# Virtual soybean—a computational model for studying autoregulation of nodulation

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## Introduction

Nitrogen fixation by legumes is the product of a symbiosis of legume plants and a group of bacteria known as rhizobia (Carroll, et al., 1985; Kinkema, et al., 2006). It has been hypothesised that when rhizobia receive flavonoid signals from the host plants, production of a chemical nodulation factor is induced. The perception of nodulation factor by legume roots then activates a signal cascade leading to division of cortical cells for nodule formation. The activated cells produce a translocatable signal (Q), sent through a root-shoot pathway to the leaves and then detected by a leucine-rich repeat receptor kinase encoded by the *NARK* gene (Searle, et al., 2003). The detection of this root-shoot signal further induces the production of a shoot-derived signal (SDI) transported to the root, which inhibits development of new nodules. These signals, operating in a growing structure of root and shoot, compose a regulatory network known as autoregulation of nodulation (AON). The overall purpose of this study is to develop a greater understanding of this system through multi-scale modelling of processes including intra- and inter-cellular signalling, long-distance signalling and phenotypic development regulated by internal control mechanisms. The first step is to build a structural framework capable of simulating soybean growth driven by empirical results and hypothetical patterns, incorporating control mechanisms modelling Q and SDI.

## Materials and Methods

Two soybean (*Glycine max* L. Merrill) genotypes—wild-type Bragg and supernodulating mutant *nts1007* (Carroll, et al., 1985)—were grown in a glasshouse with controlled temperature of 28 °C during day and 23 °C at night. The irradiance inside the glasshouse was approximately 80% of that outdoors. Experiments were carried out from November 14$^{th}$ to December 21$^{st}$ 2006, during which the sun hours in Brisbane were approximately 10 per day on average. Inoculation was conducted 5 days after planting with 75 ml of a late-log phase culture of *Bradyrhizobium japonicum* CB1809 for each pot. All plants were watered with 500 ml B&D solution (Broughton and Dilworth, 1971) supplemented with 2 mmol$L^{-1}$ $KNO_3$ twice a week and were irrigated 1-2 times a week with tap water only. This low level of nitrate stimulates plant growth but has a minimal effect on nodule number per plant, though marginal delay in nodule initiation. Positions and orientations of the pots were randomised every week to diminish the distorting influence of phototaxis.

In this study, shoot structure was mapped as sequences of symbol strings (Hanan and Room, 1996) where each symbol represents a particular type of plant component (Fig.1.a). A Model GP12-XL sonic digitizer (Freepoint 3D, Scientific Accessories Corporation / GTCO CalComp) and Floradig

software (Hanan and Wang, 2004) were used to identify topological positions and collect 3D coordinates of significant points of shoot components (Hanan and Room, 1996).

Since root development is much more irregular than in the shoot, different methods were required. A root mapping method (Fig.1.b) was used to characterize the first-order lateral roots according to their starting points on the primary root. Lateral and nodule numbers were counted for a series of 50 mm regions along the primary root (Fig.1.c). Each region was composed of a number of segments, and each segment consists of four potential branching sites for which probabilities of generating lateral roots on the right (R), down (D), left (L) and up (U) were determined. Heading behaviours of root growth were simulated using the methods from the ROOTMAP model (Diggle, 1988). Second-order laterals were not taken into account in this work. The L-system-based software L-studio (Karwowski and Prusinkiewicz, 2004) was used for simulating plant growth.

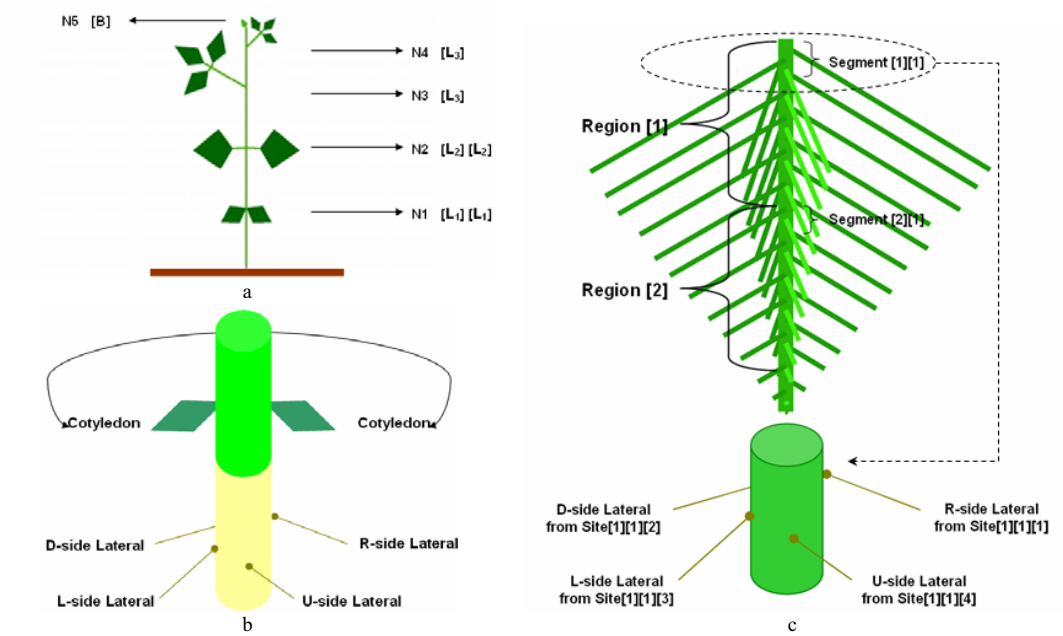



Fig. 1. Shoot and Root Mapping Methods. (a) Example of the shoot mapping method in which N means node; $L_1$ means cotyledon; $L_2$ means unifoliate leaf; $L_3$ means trifoliate leaf and B means bud. (b) The root mapping method characterizes the first-order lateral roots according to their starting points from the primary root. (c) Lateral root classification by "region", "segment" and "site".

## Results

Shoot growth is modelled by L-system production rules capturing production of opposite cotyledons and unifoliate leaves at nodes 1 and 2 and subsequent production of trifoliates at higher nodes. Leaf blade, petiole and internode expansion are modelled by positional growth functions derived from the empirical data. Internodes and petioles are represented by a user-controlled number of segments in order to allow synchronization of growth rates and signal transport rates.

Primary root elongation is simulated by adding a standard increment at each step. Increments make up segments composed of four sites of potential lateral root development. Once four sites have been created, the current segment will be finished and a new segment started. Number of segments, lengths of sites and their probability of creating a lateral are determined positionally according to region. Lateral elongation is modelled in the same manner. Nodulation can be modelled either empirically using a similar probabilistic distribution, or under control of the auto-regulation model.

Simulation results for plant structure were verified against empirical data for cumulative statistics such as shoot height, root lengths and number of laterals, with no significant differences for either Bragg or *nts1007* (Fig.2.a, b). For the empirical model of nodulation, spatial and temporal distributions of nodules are as appropriate for these two genotypes. For example, aside from agreement on the overall number itself, nodules of Bragg (Fig.2.c) concentrated more in lateral segments closer to the primary root while nodules of *nts1007* (Fig.2.d) are more well distributed, in accordance with the observed results from real plants.

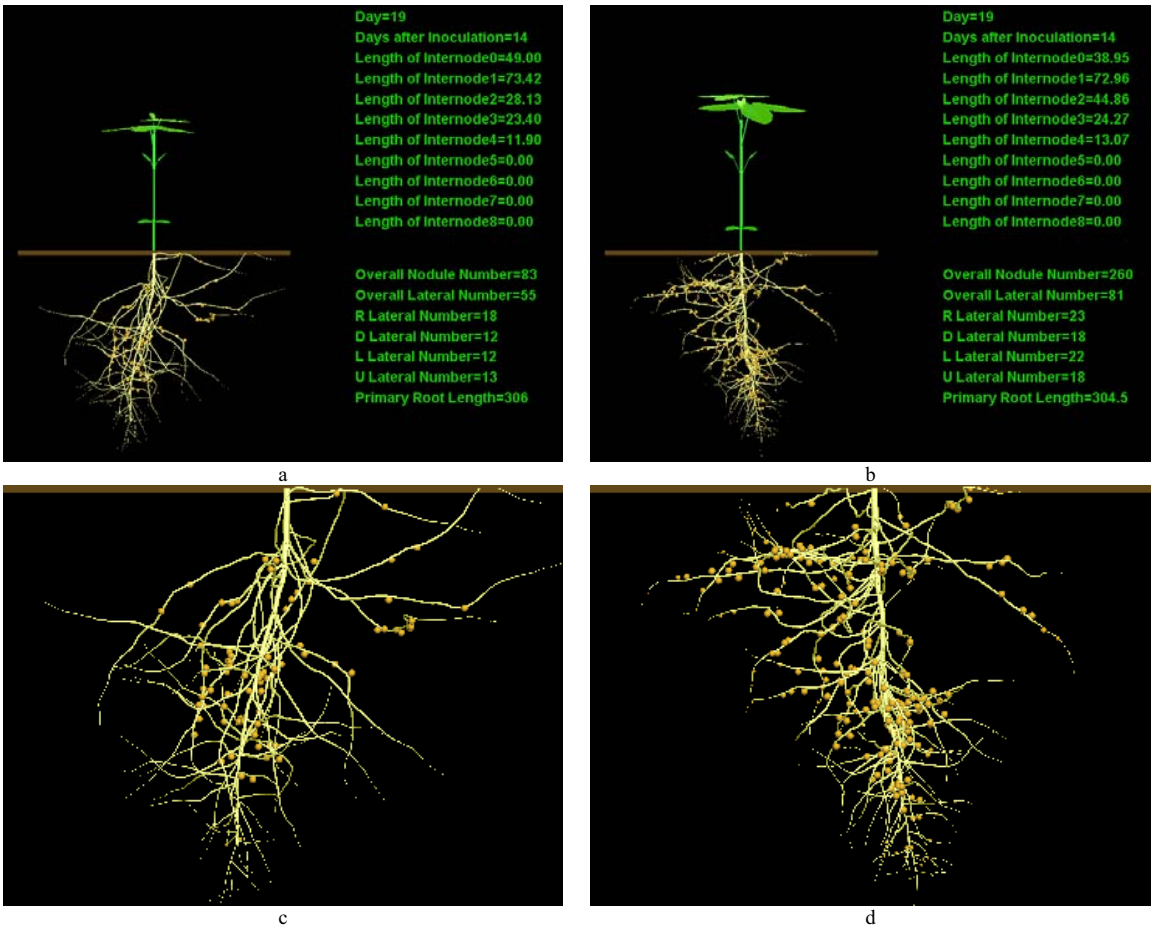
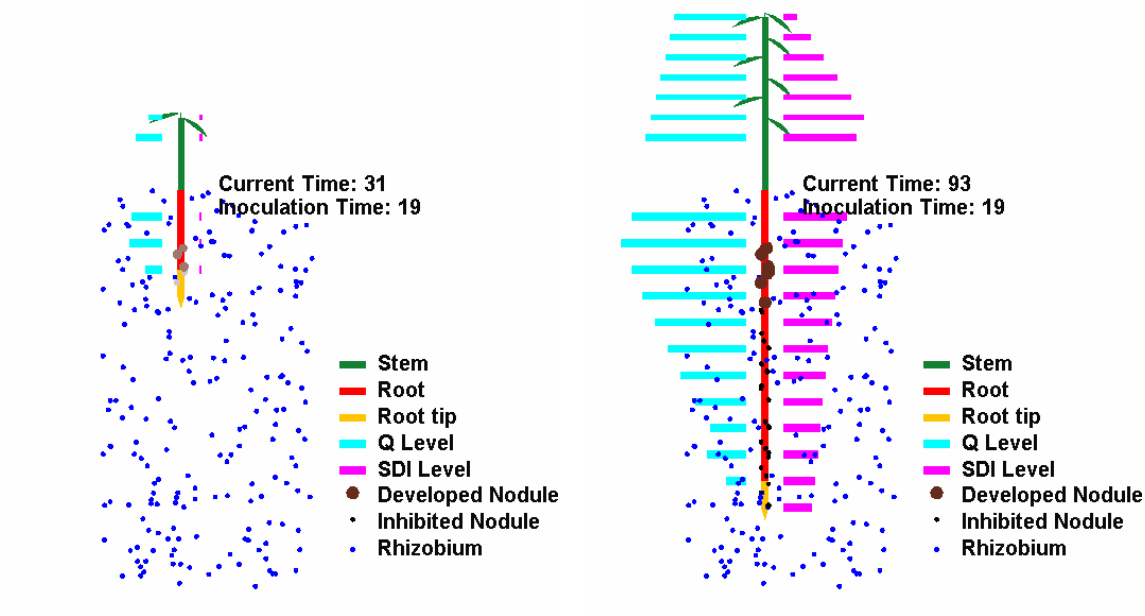



Fig. 2. Simulation of Shoot and Root Development. The results shown in (a) and (c) are from simulation of Bragg development, while those shown in (b) and (d) are from *nts1007*.

The flow of signals Q and SDI in the growing structure (Fig. 3) are modelled using context-sensitive production rules (Prusinkiewicz and Lindenmayer, 1990). The presence of rhizobia is modelled abstractly by inoculation date and concentration, probabilistically initiating nodulation and the consequent induction of the signal Q near the root tip at the appropriate time. Since the root and shoot systems are represented by L-system sub-strings that grow towards their respective growing tips, right context-sensitive productions in the root zone and left context-sensitive productions in the shoot zone move the signal "up" towards the leaves. Productions applicable to the hypocotyl transfer the signal between the root and shoot systems. If NARK is present and the Q signal is detected in the leaves, the SDI signal is generated and transmitted “down” by right and left context-sensitive rules in the shoot and root respectively. Once the level of SDI reaching the root tip is above the appropriate threshold, further nodulation is suppressed. Though currently restricted to modelling the primary

root, this model captures observed phenomena such as supernodulation in *nts1007* and autoregulation of nodulation in Bragg. Nodulation patterns as described in the literature for different inoculation dates can also be simulated appropriately.




a b

Fig. 3. Model for Predicting Nodulation Behaviours. (a) Nodulation was initiated at time 19, and the Q signal represented by the bars to the left of the plant has travelled up to the leaves, initiating the SDI signal represented by the bars to the right, but levels are not high enough to inhibit nodulation. (b) Nodulation has been suppressed.

## Discussion

The empirical architectural models of Bragg and *nts1007* successfully simulate the growth process for both shoot and root. The resulting realistic visualization integrates the different architectural attributes, allowing a holistic phenotypic representation of the differences between a wild type plant and its mutant. Since our focus is to develop computational approaches for studying the control mechanisms underlying autoregulation of nodulation, such a structural model represents target phenotypes for our mechanistic modelling. In the next step, the prototype root-shoot signalling model, will be extended to handle branching architecture and for modelling of the differing rates of hormone flow and organ growth. The integration of models of structural development with hypothesised models of long distance signalling and gene regulation will contribute to a better understanding of the complexity involved in autoregulation of nodulation.

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