Vrije Universiteit Brussel
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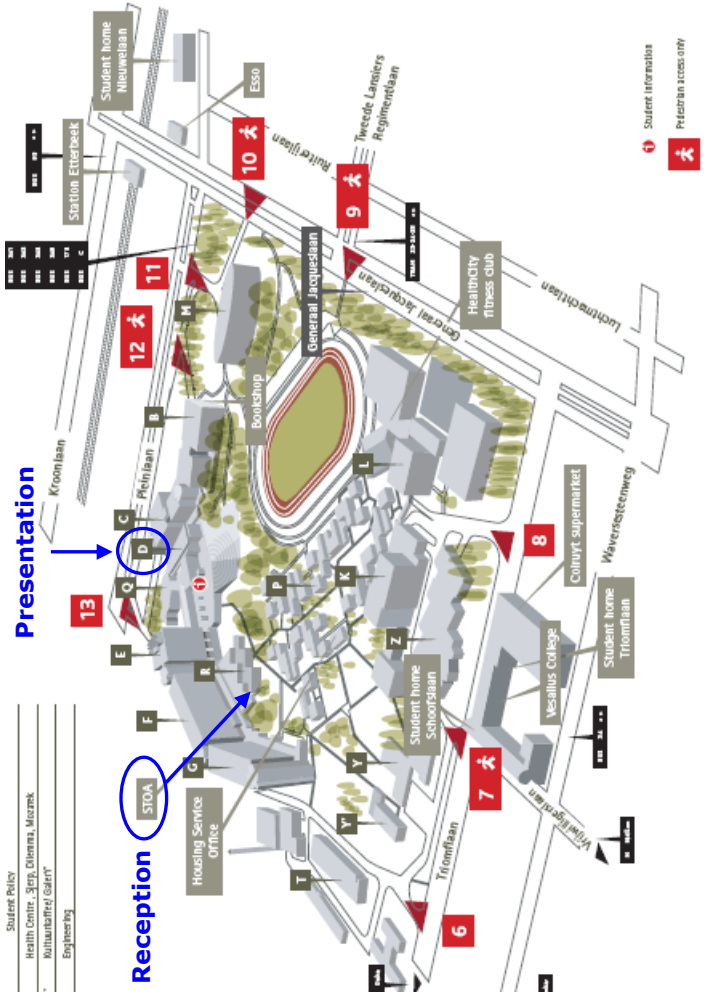

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Examination committee

Prof. Dr. Geert Angenon, promoter
Department of Applied Biological Sciences
Laboratory of Plant Genetics
Vrije Universiteit Brussel

Prof. Dr. Henri De Greve, chairman
Department of Applied Biological Sciences
Laboratory Ultrastructure
Vrije Universiteit Brussel

Prof. Dr. Danny Charlier, secretary
Department of Applied Biological Sciences
Laboratory Microbiology and Genetics
Vrije Universiteit Brussel

Prof. Dr. Jean-Pierre Hernalsteens, member
Department of Biological Sciences
Laboratory of Viral Genetics Research
Vrije Universiteit Brussel

Prof. Dr. Ann Depicker, member
Department of Plant Systems Biology
Research Group Gene Regulation
Ghent University

Prof. Dr. Danny Geelen, member
Department of Plant Production
Research Group In vitro Biology and Horticulture
Ghent University

Prof. Dr. Tom Gerats, member
Section Plant Genetics
Radboud University Nijmegen

FACULTY OF SCIENCES
Institute for Molecular Biology and Biotechnology
Department of Applied Biological Sciences
Laboratory of Plant Genetics

Characterization of putative germline-specific promoters from *Arabidopsis thaliana* and their application in DNA modification strategies

Public defense for obtaining
the academic title of
DOCTOR IN APPLIED BIOLOGICAL SCIENCES
Of Mr. **DIMITRI VERWEIRE**

Which will take place on
FRIDAY FEBRUARY 15, 2008
BUILDING D,
AUDITORIUM D0.07
CAMPUS OEFENPLEIN
AT 16H00

Promoter: **Prof. Dr. ir. GEERT ANGENON**

Afterwards a reception will be offered in the
STOA (campus Oefenplein)
AT 18H30

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Vrije Universiteit Brussel

Presentation of the manuscript

Introduction

Plant Biotechnology can play a major role in addressing some important issues which our society is confronted with. In a world with a rapidly increasing population a more sustainable and efficient agriculture is indispensable. Furthermore, Plant Biotechnology will become increasingly important in the development of "energy-crops" as the shift from an oil-based economy to a plant-based economy further unfolds. Moreover, second generation transgenic crops, producing enhanced levels of essential amino acids, vitamin A,… will create value for the consumer as well.

Therefore Plant Biotechnology offers a technological platform with high impact on different key areas of our society: health environment, economy, …. Nevertheless, the technology *an sich* faces some important challenges. Furthermore, the scientific community and the industry will have to formulate an answer to public concerns related to transgenic plants.

Objectives

• Characterization of putative germline-specific promoters to assess their applicability in new systems to remove the selectable marker (e.g. antibiotic resistance) and to introduce sequences in specific places in the genome (gene targeting).

• Development of a versatile and efficient system to remove the selectable marker (e.g. antibiotic resistance) in the same time frame as compared to transformation procedures in which the marker is not removed and this without extra handling. This could be a paradigm; technological improvements can make the technology more acceptable to the public.

• Development of an efficient gene targeting system allowing integration of the transgene in a predetermined place in the genome leading to stable and more predictable transgene expression. This would also allow to introduce specific and subtle changes in the genome sequence further optimizing transgene technology.

Thinking must never submit itself,
neither to a dogma,
nor to a party,
nor to a passion,
nor to an interest,
nor to a preconceived idea,
nor to anything whatsoever,
except to the facts themselves,
Because for it to submit to
anything else would be the end
of its existence

(H. Poincaré)

Results

• Five promoter fragments proved to be highly functional during the germline, i.e. they were able to induce a site-specific recombination reaction in cells that are progenitors of the gametes. These promoters were used in two applications: removal of the selectable marker and gene targeting.

• We were able to genetically program transgenic plants to lose their selectable marker (e.g. antibiotic resistance) when its presence in no longer required. This was achieved by introducing the Germline-Specific Auto-excision (GSA) vector which contains a site-specific recombinase under control of a promoter conferring germline functionality. This allows to obtain marker-free transgenic plants in the same time frame as in transformation procedures in which the selectable marker is not removed and without additional handling. Therefore, the removal of the selectable marker perfectly coincides with obtaining transgenic plants as such allowing faster re-transformation, field trials and/or commercialization…

• In a second application the promoters functional in the germline were used to activate a donor molecule during specific developmental stages (e.g. prophase I of meiosis). Recombination events were obtained. However, at this stage we are not able to confirm that the target locus was modified. Strategies to improve the efficiency of the system are currently developed in the Laboratory of Plant Genetics, Vrije Universiteit Brussel.

It is through science
that we prove, but
through
intuition that we
discover

(H. Poincaré)

Output

• Verweire D, Verleyen K, De Buck S, Claeys M, Angenon G (2007) Marker-free transgenic plants through genetically programmed auto-excision. Plant Physiol 145: 1220-1231.

• Patent application filed with the EPO: Preparation of transgenic plants (PCT/EP2007/060902).

• Patent application filed at the EPO: Targeted genome modifications in plants (07109346.2).

Curriculum vitae

Dimitri Verweire, born in Ghent on December 17 1978, obtained in the year 1996 his secondary school degree in Latin-Sciences at the St-Lievens college in Ghent. In the year 2000, he obtained a license degree in Sciences, option Biotechnology with distinction (great distinction in the last year) at the University of Ghent. He then became assistant in the Laboratory of Plant Genetics at the Department of Applied Biological Sciences, Vrije Universiteit Brussel. During this period he organized practical courses and guided thesis students. Furthermore, he has been a member of the Department Council for several years. The research in frame of his PhD focused on the characterization of putative germline-specific promoters of *A. thaliana* and using them in DNA modification strategies. Part of the work presented in this manuscript was published in a highly ranked international peer-reviewed journal, presented on international conferences and subject of two patent applications.