Experimental Agriculture (2020), **56**, 321–330
doi:10.1017/S0014479719000383

CAMBRIDGE UNIVERSITY PRESS

RESEARCH ARTICLE

Performance of two *Lupinus albus* L. cultivars in response to three soil pH levels

Omnia M. Arief[1,2,*], Jiayin Pang[3,4], Kamal H. Shaltout[5] and Hans Lambers[1,4]

[1]School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia, [2]Botany and Microbiology Department, Faculty of Science, Benha University, Egypt, [3]School of Agriculture and Environment, The University of Western Australia, Perth, WA 6009, Australia, [4]The UWA Institute of Agriculture, The University of Western Australia, Perth, WA, 6009, Australia and [5]Botany Department, Faculty of Science, Tanta University, Egypt
*Corresponding author. Email: omnia.arief@fsc.bu.edu.eg

(Received 06 March 2019; revised 19 July 2019; accepted 18 October 2019; first published online 14 November 2019)

Abstract
Soil alkalinity imposes important limitations to lupin productivity; however, little attention has been paid to investigate the effects of soil alkalinity on plant growth and development. Many lupins are sensitive to alkaline soils, but *Lupinus albus* material from Egypt was found to have tolerance to limed soils. The aim of this study was to compare the growth response of two cultivars of *L.albus* L. – an Egyptian cultivar, P27734, and an Australian cultivar, Kiev Mutant, to different soil pH levels and to understand the physiological mechanisms underlying agronomic alkalinity tolerance of P27734. Plants were grown under three pH levels (5.1, 6.7, and 7.8) in a temperature-controlled glasshouse. For both cultivars, the greatest dry mass production and carboxylate exudation from roots were observed at alkaline pH. The better performance of the Egyptian cultivar at high pH was entirely accounted for by its greater seed weight. From a physiological perspective, the Australian cultivar was as alkaline-tolerant as the Egyptian cultivar. These findings highlight the agronomic importance of seed weight for sowing, and both cultivars can be used in alkaline soils.

Keywords: Carboxylates; *Lupinus albus*; Root exudates; Root morphology; Seed size; Alkaline-tolerant; Soil pH

Introduction

Soil pH is a major factor affecting plant growth, development, and reproductive output (Gentili *et al.*, 2018). Lupins have been domesticated independently as a crop (food, green manure, and forage) in both the Mediterranean region and the Andes of South America (Gladstones, 1970). Their successful use in modern agriculture is based on their ability to grow on infertile, sandy, well-drained, and neutral to acidic soils (Lambers *et al.*, 2013). However, lupins are generally intolerant to alkaline soil (Tang *et al.*, 1993; Tang and Robson, 1995). Interestingly, cultivars of *Lupinus albus* in Egypt are tolerant of calcareous soils (Christiansen *et al.*, 1999) and have tolerance to limed soils similar to the level of tolerance of the calcareous soil-tolerant *Lupinus pilosus* (Kerley *et al.*, 2002). The high soil pH affects plant establishment and growth, since it affects the availability and uptake of mineral elements, especially phosphorus (P) (Lambers *et al.*, 2008).

Lupins have the ability to release carboxylates (mainly, citrate and malate) that mobilize poorly available soil P (Dinkelaker *et al.*, 1989; Gardner *et al.*, 1983). The release of carboxylates in *L. albus* L. has been extensively researched (Kidd *et al.*, 2016; Pearse *et al.*, 2007; Zhu *et al.*, 2005); however, there have been limited studies on the effect of soil alkalinity on

plant growth, such as Ding *et al.* (2019), or on the role of seed size in alkalinity tolerance in lupin cultivars. Large-seeded seedlings may perform better in tolerance to abiotic stress. For example, Benard and Toft (2007) reported that large-seeded seedlings of the sand dune plant *Ericameria nauseosa* perform better than small-seeded seedlings during summer drought in an ecosystem near Mono Lake, California. In this study, we hypothesized that two cultivars of *L. albus* L. (an Egyptian cultivar P27734, with larger seeds, and an Australian cultivar Kiev, with smaller seeds) differ in their physiological responses when grown at different soil pH. Testing this hypothesis should provide critical insights on how white lupin tolerates high soil pH. Therefore, the main objectives of the following experiment were to compare plant growth, root mass ratio, amount of rhizosheath carboxylates, and carboxylate composition of two cultivars of *L. albus* L. – an Egyptian cultivar, P27734, and an Australian cultivar, Kiev Mutant grown in sand culture with three pH treatments (5.1, 6.7, and 7.8), thus placing the results into a wider perspective relative to the Australian cultivar.

Material and Methods

Experiment 1: Assessing differences between seedlings of two cultivars

To test the germination rate of 2 cultivars of *L. albus* L. (white lupin), 10 seeds of each cultivar were planted in sand with neutral pH (6.7), without addition of nutrient solution. All seeds were obtained from the Australian Lupin Collection located at the Department of Primary industries and Regional Development, Western Australia. Germinated seeds were scored every 2 days until 14 days after sowing when seedlings were harvested. At harvest, roots were washed carefully. The number of germinated seeds, seedling height, root depth, and fresh and dry weight of shoots and roots were measured.

Experiment 2: Effects of pH levels on two L. albus L. cultivars

A glasshouse experiment was a combination of two *L. albus* L. cultivars (Egyptian cultivar P27734 and Australian cultivar Kiev Mutant); there were seven replicates in each of three pH treatments (5.1, 6.7, and 7.8, determined in 0.01-mM $CaCl_2$). Target pH values were obtained by mixing 10 g $CaCO_3$ and 0.008 g NaOH kg^{-1} river sand for the pH 7.8 treatment, 0.6 g $FeSO_4$ kg^{-1} river sand for the pH 5.1 treatment; the unamended river sand had a pH of 6.7. Seeds of the two cultivars were surface sterilized in 10% (v/v) sodium hypochlorite for 15 min, rinsed thoroughly with deionized water, and germinated on water-moistened filter paper in the dark for 3 days. Three germinated seedlings were planted in each pot and thinned to one plant after 1 week. All seedlings were inoculated with the appropriate strain of rhizobium bacteria (New-Edge Microbials Pty. Ltd., North Albury, NSW, Australia). The experiment was carried out in a temperature-controlled glasshouse at the University of Western Australia (Perth, WA, Australia) with an average daytime temperature of 20 °C and an average nighttime temperature of 15 °C and natural daylight. Pots (8.5 × 8.5 × 18 cm) were filled with 1.2-kg washed river sand; all nutrients were provided with final concentrations being (in μg g^{-1} dry soil): N 30, S 50, Ca 33, Mg 10, Cu 0.5, Zn 2, Mn 4, B0.119, Mo 0.4, Fe 5, Cl 23, and P 50 (P source was KH_2PO_4). The germination started on 10 February 2017 and harvesting was on 18 April 2017. The pots were watered to 70% of field capacity by weighing every second day for the first 6 weeks and then daily until the end of the experiment. The plants were harvested 8 weeks after sowing when visual differences in plant size among the pH treatments could be observed.

Plant measurements

At harvest, the plants were removed from the pots, and the root systems were shaken gently. The sand that was attached to the roots was defined as the rhizosheath (Pang *et al.*, 2017).

https://doi.org/10.1017/S0014479719000383 Published online by Cambridge University Press

Rhizosheath extracts for the analyses of carboxylates were collected according to Pang *et al.* (2010). The entire root system was submerged in 0.2-mM $CaCl_2$ and shaken to remove the rhizosheath sand. The volume of $CaCl_2$ needed to cover the whole root system varied according to the root size from 20 to 100 ml. Subsamples of the extracts were taken and filtered through a 0.22-µm syringe filter. One millimeter of the filtered extract was transferred into a 1-ml high-performance liquid chromatography (HPLC) vial. HPLC analysis of the extracts was performed as described by Cawthray (2003) using working standards of malic, malonic, lactic, acetic, maleic, citric, succinic, cis-aconitic, and trans-aconitic acid (ICN Biomedicals Inc., Aurora, OH, USA) to identify and quantify carboxylates.

Immediately after collection of carboxylates, plants were separated into leaves (mature and young), stems, roots, and flowers (if present). Total plant leaf area was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE, USA). Subsequently, cleaned roots were scanned with a transparent adapted tray (acrylic box) positioned in the scanner (Epson Expression Scan 1680, Long Beach, ON, Canada). Roots were spread out in the acrylic box (size A4) containing tap water to minimize the number of overlaps. Based on the scanned images of the root systems, total length, mean diameter, and total surface area were measured using the WinRHIZO™ regular V.2009 software (Regent Instruments Inc., Quebec, QC, Canada). Plant parts were dried at 70 °C for 72 h, and dry mass (DM), i.e., total (DMtot), leaf (DMl), root (DMr), shoot (DMsh) and stem (DMst) DM, was measured. Root mass ratio was calculated as the ratio of root DM to total plant DM.

To determine the leaf P concentrations, dried leaf samples were ground to a fine powder using Geno/Grinder 2010 (Spex SamplePrep, Metuchen, NJ, USA). Weighed subsamples of approximately 200 mg were digested using a hot concentrated nitric-perchloric (3:1) acid mixture. Phosphorus concentration in mature leaves was determined by the malachite green method (Motomizu *et al.*, 1983) using a UV-VIS spectrophotometer (Shimadzu Corporation, Kyoto, Japan) analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; ChemCentre, Perth, WA, Australia).

Statistical analysis

Generalized least square models were used (Zuur *et al.*, 2009) to test the responses of the two cultivars (Egyptian cultivar P27734 and Australian cultivar Kiev Mutant) at three soil pH levels and interaction between these two factors. Cultivars and pH were used as nonrandom/'fixed' factors of plant functional groups. Normality of data was checked, and transformations of $\log_{10}$ were undertaken if required. Statistical analyses were performed in 'R' using the 'NLME' package (Pinheiro *et al.*, 2017). All figures present the cultivar × pH interaction (mean ± confidence intervals). If this interaction was significant ($p < 0.0001$), then *post hoc Tukey* tests were also performed (Hothorn *et al.*, 2008). If there was no significant interaction, while pH or cultivar had a significant effect ($p < 0.0001$), then marginal means for pH are presented in the results. *t*-test was performed to assess differences between cultivars' seeds and seedlings.

Results

Shoot and root growth response

DMtot, DMst, DMsh, and DMr were significantly affected by soil pH, while mature leaf (DMl) was significantly different between cultivars, and mature leaf area, mean root diameter, and shoot P concentration were significantly affected by cultivar × pH interaction (Table 1). The Egyptian cultivar produced more DM in all pH treatments, especially at pH 7.8 (Figure 1, Supplementary Figure S1 available online at https://doi.org/10.1017/S0014479719000383). Increasing soil pH resulted in increased DMtot for both cultivars ($p \leq 0.0001$), with values approximately 1.4- and 1.3-fold higher for the Egyptian and the Australian cultivars DM at

https://doi.org/10.1017/S0014479719000383 Published online by Cambridge University Press

Table 1. Significance of characters under three pH levels (5.1, 6.7, and 8.3) in two *Lupinus albus* cultivars (P27734 and Kiev Mutant) and cultivar × pH interaction (detected by generalized least square models)

	Significant levels			Marginal means for pH		
Character	Cultivar	pH	Cultivar × pH	Acidic	Neutral	Alkaline
Total plant DM (g)	***	***	ns	2.66	2.40	3.65
Shoot DM (g)	ns	***	ns	2.09	1.84	2.72
Stem DM (g)	ns	***	ns	0.62	0.55	1.00
Mature leaf DM (g)	***	ns	ns	0.88	0.71	0.90
Mean root diameter (mm)	ns	ns	***	0.67	0.68	0.73
Root DM (g)	ns	***	ns	0.57	0.55	0.93
Malate (μmol g^{-1} root DM)	ns	***	ns	5.31	7.66	16.33
Citrate (μmol g^{-1} root DM)	ns	***	ns	2.37	3.89	21.63
Carboxylates in rhizosheath (μmol g^{-1} root DM)	ns	***	ns	7.72	11.59	38.05
Log10 (total carboxylates in rhizosheath soil +1) (μmol g^{-1} root DM)	ns	***	ns	0.85	1.02	1.52
Shoot P concentration (mg g^{-1})	ns	ns	***	7.85	7.57	5.68
Leaf P concentration (mg g^{-1})	ns	***	ns	4.20	4.23	2.67
Rhizosheath pH at final harvest	ns	***	Ns	5.48	6.43	7.46

DM, dry mass; ns, nonsignificant difference.
The marginal means are presented here when there was no significant interaction of pH × cultivar.
***$p \leq 0.0001$.

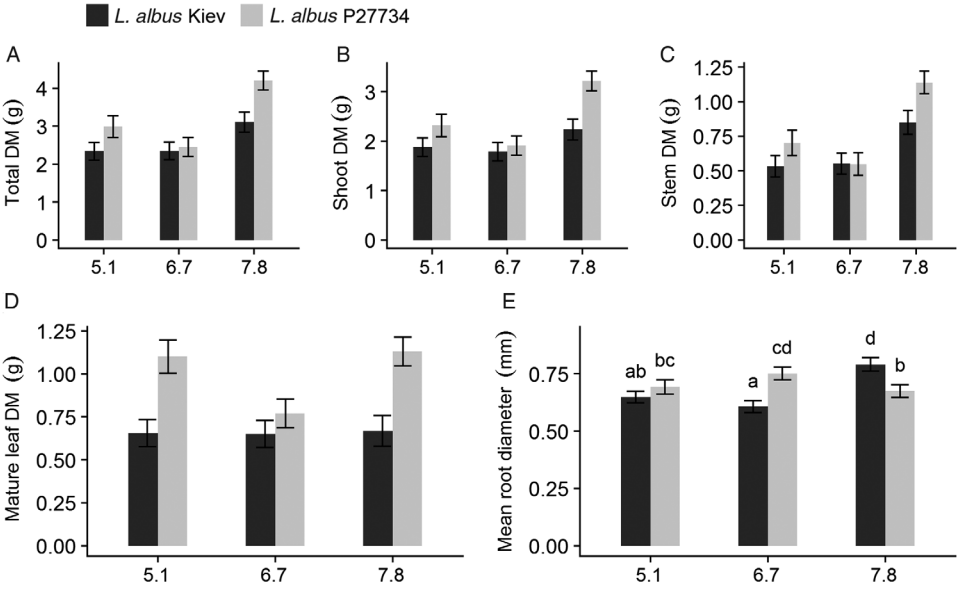

Figure 1. Biomass of two *Lupinus albus* cultivars (Egyptian genotype P27734 and Australian cv. Kiev Mutant) shown at Y-axis grown at soil pH of 5.1, 6.7, and 7.8 shown at X-axis. Bars are the means, and error bars are the standard errors of the means, derived from generalized least square models (A) total dry mass (DM), (B) shoot DM, (C) stem DM (pH, $p \leq 0.0001$); (D) mature leaf DM (Cultivar, $p \leq 0.0001$), and (E) mean root diameter (Cultivar × pH interaction, $p \leq 0.0001$). Different letters among cultivars indicate significant differences within each panel (*post hoc* Turkey test, $p \leq 0.05$). (*L. albus* Kiev: *L. albus* Kiev Mutant).

alkaline than at acidic pH, respectively (Figure 1A). Similar to the total DM, DMsh ($p \leq 0.0001$, Figure 1B) and DMst (Figure 1C) also differed significantly under different soil pH. The two cultivars differed significantly in DMl, with significantly higher values at alkaline and acidic pH for P27734 than for Kiev Mutant ($p \leq 0.0001$), while no difference was observed between

https://doi.org/10.1017/S0014479719000383 Published online by Cambridge University Press

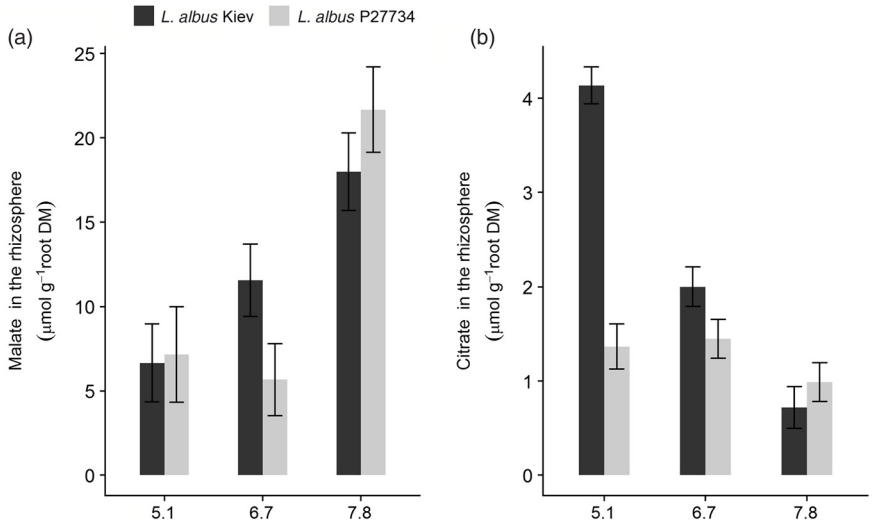

Figure 2. The most abundant carboxylates: (a) malate and (b) citrate relative to root dry mass (DM) of two *Lupinus albus* cultivars (Egyptian genotype P27734 and Australian cv. Kiev Mutant) shown at Y-axis grown at soil pH of 5.1, 6.7 and 7.8 shown at X-axis. Bars are the means, and error bars are the standard errors of the means, derived from generalized least square models (pH, $p \leq 0.0001$). Different letters among cultivars indicate significant differences within each panel (*post hoc* Turkey test, $p \leq 0.05$).

the two cultivars at neutral pH (Figure 1D). For mean root diameter, a significant interaction between cultivar and pH was found (Table 1, Figure 1E). The mean root diameter differed significantly between cultivars under each pH treatment; that of P27734 in the alkaline treatment was similar to that in the acidic pH treatment, whereas that of Kiev Mutant was 18% greater in the alkaline treatment than in the acidic treatment (Figure 1E).

The alkaline treatment caused a minor shift in the total leaf area of both cultivars; there was no cultivar and pH interaction and no significant difference was found between cultivars or pH treatments (Supplementary Figure S2). Root surface area, specific root length, and root mass fraction were not significantly affected by soil pH, cultivar, or cultivar × pH interaction ($p > 0.05$) (Supplementary Figure S3).

Rhizosheath pH and carboxylate response

Both cultivars showed the greatest amount of exuded malate and citrate per root DM at pH 7.8 ($p < 0.001$; Table 1, Figure 2.). The proportion of exuded carboxylates varied greatly among pH treatments, being ~45–77% of malate for P27734 and 30–73% for Kiev Mutant. Citrate comprised 20–55% for P27734 and 25–60% for Kiev Mutant, with values of $>18\ \mu mol\ g^{-1}$ root DM (Supplementary Figure S4). The proportion of both fumarate and cis-aconitate was <1% for both cultivars (Supplementary Figure S4).

The pH of the rhizosheath soil extracted with 0.2-mM $CaCl_2$ varied significantly among pH treatments; for P27734 at a bulk soil pH of 7.8, the rhizosheath pH was 6.7, whereas Kiev Mutant lowered the pH to only 7.1 (Supplementary Figure S5). The rhizosheath pH did not differ between the two cultivars (Table 1, Supplementary Figure S5).

Leaf phosphorus concentrations

For shoot P concentration, a significant interaction of cultivar × pH treatment was found (Table 1, Supplementary Figure S6). It showed a reduction by 9.8% for P27734 and 43% for

https://doi.org/10.1017/S0014479719000383 Published online by Cambridge University Press

Table 2. Seed and seedling characters of two *Lupinus albus* cultivars (P27734 and Kiev Mutant) at 14 days after germination grown in a glasshouse

Character	P27734	Kiev Mutant	*p* value (*t*-test)
Seed character			
Seed size (g seed^{-1})	0.32 ± 0.01	0.24 ± 0.003	<0.0001
Seed P (mg g^{-1})	2.41 ± 0.07	2.71 ± 0.19	<0.0001
Seedling character			
Seedling height (cm plant^{-1})	8.75 ± 0.80	3.5 ± 0.65	<0.0001
Shoot DM (g plant^{-1})	0.33 ± 0.02	0.23 ± 0.03	<0.0001
Root DM (g plant^{-1})	0.13 ± 0.01	0.11 ± 0.02	<0.0001
Total DM (g plant^{-1})	0.46 ± 0.02	0.33 ± 0.12	<0.001

DM, dry mass.
Data are means ± SE ($n = 10$).

Kiev Mutant cultivars due to the alkaline treatment compared with the acidic treatment. The leaf P concentration was significantly reduced by 33% for P27734, and by 40% for Kiev Mutant at pH 7.8 compared with pH 5.1 and 6.7 (Table 1, Supplementary Figure S6).

Seed characters and assessing differences between cultivars' seedlings

Seed P content of P27734 was less than that of Kiev Mutant (2.4 and 2.7 mg P seed^{-1}, respectively) (Table 2). The seed size of P27734 cultivar was larger than that of Kiev Mutant cultivar, being 0.32 and 0.24 g seed^{-1}, respectively ($p < 0.0001$; Table 2). Root DMs of 14-day-old seedlings grown in river sand (neutral pH 6.7) were 0.13 and 0.11 g plant^{-1}; total DMs were 0.46 and 0.33 g plant^{-1} for P27734 and Kiev Mutant cultivars, respectively. The seedling height showed a major difference with 8.75 cm for P27734 and 3.50 cm for Kiev Mutant. The germination percentages of P27734 and Kiev Mutant cultivars on the second day of germination were 100 and 70%, respectively; the larger seeds of the Egyptian cultivar germinated faster than the smaller ones of the Australian cultivar.

Discussion

Most lupin species prefer acidic to neutral soils, with pH 4.5–7.5 (Huyghe, 1997; Jansen, 2006; Lim, 2012; Nigussie, 2012; Wolko *et al.*, 2011). However, cultivars of *L. albus* L. in Egypt are tolerant of calcareous soils when cultivated in soils with pH 7.5 to 9.4 (Christiansen *et al.*, 1999). In Australia, some researchers reported that *L. albus* L. grows relatively well in alkaline soil (Tang *et al.*, 1993).

The most important difference between the two investigated cultivars was that the alkaline-tolerant Egyptian cultivar had larger seeds and faster germination. This difference alone accounted for its greater biomass production at pH 7.8 than that of Kiev Mutant. There were interesting physiological responses to soil pH, but these were essentially the same for both cultivars. Physiological traits that are involved in a plant's tolerance to abiotic stress, that is, alkalinity stress (pH = 7.8), of both cultivars were similar, suggesting that the Australian cultivar had an alkalinity tolerance similar to that of the Egyptian cultivar (P27734). Of all the analysed physiological traits, soil pH significantly affected rhizosheath carboxylates, mainly citrate and malate.

Our results show that the Egyptian cultivar had larger seeds than the Australian cultivar and germinated more quickly. Two weeks after germination, the Egyptian cultivar had a greater seedling height, so the difference in seed size was likely a factor of the greater alkalinity tolerance of the Egyptian cultivar. The seed size gives an indication of the amount of reserves in the

https://doi.org/10.1017/S0014479719000383 Published online by Cambridge University Press

embryo which allow it to establish itself independent of resources to be acquired from the environment (Lambers *et al.*, 2008). Typically, small-seeded species are more affected by stress than large-seeded ones (Hewitt, 1998; Leishman and Westoby, 1994; Osunkoya *et al.*, 1994). Liu and Tang (1999) compared several white lupin genotypes and noted that poor growth under alkaline conditions was associated with small-seeded plants; they also suggested that seed size might contribute to alkalinity tolerance.

The Australian cultivar exhibited a greater shoot P concentration when grown under neutral pH, while the Egyptian cultivar did so at the acidic pH. Moir *et al.* (2016) found that as the increase of lime in soil increases the phosphorus in plant tissue and that agree with our results in which both had the greatest DM at alkaline pH 7.8. The P concentrations for both cultivars were above the critical P concentration in the shoot; this critical P concentration, defined as the leaf concentration required to gaining 90% of the maximum grain yield, is 2.2 mg P g^{-1} DM for *L. albus* (Bolland, 1997). This supports the notion that both cultivars are equally alkaline-tolerant. A greater ability to produce DM combined with a lower leaf P concentration at pH 7.8 may partly explain why the Egyptian cultivar is considered alkaline-tolerant in southern Egypt.

Interestingly, the specific root length was lower at pH 7.8 for both cultivars than that at pH 5.1 and 6.7, while there was no significant difference in root mass ratio due to pH treatments for both cultivars. The observed slight increase in root surface area at alkaline conditions may contribute to higher P-uptake efficiency, suggesting that the P availability was sufficient for both cultivars (Brouwer, 1963, 1983; Kemp and Blair, 1994; Niklas, 1994). However, our results are inconsistent with studies that reported Kiev Mutant as alkaline sensitive. Tang *et al.* (1993, 1995) compared the growth of lupin genotypes in alkaline soils and found that *L. albus* was as sensitive as *Lupinus angustifolius*. Tang and Thomson (1996) investigated a range of legume adaptations to soil pH and biocarbonate and found that the growth of *L. angustifolius* and *L. albus* L. is sensitive to pH $\geq$ 6. They found that the most alkaline-tolerant lupin species is *L. pilosus*.

Lupins have the ability to release carboxylates (mainly, citrate and malate) that mobilize poorly available soil P (Dinkelaker *et al.*, 1989; Gardner *et al.*, 1983). The greatest carboxylate release for both cultivars was at pH 7.8; at this soil pH, P and several micronutrients are less available for the plant than at a neutral pH (Lambers *et al.*, 2008). Our finding that the greatest release of rhizosheath carboxylates was detected at pH 7.8 for both lupin cultivars is consistent with the study of Pearse *et al.* (2006b), who reported that *Lupinus* species acidified their rhizosheath; acidification enhances the solubility of acid-soluble Ca-phosphates (Dinkelaker *et al.*, 1989; Gahoonia *et al.*, 1992; Gerke *et al.*, 1994). To acquire micronutrients, *L. albus* L. depends on carboxylate release and cluster-root production, instead of a large root system (Johnson *et al.*, 1996; Pearse *et al.*, 2007). Both cultivars released carboxylate at pH 7.8, mainly citrate and malate; carboxylates increase not only the availability of P, but also that of Fe and Mn (Johnson *et al.*, 1994). The large proportion of citrate in rhizosheath carboxylates agrees with previous reports on *L. albus* L. (e.g., Dinkelaker *et al.* 1989; Gardner *et al.* 1983; Kidd et al. 2016; Pearse *et al.* 2006b). Malate comprises a significant fraction of exudates in *L. albus* L. as well (Neumann *et al.*, 1999; Pearse *et al.*, 2006a). Both citrate and malate are important for nutrient acquisition at alkaline pH for both cultivars. Jones and Darrah (1994) demonstrated that both citrate and malate are capable of mobilizing calcium, potassium, and magnesium from alkaline soils.

Our results of the germination test show that the Egyptian cultivar had larger seeds than the Australian cultivar and germinated more quickly. Two weeks after germination, the Egyptian cultivar had a greater seedling height. Liu and Tang (1999) compared several white lupin genotypes and noted that poor growth under alkaline conditions was associated with small-seeded plants. They suggested that seed size might contribute to alkalinity tolerance. The results from this study fully support this suggestion, and the physiological responses of both cultivars

were the same at the alkaline pH. Therefore, our study shows that both cultivars are equally alkaline-tolerant.

Concluding Remarks

Dry matter production of both cultivars was greatest at alkaline pH. From a physiological perspective, the Australian cultivar was equally alkaline-tolerant as the Egyptian cultivar. Our findings show no physiological differences in traits that might account for alkalinity tolerance. A pivotal difference between the cultivars was seed size and initial seedling weight, and that difference accounted entirely for the lime tolerance of the Egyptian cultivar. Future field tests should assess the effect of alkalinity on the growth of the cultivars under field conditions.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S0014479719000383

Acknowledgements. The first author gratefully acknowledges the financial support provided by The Egyptian Mission Sector that supported OMA with funding to conduct this work at the University of Western Australia. The authors gratefully thank lab colleagues Wenli Ding, Patrick Hayes, and Rodrigo Pires for helping with statistical analyses. Thanks to Michael Smirk for assisting with ICP-OES analysis, and to Greg Cawthray for assisting with HPLC analyses. Deep thanks for editor of Experimental Agriculture and anonymous reviewers who improve the manuscript to be in this good form.

Financial support. None.

References

Benard R. and Toft C. (2007). Effect of seed size on seedling performance in a longlived desert perennial shrub (*Ericameria nauseosa: Asteraceae*). *International Journal of Plant Sciences* **168**, 1027–1033.

Bolland M.D.A. (1997). Comparative phosphorus requirement of four lupin species. *Journal of Plant Nutrition* **20**, 1239–1253.

Brouwer R. (1963). Some aspects of the equilibrium between overground and underground plant parts. *Jaarboek van het Instituut voor Biologisch en Scheikundig Onderzoek aan Landbouwgewassen* **213**, 31–39.

Brouwer R. (1983). Functional equilibrium: sense or nonsense? *Netherlands Journal of Agricultural Science* **31**, 335–348.

Cawthray G.R. (2003). An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. *Journal of Chromatography A* **1011**, 233–240.

Christiansen J.L., Raza S. and Ortiz R. (1999). White lupin (*Lupinus albus* L.) germplasm collection and preliminary *in situ* diversity assessment in Egypt. *Genetic Resources and Crop Evolution* **46**, 169–174.

Ding W., Clode P.L. and Lambers H. (2019). Is pH the key reason why some *Lupinus* species are sensitive to calcareous soil? *Plant Soil* **434**, 185–201.

Dinkelaker B., Römheld V. and Marschner H. (1989). Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant, Cell and Environment* **12**, 285–292.

Gahoonia T.S., Claassen N. and Jungk A. (1992). Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. *Plant and Soil* **140**, 241–248.

Gardner W.K., Barber D.A. and Parbery D.G. (1983). The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant and Soil* **70**, 107–124.

Gentili R., Ambrosini R., Montagnani C., Caronni S. and Citterio S. (2018). Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. *Frontiers in Plant Science* **9**, 1–12.

Gerke J., Römer W. and Jungk A. (1994). The excretion of citric and malic acid by proteoid roots of *Lupinus albus* L.; effects on soil solution concentrations of phosphate, iron, and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. *Zeitschrift für Pflanzenernährung und Bodenkunde* **157**, 289–294.

Gladstones J.S. (1970). Lupins as crop plants. *Field Crop Abstracts* **23**, 123–148.

Hewitt N. (1998). Seed size and shade-tolerance: a comparative analysis of North American temperate trees. *Oecologia* **114**, 432–440.

Hothorn T., Bretz F. and Westfall P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal* **50**, 346–363.

Huyghe C. (1997). White lupin (*Lupinus albus* L.). *Field Crops Research* **53**, 147–160.

Jansen P.C.M. (2006). *Lupinus albus L.* Wageningen, Netherlands: PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale).

Johnson J.F., Allan D.L. and Vance C.P. (1994). Phosphorus stress-induced proteoid roots show altered metabolism in *Lupinus albus*. *Plant Physiology* **104**, 657–665.

Johnson J.F., Allan D.L., Vance C.P. and Weiblen G. (1996). Root carbon dioxide fixation by phosphorus-deficient *Lupinus albus* (contribution to organic acid exudation by proteoid roots). *Plant Physiology* **112**, 19–30.

Jones D.L. and Darrah P.R. (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil* **166**, 247–257.

Kemp P.D. and Blair G.J. (1994). Phosphorus efficiency in pasture species. VIII. Ontogeny, growth, P acquisition and P utilization of Italian ryegrass and phalaris under P deficient and P sufficient conditions. *Australian Journal Of Agricultural Research* **45**, 669–688.

Kerley S.J., Norgaard C., Leach J.E., Christiansen J.L., Huyghe C. and Römer P. (2002). The development of potential screens based on shoot calcium and iron concentrations for the evaluation of tolerance in Egyptian genotypes of white lupin (*Lupinus albus* L.) to limed soils. *Annals of Botany* **89**, 341–349.

Kidd D.R., Ryan M.H., Haling R.E., Lambers H., Sandral G.A., Yang Z., Culvenor R.A., Cawthray G.R., Stefanski A. and Simpson R.J. (2016). Rhizosphere carboxylates and morphological root traits in pasture legumes and grasses. *Plant and Soil* **402**, 77–89.

Lambers H., Chapin F.S. and Pons T. (2008). *Plant physiological ecology*. Second edition. New York: Springer

Lambers H., Clements J.C. and Nelson M.N. (2013). How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *American Journal of Botany* **100**, 263–288.

Leishman M.R. and Westoby M. (1994). The role of large seed size in shaded conditions: experimental evidence. *Functional Ecology* **8**, 205–214.

Lim T.K. (2012). *Lupinus albus*. In *Edible medicinal and non-medicinal plants*. Netherlands: Springer, pp. 763–769.

Liu A. and Tang C. (1999). Comparative performance of *Lupinus albus* genotypes in response to soil alkalinity. *Australian Journal of Agricultural Research* **50**, 1435–1442.

Moir J., Jordan P., Moot D. and Lucas R. (2016). Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand soil under glasshouse conditions. *Journal of Soil Science and Plant Nutrition* **16**, 438–460.

Motomizu S., Wakimoto T. and Toei K. (1983). Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. *Analyst* **108**, 361–367.

Neumann G., Massonneau A., Martinoia E. and Römheld V. (1999). Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta* **208**, 373–382.

Nigussie Z. (2012). Contribution of white lupin (*Lupinus albus L.*) for food security in north- western Ethiopia: a review. *Asian Journal of Plant Sciences* **11**, 200–205.

Niklas K.J. (1994). *Plant allometry: the scaling of form and process*. Chicago: University of Chicago Press.

Osunkoya O., Ash E., Hopkins S. and Graham A. (1994). Influence of seed size and seedling ecological attributes on shade-tolerance of rain-forest tree species in northern Queensland. *Journal of Ecology* **82**, 149–163.

Pang J., Ryan M., Siddique K. and Simpson R. (2017). Unwrapping the rhizosheath. *Plant and Soil* **418**, 129–139.

Pang J., Ryan M.H., Tibbett M., Cawthray G.R., Siddique K.H.M., Bolland M.D.A., Denton M.D. and Lambers H. (2010). Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant and Soil* **331**, 241–255.

Pearse S.J., Veneklaas E.J., Cawthray G., Bolland M.D. and Lambers H. (2006a). *Triticum aestivum* shows a greater biomass response to a supply of aluminium phosphate than *Lupinus albus*, despite releasing fewer carboxylates into the rhizosphere. *New Phytologist* **169**, 515–524.

Pearse S.J., Veneklaas E.J., Cawthray G., Bolland M.D.A. and Lambers H. (2006b). Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant and Soil* **288**, 127–139.

Pearse S.J., Veneklaas E.J., Cawthray G., Bolland M.D.A. and Lambers H. (2007). Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. *New Phytologist* **173**, 181–190.

Pinheiro J., Bates D., DebRoy S., Sarkar D. and **R Core Team** (2017). Package 'nlme': linear and nonlinear mixed effects models, version 3.1.

Tang C., Buirchell B.J., Longnecker N.E. and Robson A.D. (1993). Variation in the growth of lupin species and genotypes on alkaline soil. *Plant and Soil* **155**, 513–516.

Tang C., Robson A.D., Longnecker N.E. and Buirchell J. (1995). The growth of *Lupinus* species on alkaline soils. *Australian Journal of Agricultural Research* **46**, 255–268.

Tang C. and Robson A.D. (1995). Nodulation failure is important in the poor growth of two lupin species on an alkaline soil. *Australian Journal of Experimental Agriculture* **35**, 87–91.

Tang C. and Thomson B.D. (1996). Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legume species. *Plant and Soil* **186**, 321–330.

Wolko B., Clements J., Naganowska B., Nelson M. and Yang H. (2011). *Lupinus*. In Kole C. (ed), *Wild crop relatives: genomic and breeding resources: legume crops and forages*. Heidelberg: Springer, pp. 153–206.

Zhu Y., Yan F., Zörb C. and Schubert S. (2005). A link between citrate and proton release by proteoid roots of white lupin (*Lupinus albus* L.) grown under phosphorus-deficient conditions? *Plant and Cell Physiology* **46**, 892–901.

Zuur A., Ieno E.N., Walker N., Saveliev A.A. and Smith G.M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY, USA: Springer Science & Business Media.

Cite this article: Arief OM, Pang J, Shaltout KH, and Lambers H (2020). Performance of two *Lupinus albus* L. cultivars in response to three soil pH levels. *Experimental Agriculture* **56**, 321–330. https://doi.org/10.1017/S0014479719000383