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RESEARCH PAPER

Sugar signalling mediates cluster root formation and phosphorus starvation-induced gene expression in white lupin

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Abstract

Cluster root (CR) formation contributes much to the adaptation to phosphorus (P) deficiency. CR formation by white lupin (*Lupinus albus* L.) is affected by the P-limiting level in shoots, but not in roots. Thus, shoot-derived signals have been expected to transmit the message of P-deficiency to stimulate CR formation. In this study, it is shown that sugars are required for a response to P starvation including CR formation and the expression of P starvation-induced genes. White lupin plants were grown *in vitro* on P-sufficient or P-deficient media supplemented with sucrose for 4 weeks. Sucrose supply stimulated CR formation in plants on both P-sufficient and P-deficient media, but no CR appeared on the P-sufficient medium without sucrose. Glucose and fructose also stimulated CR formation on the P-sufficient medium. On the medium with sucrose, a high concentration of inorganic phosphate in leaves did not suppress CR formation. Because sorbitol or organic acid in the media did not stimulate CR formation, the sucrose effect was not due to increased osmotic pressure or enriched energy source, that is, sucrose acted as a signal. Gene transcription induced by P starvation, *LaPT1* and *LaPEPC3*, was magnified by the combination of P limitation and sucrose feeding, and that of *LaSAP* was stimulated by sucrose supply independently of P supply. These results suggest that at least two sugar-signalling mediating systems control P starvation responses in white lupin roots. One system regulates CR formation and *LaSAP* expression, which acts even when P is sufficient if roots receive sugar as a signal. The other system controls *LaPT1* and *LaPEPC3* expression, which acts when P is insufficient.

Key words: Hexose, *Lupinus albus* L., phosphorus deficiency, proteoid root, sucrose.

Introduction

White lupin (*Lupinus albus* L.) plants have a great ability to adapt to a phosphorus (P)-deficient environment (Vance *et al*., 2003). This adaptation may be attributed mainly to the formation of special roots with closely spaced lateral roots (rootlets) of determinate growth, called cluster (or proteoid) roots (Watt and Evans, 1999*b*). Cluster roots (CR) increase P uptake from the soil by expanding the root surface area and increasing the release of proton, acid phosphatase, and citrate (Johnson *et al*., 1994, 1996*a*, *b*; Keerthisinghe *et al*., 1998; Newmann *et al*., 1999; Watt and Evans, 1999*a*, *b*; Yan *et al*., 2002). The released acid phosphatase and citrate effectively change P compounds in the rhizosphere into an absorbable form. The P-deficient condition activates phosphate transporters in CR (Liu *et al*., 2001).

The P deficiency level in plants is the predominant inducer of CR formation, the release of acid phosphatase and citrate (Johnson *et al*., 1994, 1996*b*; Keerthisinghe *et al*., 1998; Newmann *et al*., 1999; Yan *et al*., 2002), and the expression of genes, such as the secreted acid phosphatase, *LaSAP* (Wasaki *et al*., 1999; Miller *et al*., 2001), the phosphate transporter, *LaPT* (Liu *et al*., 2001), the multidrug and toxin efflux, *LaMATE* (Uhde-Stone *et al*., 2005), and the phosphoenolpyruvate carboxylase,

LaPEPC (Uhde-Stone *et al.*, 2003*a*; Peñaloza *et al.*, 2005). PEPC activity was thought to be essential for citrate biosynthesis (Johnson *et al.*, 1994; 1996*a*, *b*). Iron deficiency also induces CR formation (Hagström *et al.*, 2001). In studies on the effect of P level in plants on CR formation and citrate exudation, foliar P application and high internal P concentration in shoots suppressed CR formation and citrate exudation (Keerthisinghe *et al.*, 1998; Shane *et al.*, 2003). Split-root system studies indicated that induction of CR is suppressed in that half of the root grown in the nutrient solution without P (–P) by the high P supply to the other root half, and P concentrations in roots show no correlation with CR formation and citrate exudation (Shane *et al.*, 2003; Shen *et al.*, 2005). These studies indicate that induction of CR formation is systemic, that is, the physiological condition in shoots leads to CR formation and to organic acid and acid phosphatase exudation. Therefore, some signals and signalling cascades are expected to transport the message of P deficiency from the shoots to the roots to stimulate CR formation and organic acid and acid phosphatase release. However, such signalling mechanisms are not fully understood. Auxin is one candidate that participates in such signalling cascades. Auxin applied to leaves stimulates CR formation but does not stimulate PEPC and malate dehydrogenase activities (Gilbert *et al.*, 2000; Newmann *et al.*, 2000). Exogenously supplied cytokinin suppresses CR formation (Newmann *et al.*, 2000), suggesting cytokinin negatively correlates with such signalling pathways.

Sugars act as signals that mediate plant metabolism, growth, development, ion transport, and responses to biotic and abiotic stresses. Many sugar signal sensors and sugar signal transduction pathways have been investigated (Gibson, 2005; Rolland *et al.*, 2006), among which the hexokinase sugar sensor (Moore *et al.*, 2003) and the SnRK/SNF4-dependent signal transduction pathway (Gissot *et al.*, 2006; Lu *et al.*, 2007) have been well investigated. In white lupin, exogenously supplied sugars and photosynthates stimulate the transcription of *LaSAP*, *LaPT1*, and *LaMATE*, indicating that sugar signalling mediates the expression of P starvation-induced genes (Liu *et al.*, 2005). Liu's study predicted that sugar signalling should participate in other P starvation responses, such as CR formation in white lupin, but we believe the effect of sugar on CR formation has not been examined. In this study, the effect of sugars on the formation of cluster roots was examined to determine the signal that stimulates CR formation. CR formation and the expression of P starvation-induced genes, *LaSAP*, *LaPT1*, and *LaPEPC3*, were examined.

Materials and methods

Plant growth

The following culture media were used. 1P medium consisted of 2 mM KNO_3, 3 mM $Ca(NO_3)_2.4H_2O$, 1 mM KH_2PO_4, 1 mM $MgSO_4.7H_2O$, 0.01 mM Fe(III)-EDTA, 0.023 mM H_3BO_3, 4 µM $MnCl_2.4H_2O$, 0.46 µM $ZnSO_4$, 0.1 µM $CuSO_4$, 0.1 µM $NaMoO_4$, and 0.6% agarose (Agarose L03, Takara, Japan) at pH 5.5. –P medium contained 1 mM KCl instead of 1 mM KH_2PO_4. To 2P, 5P or 10P media, 1, 4, or 9 mM NaH_2PO_4, respectively, was added in addition to 1 mM KH_2PO_4. To avoid precipitation, these phosphate solutions were autoclaved separately from other medium components and were then mixed just before pouring onto culture plates. Sucrose, glucose, fructose, sorbitol, or organic acid at 25 mM concentration were added to the medium. Seeds of white lupin (*Lupinus albus* L. cv. Kievskij mutant) were sterilized by chlorine gas exposure for 16 h by using the same method as that applied to soybean tissue culture (Olhoft *et al.*, 2003). Sterilized seeds were pre-cultured on a –P medium for 2 or 3 d. Germinated seeds were transplanted onto 240×240 mm plates (20 mm depth) containing 250 ml medium. The plates were sealed with Parafilm M (Alcan Packaging, WI), and about two-thirds of each plate was covered with aluminium foil to shade the roots. They were kept in a growth incubator at 20 °C under a 16/8 h light/dark condition. The position of the plates was changed daily to minimize any surrounding influences.

Plants were harvested 4 weeks after the transplanting and were divided into CR, normal roots (non-cluster roots), and shoots. Their fresh weight (FW) was measured and the number of CR was counted. CR was defined as the parts of primary lateral roots bearing clusters of rootlets having a density of 10 rootlets or more per cm (Johnson *et al.*, 1994, 1996*a*). The roots and leaves were frozen at –80 °C until the inorganic phosphate determination and RNA extraction.

Fluorescence microscopy

Whole cluster rootlets and the secondary lateral roots generated on the 5P medium with sucrose were fixed in an ethanol-acetic acid solution (3:1 v:v) at 4 °C overnight, were rinsed with tap water, and were stained with 0.1 µg ml^{-1} 4′,6-diamino-2-phenylindole (DAPI) solution for more than 1 h. They were examined by using a microscope with epi-fluorescence optics.

Inorganic phosphate determination

The method for assaying inorganic phosphate was modified from Ames (1966). About 1 g FW leaves was weighed and was homogenized with 4 ml of 10% perchloride acid solution by using a mortar and pestle. After centrifugation at 15 000 rpm, 400 µl of supernatant was mixed with 4 ml solution consisting of 15 mM ammonium molybdate and 100 mM zinc acetate (pH 5.0) and then with 1 ml of fresh ascorbic acid (10%, pH 5.0). The mixture was incubated at 30 °C in a water bath for 15 min. Absorbance at 850 nm was measured.

Statistics

The number of CR and the inorganic phosphate concentration are shown as averages and standard errors (SE) of three to eight replications (most were six). Differences between treatments were tested by using the Tukey compromise test at the 5% probability level.

Expression analysis of P starvation-induced genes (quantitative RT-PCR)

Total RNA from CR and normal roots was extracted and purified by using the RNeasy Plant Mini Kit in combination with RNase-free DNase (Quagen, Germany). cDNA was synthesized by using Super Script III reverse transcriptase (Invitrogen) and the oligo dT primer. In quantitative PCR, SYBR Premix Ex Taq (Takara, Japan) was

used to intercalate SYBR Green I in amplified products, and signals were monitored by using the Chromo4 real time PCR system (Bio-Rad Japan). The amount of *LaUbiquitin* (CA410752, Uhde-Stone *et al.*, 2003*b*) mRNA in each sample was determined to normalize the differences in mRNA amount of *LaSAP* (AF309552, Miller *et al.*, 2001), *LaPT1* (AF305623, Liu *et al.*, 2001), and *LaPEPC3* (AY663387, Peñaloza *et al.*, 2005). Primer sequences were: *LaUbiquitin*, 5′-TCTTTGTGAAGACCCTCACC-3′ and 5′-CTG-CTGGTCCGGAGGAATG-3′; *LaSAP*, 5′-TCCACTCGTTACCA-TACTCC-3′ and 5′-CCTTCTAGGTTTCCTCCATCC-3′; *LaPT1*, 5′-ATAGTCCAAATTCTGTGTTGGC-3′ and 5′-ATGGTTTTCC CTGCGCCTCTTC-3′; *LaPEPC3*, 5′-TCGTGACCCGAACTT-TAATGTG-3′ and 5′-TTTTGGTGAGTGCAACTATGAT-3′.

Results

To examine the effect of sucrose on CR formation, white lupin plants were grown for 4 weeks on medium with or without sucrose *in vitro*. CR appeared on some of the primary lateral roots on the medium containing sucrose. Rootlet density and rootlet length of the CR were the same as those grown on the –P medium without sucrose (Fig. 1a, b). No CR appeared on the 1P medium without sucrose (Fig. 1c). To clarify if the CR grown on the medium containing sucrose was typical, rootlets of CR and secondary lateral roots were stained with DAPI and were observed under a fluorescence microscope. The meristem is not present in rootlets of mature CR, which is a typical feature of CR rootlets with determinate elonga-tion (Watt and Evans, 1999*a*, *b*). The secondary lateral roots (i.e. normal root) had a dense region of nuclei near the root tips, which indicated a meristematic zone (Fig. 2a) that indicated indeterminate growth. Relatively young CR rootlets that were approaching their final length showed a smaller dense region of nuclei near the root tips (Fig. 2b). Mature rootlets that reached the final length no longer showed a dense region of nuclei (Fig. 2c), that is, the apical meristem was not present. These results indicate that CR grown on the medium containing sucrose were typical CR with determinate elongation of rootlets.

In addition to relatively thick and long primary lateral roots, short (about 5 mm) and thin primary lateral roots emerged from the taproot on medium containing sucrose, resulting in a higher density of primary lateral roots than by plants on the medium without sucrose (Fig. 1). Short and thin primary lateral roots were stained with DAPI and were observed under a fluorescence microscope. These roots showed no dense region of nuclei near the root tips, indicating determinate growth (data not shown). That is, these short primary lateral roots were rootlet-like, but these CR-like taproot portions with rootlet-like lateral roots were not included when the number of CR was counted.

The effect of 25 mM sucrose, sorbitol and organic acid on CR number was examined. Fresh weight of roots and shoots was not changed significantly by the supply of
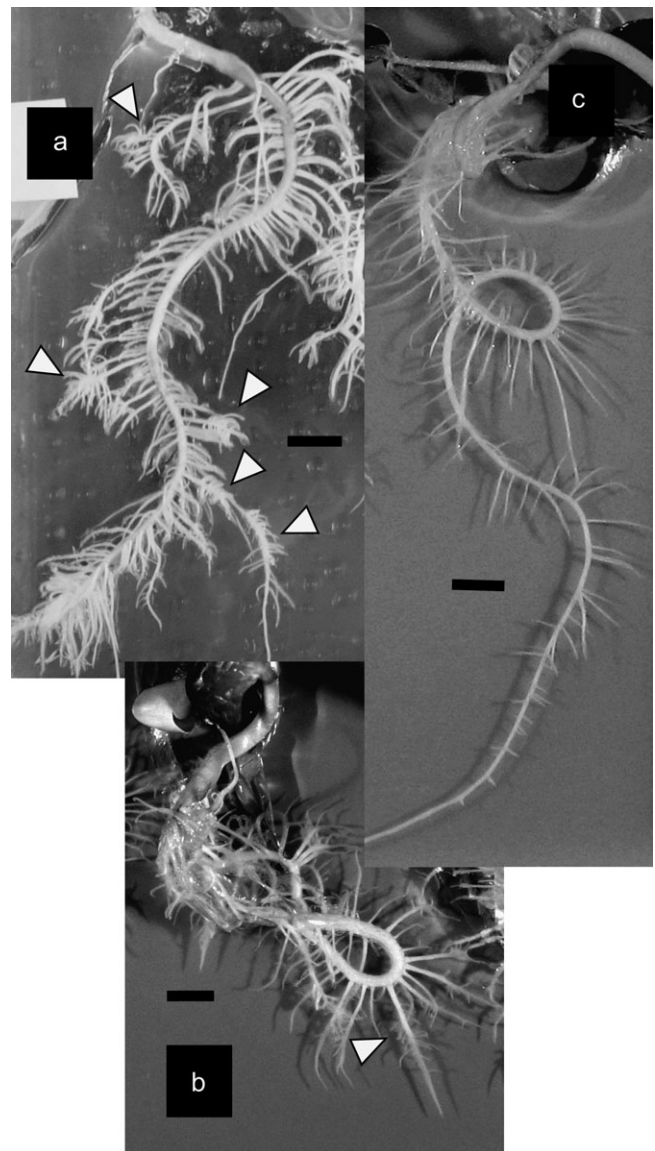

Fig. 1. White lupin roots cultured on media with 1 mM P and 25 mM sucrose (a), without P or sucrose (b), and with 1 mM P without sucrose (c) for 4 weeks. Arrowheads indicate CR (bar=1 cm).

these organic compounds (Table 1). Sucrose on media stimulated CR formation at seven and six CR under –P and 1P conditions, respectively, but no CR appeared on 1P medium without sucrose (Fig. 3). On –P medium without sucrose, 3.2 CR grew, but it was not significantly different from the no CR on the 1P medium without sucrose due to extensive variability in CR number from zero to seven. Sorbitol with or without P did not stimulate CR formation, indicating that the effect of sucrose on CR formation is not due to an increase in osmotic pressure. Sucrose acts as an energy source, so the effect of organic acids, 2-oxo-glutaric acid and malate, which can act as an energy source, on CR formation was investigated. CR numbers in plants on media containing 2-oxo-glutaric acid
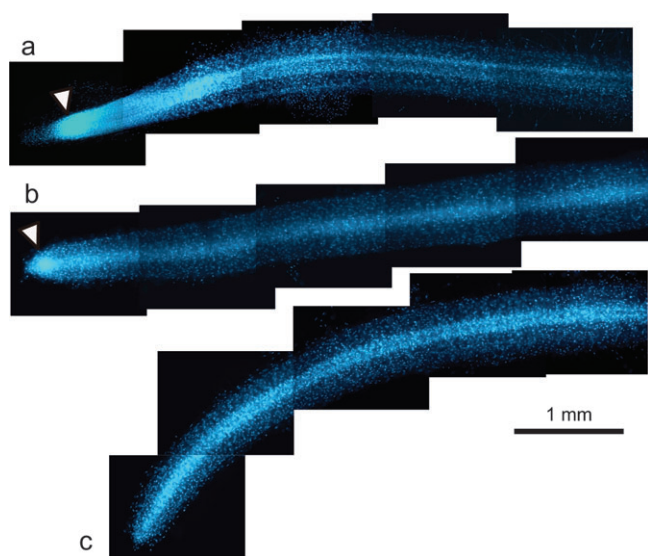
Fig. 2. Whole mounts of CR rootlets and a secondary lateral root of white lupin stained with DAPI. A secondary lateral root, i.e. a normal root (a), a young CR rootlet that was approaching its final length (b), and a mature rootlet that had reached its final length (c) were collected from plants cultured on a medium containing both P and sucrose. Arrowheads indicate a dense region of nuclei (bar=1 mm).

Table 1. *Fresh weight (average ±standard error) of white lupin plant cultured on media with (1P) or without (–P) P supplemented with sucrose, sorbitol, 2-oxo-glutaric acid or malate*

Culture condition	Root fresh weight (g)[a]	Shoot fresh weight (g)[a]
–P	0.91±0.11	2.88±0.27
1P	0.85±0.12	2.71±0.25
–P, Sucrose	1.01±0.12	2.31±0.20
1P, Sucrose	0.74±0.15	2.26±0.17
–P, Sorbitol	0.92±0.22	2.52±0.35
1P, Sorbitol	0.87±0.25	2.45±0.29
–P, 2–oxo–glutaric acid	0.66±0.20	1.95±0.23
1P, 2–oxo–glutaric acid	0.68±0.02	1.95±0.14
–P, Malate	1.20±0.31	2.51±0.29
1P, Malate	1.17±0.08	2.45±0.13

[a] After ANOVA, no significant difference was detected at the 5% level.

were almost the same as those on media without sucrose (Fig. 3). Although the primary lateral roots on –P and 1P media with malate elongated well (data not shown) resulting in a somewhat increased root fresh weight (Table 1), malate showed no effect on CR number (Fig. 3). These results indicate that stimulation of CR formation by sucrose supply is not due to an enriched energy source.

Because sugars can sequester inorganic phosphate during phosphorylation reaction, it was thought that the inorganic phosphate concentration in shoots should be low when sucrose is supplied. Therefore, the inorganic
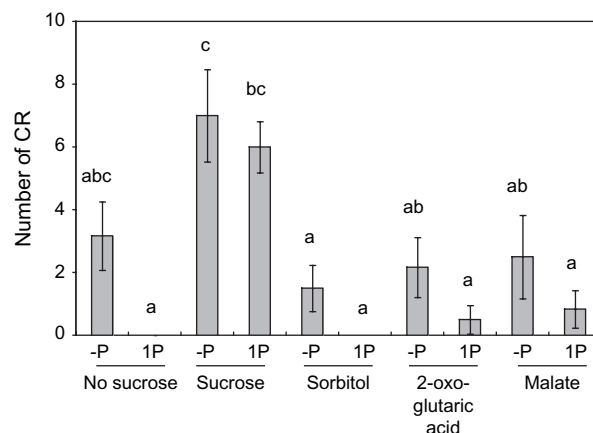

Fig. 3. Effect of sucrose, sorbitol, and organic acid on CR formation by white lupin. White lupin plants were cultured on solid media containing zero (–P) or 1 mM (1P) phosphate supplemented with 25 mM sucrose, sorbitol, 2-oxo-glutaric acid, or malate for 4 weeks. Vertical bars indicate the standard error. Histograms with different letters are significantly different at the 5% level.

phosphate concentration in leaves was determined. On both 1P-sucrose and 1P-malate media, the inorganic phosphate concentration in leaves was almost at the same level as that on the –P medium without sucrose (Fig. 4). Therefore, there was a possibility that the low level of inorganic phosphate in shoots should stimulate CR formation on medium containing sucrose. To exclude this possibility, it was investigated whether CR formation is stimulated by sucrose with a high inorganic phosphate concentration in leaves. Phosphate (0, 1, 2, 5, or 10 mM) was added to both the medium with and without sucrose, and the CR number and inorganic phosphate concentration in leaves were determined. When 5 or 10 mM phosphate was added to the medium that contained sucrose, the inorganic phosphate concentration in leaves was the same as or higher than that in leaves of plants grown on 1P medium without sucrose (Fig. 5a). In these plants, the number of CR was greater than in plants on media without sucrose (Fig. 5b), indicating that sucrose stimulated CR formation independently of inorganic phosphate level in leaves. These results indicate that sucrose stimulates CR formation as a signal, not as indirect effects.

Sucrose can be readily hydrolysed in glucose and fructose in sink organs. The effect of glucose and fructose on CR formation was examined. Supplementation of 25 mM glucose or fructose stimulated CR formation on 5 mM P containing medium (Fig. 6), indicating that these hexoses are as valuable as signal molecules as sucrose is.

The transcript accumulation of P starvation-induced genes in white lupin roots was estimated by using quantitative RT-PCR. White lupin roots secrete acid phosphatase, and the expression of the secreted acid phosphatase gene *LaSAP* can be detected in roots only
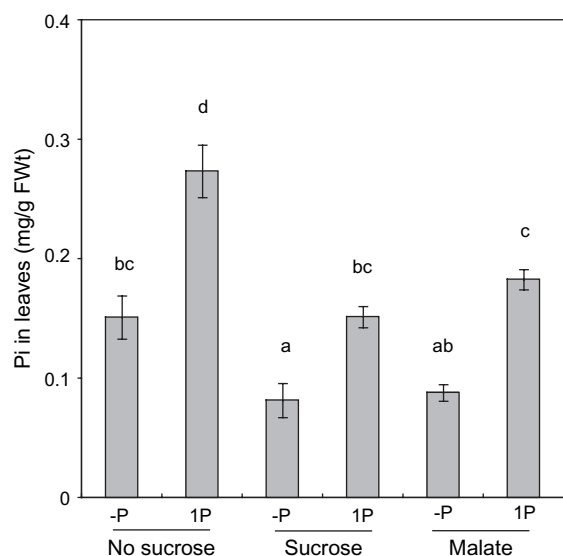
Fig. 4. Inorganic phosphate (Pi) concentration in leaves of white lupin cultured on solid medium containing zero (–P), or 1 mM (1P) phosphate supplemented with 25 mM sucrose or malate for 4 weeks. Vertical bars indicate the standard error. Histograms with different letters are significantly different at the 5% level.
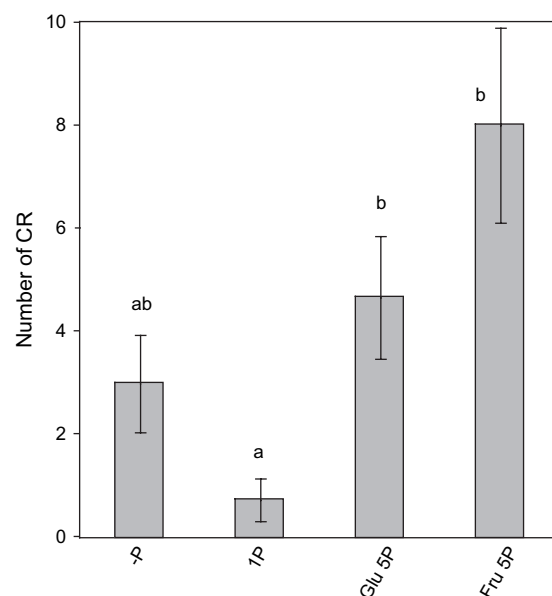

Fig. 6. Effect of 0 mM P (–P), 1 mM P (1P), 25 mM glucose (Glu) plus 5 mM P, and 25 mM fructose (Fru) plus 5 mM P on CR formation by white lupin. Vertical bars indicate the standard error. Histograms with different letters are significantly different at the 5% level.
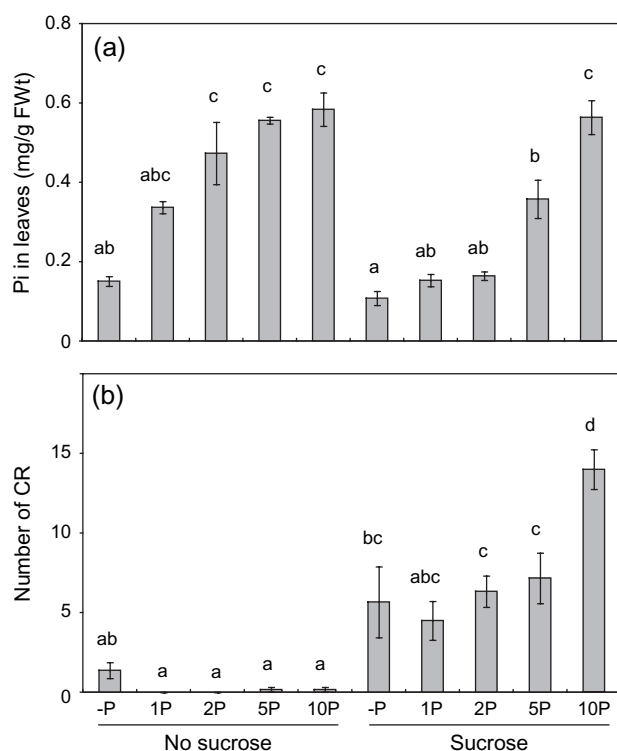

Fig. 5. Effects of sucrose on inorganic phosphate (Pi) concentration in leaves (a) and cluster root number (b) of white lupin on culture media containing zero (–P), 1 (1P), 2 (2P), 5 (5P), or 10 (10P) mM phosphate. White lupin plants were cultured on the solid media for 4 weeks. Vertical bars indicate the standard error. Histograms with different letters are significantly different at the 5% level.

under the P starvation condition (Miller *et al.*, 2001). In white lupin roots *in vitro*, transcription of *LaSAP* was nearly zero on the 5P medium without sucrose and was detected more on the –P medium without sucrose. Sucrose, however, stimulated *LaSAP* expression independently of the presence or absence of P in the medium (Fig. 7a). Both *LaPT1* and *PEPC3* are expressed weakly under normal conditions in white lupin roots, and their expression increases by P limitation (Liu *et al.*, 2001; Peñaloza *et al.*, 2005). *In vitro* white lupin in this study showed that the expression of *LaPT1* and *PEPC3* was high on the –P medium containing 25 mM sucrose, but the expression was weak in the other media, indicating that both a P-limiting condition and sucrose are necessary to increase the expression of *LaPT1* and *LaPEPC3* (Fig. 7b, c).

Discussion

Sugars act as signal molecules that regulate gene expression, growth and development in plants (Gibson, 2005; Rolland *et al.*, 2006). P starvation responses are mediated by sugar signalling, that is, sugar signalling participates in the expression of P starvation-induced genes and in the changes in the root system architecture caused by P limitation (Jain *et al.*, 2007; Karthikeyan *et al.*, 2007; Hammond and White, 2008). In white lupin, the expression of P starvation-induced genes is stimulated by endogenously supplied sugars and is suppressed under photosynthate-limiting conditions (Liu *et al.*, 2005). This study showed that endogenously supplied sucrose
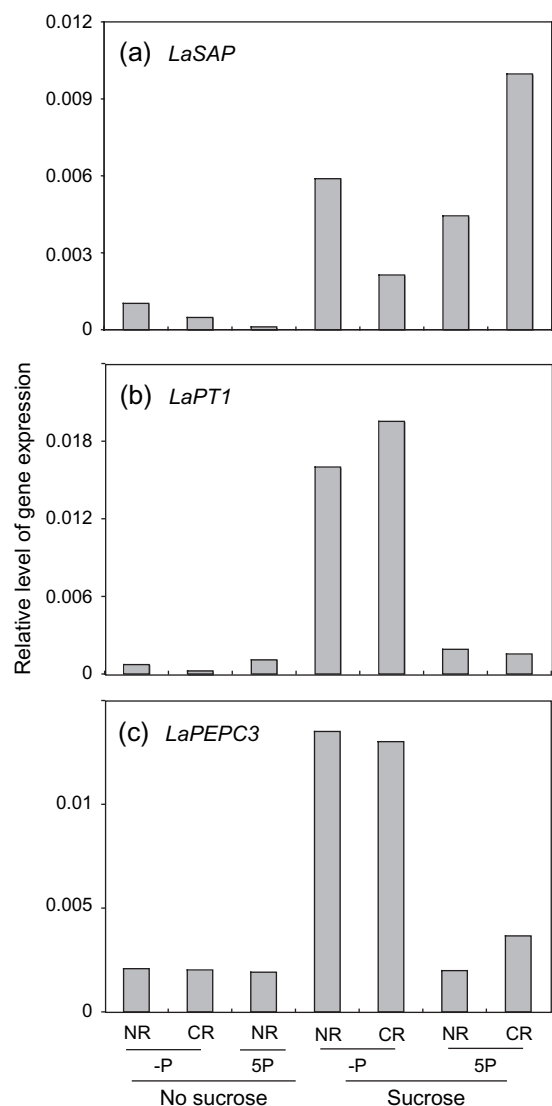
Fig. 7. Effect of sucrose and 5 mM P (5P) in media on the relative expressions of *LaSAP* (a), *LaPT1* (b), and *LaPEPC3* (c) genes in white lupin roots. *LaUbiquitin* was used to normalize the expression of these genes. NR, normal roots; CR, cluster roots.

stimulated CR formation as a signal, not as an energy source or secondary effects (Figs 3, 5). Sucrose supply induced typical CR with determinate elongation of rootlets (Fig. 2) and with increased expression of *LaPT1*, *LaSAP*, and *LaPEPC3* (Fig. 6). This sucrose effect on CR formation was independent of the presence or absence of P on the medium and of inorganic phosphate concentration in leaves (Fig. 5). The root system architecture in *Arabidopsis* is modulated by P limitation: suppressed taproot elongation and increased lateral root density. Exogenously supplied sugars stimulate *Arabidopsis* roots to increase lateral root density independently of the presence or absence of P in media and, however, have no effect on taproot length (Jain *et al.*, 2007; Karthikeyan *et al.*, 2007). Although CR is the special root architecture

in white lupin, the regulation system to stimulate CR formation mediated by sugar signalling should be similar to that to increase lateral root density in *Arabidopsis*.

Although both hexose and sucrose are usually valuable as a signal, sucrose-specific induction of gene expression is reported in some sugar signalling pathways and glucose and fructose are less effective in them (Smeekens, 2000; Rolland *et al.*, 2006). In this study, glucose and fructose showed an effect on CR formation (Fig. 6). The expression of P starvation-induced genes, *LaPT1* and *LaSAP*, is enhanced by the supply of sucrose, glucose, and fructose in white lupin seedlings (Liu *et al.*, 2005). This evidence suggests that hexose acts as the signal molecules in the signalling of P starvation responses in white lupin roots. There are at least three systems for hexose sensing; a hexokinase-dependent system, a glycolysis-dependent system and a hexokinase-independent system (Xiao *et al.*, 2000; Rolland *et al.*, 2006). Further studies to determine the sugar-sensing system are necessary in white lupin roots. Exogenously supplied sugars stimulate *Arabidopsis* roots to increase lateral root density, and Karthikeyan *et al.* (2007) showed by using the *gin2* hexokinese mutant that a hexokinese sugar sensor participates in some aspects of the P deficiency response mechanism in lateral roots.

In this study, sugar signalling of the expression of P starvation-induced genes was investigated. The expression of *LaSAP* increased on the sucrose-containing media with or without P (Fig. 7). Liu *et al.* (2005) showed in their northern blot analysis that *LaSAP* gene expression in white lupin seedlings is induced by sugar supply even with sufficient P. In this study, in contrast to *LaSAP*, both P deficiency and sucrose supply were necessary to increase *LaPT1* and *LaPEPC3* expressions, but the presence of P and sucrose together had no or little effect on the increase (Fig. 7). Liu *et al.* (2005) showed that the expressions of *LaPT1* and *LaMATE* in seedlings are stimulated by the combination of P limitation and sugars. That is, regulations of *LaSAP* expression and *LaPT1* and *LaPEPC3* expressions differ but sugar signalling participates in both. Many genes induced by P starvation in *Arabidopsis* have been examined. Among them, the transcription of some genes, such as phosphate transporters *Pht1;1* and *Pht1;4* and purple acid phosphatase *PAP*, increases by sugar treatment when P is insufficient (Müller *et al.*, 2005; Karthikeyan *et al.*, 2007). However, sugar supply stimulates the expression of the acid phosphatase *ACP5*, even when P is sufficient (Müller *et al.*, 2005). That is, several pathways mediated by a sugar signal exsist that regulate P starvation responses. DNA microarray analysis of *Arabidopsis* showed that transcription of 187 of 21500 genes changes more than 2-fold by P starvation: 171 were induced and 16 were repressed (Müller *et al.*, 2007). In that DNA microarray analysis, 149 genes that were synergistically

or antagonistically regulated by P limitation and sugar supply were selected and categorized into three clusters (clusters 1, 2, and 3) according to the expression profile, and possible *cis*-regulatory elements in these genes were identified. P starvation stimulated the transcription of the cluster 3 genes, which was magnified by sucrose feeding, and 43% (20 of 47) of the cluster 3 genes contained the *PHR1* binding site in their 3′ upstream region (Müller *et al.*, 2007). *PHR1* is the MYB-CC transcription factor that regulates P starvation responses (Rubio *et al.*, 2001). This observation indicates that, in addition to sugar signals, many kinds of transcription factors as well as other signals and sensors participate in the regulation of gene expression induced by P deficiency.

DNA microarray and metabolome analyses in rice, common bean, and *Arabidopsis* reveal that gene transcription and enzyme activities in carbohydrate synthesis are increased in P-starved tissues that accumulate sucrose, hexoses, and starch (Wasaki *et al.*, 2003, 2006; Hernández *et al.*, 2007; Morcuende *et al.*, 2007). Allocation of dry matter and ^{13}C changes when P is insufficient, and roots receive increased allocation of photosynthates in common bean, *Arabidopsis* and tomato (Nielsen *et al.*, 2001; López-Bucio *et al.*, 2002; Fujita *et al.*, 2003). The *PHO3* gene in *Arabidopsis* encodes *SUC2*, the sucrose transporter that participates in phloem loading in shoots, and the *pho3* mutant has limited ability to transport sucrose from leaves to roots (Lloyd and Zakhleniuk, 2004). Interestingly, the *pho3* mutant is thought to be impaired in the expression of P starvation-induced genes in roots because this mutant was originally identified as a mutant that suppresses root phosphatase (Hammond and White, 2008). Thus, sugars have been proposed to be a long-range signal molecule synthesized mainly in the shoot and transported from the shoot to the roots to trigger plant responses to P limitation (Hermans *et al.*, 2006; Hammond and White, 2008). Therefore, it is postulated that white lupin uses sugars as a systemic signal exported from shoots. This idea was supported by the stem girdling experiment using white lupin in which the expression of P starvation-induced genes in roots was repressed 24 h after the girdling (Liu *et al.*, 2005). At least two sugar signal-mediating regulation systems control P starvation responses in white lupin roots. One system regulates CR formation and *LaSAP* expression and acts even when P is sufficient if roots receive a sugar signal. The other system regulates *LaPT1* and *LaPEPC3* gene expression and acts when P is limited, suggesting that sugar signalling interacts with other signalling networks of signals such as a local P-starvation signal or another systemic signal or both. Both the mechanism that senses low P in the rhizosphere (Svistoonoff *et al.*, 2007) and the micro-RNA399-mediating signalling cascade that regulates P starvation responses (Bari *et al.*, 2006; Chiou, 2007) have been clarified for *Arabidopsis*. Further analysis is necessary to examine how sugar signalling interacts with other signalling networks.

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