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## ORIGINAL ARTICLE

Ionic response of *Lotus japonicus* to different root-zone temperaturesQuazi Forhad QUADIR<sup>1</sup>, Toshihiro WATANABE<sup>2</sup>, Zheng CHEN<sup>1</sup>, Mitsuru OSAKI<sup>2</sup> and Takuro SHINANO<sup>3</sup><sup>1</sup>Graduate School of Agriculture, <sup>2</sup>Research Faculty of Agriculture, Hokkaido University, Sapporo and<sup>3</sup>National Agricultural Research Center for Hokkaido Region, Sapporo 062-8555, Japan**Abstract**

The effects of root-zone temperature (RZT) on the ion uptake, distribution and interactions of 21 (11 essential and 10 non-essential) elements using *Lotus japonicus* were studied. After acclimation in hydroponics at ambient (25°C) RZT for three weeks, seedlings were exposed to sub-optimal (15°C and 20°C) and ambient RZTs. After one week, plants were harvested and then growth and mineral elements were analyzed. The highest shoot and root biomass was at 25°C and lowest at 15°C RZT. In shoots, the highest concentrations of all elements, except for nickel (Ni), were at 25°C RZT. At lower RZTs, the reductions in concentrations were more evident especially for trace elements and heavy metals. Except for magnesium (Mg), the highest root concentrations were at 15°C RZT for all essential elements. Unlike other non-essential elements, the highest cadmium (Cd) and cobalt (Co) concentrations in roots were at 25°C RZT. The shoot/root ratios of almost all elements were affected by sub-optimal RZTs. Only manganese (Mn) and Co showed increased shoot/root ratio at sub-optimal RZTs; and this ratio decreased for other elements. Nickel in shoots and Mn, Co and Cd in roots tended to be negatively correlated with most other elements (either, essential or non-essential) in each respective organ. The present study showed that RZT significantly changed the ion profile of *L. japonicus* and suggests possible connection between global warming and phyto-accumulation of heavy metals, especially in temperate regions, since most metals tended to accumulate in shoots at higher RZT.

**Key words:** element interactions, heavy metal phyto-accumulation, ionome, principal component analysis, root-zone temperature.

**INTRODUCTION**

The mineral nutrition of higher plants is of fundamental importance to agriculture and human health. Apart from macronutrients, the awareness of the importance of micronutrients (most of which are heavy metals) to agriculture has been increasing, since in many soils micronutrient availability limits crop production and influences nutritional quality (Williams and Pittman 2010). The management of micronutrients in soil is crucial, since insufficient quantities of these elements

result in plants with deficiency symptoms and excess amounts are toxic to plants. Furthermore, many elements which are not essential and sometimes toxic to plants and to humans [e.g. cadmium (Cd), chromium (Cr) and arsenic (As)] are present in soil, mostly due to anthropogenic activity (Chen Z. *et al.* 2009a). Being present at the beginning of the food chain, uptake of these elements by plants causes food chain contamination. In this context, it is crucial to understand the mechanisms that regulate absorption and storage of all metals in plants.

The content of these mineral nutrients and trace elements (whether essential or not) represents the ionome of a plant (Salt *et al.* 2008), which is a dynamic network of elements in plants (Baxter 2010). It is generally perceived that all physiological processes within the plant influence the plant's ionome and

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alterations in any processes that transport inorganic ions from the soil solution to the shoot could potentially affect the plant's ionome (Baxter *et al.* 2008). Thus, the ionome of a plant is likely to be very sensitive to the physiological state of the plant as well as the environmental conditions it is exposed to, and so different ionic signatures should reflect different physiological states of the plant. Such ionic signatures may be useful as markers for the particular physiological and/or environmental conditions with which they are associated (Baxter *et al.* 2008). So far, studies of the ionome of a plant have mostly overlooked the effect of environmental factors.

Two studies on *Arabidopsis* (Lahner *et al.* 2003) and yeast (Eide *et al.* 2005) have revealed the connection between the ionome and the genome and concluded that approximately 2–4% of all genes contributed to ion uptake control (Lahner *et al.* 2003).

Since gene expressions are influenced by environmental factors, the ionome of a plant should also respond to environmental stimuli. Revealing the linkages between the ionome and environmental parameters such as light, soil and air temperatures, water, humidity and carbon dioxide (CO<sub>2</sub>) concentrations may help in developing strategies for better nutrient management in the ever-changing environment. Improvements in inductively coupled plasma (ICP) spectroscopy techniques have enabled the expansion of this ionic approach to characterize mutants (e.g. Chen Z. *et al.* 2009b) in element interaction studies under stresses generated by toxic elements (Chen Z. *et al.* 2009a). However, reports on the ionic response of plants to different environmental parameters are still unavailable.

Temperature is a principal environmental factor. It is generally recognized that root-zone and/or air temperature influences all aspects of the growth and development of plants (Awal *et al.* 2003). Temperature also controls the physiological and metabolic processes of plant such as photosynthesis, respiration, ion and water uptake, antioxidant metabolism, and signal transduction (Erice *et al.* 2006; Hewezi *et al.* 2006). The uptake and translocation of essential nutrients, root growth and development, and root-cell differentiation of plants are directly influenced by temperature of the root zone (Marschner 1995).

There are several reports on the effect of root-zone temperatures (RZTs) on individual or groups of nutrient elements; however, these reports do not consider how this important environmental parameter influences the entire network of different elements. Indeed, a number of studies have demonstrated that changes in soil temperature can directly affect plant nutrient acquisition by changing root transport properties for nitrogen (N) (BassiriRad *et al.* 1993), phosphorus (P) and potassium (K) (Siddiqi *et al.* 1984; Adebooye *et al.* 2010).

Baghour and co-workers demonstrated that 23–27°C soil temperature was optimum for the uptake of most essential and some non-essential elements such as aluminum (Al), As, barium (Ba), Cd, nickel (Ni) and lead (Pb) (Baghour *et al.* 2001, 2002a, 2002b, 2002c, 2003). These studies also showed that RZT < 20°C, considered sub-optimal for most plants (Zhang *et al.* 2008; Adebooye *et al.* 2010), exacerbates growth as well as nutrient uptake. However, no reports have considered how this important environmental parameter influences the entire network of different elements.

Under these circumstances, we undertook the present study emphasizing the effect of RZT on the ionome of plants. Here we selected the legume *Lotus japonicus* as a model plant. Although legumes are one of the important plant families for human/animal nutrition, they are little studied compared to cereals and other crops. The objective of this study was to investigate how the ion profile and distribution of 21 elements – essentials N, P, K, calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo) and boron (B); and non-essentials Al, As, Ba, Cd, cobalt (Co), Cr, cesium (Cs), sodium (Na), Ni and strontium (Sr) – changed with changing RZT.

## MATERIALS AND METHODS

### Experimental material and cultivation procedure

The study was conducted in the Laboratory of Plant Nutrition, Hokkaido University, Japan using *Lotus japonicus* (Regel) Larsen ecotype Miyakojima (MG-20) seeds collected at the National BioResource Project in Miyazaki University.

In this experiment, we followed the modified high-throughput cultivation procedure described by Chen Z. *et al.* (2009a). Initially, seeds were soaked in 98% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 20 min; rinsed with deionized water several times and then placed in incubators for germination in 0.5-mL micro-centrifuge tubes containing half-strength nutrient-agar media (0.9% agar containing half-strength standard nutrition solution) and kept in the dark at 25°C for three days. Germinated seedlings were then transplanted to hydroponics containing standard nutrient solution. The standard nutrition solution contained 2.14 mM N (NH<sub>4</sub>NO<sub>3</sub>), 0.30 mM P (NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O), 0.77 mM K (K<sub>2</sub>SO<sub>4</sub>:KCl = 1:1), 1.25 mM Ca (CaCl<sub>2</sub>·2H<sub>2</sub>O), 0.82 mM Mg (MgSO<sub>4</sub>·7H<sub>2</sub>O), 35.8 μM Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 9.1 μM Mn (MnSO<sub>4</sub>·4H<sub>2</sub>O), 46.3 μM B (H<sub>3</sub>BO<sub>3</sub>), 3.1 μM Zn (ZnSO<sub>4</sub>·7H<sub>2</sub>O), 0.16 μM Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O) and 0.05 μM Mo [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O]. The pH was adjusted to 4.8 ± 0.1 with 0.05 mol L<sup>-1</sup> hydrochloric

acid (HCl) or  $0.5 \text{ mol L}^{-1}$  *N*-methyl-D-glucamine (99%, Acros Organics, NJ, USA) daily. Since the present study concerns other alkali metals (e.g. Na and K), *N*-methyl-D-glucamine (an organic buffer) was used instead of common inorganic alkalis to adjust solution pH. Nutrient solution was replaced every seven days. After 21 days of hydroponics cultivation in a temperature chamber (LPH-4P-NC, WT-0040; Nippon Medical and Chemical Instruments, Osaka, Japan) ( $25^\circ\text{C}$  with 16/8 h day/night, 70% humidity and light intensity  $140 \mu\text{mol m}^{-2} \text{ s}^{-1}$  at the plant top) the plants were subjected to RZT treatments.

### RZT treatments and addition of non-essential elements in growth solution

After acclimatization for 21 days we transferred the seedlings to three different RZT treatments:  $25^\circ\text{C}$  (ambient RZT),  $20^\circ\text{C}$  and  $15^\circ\text{C}$  (sub-optimal RZTs) each with 24 replications in a growth chamber (EYELA FLI-2000; Tokyo Rikakikai Co Ltd, Japan) at  $25^\circ\text{C}$  air temperature with 16/8 h day/night, with light intensity of  $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and 70% humidity. RZTs were maintained using a thermo-electric cooler (AS ONE, Model UCT-1000). In addition to standard nutrient solution 10 additional non-essential elements of current environmental concern (i.e.  $5 \mu\text{M}$  Al as  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ;  $10 \mu\text{M}$  As as  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ;  $5 \mu\text{M}$  Ba as  $\text{BaCl}_2$ ;  $5 \mu\text{M}$  Cd as  $\text{CdCl}_2$ ;  $5 \mu\text{M}$  Co as  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ;  $5 \mu\text{M}$  Cr as  $\text{CrCl}_2$ ;  $50 \mu\text{M}$  Cs as  $\text{CsCl}$ ;  $2500 \mu\text{M}$  Na as  $\text{NaCl}$ ;  $10 \mu\text{M}$  Ni as  $\text{NiCl}_2$ ; and  $250 \mu\text{M}$  Sr as  $\text{SrCl}_2$ ) were added to the culture solution. The choice of concentrations was based on Chen (2009), who showed no significant reduction in biomass growth of *Lotus japonicus* grown at the selected concentrations. Treatments were continued for seven days, and during this period each day solution pH was maintained at  $4.8 \pm 0.1$ . Milli-Q water was replenished every second day to replace any evaporative water loss.

### Sample digestion and chemical analysis

One week after treatment, plant roots and shoots were harvested using ceramic scissors (CS-250; Kenis, Osaka, Japan). After harvest, root and shoot samples were oven-dried at  $60^\circ\text{C}$  for 72 h and then weighed. During digestion individual shoot samples and two root samples pooled as one in each replicate. For ionic analysis by ICP mass spectroscopy (ICP-MS), dried shoot ( $n=16$ ) and root ( $n=8$ ) samples were digested by 1.5 mL of 61% nitric acid ( $\text{HNO}_3$ ) (EL grade; Kanto Chemical, Tokyo, Japan) at  $110^\circ\text{C}$  in a DigiPREP apparatus (SCP Science, Quebec, Canada) for 2 h, then 0.5 mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (semiconductor grade; Santoku Chemical, Tokyo) was added and heated at  $110^\circ\text{C}$  for

a further 30 min. After the solution was cooled to room temperature, it was diluted to 10 mL with 2%  $\text{HNO}_3$  and analyzed for 20 elements (P, K, Mg, Ca, Fe, Cu, Zn, Mn, Mo, B, Ni, Sr, Cd, Al, Ba, Cr, As, Na, Co, and Cs) by ICP-MS (ELAN DRC-e; Perkin Elmer, Waltham, MA, USA) according to the manufacturer's instructions. After Kjeldahl decomposition total N of shoot ( $n=8$ ) and root ( $n=3$ ) samples were analyzed by auto analyzer (Bran + Luebbe, AACS-III).

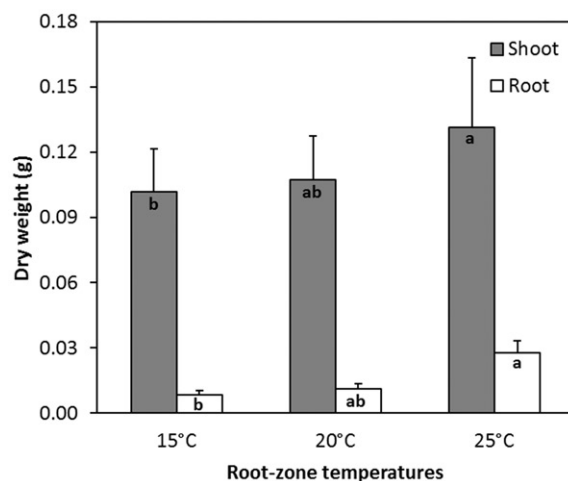
### Data analysis

All data collected were subjected to analyses of variance using the SPSS statistical program version 16.0 (SPSS Inc, Chicago, IL, USA). When significant differences were found, the means were separated at  $P < 0.05$  by least significant difference (LSD). Principal-component analysis (PCA) of 21 elements was carried out using MiniTable 15 (MiniTable, State College, PA, USA).

## RESULTS

### RZTs and shoot and root dry weights

Both root and shoot dry weights were significantly affected by RZT, with greater effects on roots (Fig. 1). The highest shoot (0.131 g) and root (0.028 g) dry weights were at  $25^\circ\text{C}$  RZT, significantly higher than at  $15^\circ\text{C}$  for which shoot (0.102 g) and root (0.009 g) dry weights were the lowest. However, shoot/root dry weight ratio increased (5, 9.5 and  $12 \text{ g g}^{-1}$ ) with decreased RZT ( $25, 20, 15^\circ\text{C}$ ). It is noteworthy that plant roots grown at



**Figure 1** Effect of root-zone temperature (RZT) on the shoot and root growth ( $n=24$ ) of *Lotus japonicus*. All data are means of 24 replications. Bars indicate standard deviations. Columns with different letters are significantly different according to least significant difference (LSD) at  $P < 0.05$ .

**Table 1** Effect of root-zone temperature (RZT) on the essential nutrient element concentration means of shoots and roots of *Lotus japonicus*<sup>†</sup>

RZT	N (mg g <sup>-1</sup> )	P (mg g <sup>-1</sup> )	K (mg g <sup>-1</sup> )	Mg (mg g <sup>-1</sup> )	Ca (mg g <sup>-1</sup> )	Fe (mg g <sup>-1</sup> )	Cu (mg g <sup>-1</sup> )	Zn (mg g <sup>-1</sup> )	Mn (mg g <sup>-1</sup> )	Mo (μg g <sup>-1</sup> )	B (μg g <sup>-1</sup> )
<i>Shoot</i> (n = 16; for nitrogen, n = 8)											
25°C	46.2 <sup>a‡</sup>	4.77 <sup>a</sup>	20.6 <sup>a</sup>	2.55 <sup>a</sup>	6.93 <sup>a</sup>	0.09 <sup>a</sup>	0.042 <sup>a</sup>	0.086 <sup>a</sup>	0.092 <sup>a</sup>	28.1 <sup>a</sup>	62.3 <sup>a</sup>
20°C	18.5 <sup>c</sup>	3.05 <sup>b</sup>	7.74 <sup>c</sup>	1.23 <sup>b</sup>	3.01 <sup>b</sup>	0.04 <sup>b</sup>	0.024 <sup>c</sup>	0.064 <sup>b</sup>	0.030 <sup>c</sup>	18.5 <sup>b</sup>	23.7 <sup>b</sup>
15°C	21.5 <sup>b</sup>	2.59 <sup>c</sup>	10.5 <sup>b</sup>	1.16 <sup>b</sup>	2.62 <sup>c</sup>	0.04 <sup>b</sup>	0.031 <sup>b</sup>	0.067 <sup>b</sup>	0.038 <sup>b</sup>	26.8 <sup>a</sup>	24.6 <sup>b</sup>
<i>Root</i> (n = 8; for nitrogen, n = 3)											
25°C	41.9 <sup>ab</sup>	7.40 <sup>c</sup>	3.79 <sup>b</sup>	1.58 <sup>a</sup>	3.67 <sup>c</sup>	4.62 <sup>b</sup>	0.19 <sup>c</sup>	0.31 <sup>c</sup>	1.33 <sup>a</sup>	7.83 <sup>b</sup>	17.9 <sup>b</sup>
20°C	41.1 <sup>b</sup>	11.8 <sup>b</sup>	12.7 <sup>a</sup>	1.19 <sup>b</sup>	5.52 <sup>b</sup>	15.1 <sup>a</sup>	6.87 <sup>b</sup>	4.32 <sup>b</sup>	0.98 <sup>b</sup>	38.1 <sup>a</sup>	14.3 <sup>b</sup>
15°C	47.2 <sup>a</sup>	20.3 <sup>a</sup>	10.4 <sup>a</sup>	1.41 <sup>ab</sup>	6.70 <sup>a</sup>	22.4 <sup>a</sup>	16.4 <sup>a</sup>	5.62 <sup>a</sup>	0.33 <sup>c</sup>	39.2 <sup>a</sup>	25.3 <sup>a</sup>

Essential elements: nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), boron (B).

<sup>†</sup>Values followed by the same letter within a column were not significantly different at  $P < 0.05$  according to least significant difference (LSD) and vice versa.

**Table 2** Effect of root-zone temperature (RZT) on the non-essential element concentration means of shoots and roots of *Lotus japonicus*<sup>†</sup>

RZT	Ni (μg g <sup>-1</sup> )	Sr (μg g <sup>-1</sup> )	Cd (μg g <sup>-1</sup> )	Al (μg g <sup>-1</sup> )	Ba (μg g <sup>-1</sup> )	Cr (μg g <sup>-1</sup> )	As (μg g <sup>-1</sup> )	Na (μg g <sup>-1</sup> )	Co (μg g <sup>-1</sup> )	Cs (μg g <sup>-1</sup> )
<i>Shoot</i> (n = 16)										
25°C	3.83 <sup>c</sup>	26.3 <sup>a</sup>	0.900 <sup>a</sup>	31.4 <sup>a</sup>	0.230 <sup>a</sup>	0.400 <sup>a</sup>	8.97 <sup>a</sup>	0.47 <sup>a</sup>	0.709 <sup>a</sup>	7.13 <sup>a</sup>
20°C	68.7 <sup>a</sup>	2.84 <sup>b</sup>	0.061 <sup>c</sup>	8.47 <sup>b</sup>	0.042 <sup>c</sup>	0.165 <sup>b</sup>	5.18 <sup>b</sup>	0.11 <sup>b</sup>	0.085 <sup>b</sup>	0.68 <sup>c</sup>
15°C	29.0 <sup>b</sup>	2.91 <sup>b</sup>	0.273 <sup>b</sup>	11.9 <sup>b</sup>	0.082 <sup>b</sup>	0.154 <sup>b</sup>	7.29 <sup>ab</sup>	0.15 <sup>b</sup>	0.090 <sup>b</sup>	1.47 <sup>b</sup>
<i>Root</i> (n = 8)										
25°C	45.7 <sup>c</sup>	40.3 <sup>c</sup>	38.5 <sup>a</sup>	105	2.99 <sup>b</sup>	19.05 <sup>b</sup>	481	0.97 <sup>c</sup>	10.64 <sup>a</sup>	1.58 <sup>c</sup>
20°C	1670 <sup>a</sup>	66.0 <sup>b</sup>	4.26 <sup>c</sup>	81.5	10.8 <sup>a</sup>	51.13 <sup>a</sup>	604	3.46 <sup>a</sup>	1.19 <sup>c</sup>	4.15 <sup>b</sup>
15°C	1273 <sup>b</sup>	125 <sup>a</sup>	14.9 <sup>b</sup>	100	13.8 <sup>a</sup>	60.04 <sup>a</sup>	709	1.94 <sup>b</sup>	2.87 <sup>b</sup>	5.88 <sup>a</sup>

Non-essential elements: nickel (Ni), strontium (Sr), cadmium (Cd), aluminum (Al), barium (Ba), chromium (Cr), arsenic (As), sodium (Na), cobalt (Co), cesium (Cs).

<sup>†</sup>Values followed by the same letter within a column were not significantly different at  $P < 0.05$  according to least significant difference (LSD) and vice versa.

sub-optimal RZTs (especially at 15°C) were long, thin and darker compared to roots grown at 25°C (data not shown). Lateral roots were also significantly shorter at sub-optimal RZT.

### Effect of RZTs on the element concentrations

Generally, for most essential nutrients analyzed, the concentrations were highest in shoots at 25°C RZT and in roots at 15°C RZT (Table 1). However, each nutrient displayed different characteristics with respect to concentration trends as well as the quantity partitioned into shoots and roots at the different RZTs.

The shoot N, K, Cu and Mn concentrations at different RZTs followed the trend  $25 > 15 > 20^\circ\text{C}$ ; and Mg, Fe, Zn and B followed  $25 > 15 = 20^\circ\text{C}$ . For P and Ca, shoot concentrations gradually decreased with increased RZTs. In the case of Mo, the shoot concentration was highest at 25°C, but not significantly different from the concentration at 15°C. The trend of mineral concentrations in roots was less consistent than that for shoot; the highest concentrations of N, P, Ca, Fe,

Cu, Zn, Mo and B were at 15°C RZT; for Mg and Mn at 25°C; and for K at 20°C (Table 1). At 25°C RZT the root P, K, Ca, Fe, Cu, Zn and Mo concentrations were lowest, while at 20°C the N, Mg and B concentrations were lowest. In roots only was Mn concentration lowest at 15°C.

RZT had a profound impact on both shoot and root concentrations of non-essential elements (Table 2). There were significantly highest shoot concentrations for all elements in plants at 25°C RZT, with the exception of Ni for which the highest concentration was at 20°C and lowest at 25°C. Strontium, Cd, Ba and Cs concentrations were lowest at 20°C RZT. Although not significantly different to values of 15°C RZT, 20°C produced the lowest concentrations for Sr, Al, As, Na and Co; and for Cr, this was at 15°C. It is interesting to note that (except Al and As) concentrations of most elements changed significantly with the introduction of sub-optimal RZT.

Unlike essential elements, root data for non-essential elements did not follow any specific trend. Strontium and Cs concentrations were highest at 15°C and lowest at 25°C RZT. The highest Ba and Cr concentrations were

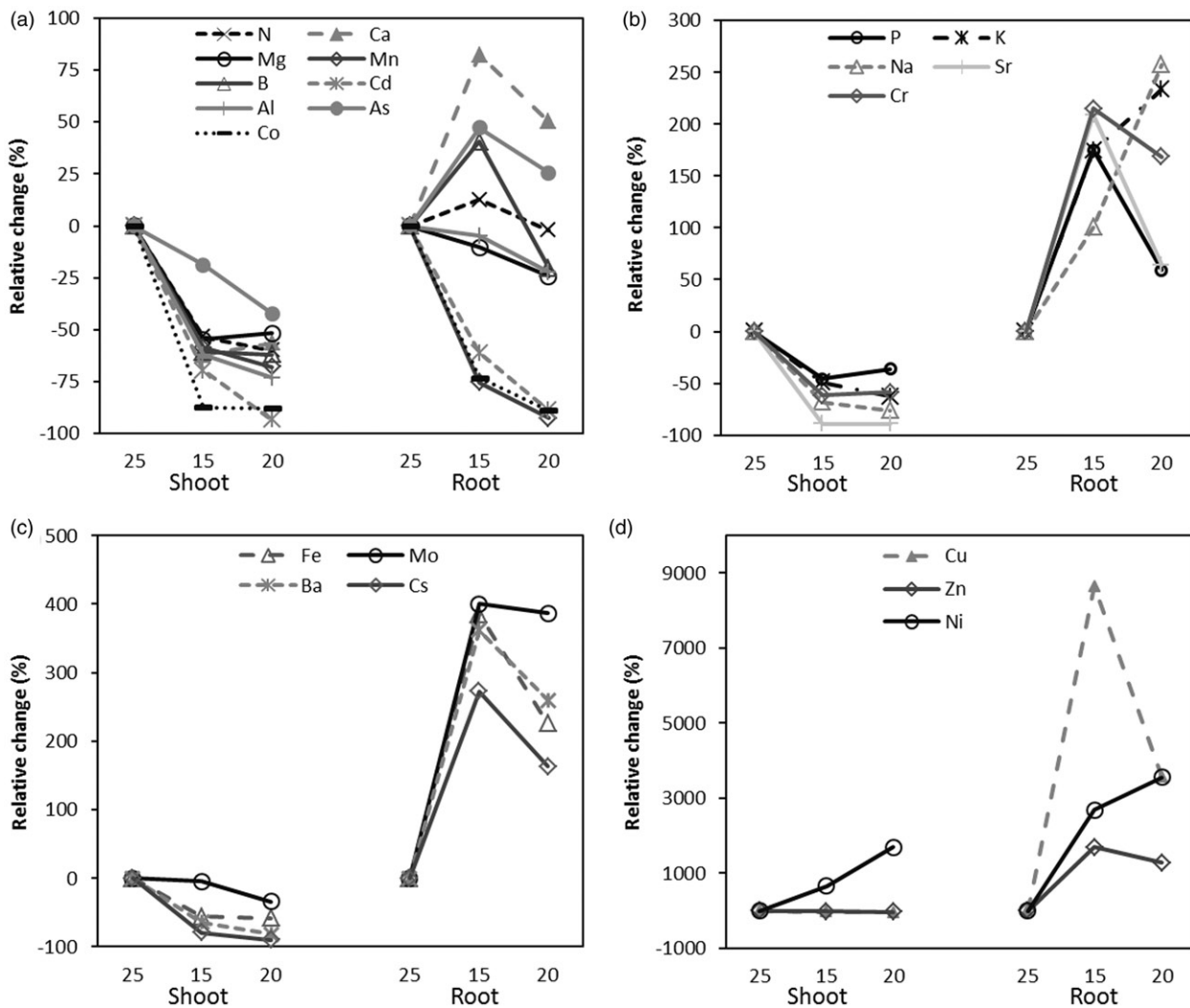


Figure 2 Relative change in *Lotus japonicus* shoot and root ion profiles of 21 elements as influenced by different root-zone temperatures (RZTs). Relative change for each element either in root or shoot was calculated by the following equation: Relative change (%) =  $\{(x - a) \times 100\} / a$ ; where  $x$  is element concentration at low RZTs and  $a$  is element concentration at 25°C RZT. The elements are arranged (a)–(d) based on the magnitude of increase/decrease of the relative concentration change: e.g. in (a) elements had  $< 100\%$  change in concentration, in (b)  $< 300\%$  change, and so on. Magnesium (Mg), manganese (Mn), aluminum (Al), cobalt (Co), nitrogen (N), calcium (Ca), cadmium (Cd), arsenic (As), boron (B), potassium (K), chromium (Cr), phosphorus (P), strontium (Sr), sodium (Na), iron (Fe), cesium (Cs), barium (Ba), molybdenum (Mo), nickel (Ni), copper (Cu), zinc (Zn).

also at 15°C RZT but not significantly different to that of 20°C RZT. Cadmium and Co concentrations in roots were highest at 25°C and lowest at 20°C RZT; the exact reverse of Ni and Na, where highest concentrations were at 20°C and lowest at 25°C RZT. Aluminum and As concentrations seemed unaffected by RZT treatments.

#### Profile variation of different elements due to RZT treatments

The effect of sub-optimal RZTs on the shoot and root concentrations of the 21 elements analyzed were compared with that of ambient (i.e. 25°C) RZT. The relative change of concentration for each element was calculated

by subtracting the element concentrations at sub-optimal RZT levels from the respective concentration at 25°C (either in roots or shoots) then dividing it by ambient RZT element concentration (expressed in percentage; Fig. 2a–d). In response to lower RZTs, element concentrations fluctuated more in roots than in shoots.

Except for Ni, all mineral concentrations in shoots decreased with the sub-optimal RZTs. However, the decline never exceeded 100%. The Ni concentration in shoots increased 17-folds at 20°C and seven-fold at 15°C RZT. Non-essential elements, most of which are heavy metals, showed considerable reductions in shoot concentrations at sub-optimal RZTs. For instance,

there were declines in Sr (89% in both cases), Cd (93 and 70%), Al (73 and 62%), Ba (82 and 65%), Cr (59 and 61%), As (42 and 19%), Na (76 and 68%), Co (88 and 87%) and Cs (90 and 79%) concentrations for the sub-optimal RZT treatments (20 and 15°C, respectively). Among the essential nutrient elements, declines in concentration were minimal in Mo (34 and 5%), Zn (25 and 22%) and Cu (43 and 28%) for 20 and 15°C RZTs, respectively. For other elements there were approximately 50% reductions. Amid all elements analyzed, only P, Ca and Cr concentrations showed gradual decreases in the relative concentration in shoots and increases in roots with decreased RZT.

There were very interesting results in the element concentrations in roots. Except for N, Mg, Mn, B, Cd, Al and Co, there was a positive change in the ion profiles for all other elements. Relative concentrations of Mn decreased by 93 and 75%, Cd by 89 and 61%, Co by 89 and 73% at 20 and 15°C RZTs, respectively. Although small, the patterns of relative changes in N and B were interesting; they were negative (−2 and −20%, respectively) at 20°C and then became positive (13 and 41%, respectively) at 15°C RZT.

There was a dramatic increase in the relative concentrations of Cu (36- and 87-folds), Zn (13- and 17-folds) and Ni (36- and 27-folds) in roots at 20 and 15°C RZT, respectively, compared to 25°C (Fig. 2d). Copper and Zn concentrations tended to increase in roots, possibly indicating a lack of mobilization of ions at lower root temperatures. At 20 and 15°C RZT, there was a significant increase in roots of the respective relative concentrations of Fe (two- and four-fold), Ba (three- and four-fold), Cs (two- and three-fold) and Mo (four-fold in both treatments) compared to 25°C (Fig. 2c).

Among other elements analyzed, in response to sub-optimal temperature treatments, there were approximately two-fold increase in roots of K, Cr, Sr, Na and P (Fig. 2b) and 100% increases/decreases in Mn, Ca, Cd and Co (Fig. 2a). The changes were not significant for other elements.

### Phyto-accumulation of mineral elements

The main accumulation of all mineral elements in shoots and roots was at 25°C RZT (Fig. 3a–d). However, the exceptions to this generalization were Ni (both shoots and roots) and Fe, Cu, Zn, Mo, Cs and Ba (in roots only).

In the case of Sr, Cd, Ba, Na, Co and Cs there were significant reductions (>70%) in shoot accumulation. Both the sub-optimal RZTs reduced Sr accumulation in shoots by 91% and in Co by 90% compared to 25°C. Cadmium in shoots was reduced by 94 and 76%; Ba by 85 and 72%; Na by 80 and 75%; and in Cs by 92 and

16% due to the introduction of 20 and 15°C RZTs, respectively. There were >70% reductions in root uptake for Mg (70 and 73%), Mn (97 and 92%), Cd (95 and 88%), Al (70 and 71%) and Co (95 and 92%) due to 20 and 15°C RZTs, respectively.

Nickel content in shoots increased to 7.23  $\mu\text{g plant}^{-1}$  (1400% increase compared to 25°C RZT) at 20°C; at 15°C it declined to 2.86  $\mu\text{g plant}^{-1}$ , although still a 500% net increase compared to ambient RZT (0.48  $\mu\text{g plant}^{-1}$ ). There was a similar trend of Ni uptake also in roots with a net increase of 1395% at 20°C and 747% at 15°C. This enhanced phyto-accumulation of Ni both in shoots and roots greatly contributed by the high concentrations observed in the respective plant parts. The pattern of Zn accumulation in roots was also similar to that of Ni, the highest total uptake was at 20°C (23.55  $\mu\text{g plant}^{-1}$ , 465% increase compared to 25°C RZT) followed by 15°C (22.77  $\mu\text{g plant}^{-1}$ , 446% increase compared to 25°C). Copper accumulation increased with decreased RZTs; the highest accumulation was at 15°C (66.30  $\mu\text{g plant}^{-1}$ , 2561% increase compared to 25°C RZT) and then at 20°C (37.45  $\mu\text{g plant}^{-1}$ , 1403% increase compared to 25°C).

Net root uptake of K, Fe, Mo, Na, Cr, Cs and Ba also increased at 20°C by 36, 33, 99, 46, 10, 115 and 47%, respectively, compared to ambient RZT. However, only Fe (47%), Mo (52%), Cs (127%) and Ba (40%) had greater root accumulation at 15°C.

Apart from the mineral elements discussed earlier, there were noticeable reductions in shoot accumulation for K (69 and 70%), B (68 and 69%), P (46 and 58%) and Ca (64 and 70%); and root accumulation for As (49 and 55%) and B (67 and 57%) due to low RZT (20 and 15°C, respectively).

### Shoot/root partitioning of minerals

The shoot/root ratio of each element indicates the translocation of ions within the plant from roots to shoots. In the present study, sub-optimal RZT greatly altered the partitioning of most elements. The extent of change in the shoot/root ratio varied with the element considered. In general, the shoot/root ratio decreases at sub-optimal RZT (Fig. 4a–c). There were two distinct types of decreasing pattern. In the first pattern, the shoot/root ratio gradually decreased with decreased RZT; this included P, Ca, B, Cu, Fe, and Sr. The second pattern included K, Mo, Zn, Cs, Na, Ba, and Cr in which the shoot/root ratio decreased at 20°C RZT then again increased at 15°C, but did not exceed the ratio values at 25°C. A decrease in shoot/root ratio indicates low mobility of an element from root to shoot.

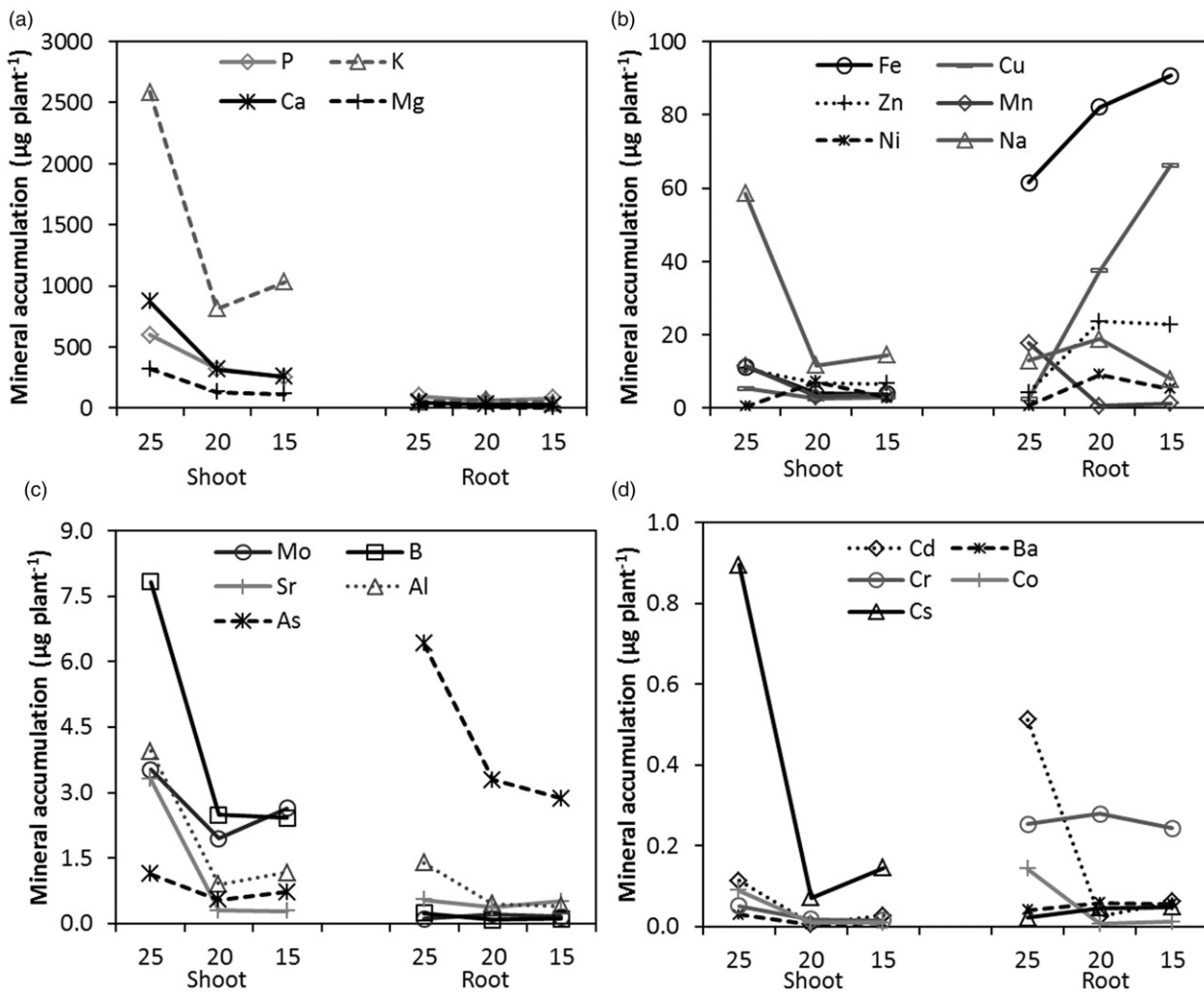


Figure 3 Accumulation of essential and non-essential mineral nutrients in *Lotus japonicus* shoots and roots at different root-zone temperatures (RZTs). Phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), nickel (Ni), copper (Cu), manganese (Mn), sodium (Na), molybdenum (Mo), strontium (Sr), arsenic (As), boron (B), aluminum (Al), cadmium (Cd), chromium (Cr), cesium (Cs), barium (Ba), cobalt (Co).

Interestingly, in the case of Mn, Mg, Co and Cd the shoot/root ratio increased at sub-optimal RZT, with most obvious change for Mn. Manganese and Co showed a similar pattern of increase: where the ratio increased at 20°C RZT and then decreased at 15°C, but still above the 25°C value. This shows that low RZT (especially 20°C) favored the mobility of Mn and Co.

The change in shoot/root ratio was not quite obvious in N, Ni, Al and As. It is noteworthy that >80% of Fe, As, Cd and Cr accumulated in roots.

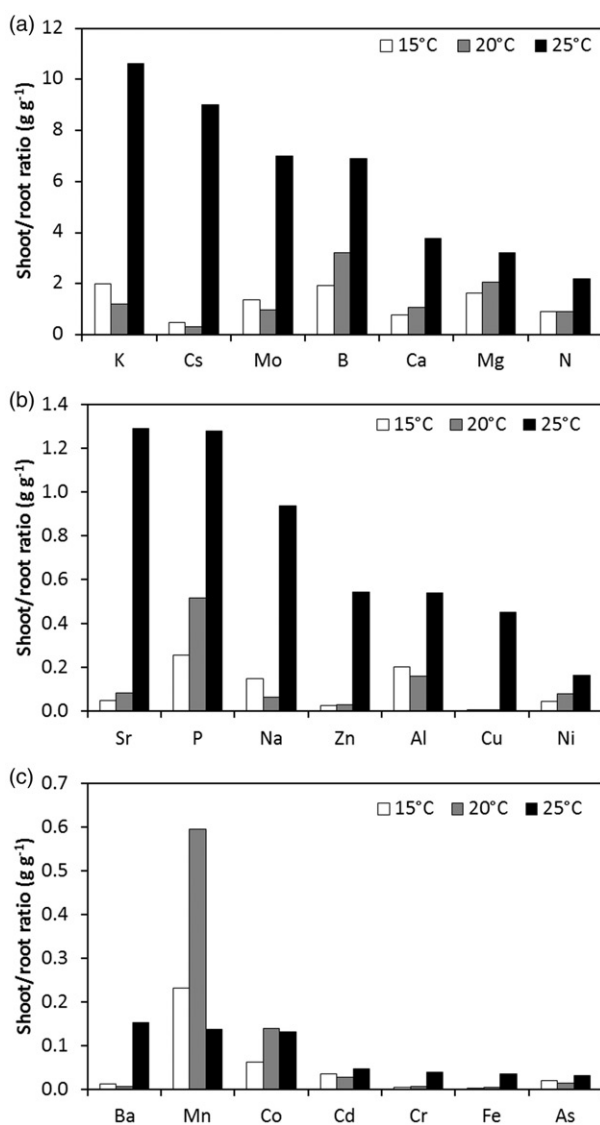
## DISCUSSION

### RZT and plant growth

In the present experiment, both root and shoot growth was reduced by sub-optimal RZT, despite uniform

favorable air temperatures (Fig. 1). Similar results were also reported for potato (Baghour *et al.* 2001, 2002a, 2002b, 2002c, 2003), *Brassica napus* (Ye *et al.* 2006) and tropical cucurbitaceous plants (Zhang *et al.* 2008): within the RZT range of 20–27°C, the total biomass increased in plants, while outside this range the dry weight fell. Effect of low RZT has been previously emphasized; Engels and Marschner (1990) found that low root temperatures not only delayed root growth but also reduced the shoot growth of corn plants. Below 20°C RZT, the growth of cucumber plants was adversely affected (Tachibana 1987). It has been suggested that shoot growth, after exposure to low RZT, is reduced mainly by altered uptake and transport of nutrients; supply of water and/or phyto-hormones from roots (Ali *et al.* 1998); and other physiological and biochemical properties of the plant (Klepper 1991).





**Figure 4** Effect of root-zone temperature (RZT) on the shoot/root ratio of different mineral elements in *Lotus japonicus*. In (a)–(c), elements are arranged in the decreasing order of shoot/root ratio at 25°C RZT. Potassium (K), cesium (Cs), molybdenum (Mo), boron (B), calcium (Ca), magnesium (Mg), nitrogen (N), strontium (Sr), phosphorus (P), sodium (Na), zinc (Zn), aluminum (Al), copper (Cu), nickel (Ni), barium (Ba), manganese (Mn), cobalt (Co), cadmium (Cd), chromium (Cr), iron (Fe), arsenic (As).

The higher shoot/root dry weight ratios at low RZT indicated a relative increase in dry matter partitioning towards shoots. Such an effect has been attributed to the maintenance of a functional equilibrium between shoot and roots (Brouwer 1983; Engels and Marschner 1990). Under normal growing conditions, plants maintain functional equilibrium; i.e. root growth is regulated by the rate of supply of carbon from shoots, whereas shoot growth is limited by the supply of water or nutrients by

the roots. However, a shift in functional equilibrium in plants occurs when the growth conditions of the surrounding environment change. There may have been such a shift in the present study, where low RZT not only affected carbohydrate production but also the distribution of photosynthate, thus leading to the increased shoot/root ratio of dry matter and probably decreased nutrient elements.

### RZT and ion uptake

In general, the highest shoot concentrations of most essential elements were at 25°C RZT, while at 15°C the concentrations were the lowest. Baghour and coworkers reported that 23–27°C was ideal for uptake of most element (Baghour *et al.* 2001, 2002a, 2002b, 2002c, 2003). Engels and Marschner (1990) reported that N, P and K concentrations in shoots of maize decreased at low RZT (12–18°C). This decline in concentration, especially for P, was probably caused by inhibition of root growth at low RZT (Mackay and Barber 1984).

Nutrient concentrations in the shoot tissue at low RZT is not only determined by nutrient availability in the culture medium but also nutrient dilution by growth. It is assumed that at ambient RZT all enzymatically mediated mechanisms with respect to mineral uptake remained optimal, thus enabling this increased mineral uptake; however, sub-optimal temperature may disrupt this balanced state.

In the present study, element concentrations fluctuated more in roots than in shoots, especially at lower RZTs. In fact, low RZT affects the carbon allocation in roots which ultimately reduces root growth. Clarkson *et al.* (1988) suggested that root growth controls the rate of nutrient uptake rather than controlled by the root itself. Again, the membrane permeability of certain elements such as P is hindered by low RZT (Lambers *et al.* 2008). All these may contribute to the significant alterations in ion concentrations in the root. Moreover, there were very high concentrations of Fe, Cu, Zn and Ba in roots compared to shoots at sub-optimal RZTs (Tables 1 and 2). In hydroponic culture, measurement of cationic micronutrients such as Fe, Zn, Cu and Mn are frequently overestimated due to strong adsorption in the root apoplast (Marschner 1995; Strasser *et al.* 1999). Many cations such as Cu<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup> are also reported to be adsorbed to the cell walls of the root tissues (Allan *et al.* 1989; Baig *et al.* 1999). This may explain the exceptionally high concentrations of such elements in roots, especially at sub-optimal RZTs.

Nickel, Cu, Zn and possibly Mn uptake and translocation are reported to be pH-dependent and is enhanced at pH <5 (Kukier *et al.* 2004; Antoniadis *et al.* 2008;

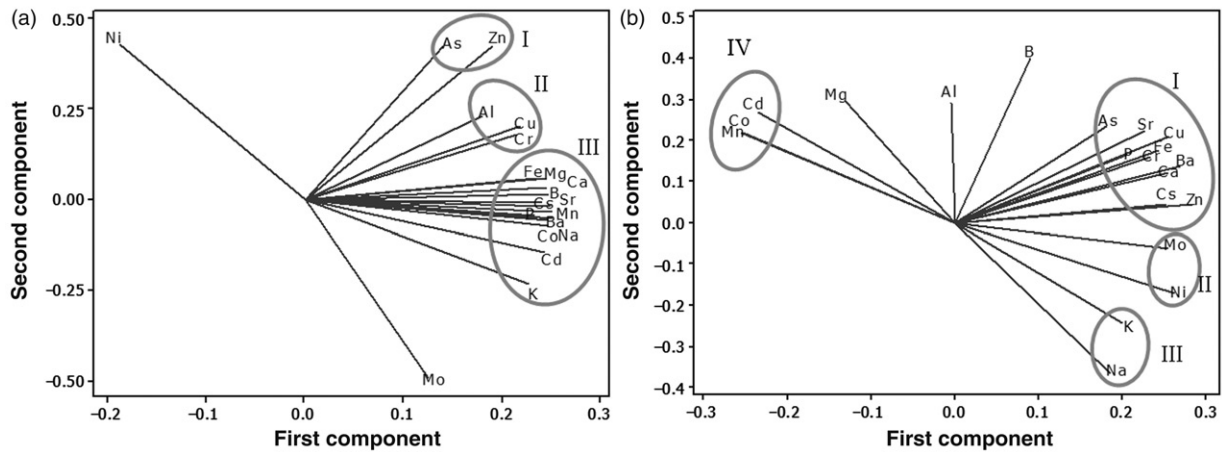


Figure 5 Biplot graphs of 20 elements prepared from the ionome of *Lotus japonicus* (a) shoots and (b) roots at different root-zone temperatures (RZTs). The length of each eigenvector is proportional to the variance in the data for that element. The angle between the eigenvectors represents the correlations among the different elements. In each figure, the circled groups of elements show strong positive correlations with each other. Since nitrogen data was obtained from a different set of samples, it was excluded from this graphical presentation. Phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), nickel (Ni), copper (Cu), manganese (Mn), sodium (Na), molybdenum (Mo), strontium (Sr), arsenic (As), boron (B), aluminum (Al), cadmium (Cd), chromium (Cr), cesium (Cs), barium (Ba), cobalt (Co).

Chen *C. et al.* 2009). In the present experiment, solution pH was 4.8 and root uptake of these ions was evident at 25°C RZT. However, at lower RZTs the mobility of these two ions seemed to be altered (Tables 1 and 2 and Fig. 3). This may be due to immobilization of these ions in the vacuoles of roots when exposed to low root temperatures. Seregin and Kozhevnikova (2006) described such immobilization of Ni in higher plants as a defense mechanism to avoid Ni toxicity in aerial parts.

It was observed that shoot/root ratio of all elements was influenced by RZTs, and in most elements the ratio decreased at lower RZTs. In *Brassica napus* at RZT as low as 10°C there were decreased shoot/root ratios of B (Ye *et al.* 2006). The low level of mineral uptake and the subsequent partitioning into root and shoot may be associated with reduced root function, which can significantly limit solute uptake (George *et al.* 2002a, 2002b) due to reduced root hydraulic conductivity and/or transpiration rate (Marschner 1995). Hydraulic conductivity is one process regulating water and nutrient uptake into roots from soil and the transpiration rate determines subsequent shoot transport of ions.

### RZT and mineral phyto-accumulation

All elements in shoots tended to accumulate at 25°C RZT while in roots only Fe, Cu, Zn and Ni tended to accumulate more at sub-optimal RZT (Fig. 4). Usually the accumulation of nutrients depends on their mobility and on the size of the sink. Since shoot growth was

enhanced by ambient RZT and the concentrations of most elements were highest at this temperature, it is very likely that phyto-accumulation was highest at ambient RZT. Similarly, reductions in phyto-accumulation of Zn, Cu, Mo, B, Al, Ba and Ni at RZT < 20°C have been reported (Baghour *et al.* 2002c, 2002d; Ye *et al.* 2006)

### Interaction among elements

The mechanisms that control ion homeostasis in an organism are reportedly interrelated (Eide *et al.* 2005). Such connections are easily revealed by PCA of the ionome data. Biplot representation of the ionome results of shoot and root showed both positive and negative correlations (Fig. 5a and b); however, the interconnection between elements varied depending on the organ concerned.

In root, Mn, Cd and Co were negatively correlated with most other elements (Fig. 5b). Elements with positive correlation were grouped together (Fig. 5b): As, P, Ca, Ba, Sr, Fe, Cr, Cu, Zn and Cs in group I; Mo and Ni in group II; and K and Na in group III.

In *Lotus japonicus*, a strategy-one plant, the uptake of Mn, Cd and Co in association with Fe is carried out mainly via root-specific ferric-chelate reductase FRO2 and IRT1 family (Baxter *et al.* 2008; Palmer and Guerinot 2009). There may be a negative correlation between these ions and Fe as IRT1 is up-regulated, especially for Mn, during Fe limitation (Williams and Pittman 2010). As in the present study, Chen *et al.*

(2007) also reported a negative correlation between Cd and K, with a reduced uptake of Cd by wheat roots due to K fertilizer application.

In the case of shoots, only Ni was negatively correlated with other elements (Fig. 5a). Whereas, As and Zn (group I); Al, Cu and Cr (group II); and Fe, Ca, Mg, Sr, B, Ba, Mn, Cs, Na, Co and Cd (group III) showed similar correlations and so were assigned to their respective groups.

The uptake of Ni<sup>2+</sup> was reportedly inhibited by Ca<sup>2+</sup> and Mg<sup>2+</sup> (Robinson *et al.* 1999). Both Ca<sup>2+</sup> and Mg<sup>2+</sup> are non-competitive inhibitors of Ni<sup>2+</sup> influx in excised barley roots; and competitive inhibition of Ni<sup>2+</sup> influx by Zn<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup> was found in barley (Körner *et al.* 1987).

The present ionome results are not entirely in accordance with the findings of Chen *Z. et al.* (2009a). In a separate study with *Lotus japonicus*, with different growing conditions, they found considerably higher concentrations of Mo, Ni, Sr and Cs but very low Mn and Cu. The shoot/root distribution of K, Mn, Zn and Sr was also of a different pattern compared with the present study. Moreover, they found a strong positive correlation between Ni and Mg and a negative correlation between Na and Cs, which is opposite to the present study. In the present study there was a positive correlation among similar groups of metals (e.g. K and Na; or Ca, Sr and Ba); however, this was not the case in the previous study. This suggested that growing conditions may alter the ionic profile of a plant and that particular environmental stimuli (RZT in the present case) may determine specific ionic signatures.

In conclusion, the present study reports the first evidence of RZT effects on the ionome of a plant. Sub-optimal RZT altered ion uptake, as well as the distribution of all 21 elements studied. RZT also influenced the interconnection among different elements, which varied with the plant part and also under different growing conditions. This suggested that environmental parameters (such as temperature) should be considered carefully in ionic studies, since ionic signatures change with particular growing conditions. Finally, since most toxic elements (e.g. Cd, Co and As) accumulated more at higher RZT, this study indicated an interesting connection between global warming and heavy metal phyto-extraction.

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