A structured growth model for Hairy roots of *Beta vulgaris*

Growth morphology and secondary metabolite distribution in dense root networks

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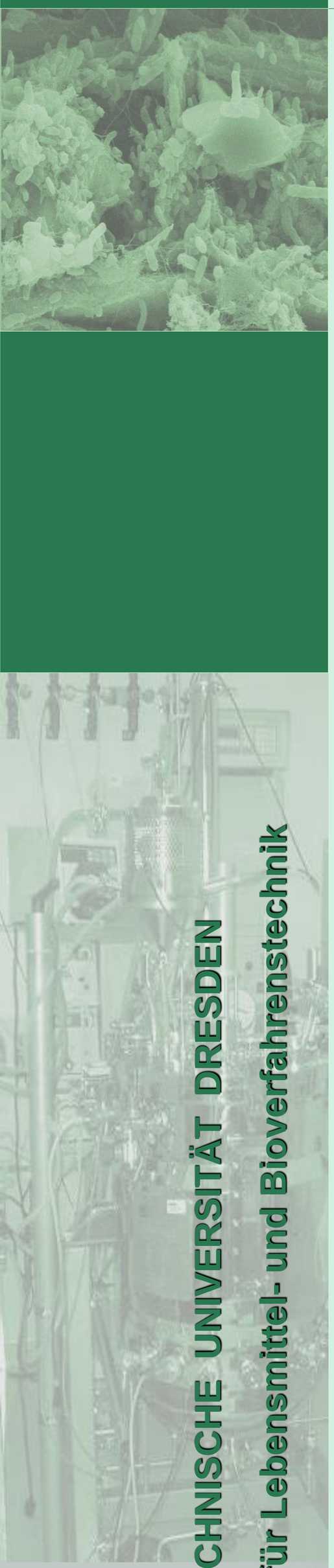

Introduction and Aims:

Plant cells can be used to produce a wide range of nutritional-physiological and pharmaceutical relevant secondary metabolites. While usual suspended *in vitro* cultures need a constant level of different hormone concentrations, *Agrobacterium rhizogenes* induced Hairy roots can be cultivated in hormone free media. In general the growth of these tissue cultures on agar plates, in shaking flasks or bioreactors for industrial use has been heavily investigated experimentally but only limited theoretical descriptions of the growth process exist.

In order to model the growth morphology and the distribution of secondary metabolites beetroot (*Beta vulgaris*) was chosen as a model system. It produces the red dye *Betanin* which is used as a food color and is also responsible for the red color of the root network. Therefore it can be used to identify the distribution of *Betanin* optically.

The presented structured growth model is an approach to simulate and visualize the growth of dense root networks in different environments.

While the model kinetics can be changed to adapt to other species, the gained knowledge can therefore be used by other scientists to improve their cultivation protocols and to simulate growth of their own cultures by amending the parameters of the model.

Fig. 1 *Beetroot* plant

Fig. 2 Hairy roots of *Beetroot*

Materials and Methods:

The proposed model uses an individual-based matrix approach for growth simulations on agar plates. It consists of a 2-dimensional **organ matrix** containing a vector with information about each state of a cell (e.g. age, size, metabolite concentrations) and a **nutrient matrix** which represents the composition of the nutrient media (e.g. carbon source, solved oxygen etc.).

In dense root networks growth can be determined spatially at three different parts of the organ complex (see Fig. 4):

- Tip movement (a: elongation)
- Branching (b)
- Overall biomass growth (c: secondary thickening)

The mentioned growth processes can be structured further. At first the type of growth must be identified (a, b or c). For case a kinetics and directions of growth

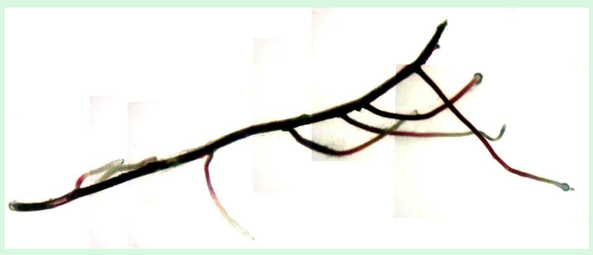

Fig. 3 Hairy root complex under the microscope

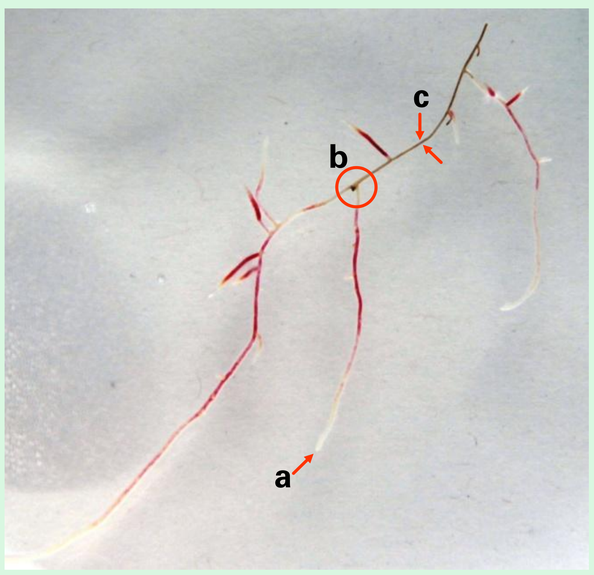

Fig. 4 growth processes in dense root networks

(angle τ) are used while in case of b it is stochastically determined how often cells form a new branch. Secondary thickening (case c) is carried out with all existing cells with respect to kinetics and age. All parameters depend on the species and more often even on the specific line. The production of the secondary metabolite *Betanin* starts after a distinct threshold of age and size is crossed. The nutrient concentration in the media must still be sufficient.

For the simulation of all underlying growth processes a recursive algorithm which only uses the former state of organ and nutrient matrix is recalculated for a given number of defined time steps. For each time step the implemented differential equations (ODE's and PDE's) for model kinetics and diffusion processes are solved numerically.

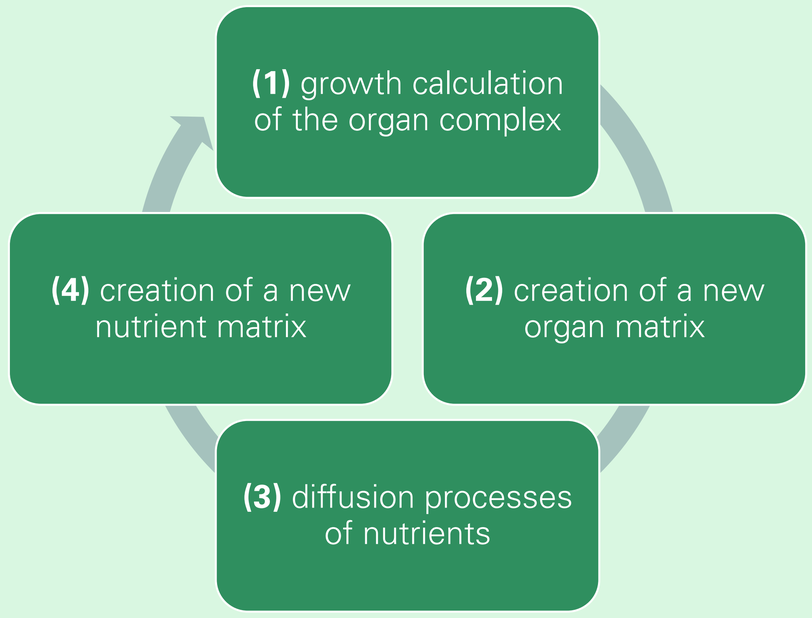

Fig. 5 schematics of recursive algorithm

Results and future prospects:

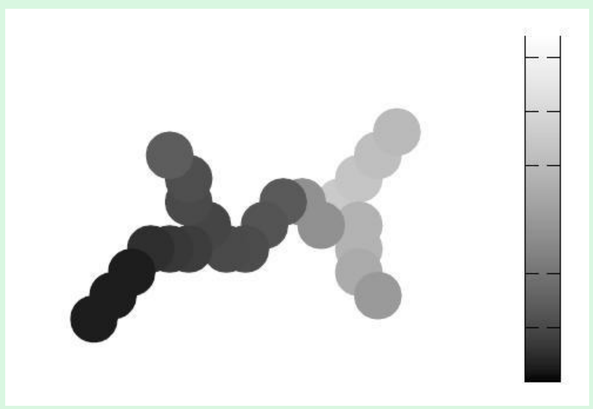

Fig. 6 simulation results for Hairy root growth

A model grid for the simulation of Hairy root growth was established and the three main forms of growth in dense root networks were identified and structured into the growth process algorithm.

Field studies to quantify the distributions of the growth parameters have been conducted and will be the basis for systematical investigations. For comparable simulation results information about morphology and distribution of secondary metabolites will also be taken from automatically analyzed images of the root networks during the cultivation process. Therefore a customized solution of picture recognition will be developed.

Results shown in Figure 6 are a very limited simulation of growth with an exemplary distribution of distribution of the darkness depending on the age of each cell. Each dot represents one cell which forms the root network.

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Finanzierung:

Europäischer Sozialfonds und der Freistaat Sachsen
Projektnummer: 080938406
Projektlaufzeit: 01.10.2009 - 30.09.2012