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Characterisation of the Genetic and Hormone controls of Branching in Petunia.

» J. Simons[1,2] jsimons@hortresearch.co.nz
» K. Templeton[1]
» K. Plummer[2]
» C. Beveridge[3]
» K. Snowden[1]

HortResearch, New Zealand[1], Auckland University, New Zealand[2] and University of Queensland, Australia[3]

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Introduction

Branching is one of the fundamental developmental processes that determines the architecture of a plant. Lateral branching is the outgrowth of buds in leaf axils to form axillary branches. The work presented here uses petunia as a model plant to study branching (Fig. 1). This work focuses on the study of the *decreased apical dominance* (*dad*) mutants (Fig. 2), three non-pleiotropic increased branching mutants isolated in petunia (Napoli, 1996; Napoli and Ruehle, 1996).

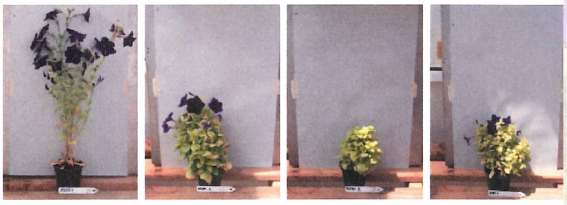

Figure 2. Wild type and *dad* mutant petunia used in morphological phenotyping experiment. The *dad* mutants show increased levels of basal branching, and a decrease in plant height compared to wild type petunia.

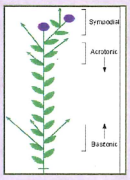

Figure 1. Branching in Petunia. Petunia is an herbaceous perennial that displays several different types of branches. Branching in petunia is also affected by environmental conditions such as day length (Snowden and Napoli, 2003).

Phenotyping of the *dad* double mutants to determine interactions of the gene products in branching

- Characters including plant height, number and length of basal branches were measured (Fig. 3)
- Some characters measured were different to wild type but not informative for differentiating *dad* mutants
- *dad1dad3* is additive, but neither *dad1dad2* or *dad2dad3* are additive with regards to morphology
- *Dad2* could be part of a receptor or signal transduction pathway downstream of the branching signal controlled by *Dad1* and *Dad3* (Fig. 4)

Hormone analysis of the *dad* mutants

- *Dad1* and *Dad3* control the levels of a graft transmissible substance that affects branching (Fig. 5)
- Is this substance auxin or a cytokinin? Both hormones have important roles in branching and are graft transmissible.
- IAA levels in *dad* mutants are mostly similar to than wild type (Fig. 6)
- Total cytokinin levels are lower in *dad* mutants than wildtype (Fig. 7)
- *Dad1* and *Dad3* control some other branching substance

Real-time PCR analysis of *Dad1* indicates expression in roots and stem

- *Dad1* expression is high in stem and root tissue, reflecting the role of the gene in our model (Fig. 8).
- In *dad* mutants *Dad1* expression is up-regulated in the stems, indicating a feedback mechanism controlling expression.
- This feedback does not occur in roots where expression may be independently controlled.

Development of *PhMax2* transgenic plants to investigate its role in petunia

- *MAX2* originally isolated from increased branching mutant in *Arabidopsis* with similar morphological phenotype to *dad* mutants (Stirnberg *et al.*, 2002).
- *dad* mutants contain no mutations in *PhMax2* coding sequence, no changes in expression in mutant seedlings (Fig. 9).
- Constructs to misexpress *PhMax2* (Fig. 10) were used to generate transgenic plants.
- RNAi plants silencing *PhMax2* show decreased height and increased branching (Fig. 11).

Summary

We are using several different methods to study the genetic and hormonal controls of plant branching, using petunia as a model. Phenotyping and gene expression analyses have suggested a loose model for the interactions of the *Dad* genes in controlling branching with *Dad2* possibly acting as a receptor, or in the signal transduction pathway, of the branching signal regulated by *Dad1* and *Dad3*. Analysis of IAA and cytokinin levels indicates that these are probably not the graft transmissible substance that is being controlled by *Dad1* and *Dad3*. Early analysis of transgenic plants misexpressing *PhMax2* indicates a role for this gene in control of branching in petunia. Current work focuses on construction of plants overexpressing *PhMax2*, and further analysis of hormone levels in *dad* mutant plants.

Acknowledgements

We would like to acknowledge Dr John Ross (University of Tasmania) and Chuong Ngo (University of Queensland) for mass spectrometry analysis of the plant extracts for IAA and cytokinins respectively. The binary vectors used toconstruct the RNAi and OX constructs were created by Dr Andrew Gleave (HortResearch). This work was supported by the New Zealand Foundation for Research, Science and Technology as a subcontract from AgResearch.

References

Napoli, C. (1996) Highly branched phenotype of the petunia *dad1*-1 mutant is reversed by grafting. *Plant Physiology* 111:27-37.
Napoli, C. A. and Ruehle, J. (1996) New mutations affecting meristem growth and potential in *Petunia hybrida Vilm. Journal of Heredity* 87:371-377.
Snowden, K. C. and Napoli, C. A. (2003) A quantitative study of lateral branching in Petunia. *Functional Plant Biology* 30(9):987-994.
Stirnberg, P., van de Sande, K. and Leyser, H. M. O. (2002) *MAX1* and *MAX2* control shoot lateral branching in *Arabidopsis*. *Development* 129:1131-1141.

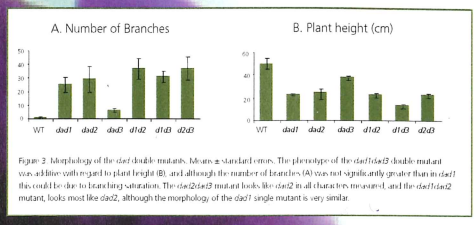

Figure 3. Morphology of the *dad* double mutants. Means ± standard errors. The phenotype of the *dad1dad3* double mutant was additive with regard to plant height (B), and although the number of branches (A) was not significantly greater than in *dad1* this could be due to branching saturation. The *dad2dad3* mutant looks like *dad2* in all characters measured, and the *dad1dad2* mutant, looks most like *dad2*, although the morphology of the *dad1* single mutant is very similar.

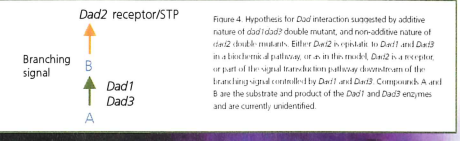

Figure 4. Hypothesis for *Dad* interaction suggested by additive nature of *dad1dad3* double mutant, and non-additive nature of *dad2* double mutants. Either *Dad2* is epistatic to *Dad1* and *Dad3* in a biochemical pathway, or as in this model, *Dad2* is a receptor, or part of the signal transduction pathway downstream of the branching signal controlled by *Dad1* and *Dad3*. Compounds A and B are the substrate and product of the *Dad1* and *Dad3* enzymes and are currently unidentified.

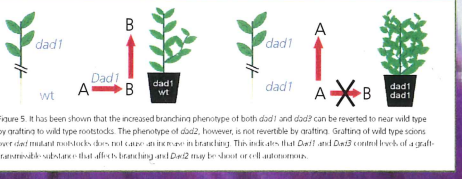

Figure 5. It has been shown that the increased branching phenotype of both *dad1* and *dad3* can be reverted to near wild type by grafting to wild type rootstocks. The phenotype of *dad2*, however, is not revertible by grafting. Grafting of wild type scions over *dad* mutant rootstocks does not cause an increase in branching. This indicates that *Dad1* and *Dad3* control levels of a graft-transmissable substance that affects branching and *Dad2* may be shoot or cell autonomous.

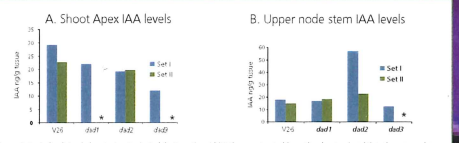

Figure 6. Auxin levels in *dad* mutant petunia. Indole-3-acetic acid (IAA) was extracted from the shoot apices (A) and upper nodes (B) of young plants then measured by GC-MS against known levels of deuterated internal standards (* indicates sample not determined). As IAA levels do not drop significantly, it is unlikely they are the branching substance controlled by *Dad1* and *Dad3*.

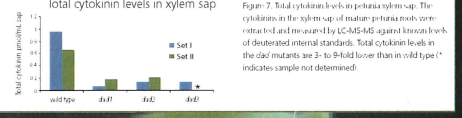

Figure 7. Total cytokinin levels in petunia xylem sap. The cytokinins in the xylem sap of mature petunia roots were extracted and measured by LC-MS-MS against known levels of deuterated internal standards. Total cytokinin levels in the *dad* mutants are 3- to 9-fold lower than in wild type (* indicates sample not determined).

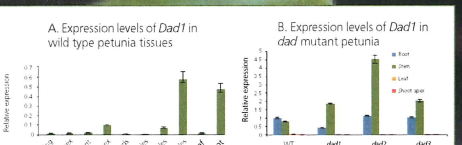

Figure 8. Expression levels of *Dad1* (a homologue of MAX4) in wild type petunia. We used quantitative real time PCR from a range of tissues of wild type V26 (A) and dad mutant plants (B) to detect expression of the *Dad1* gene.

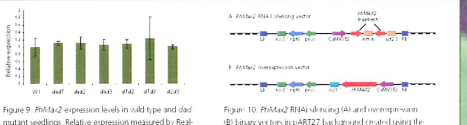

Figure 9. *PhMax2* expression levels in wild type and *dad* mutant seedlings. Relative expression measured by Real-time PCR. No significant differences in expression.

Figure 10. *PhMax2* RNAi silencing (A) and overexpression (B) binary vectors in pART27 background created using the GATEWAY™ system. Expression in both cases is under the control of a modified CaMV35S promoter.

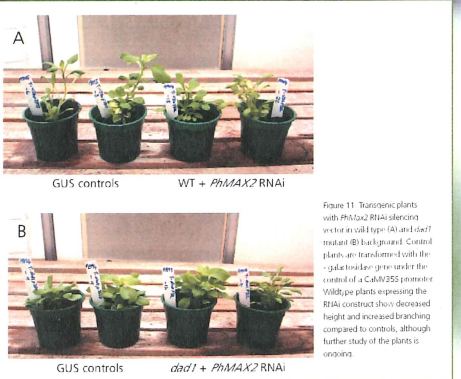

Figure 11. Transgenic plants with *PhMax2* RNAi silencing vector in wild type (A) and *dad1* mutant (B) background. Control plants are transformed with the β-galactosidase gene under the control of a CaMV35S promoter. Wild type plants expressing the RNAi construct show decreased height and increased branching compared to controls, although further study of the plants is ongoing.