FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

GRS10329

Investigating the role of root architecture regulators as mediators of environmental information in root development

PROJECT DETAILS

PROJECT CODE:	GRS10329
PROJECT TITLE:	INVESTIGATING THE ROLE OF ROOT ARCHITECTURE REGULATORS AS MEDIATORS OF ENVIRONMENTAL INFORMATION IN ROOT DEVELOPMENT
START DATE:	13.02.2012
END DATE:	12.08.2015
SUPERVISOR:	MICHAEL DJORDJEVIC (LAB LEADER)
ORGANISATION:	AUSTRALIAN NATIONAL UNIVERSITY
CONTACT NAME:	MICHAEL DJORDJEVIC

Summary

Progress summary

The project has been completed and the thesis was submitted for examination on 23/09/2015.

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Conclusions

Regulatory peptides are being increasingly recognised for playing key roles in plant development. Here, the analysis of the C-terminally encoded peptide (CEP) family of regulatory peptides has been extended. Two distinct classes of CEP genes were identified. Phylogenetic analysis has been used to explore relationships between CEPs and this was complemented by expression analysis. The expression of eight of the nine CEPs tested was perturbed by environmental cues such as decreased nitrogen (N) levels, increased salt levels, increased osmotic strength and increased CO_2 levels. The importance of individual residues in biological activity was explored. To initiate the study of CEPs in *Arabidopsis*, the relationships between the 15 genes, their protein and peptide products and their expression patterns were explored using *in silico* tools. This formed the basis for experiments examining the expression of CEP genes. It was found that CEPs are induced by abiotic stress conditions, particularly N limitation, and their spatial expression is tightly regulated. To gain an understanding of the developmental pathways that were affected by CEPmis-expression, transgenic plants over-expressing CEP genes were examined. It was found that CEPs play a significant role in determining root system architecture and also affect shoot morphology. Synthetic peptide assays were used to corroborate these results and to further examine the importance of the amino acid sequence and post-translational modifications of the peptide ligand. After confirming that CEPs are negative regulators of primary and lateral root development, CEP3 was chosen for further in-depth analyses.

A CEP3 T-DNA insertion mutant was isolated and characterised. It was found that this mutant was more resistant to a range of abiotic stresses, including N limitation. The roles of CEP3 in lateral root and primary root development were then examined. The effect of CEP3 peptide on lateral root prebranch site formation, founder cell specification and lateral root primordia development was examined. This revealed that CEP3 reduces lateral root number, probably at the initiation stage. In the primary root, excess CEP3 caused a slowing of root growth and smaller root meristem. Reporter construct analysis revealed this was due to a perturbation in cell cycle progression. Flow cytometry was used to show that CEP3 peptide reduced the number of root tip cells in the synthesis phase of the cell cycle, whereas in the CEP3 mutant, more cells were in this phase.

Transcriptomic analysis was then used to explore the role of CEP3 in altering growth. The results indicated that CEP3 affects the expression of genes involved in N uptake, transport and assimilation, possibly controlling the stress induced nitrate allocation to roots (SINAR) response. A model for CEP3 activity was then proposed where CEP3 is induced in the roots by low N and is perceived by one or more receptors, presumably triggering a signalling cascade that ends in activation or repression of specific N uptake, assimilation and allocation genes. Due to the changes in expression of these genes, there may be a decrease in nitrate (and N assimilate) in the roots, resulting in decreased supply of resources to the Regulation of Ace2 and Morphogenesis (RAM) where cell cycle progression is consequently affected. The alteration in cell cycle results in drastically slowed root growth.

Achievement/Benefit

Overview of project achievements

This project has resulted in gains in the understanding of how root development is affected by different

environmental stimuli. New research findings include:

- A family of signalling peptides (CEPs) has been described and characterised.
- This peptide family is responsive to stress conditions including nutrient, drought and salt stresses.
- Members of this family negatively regulate root development.
- A detailed study of one member of this peptide family (CEP3) was undertaken, resulting in the first description of the mode-of-action of any family member in *Arabidopsis*.
- CEP3 may act in controlling nutrient allocation and use efficiency by perturbing the expression of key genes involved in this process.

It has also resulted in a number of publications, with more planned in the near future.

Additional Information

Publications

Delay, C., Imin, N., and Djordjevic, M.A. (2013). Regulation of *Arabidopsis* root development by small signalling peptides. Frontiers in Plant Science 4, 352.

Delay, C., Imin, N., and Djordjevlc, M.A. (2013). CEP genes regulate root and shoot development in response to environmental cues and are specific to seed plants. Journal of Experimental Botany 64, 5383-5394.

Work presented in this thesis has also resulted in the following patent.

Delay, C., Imin, N., and Djordjevic, M.A. (2013). Method for modulating plant growth. Australian patent AU2013903988.

This work has also been presented in various forms at the following international conferences.

Delay. C., Imin, N., and Djordjevic, M.A. (2015). Poster, 3rd European Workshop on Peptide Signalling and Activity in Plants.

Delay. C., Imin, N., and Djordjevic, M.A. (2015). Poster, International Conference on *Arabidopsis* Research.

Delay. C., Imin, N., and Djordjevic, M.A. (2014). Colloquium Presentation, ComBio.

Delay, C., Imin, N., and Djordjevic, M.A. (2014). Seminar, International Symposium on Root Development.

Delay, C., Imin, N., and Djordjevic. M.A. (2014). Seminar, European Workshop on Peptide Signalling in Plants.