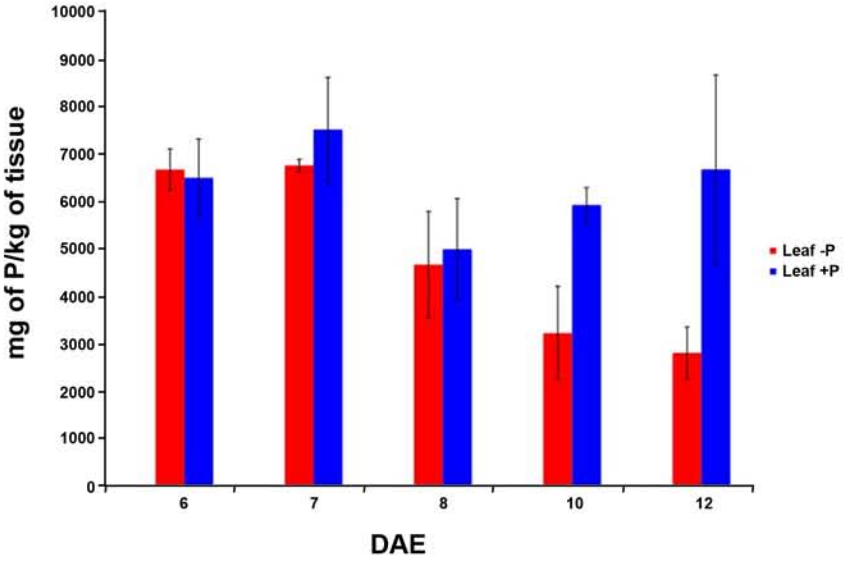
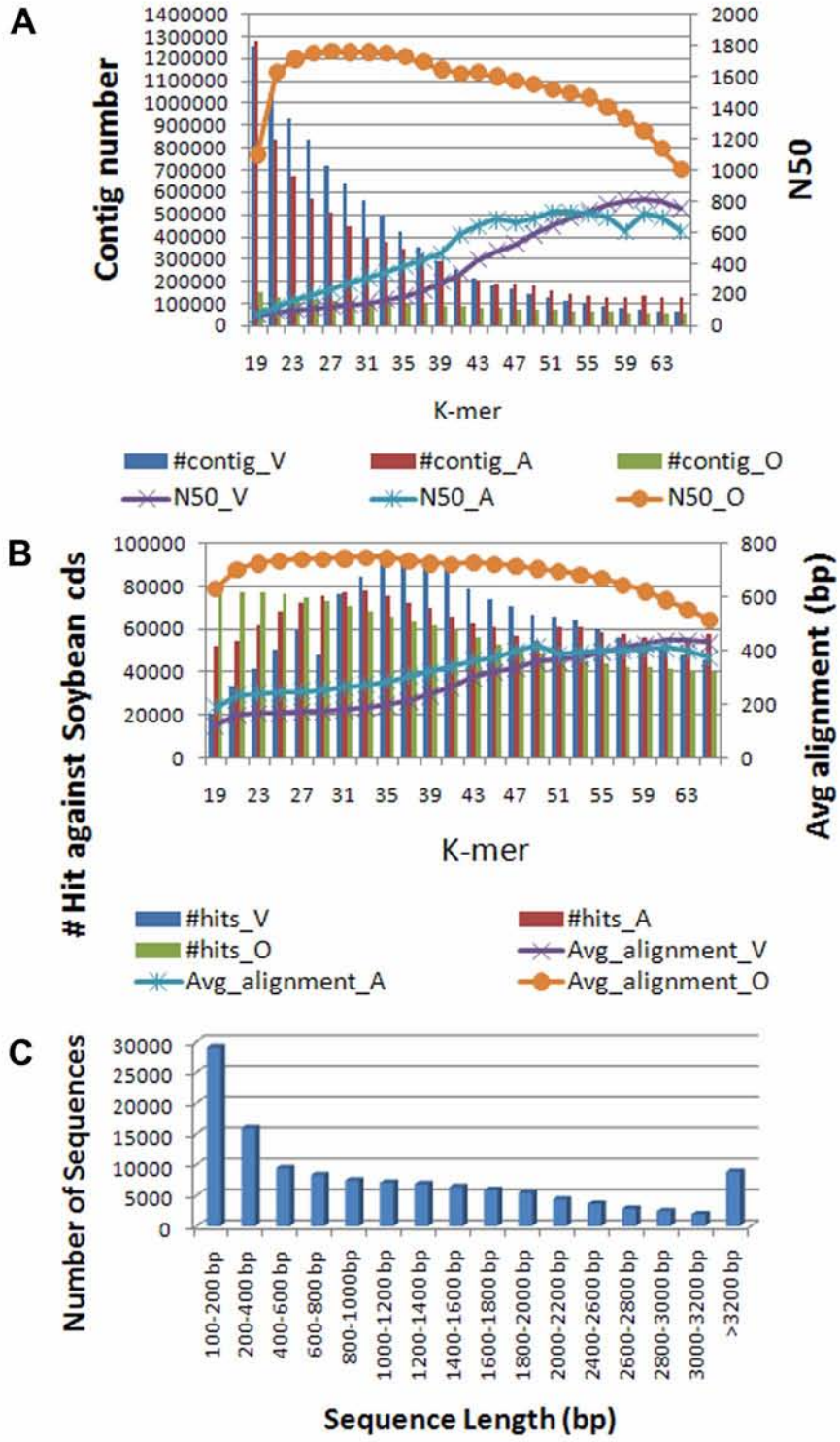
Supplemental Figure 1



Supplemental Figure 1. Total phosphorus content in developing white lupin shoot tissue.

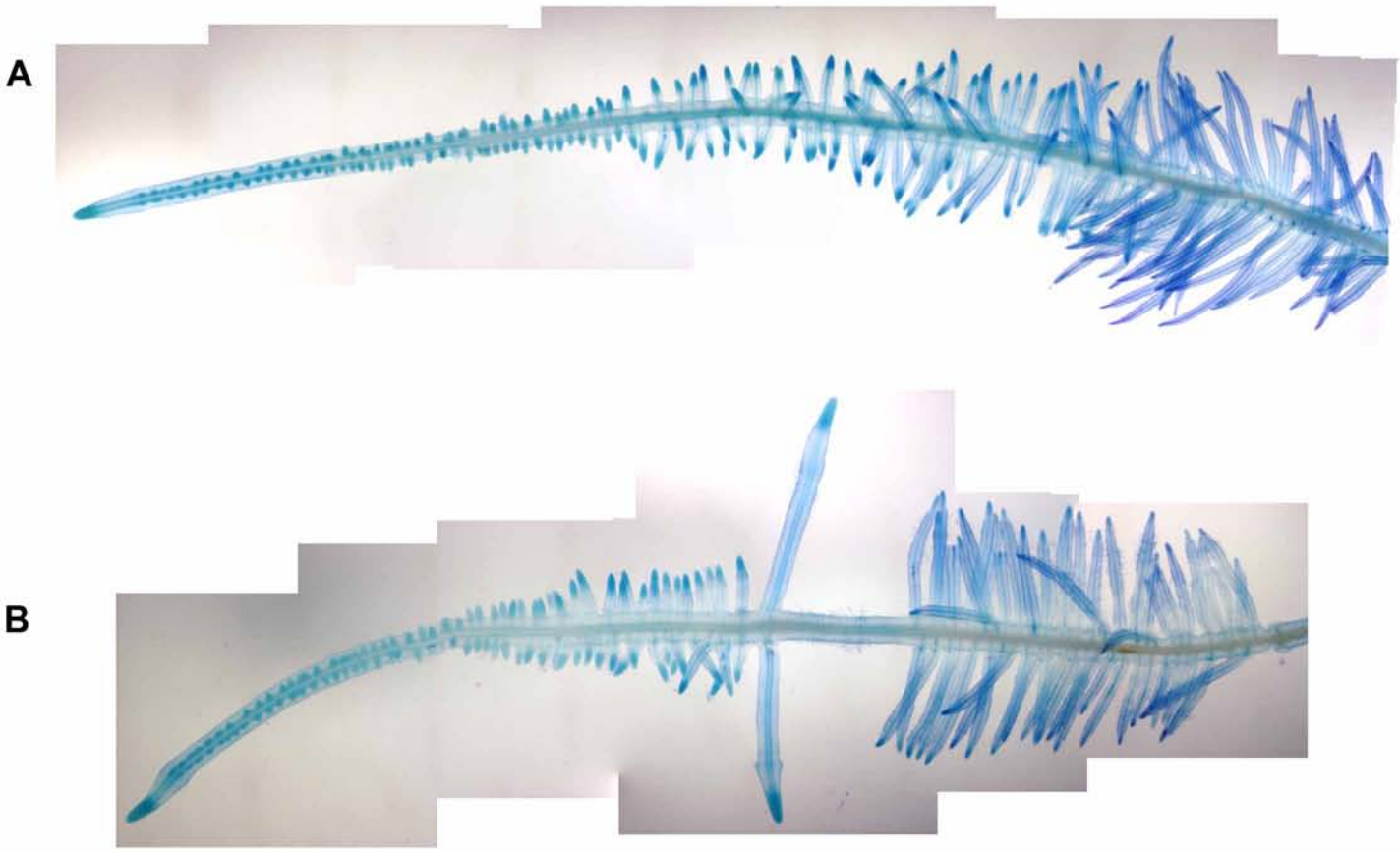
Seeds were germinated and treated as described in the materials and methods. Three seedlings were harvested at 6, 7. 8, 10, and 12 days after emergence (DAE) and total leaf phosphorus content of each were measured (mg of P / kg of tissue) by ICP (inductively coupled plasma) analysis. Note, seedlings placed in P_i sufficient and P_i deficient growth conditions show no difference in total plant P content until 10 DAE.

Supplemental Figure 2



Supplemental Figure 2. Optimization of the *de novo* Assembly.
A, comparison of number of contigs generated (Y-axis, Contig number) and the contig contiguinity (Y-axis, N50) using a variety of K-mers (X-axis) and three programs. V, Velvet alone; A, ABySS; O, Velvet/Oases.
B, Comparing contigs generated by three programs to the soybean CDS. Number of contigs showing with BLAST homology to soybean hit using a variety of K-mers (X-axis), Y-axis, # hit against soybean CDS. Average length of the alignment hitting the soybean CDS, Y-axis Av. Alignment bp. V, Velvet alone; A, ABySS; O, Velvet/Oases.
C, Sequence size distribution using optimal K-mer (29) and program (Velvet/Oases) for LAGI 1.0. Length of sequences (X-axis) and the number of sequences of each length (Y-axis) in the LAGI 1.0 assembly.

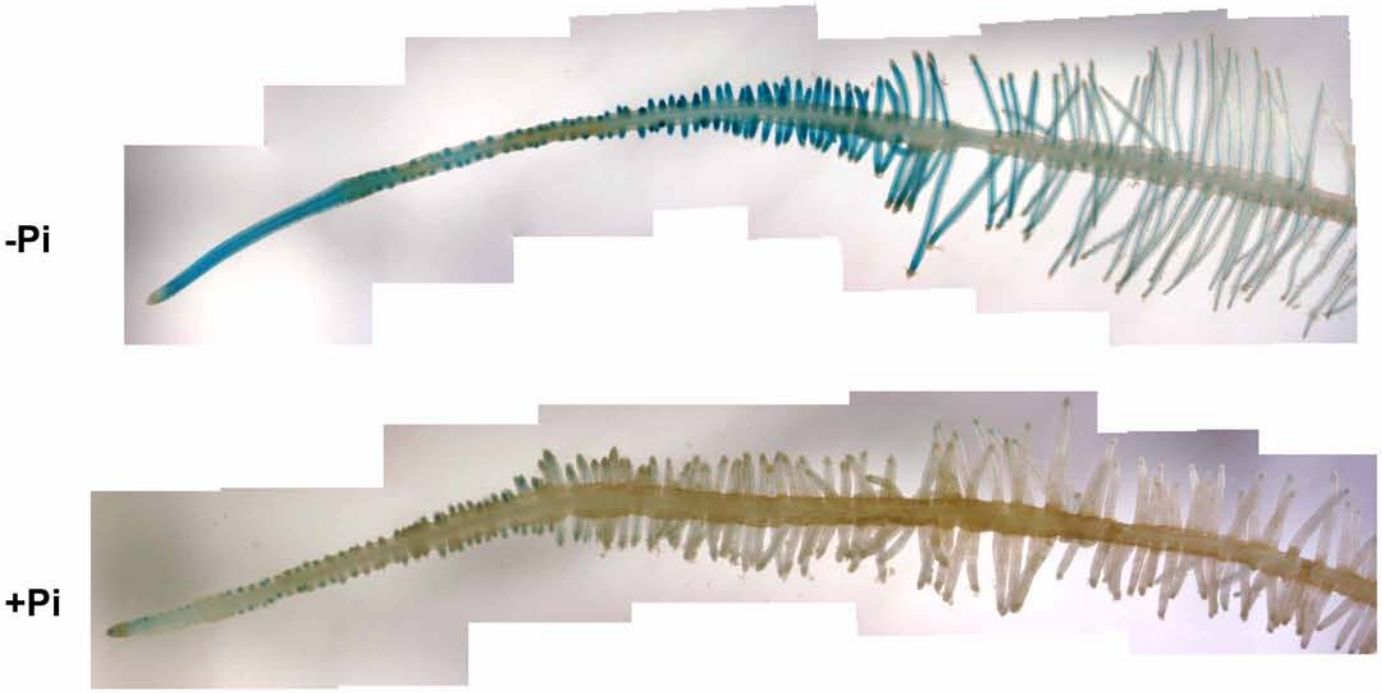
Supplemental Figure 3



Supplemental Figure 3. Phenotype of cytokinin oxidase RNAi (CKXi) white lupin P_i deficient cluster root.

Methylene blue staining was used to visualize P_i deficient cluster root development. Four roots per plant from a total of 16 independent transformation events were evaluated. A, Control white lupin cluster root. Rootlets emerge along a primary lateral root. B, CKXi cluster root. Note the interruption in rootlet emergence in the developing cluster root and elongation of first lateral rootlet after interruption. This altered phenotype emphasizes the importance of cytokinin, and cytokinin breakdown, in regulating lateral root development.

Supplemental Figure 4



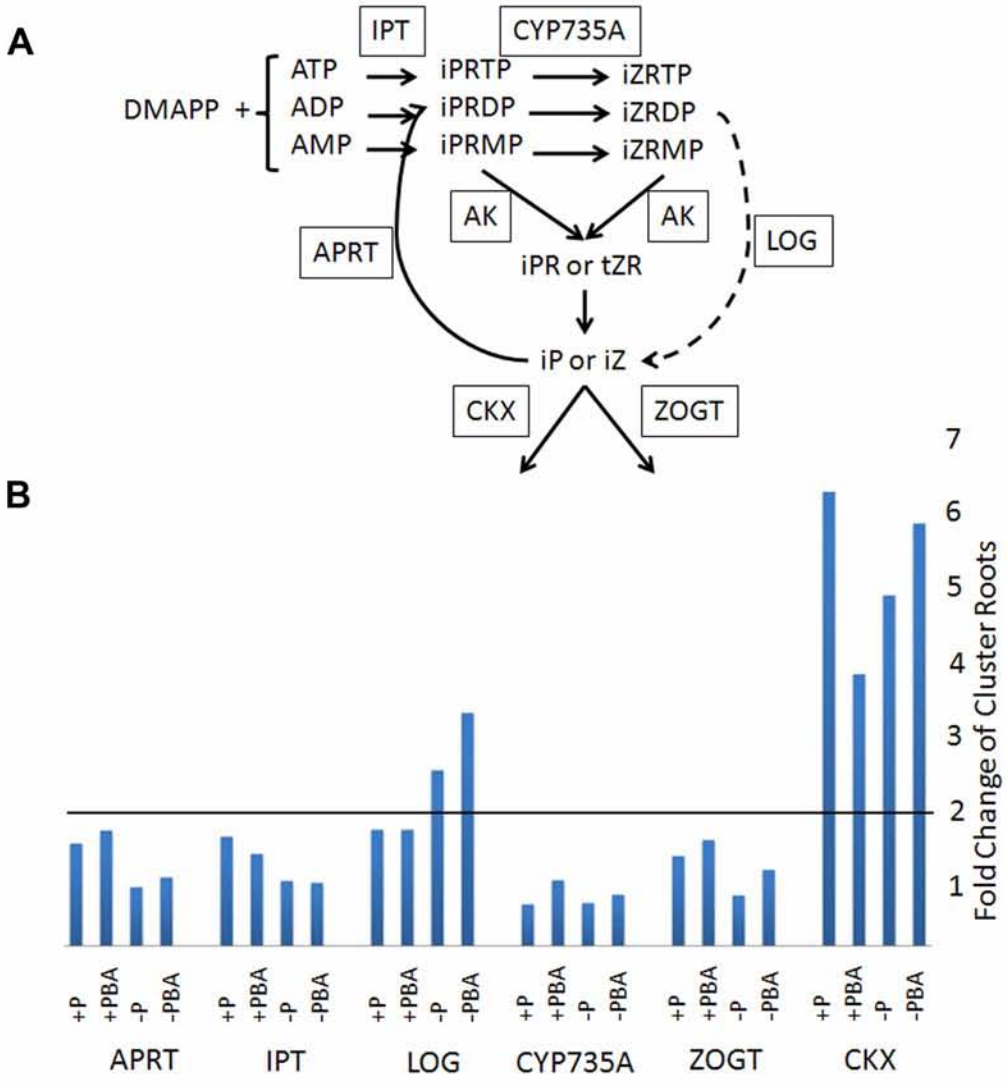
Supplemental Figure 4. ß-glucuronidase (GUS) staining of cytokinin receptor (CRE) expression.
Transgenic white lupin roots with the CRE promoter driving GUS expression were generated via *A. rhizogenes* mediated hairy root transformation. Transgenic roots from both $+P_i$ and $-P_i$ plants were collected at 14 days after emergence. A minimum of four roots per plant from a total of 16 independent transformation events were evaluated. CRE: GUS expression is visible throughout the entire transgenic root in $-P_i$ plants, but is almost completely lacking in $+P_i$ grown plants, illustrating the importance of cytokinin signaling both on lateral root growth and in the P_i deficiency acclimation.

Supplemental Figure 5

Supplemental Figure 5. ß-glucuronidase (GUS) staining of cytokinin oxidase (CKX) expression

Transgenic white lupin roots containing the CKX promoter driving GUS expression were generated via *A. rhizogenes* mediated hairy root transformation. Transgenic roots from both $+P_i$ and $-P_i$ plants were evaluated 14 days after emergence. A minimum of four roots per plant from a total of 16 independent transformation events were evaluated. In P_i deficient ($-P_i$) plants GUS expression is intense in meristematic and elongating regions of transgenic rootlets (inset panel A). In contrast, P_i sufficient ($+P_i$) plants exhibit more diffuse GUS expression (inset panel B).

Supplemental Figure 6

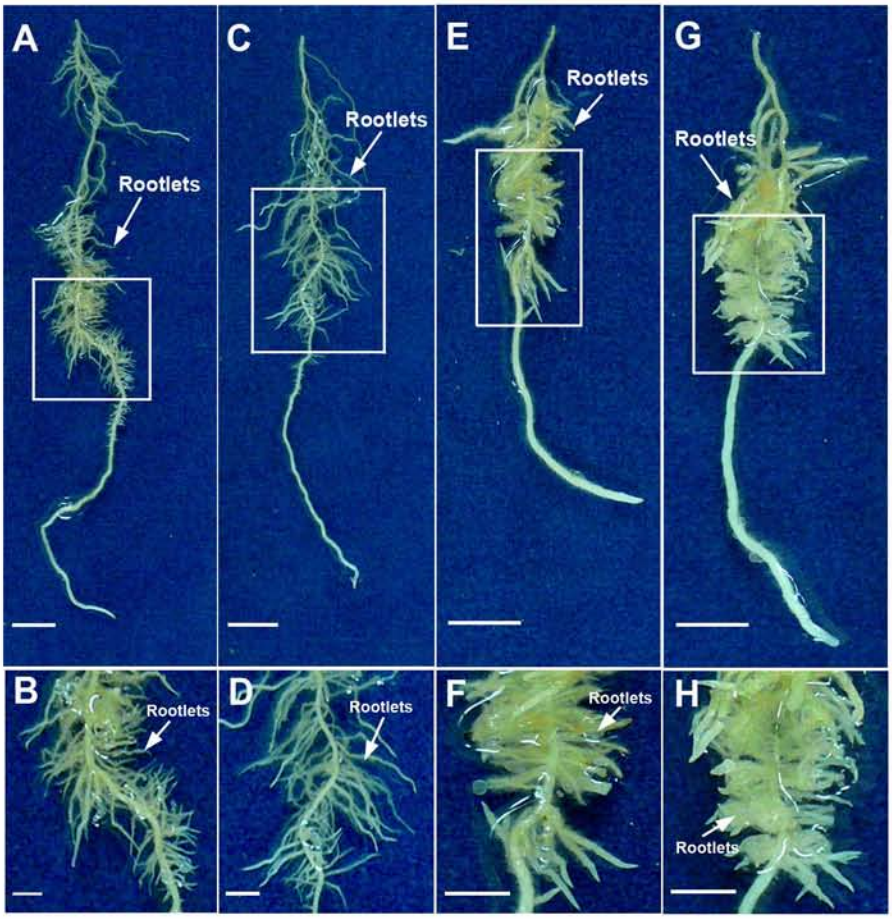


Supplemental Figure 6. Gene expression patterns for cytokinin activation and degradation.

A, Simplified cytokinin pathway illustrating the important steps in cytokinin activation and degradation adapted from Kurakawa et al. (2007).

B, Gene expression patterns as determined by qPCR analysis for the genes involved in cytokinin biosynthesis and breakdown from the cytokinin hormone treatment experiment for cluster roots grown in $+P_i$ and $-P_i$ conditions (no effect of cytokinin treatment on gene expression was seen in normal roots). Plants grown under normal (no BA application) P_i sufficient conditions (+P). Plants grown in P_i sufficient conditions with BA application (+PBA). Plants grown in P_i deficient conditions (no BA application), -P. Plants grown in $-P_i$ conditions with BA application (-PBA). P_i deficient cluster roots show a greater than two fold increase in expression of the one-step cytokinin activation pathway gene, LOG. Additionally, cluster roots from both P_i sufficient and P_i deficient conditions, regardless of the application of exogenous BA, show increased expression of CKX (cytokinin oxidase). Under P_i sufficient conditions, the application of BA results in reduced CKX expression while in P_i deficient conditions the application of BA induces CKX expression. This analysis suggests cluster roots invoke CKX to breakdown bioactive cytokinins, which would inhibit lateral root growth, especially under P_i deficient conditions.

Supplemental Figure 7



Supplemental Figure 7. The effect of gibberellic acid (GA) and paclobutrazol (Paclo) on rootlet density of cluster roots.

All root samples were collected at 14 days after emergence (DAE) of shoots from quartz sand growth media. GA treated plants were given 10^{-6} M GA at 3, 6, 9, and 12 DAE. Paclobutrazol treated plants were given 1mg Paclo /6L volume pot at 5 DAE. Panels A-G, cluster roots. Panels B-H, enlargement of boxed area showing rootlets. Panel A and B, cluster roots from P_i deficient plants. Panels C and D, cluster roots from P_i deficient plants treated with GA. Panels E and F, cluster roots from P_i deficient plants treated with Paclo. Panels G and H, cluster roots from P_i sufficient plants treated with Paclo. Note, GA treatment reduces rootlet density while Paclo increases rootlet density. Paclo treatment induces cluster roots on P_i sufficient plants, mimicking the P_i deficient cluster root response. Scale bar for A, C, E, and G = 1 cm; for B, D, F, and H = 0.5 cm.