher vacuolar pH would tend to favour the retention of the ABA in the root tissue (Daeter et al., 1993)

The comparison of the ABA content of chickpea and lupin roots grown hydroponically, aeroponically and in soil did not provide an explanation of the differences in growth of lupin in acid compared to alkaline soil and the poor growth of lupin on alkaline soils. Small differences in suberization of the endodermal cells and in vacuolar pH are unlikely to have a major effect on root growth and plant function in alkaline compared to acid soils. The high concentrations of ABA in the acid soils, particularly the soil surrounding chickpea roots suggest that the simulations of Daeter et al. (1993) and Slovik et al. (1995) are not confirmed in these two cool-season legumes.

Acknowledgements

The research collaboration was made possible by the award of a Grains Industry Senior Visiting Fellowship by the Grains Research and Development Corporation to WH. WH and AS are also grateful to the Deutsche Forschungsgemeinschaft (SFB 251; TP A3) for financial support. Mike Barr, Christiane Ludwig and Barbara Dierich provided expert technical assistance and Drs Amanda Ellery, Jairo Palta and Mingtan Liao are thanked for comments on the manuscript.

References

Atwell B J 1991 Factors which affect the growth of grain legumes on a solonized brown soil. II. Genotypic responses to soil chemical factors. Aust. J. Agric. Res. 42, 107–119.

Brundrett M C, Enstone D E and Peterson C A 1988 A berberine-aniline blue fluorescent staining procedure for suberin, lignin, and callose in plant tissue. Protoplasma 146, 133–142.

Cutler A J and Kronchko J E 1999 Formation and breakdown of ABA. Trends Plant Sci. 4, 472–478.

Daeter W, Slovik S and Hartung W 1993 The pH gradients in the root system and the abscisic acid concentration in xylem and apoplastic saps. Philos. Trans. R. Soc. London, Ser. B 341, 49–56.

Degenhardt B, Gimmler H, Hose E and Hartung W 2000 Effect of alkaline and saline substrates on ABA-content, -distribution and -transport in plant roots. Plant Soil 225, 83–94.

Dracup M, Turner N C, Tang C, Reader M and Palta J 1998 Responses to abiotic stresses. *In* Lupins as Crop Plants: Biology, Production and Utilization. Eds. J S Gladstones, C A Atkins and J Hamblin. pp 227–262. CAB International, Wallingford.

Fox G G and Ratcliffe R G 1990 ^{31}P NMR observations on the effect of the external pH on the intracellular pH values in plant cell suspension cultures. Plant Physiol. 93, 512–521.

Freundl E, Steudle E and Hartung W 2000 Apoplastic transport of abscisic acid across maize roots. The role of the exodermis. Planta 210, 222–231.

Gout E, Bligny R and Douce R 1992 Regulation of intracellular pH values in higher plant cells. Carbon-13 and phosphorus-31 nuclear magnetic resonance studies. J. Biol. Chem. 267, 13903–13909.

Hartung W, Zhang J and Davies W J 1994 Does abscisic acid play a stress physiological role in maize plants growing in heavily compacted soil? J. Exp. Bot. 45, 221–226.

Hartung W, Sauter A, Turner N C, Fillery I and Heilmeier H 1996 Abscisic acid in soils: What is its function and which factors and mechanisms influence its concentration? Plant Soil 184, 105–110.

Hartung W and Turner N C 1997 Abscisic acid relations in stressed roots. *In* The Biology of Root Formation and Development. Eds. A Altman and Y Waisel. pp 125–132. Plenum Press, New York.

Jayasundara H P S, Thomson B D and Tang C 1998 Responses of cool season grain legumes to soil abiotic stresses. Adv. Agron. 63, 77–151.

Lee R B and Ratcliffe R G 1983 Development of an aeration system for use in plant tissue NMR experiments. J. Exp. Bot. 34, 1213–1221.

Perumalla C J, Peterson C A and Enstone D E 1990 A survey of angiosperm species to detect hypodermal Casparian bands. I. Roots with a uniserate hypodermis and epidermis. Bot. J. Linn. Soc. 103, 93–112.

Ratcliffe R G 1994 *In vivo* NMR studies of higher plants and algae. Adv. Bot. Res. 20, 43–123.

Saab I N, Sharp R E, Pritchard J and Voetberg G S 1990 Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol. 1329–1336.

Siddique K H M, Loss S P, Regan K L and Jettner R L 1999 Adaptation and seed yield of cool season grain legumes in Mediterranean environments of south-western Australia. Aust. J. Agric. Res. 50, 375–387.

Slovik S, Daeter W and Hartung W 1995 Compartmental redistribution and long distance transport of abscisic acid (ABA) in plants as influenced by environmental changes in the rhizosphere. A biomathematical model. J. Exp. Bot. 46, 881–894.

Spickett C M, Smirnoff N and Ratcliffe R G 1993 An *in vivo* nuclear magnetic resonance investigation of ion transport in maize (*Zea mays*) and *Spartina anglica* roots during exposure to high salt concentrations. Plant Physiol. 102, 629–638.

Tang C, Longecker N E, Thompson C J, Greenway H and Robson A D 1992 Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in their sensitivity to pH above 6.0. Plant Physiol. 140, 715–719.

Tang C, Kuo J, Longecker N E, Thompson C J and Robson A D 1993a High pH causes disintegration of the root surface in *Lupinus angustifolius*. Ann. Bot. 71, 201–207.

Tang C, Cobley B T, Mokhtara S, Wilson C E and Greenway H 1993b High pH in the nutrient solutions impairs water uptake in *Lupinus angustifolius*. Plant Soil. 155/156, 517–519.

Tang C, Robson A D, Longecker N E and Buirchell B J 1995 The growth of *Lupinus* species on alkaline soils. Aust. J. Agric. Res. 46, 255–268.

Tang C, Longecker N E, Greenway H and Robson A D 1996 Reduced root elongation of *Lupinus angustifolius* L. by high pH is not due to decreased membrane integrity of cortical cells or low proton production by the roots. Ann. Bot. 78, 409–414.

Tang C and Turner N C 1999 The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus augustifolius* L. and *Lupinus pilosus* Murr. Aust. J. Exp. Agric. 39, 457–464.

Tennant D, Schulz G, Dixon J and Purdie B 1992 Physical and chemical characteristics of duplex soils and their distribution in the south-west of Western Australia. Aust. J. Exp. Agric. 32, 827–843.

Thomson B D, Siddique K H M, Barr M D and Wilson J M 1997 Grain legume species in low rainfall Mediterranean-type environments. I. Phenology and seed yield. Field Crops Res. 54, 173–187.

Weiler E W 1986 Plant hormone immunoassay based on monoclonal and polyclonal antibodies. *In* Modern Methods of Plant Analysis. Vol. 4. Eds. H F Linskens and J P Jackson. pp 1–17. Springer Verlag, Berlin.

Wolf O, Jeschke W D and Hartung W 1990 Long distance transport of abscisic acid in salt stressed *Lupinus albus* plants. J. Exp. Bot. 41, 593–600.

Section editor: P. Ryan