# Hypothesis-driven computational modelling of branching control in pea

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## Background

The control of shoot architecture is a multi-factorial process, involving long-distance signalling and coordination between distinct and spatially separated plant tissues. Multiple plant hormones appear to be involved in the regulation of axillary bud outgrowth. For example, auxin is widely recognised as a repressor of bud outgrowth, while cytokinin is acknowledged as an inducer of bud outgrowth. Auxin, however, may not directly regulate bud outgrowth, but may require an alternative messenger (reviewed in Dun *et al*., 2006). Candidates for auxin's secondary messenger include cytokinin (Bangerth, 1994; Li *et al*., 1995), in addition to a novel signal (Foo *et al*., 2005; reviewed in Leyser, 2005) named SMS, for Shoot Multiplication Signal (Beveridge, 2006). Cytokinin is thought to promote bud outgrowth, though its exact role remains unknown. The application of cytokinin directly to axillary buds, and the over-expression of cytokinin biosynthesis genes sometimes, but not always, induces bud outgrowth (King and Van Staden, 1984; Medford *et al*., 1989).

A number of genes involved in the control of shoot branching have been identified in pea (*Pisum sativum*). These include five *Ramosus* (*RMS*) genes, *RMS1* through *RMS5* (reviewed in Beveridge, 2006). Grafting studies have demonstrated a role for these genes in shoot and rootstock tissues. The inability of exogenous auxin applications to rescue the increased branching phenotypes of the *rms* mutants (Beveridge *et al*., 2000), and the auxin-inducible *RMS1* and *RMS5* gene expression (Foo *et al*., 2005; Johnson *et al*., 2006), suggests these genes are involved in the synthesis of auxin's secondary messenger.
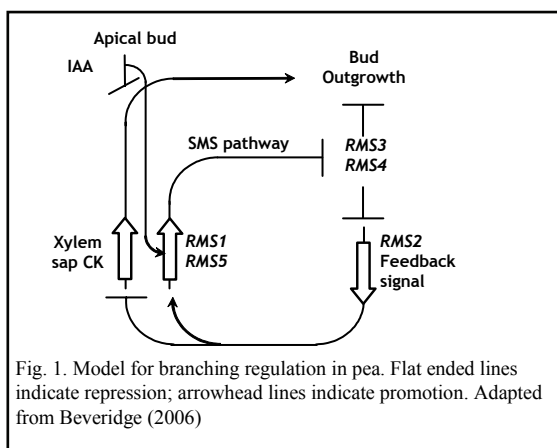


Fig. 1. Model for branching regulation in pea. Flat ended lines indicate repression; arrowhead lines indicate promotion. Adapted from Beveridge (2006)

Auxin and cytokinin analyses, together with grafting studies and putative enzymatic functions, suggest novel hormone-like signals, other than auxin and cytokinin, are involved in the regulation of shoot branching, and that their synthesis and/or level is regulated by the *RMS* genes. At least two novel signals are thought to exist, SMS moving from rootstock to shoot, and a *RMS2*-mediated feedback signal, moving from shoot to rootstock (Fig. 1). Evidence for the existence and regulation of the novel signals, both SMS and feedback signal, will be presented and explored using computational modelling techniques.

## Research methodology

The research strategy employed essentially consists of two parts: computational modelling of the *RMS* branching regulatory network and biological experiments aimed to test features and/or predictions of the computational models. In the computational models, gene action was spatially separated into rootstock(s) and shoot(s), with directional flow of long-distance signals between the compartments. Genotypes were represented with a value of 1 if wild type, and 0 if mutant. The computational models were created in L-Studio using algebraic equations and simple mass action and conservation principles to represent the hypotheses about the relationships and interactions between model components. Only three parameters were utilized in the models, representing the proportion of signal made in the rootstock that is transported to the shoot, the proportion of signal made in the shoot that is transported to the rootstock, and the proportion of functional RMS2 product made in the *rms2* mutant. Many parameter value combinations were tested, and if a broad range of sensible parameters produced acceptable output, this was taken as support that the network interactions were responsible for the emergent behavior rather than the specific parameter values. In order to assess whether model output was acceptable, biological data were categorized, and the model output compared to the biological categories. If the computational model output did not correspond with the biological datasets, then the hypotheses were further adjusted. However, if the model output corresponded with the biological results, then predictions could be made for new biological plant-based experiments, and the experiments conducted. These new biological results could then be utilized to further refine hypotheses, making the approach iterative.

## Directions

Computational models have been created that explain all the published experimental datasets. The models have been utilised to aid experimental design and result prediction. For example, based on a new proposed function for the gene *RMS4* in the rootstock, the model predicted that an *rms4* rootstock would be more inhibitory of branching in a wild-type scion than a wild-type rootstock. This was confirmed experimentally (Johnson *et al*., 2006). New plant-based experimental results testing aspects of the *RMS2*-mediated feedback signal's regulation and action will be presented, in addition to the implications to the regulation of branching in pea.

The results of this study indicate that systems with many unknowns can be modelled computationally based on hypotheses, and the effort is worthwhile, yielding both an improved understanding of the system and experimental predictions that can be tested. The strength of the approach is that it highlights data that are not explained by the hypotheses, triggering much thought to create alternate hypotheses that can be tested against the entire dataset computationally. The computational models can also be utilised to predict the results of new experiments, so it can be determined whether the experiment is worthwhile and indeed tests the hypothesis.

## Acknowledgements

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