Final Report – Return Grant Dirk De Vos

'Multiscale' mathematical modelling of root growth in *Arabidopsis thaliana*

1. Doelstellingen - Objectives

This project aimed to produce mathematical models of root development of *Arabidopsis thaliana,* integrating the molecular regulation of cell division activity, cell elongation and transport of hormones with growth and architectural and mechanical characteristics of roots. The project is closely related to the Belgian Arabidopsis Root Network (BARN) that was set up to study the Growth and Development of Higher plants (Onderzoeksproject P6/33). By enhancing our still limited understanding of those processes mentioned the project reponded to the (growing) importance of plants as food (and feed) sources for a fast expanding world population. In fact, more food production is needed on less arable land. The computational model(s) produced thanks to this project represent small yet important steps in the development of software tools that can predict strategies for improved crop yields or increased resistance to environmental stress conditions like drought and heat, which are known to have potentially devastating effects (think about famine in the developing countries). This aspect is even more pressing given the potentially negative impact of Global Climate Change and Global Warming on those issues.

Relatively few studies have reported mechanistic models of root growth. The landmark study of Grieneisen *et al.* (2007) used experimental findings on PIN exporter distributions to reproduce realistic auxin patterns in the root apex. Laskowski *et al.* (2008) extended this model to account for lateral root initiation based on auxin maxima induced by root bending. Mironova *et al.* (2010) presented the so called ’reflected flow’ mechanism as an alternative for the ‘reverse fountain’ mechanism (Grieneisen *et al.*, 2007) to explain auxin self-organisation,. Band *et al.* (2012) have modelled root growth as a single file of cells with growth-driven dilution of gibberellins providing an explanation for growth arrest toward the end of the elongation zone. What is still lacking in these models is a mechanical simulation framework to represent symplastic growth and realistic representations of growth and division. The aim of this project was to deploy advanced computational methods to better grasp the complexity of the biochemical and biophysical interactions involved in root growth and regulation. The main focus was to construct sufficiently detailed models of cell division, cell expansion and hormone signaling and integrate them in a multi-scale model that describes root growth to cellular resolution. After completion such a model can be validated by comparison with experimental data and subsequently used to make testable predictions.

2. Methodologie en resultaten – Methods and results

To construct (next-generation) high-resolution, multi-cellular models of root growth and development of *Arabidopsis thaliana* a sequence of tasks was proposed in the technical annex of the original project's proposal. We will evaluate and discuss below briefly the various tasks that were engaged during the period of the mandate. Unsurpisingly -and probably unavoidably- in a number of instances the actual technical or even scientifical implementation deviates more or less from the proposed working plan. The plant modelling software VirtualLeaf (Merks et al, 2011), written in the programming language C++, was used as a starting point for model building. This program provides interactive control to simulate, produce visual and numerical output of models and even modify the available models. To this end a graphical user interface is provided with pull-down menus and an interactive simulation display. Menus allow parameter settings to be changed and more advanced modifications of the model require the user to edit a so-called 'plugin' that contains the relevant biological rules, i.e. biochemical reaction and transport kinetic equations, as well as rules for cell division and cell growth. Whereas this provides some flexibility, to construct new models, as in this case for the root, requires more extensive modifications to the code base. Among other changes, in particular anisotropic root growth demands changes to the bio-mechanical framework which are not possible via the model interface. An essential part of a model definition is the description of the starting conditions including the initial cellular geometry and the starting concentrations of the various biochemical compounds. To describe this, a so-called LeafML input file (in xml format) is used. An extra program ('leafgen') was developed to allow generation of custom imput files (representing diverse starting situations (states)) and was eventually integrated into VirtualLeaf. From a technical perspective this level of development takes place in the context of and Integrated Development Environment (IDE), called Eclipse (www.eclipse.org), which facilitates code writing, compiling, installing, debugging, etc. Furthermore, for development of moderate-scale applications like VirtualLeaf, a version control system is strongly recommended, in this case the program Mercurial (mercurial.selenic.com). The current research project, embedded in the research group of Prof. Kris Vissenberg (Plant Growth and Development (PGO) – UA), was supported by an interdisciplinary team of scientists composed from the groups of Prof. Gerrit Beemster (Molecular Plant Biotechnology (MPB) - UA), Prof. Jan Broeckhove and Prof. Wim Vanroose (both from Computationeel Modelleren en Programmeren (CoMP) - UA) working directly or indirectly with VirtualLeaf, sharing the same development environment. This will shortly lead to the release of a new version of VirtualLeaf, in collaboration with the original developer of the first version, Dr. Roeland Merks (Centrum voor Wiskunde en Informatica, NL).

Next follows an overview of the original objectives and results.

Objective 1

“Constructing a 2D model of steady-state root growth focused on auxin transport and PIN localization, based on the cell-based model of Merks *et al.* (2007).” In other words: adapting a leaf growth model to the quasi linear (one-directional) mode of root growth.

>>*Results*

A somewhat different approach was taken translating the root model described in the seminal paper by Grieneisen *et al*. (2007) into the VirtualLeaf modelling framework. It appears that starting from the best current root model is a better strategy than starting from a leaf model. However, while the latter was readily available, the former had to be adapted from a Cellular Potts-type model (which uses fixed lattice or grid points with changing properties) to a vertex-based model (with dynamically moving nodes and connecting walls). An additional advantage of a vertex-based modelling approach is that it more closely resembles the physical structure of the plant tissue which can be seen as an interconnected complex of cells (symplast) with the apoplast being the connection. As described above a separate C++ program, ‘leafgen’, was written to produce the necessary input xml files (in this case custom rectangular grids with specific intracellular and wall-specific biochemicals; *e.g.* Fig. 1). It was initially produced and tested in the XCode (https://developer.apple.com/technologies/tools/) IDE of MacOSX and then integrated in the VirtualLeaf framework.

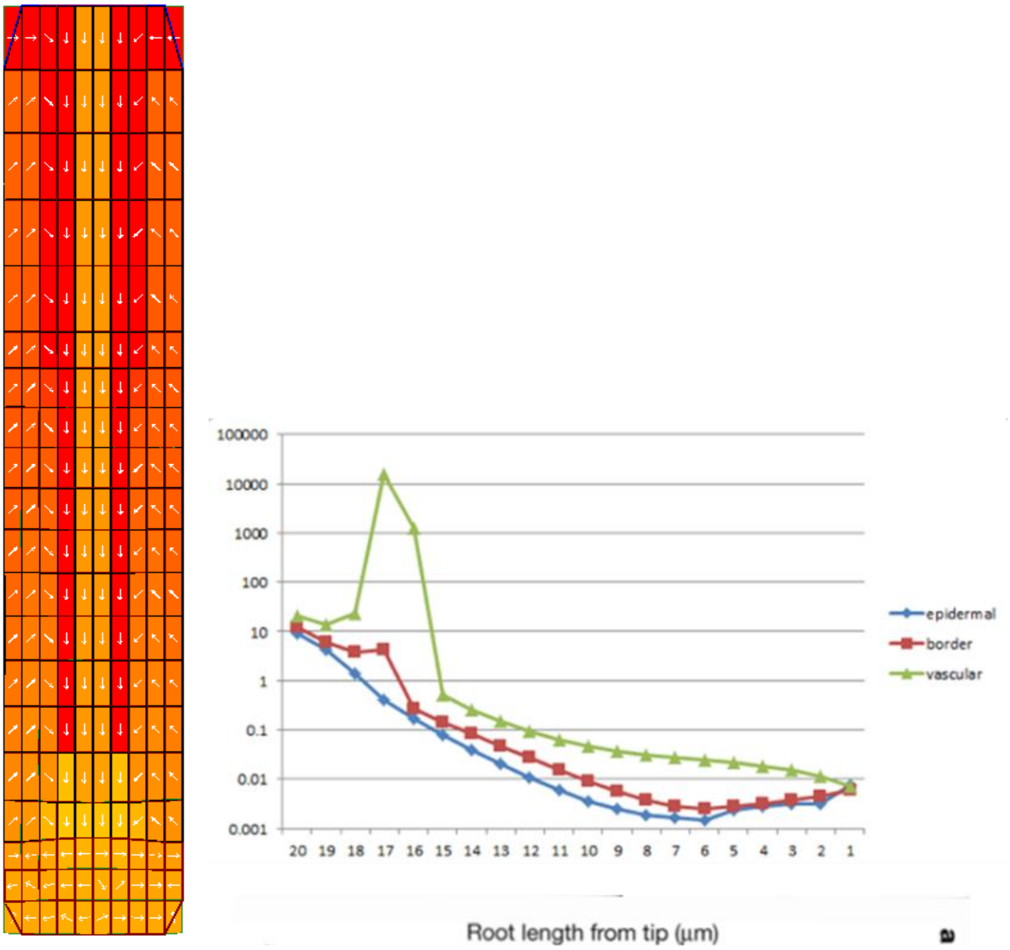

Figure 1.

Left plot: steady auxin levels in a static root tip represented by a rectangular grid of cells. The more yellow the coloring the higher the auxin level is. Right plot: auxin levels corresponding to different cell layers along the longitudinal axis. The most central layer shows a strong auxin maximum.

Objective 2

"Implement in C++ (for efficient numerical integration) and python (for coordination of different parts of software) using (*e.g.*) QT3 (toolkit for user interface design)."

>>*Results*

All model code was implemented in C++, with he graphical representation of the model simulations based on the Qt3 (later Qt4; doc.qt.nokia.com) cross-platform application development framework. The philosophy was to make maximal use of functionality in the existing Virtualleaf and therefore take that as a starting point to develop new graphical or input/output functionality. Figure 3 is an example of a graphical representation of a growing root tip with the calculated areal strain rates of the cells, based on modifications to the original program. However, some bash shell and python scripts were used for output data processing, analysis or batch running of VirtualLeaf.

Objective 3

"Evaluation of parameters and boundary conditions."

>> *Results*

The model parameters and boundary conditions of Grieneisen *et al.* (2007) were adapted if possible (in absence of apoplastic compartments or absolute auxin levels). A collaboration was initiated with Sean Stijven (from CoMP - UA) to analyse the sensitivity to model parameter changes over large parameter spaces. The results are in line with the expectations that follow from the mostly simple linear reaction and transport kinetics in the Grieneisen auxin dynamics model. A future step will be to introduce more realistic non-linear kinetics for comparison with the first results and as an extra tool to aid in model analysis. In a next step these results can be compared to a root tip model including auxin transporter (PIN) dynamics.

Objective 4
“Model prediction and validation”
>> *Results*
Simulation output from the Virtual Leaf static root model corresponds to what has been reported in studies with auxin(-signaling) molecular markers and other experimental work (cf. Fig 1 for an example). Concretely a stable auxin gradient is formed with a maximum near the quiescent center (QC) of the root apex.

Objective 5
“Analyze the model in terms of sensitivity-robustness-stability.”
>> *Results*
The stability of the auxin gradient to changes in parameter values has been investigated in more depth and indicate robust pattern formation. Simulating a more drastic operation like cutting off the upper parts (and therefore the corresponding auxin source) still conserves the same pattern.

Objective 6
“Integrate real microscopy images of cells, PIN and auxin distribution (template data extraction).”
>> *Results*
A simplified representation of *in planta* PIN distribution has been *a priori* used (=input) to produce realistic auxin patterns (=output). A joint workshop with the plant modellers from the Centre for Plant Integrative Biology in Nottingham (CPIB) has lead to the introduction of the software CellSet for extraction of cell geometries from microscopic images. The first steps to translate the images to VirtualLeaf input files have been successfully undertaken with the people from CoMP (Fig. 2).

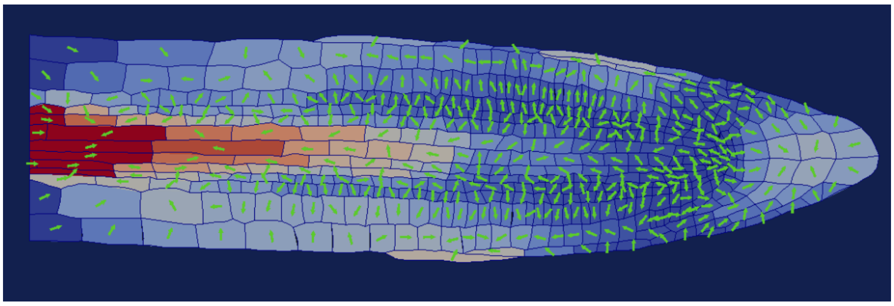

Figure 2.
Arabidopsis thaliana root tip structure extracted from microscopic image using the CellSet program (CPIB) and used as a template for auxin dynamics in a static grid.

Objective 7
“Extending model with cell cycle model (interaction with hormone signalling).”
>> *Results*
This objective is central to the project and goes hand in hand with implementation of the rules for cellular growth and underlying cell wall mechanics. Initially cell division was implemented as basic cellular timers or counters. Subsequently, a detailed cell cycle based on molecular interactions (based on Beemster *et al.*, 2006) was integrated and tested in VirtualLeaf. This successfully produced localized cell proliferation in the root apical meristem and hormone dependent exit from proliferation. In this cell cycle model the main regulators of DNA duplication and mitosis (both essential components of the cell cycle) are the so called S-phase and M-phase promoting factors

(SPF and MPF, resp.), consisting of CyclinD:CDKA and CyclinB:CDKB complexes. This still represents a very basic cellular oscillator and, despite its usefulness as a first step in building an integrated root growth model, a more advanced cell cycle model has been developed. This model is based on canonical models of the cell cycle of yeast and other eucaryotic organisms (Tyson *et al.*, 2003; Csikasz-Nagy *et al.*, 2006), with plant-specific adaptations. Tests have shown that the model could be succesfully integrated in the context of VirtualLeaf. However, further model refinement is needed to respect the most recent advances in plant cell cycle research. This includes a detailed analysis of its properties at the single cell level.

Objective 9
“Extend model with biomechanical factors: cell wall (– cytoskeleton – vacuole) (modeled as spring forces...).”
>> *Results*
In VirtualLeaf (v.1) cellular growth is currently represented as follows. Cells are modelled as polygons and the nodes (vertices) are randomly displaced. This displacement is translated as a change in a so-called Hamiltonian or generalized energy function. This function expresses the balance between the tendency for cells to increase their area (in 2 dimensions, equivalent to volume changes in reality) and the tendency of cell walls to oppose this expansion by visco-elastic forces. During the first year of the project this Hamiltonian was succesfully modified to produce the anistropic (almost linear) growth of the primary root. Moreover, several variants of cellular growth rules were implemented to predict the effect on growth of varying these rules.

Objective 8
“Model prediction and validation.”
>> *Results*
Relatively detailed implementations of cell growth and division were combined to produce root simulations approximating microscopic images of real longitudinal root sections. These developmental mechanisms were evaluated in terms of their potential for robust pattern generation versus developmental plasticity w.r.t. changing conditions. An important perturbation consists of the effect of growth itself on the signals regulating growth (feed-back). In that context it was demonstrated that the exact allocation of auxin sources can be critical for sustaining steady state root tip growth.

Objective 10
“Incorporating influence of aquaporins, H^+-ATPase polarisation, ... in biomechanical model.”
>> *Results*
Priority was given to support further development of the mechanical basis of the organ growth models in VirtualLeaf in collaboration with the CoMP and MPB groups. This work consists of adapting a published model (Corson *et al.*, 2009) for plant cell growth in the shoot apical meristem (based on soap froth mechanics) and integrating it into VirtualLeaf. This model has a detailed representation for cell walls as visco-elastic elements and also contains a more realistic description of the influence of turgor pressure on cell growth. Importantly, as soon as this is integrated in the root model it can be extended to model the effects of the regulatory processes mentioned in Objective 10.

Objective 11
“Model prediction and experimental validation (spatio-temporal patterns of cellulose deposition).”
>> *Results*
Since the above mentioned experimental data were not readily available, the focus was turned towards a more conceptual approach. Briefly, starting from a minimal number of mechanical constraints, how close can we approximate anistropic root tip growth (e.g. Fig. 3)? Importantly, this provides a solid base for future inclusion of experimentally determined cell wall properties into

these models.

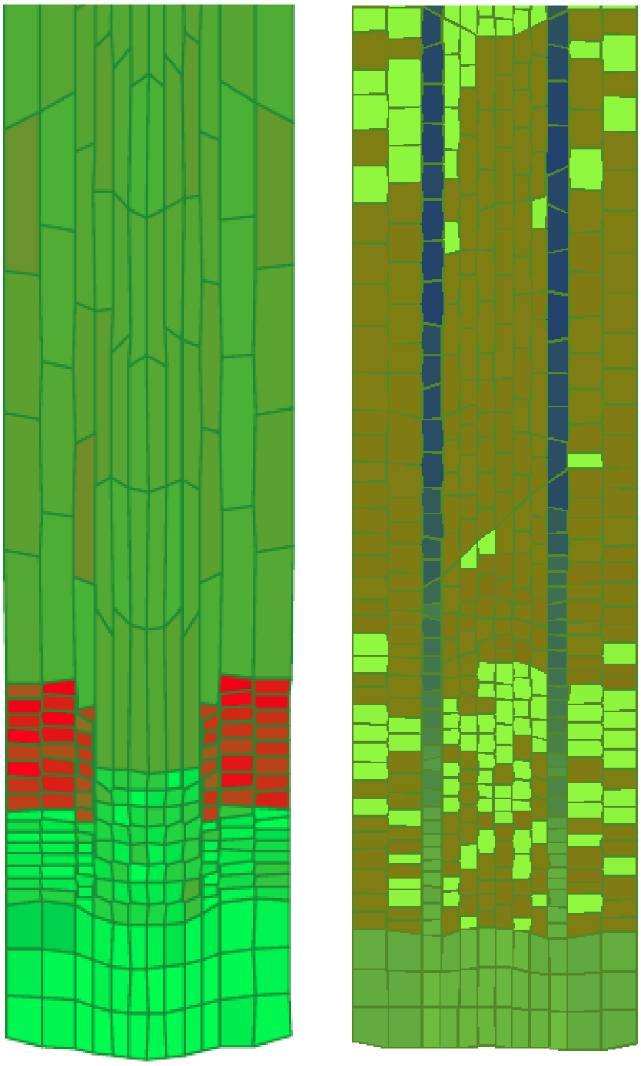

Figure 3.

Two different simulations of a growing root tip. The left is colored according to areal strain rates (red indicating high strain rates) and is illustrative for non-synchronous cell expansion in the case of a specific cell-autonomous mechanism for root development. The right is colored according to growth ('water') potential (blue indicates highly negative values) to illustrate the concept of layer-driven growth.

Objective 12

"Upscaling and extension model to 3D (code optimization, parallel computing)."

\>> *Results*

Starting from a few cells in the initial models, the current models of root tip growth consist of hundreds of cells scaling up to the millimeter range needed to represent root growth over a time-scale of several days. I have been actively involved in the development of VirtualLeaf towards more efficient (faster) simulations by collaborating with the CoMP and MPB groups. Steps towards 3D models are still at the conceptual level but will become an integral part of future projects.

Objective 13

"Integrate in plant modelling frameworks and with existing models to describe whole organ morphogenesis."

\>> *Results*

The potential to include the developed models into multi-scale single or multi-organ models was investigated. During two workshops at CPIB the complementarity to the local modellers' work was discussed. A 5 day workshop set up by Prof. Beemster (MPB) was attended to familiarize myself with the plant modelling software L-studio/VV (algorithmicbotany.org/lstudio/). Finally, a collaboration has been set up with X. Drayes (UCL, partner in BARN), to integrate the developed root tip model in a higher level functional-structural plant model (in CrossTalk framework; Draye and Pages, 2006) to investigate the root-shoot interaction in the framework of the IAP MARS project (http://www.iuap-mars.be/).

Objective 14
"Visualization of dynamically changing cellular structures (OpenGL for movies...)."
>> *Results*
The available graphical capabilities of VirtualLeaf were optimally used and extended if possible (cf. Objective 2). Support was given to the software development in the CoMP and MPB groups focusing on more advanced, more standardized and less platform dependent data output/visualization.

Objective 15
"Implement perturbations: gravitropism (root bending), mutants, hormone and inhibitor treatments."
>> *Results*
Some perturbations were already discussed in relation to Objectives 5 and 8. The effect of the cell cycle factor cyclin-D has been investigated, as well as varying the supply of auxin to the root. Whereas most of the simulations with the constructed models assume regular vertical growth of the root tip, a set of simulations were done with less constrained movement to reproduce a gravitropic response (Fig. 4).

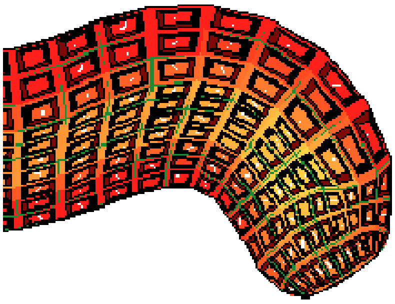

Figure 4.
Root tip tropism based on differential growth of cell layers. Coloring according to auxin levels (yellow for high levels).

Objective 16
"Model prediction and experimental validation."
>> *Results*
The perturbations described above are generally in accordance with published experimental data. The focus was on better approximation of microscopic images (qualitative) but also of 'hard' kinematic data (quantitative) of the growing root. The future integration of a detailed and updated plant cell cycle model is promising as it will allow a comparison of spatio-temporal distribution of cell cycle factors between *in silico* and experimental data.

Objective 17 and 18
"Final curation and possibly deposition."
"Publication"
>> *Results*

We published a paper which reviews mechanistic (bottom-up) modelling approaches in plant growth (De Vos *et al.*, 2012). This article highlights developments in the modelling of hormone transport, cell expansion and the cell cycle, in accordance to this project. Data from simulations in relation to the previous objectives were put into a manuscript that is under preparation for submission as a concept paper on regulation of root growth and development (preliminary title: “Complex networks for simple growth: a computational approach”). Currently, no public repository is available to deposit such spatio-temporal models as have been developed within this project. However, in collaboration with Prof. Beemster a COST (European Research Network for Mechanistic Modelling of Plant Growth and Development) Action proposal was prepared entitled: “European Research Network for Mechanistic Modelling of Plant Growth and Development.” This exactly responds to the need to advance collaboration in this field and includes setting up a public repository of models.

Objective 19
“Further possible extensions of the model (signaling, gene expression, environmental factors, ...).”
>> *Results*
Next to upscaling or integrating into whole-plant models, many further extensions to the developed models are possible. Some steps already have been taken e.g. including a second morphogen. However, these developments (including more complex signaling, molecular aspects of cell wall mechanics (together with PGO) and water relations) will likely become part of a follow up of this study (cf. Concluding Remarks below)

3. Verspreiding en valorisatie – Dissemination and valorization

Publications

+De Vos, D., Frederiks, F., Terweij, M., van Welsem, T., Verzijlbergen, K.F., Iachina, E., de Graaf, E.L., Altelaar, M.A.F., Oudgenoeg, G., Heck, A.J., Krijgsveld, J., Bakker, B.M. and van Leeuwen, F. (2011) Progressive methylation of ageing histones by Dot1 functions as a timer. EMBO Rep. 12, 956-962. (Impact Factor 7.8).

+De Vos, D., Bruggeman, F.J., Westerhoff, H.V., and Bakker, B.M. (2011) Molecular competition influences fluxes in gene-expression networks. PLoS One. 2011;6(12):e28494 (Impact Factor 4.4).

+Van Leeuwen F, Frederiks F, Terweij M, De Vos D, Bakker BM.
News about old histones: a role for histone age in controlling the epigenome.
Cell Cycle. 2012 Jan 1;11(1):11-2. (Impact Factor 5.4).

+Stout J, De Vos D, Vergauwen B, Savvides SN. Glutathione biosynthesis in bacteria by bifunctional GshF is driven by a modular structure featuring a novel hybrid ATP-grasp fold.
J Mol Biol. 2012 Mar 2;416(4):486-94. (Impact Factor 4.0).

+De Vos D, Dzhurakhalov A, Draelants D, Bogaerts I, Kalve S, Prinsen E, Vissenberg K, Vanroose W, Broeckhove J, Beemster GT. Towards mechanistic models of plant organ growth.J Exp Bot. 2012 May;63(9):3325-37 (Impact Factor 5.4).

(**bold:** within the framework of this project)

Conferences and workshops

+CPIB (Center for Plant Integrative Biology) - Modelling Cell Elongation Workshop, 7-8 Feb 2011
The main objective was to compare modelling methods and identify complementary approaches to root modelling at CPIB (Prof. M. Bennett from CPIB is member of the IUAP project BARN)

+CPIB (Center for Plant Integrative Biology) - Modelling Workshop, 23 May 2012
The main objective was to give an update on the state of the root and leaf modelling and analysis at the UA and CPIB and identify complementarity.

+Plant Modelling with L-studio Workshop (by Dr. P. Barbier de Rueille and Prof. R.S. Smith from the University of Bern, CH). Crash course into model building with software related to VirtualLeaf which was important to be in touch with the state of the art of the field and to network with peers.

Other
Further relevant activities include computational and mathematical support for biologists and biological support for mathematicians and computer scientists from the research groups of K. Vissenberg, G. Beemster, J. Broeckhove, and W. Vanroose. Moreover, teaching support was provided for Prof. Beemster for the course 'Wiskunde in de Biologie' (3^e Bachelor Biology) in 2011 and 2012.

CONLUDING REMARKS

As can be seen from the detailed listing of section 2 most of the objectives have been met during the contract, although the technical implementation is sometimes different than wath was anticipated. Briefly, the modelling software VirtualLeaf was developed and effectively used to construct succesful cellular-resolution computational models of root tip growth. Apart from one published article sufficient results are on the table that can form the basis for more research papers. As a post-doctoral scientist I have been able to play a central role in an interdisciplinary team of scientists translating and integrating information from different fields in the true sense of Systems Biology. Apart from the 'technical' contribution as a bio-modeller I have therefore employed my experience gained abroad to catalyze scientific progress in the context of the research groups mentioned above and in a broader sense of the UA. Teaching and writing or editing research proposals are equally important in that respect. The COST Action proposal mentioned above is an example of setting up collaborations in a European context.

In terms of continuity of this project and more generally my career I have accepted the offer of Prof. Beemster to continue working along the same way within the new IAP project MARS (Study of Maize-Arabidopsis-Root-Shoot growth; http://www.iuap-mars.be/). Future developments will be focusing on extending the existing root models to include more detail on signalling networks, cell wall mechanics (together with PGO) and especially the cross-talk between those processes and the cell cycle. Furthermore, the idea is to transform the root models to build a maize leaf model. Other promising plans include connecting several submodels (for example root and shoot or leaf) into larger-scale models and relating the models to environmental perturbations like drought and cold.

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