Nederlandse Vereniging voor Plantenbiotechnologie en -Weefselkweek
Netherlands Society for Plant Biotechnology and Tissue Culture
KvK nr. 40121960 NL42INGB0004240007 www.nvpw.nl info@nvpw.nl

NVPW spring symposium

Friday, June 3rd, 2022
Wageningen UR Campus, OMNIA (building 105, Hoge Steeg 2)

09:30 **Registration and coffee / tea**

09:55 **Opening by prof. Evert Jacobsen**

10:00 **Prof. dr. Mark Aarts – Wageningen UR, Laboratory of Genetics**
NPEC, the Netherlands Plant Eco-Phenotyping Centre, a novel facility for Plant Sciences

10:35 **William de Martines MSc. – Wageningen UR, Department of Plant Breeding**
New approaches to gene targeting in plants by exploiting the unique characteristics of CRISPR-Cas12a

11:10 **Elevator pitch by exhibitors**
11:15 **Coffee / tea break**

11:30 **Dr. Rob Dirks – Managerial Genetics Consulting BVBA**
Doubled Haploids: methods and applications.

12:05 **Dr. ir. Jaap Wolters - Wageningen UR, Department of Plant Breeding**
Glycoalkaloids from wild *Solanum* provide broad-spectrum disease resistance

12:40 **Lunch**
13:10 **General members meeting**

13:40 **Dr. Siel Desmet – Ghent University, Department of Plants and Crops; ILVO, Plant Sciences Unit**
Compact ornamentals by means of Rhizobium rhizogenes transformation and regeneration of Ri lines

14:15 **Dr. Katrijn van Laere – VIB, UGent Center for Plant Systems Biology; ILVO, Plant Sciences Unit**
Identification of bitterness related biosynthesis genes in Cichorium using CRISPR/Cas9 genome editing

14:50 **Coffee / tea break**

15.05 **Maria Chiara Piro MSc. - Ghent University, Department of Plants and Crops; ILVO, Plant Sciences Unit**
Combining genomic and molecular cytogenetic tools to unlock triticale's potential for wheat breeding

15:40 **Dr. Jack H. Vossen - Wageningen UR, Department of Plant Breeding**
How to turn late blight susceptible potato varieties resistant; Insertion and repair of native resistance genes through novel plant breeding techniques

16:15 **Closing drinks**

The costs for attending the symposium are € 30, to be paid by smartphone or in cash. This includes the lunch, coffee/tea and closing drinks. The printed day programme and abstracts will be handed out at the symposium.

Please subscribe **before Monday, May 30th** via **info@nvpw.nl**

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Summaries of the lectures on the NVPW spring symposium, Friday, June 3rd, 2022
Wageningen UR Campus, OMNIA (building 105, Hoge Steeg 2)

NPEC, the Netherlands Plant Eco-Phenotyping Centre, a novel facility for Plant Sciences

Prof. dr. Mark Aarts - Wageningen UR, Laboratory of Genetics

The rapid technological advances in DNA sequencing provided plant breeding with a new paradigm: genomics as a way to uncover all genetic variation and select for genotypes, rather than phenotypes, in breeding new varieties. Now plant breeding faces another paradigm: phenomics, as a systematic analysis of the biology of plants in an environmental context. Plant phenomics comprises the phenotypic diversity provided by plant genotypes in response to and in interaction with their environment. It requires advanced technology to accurately and reproducibly capture detailed plant phenotypes in a high-throughput manner over time and space. The Netherlands Plant Eco-phenotyping Centre is a national facility, funded through the National Roadmap for Large Scale Research Infrastructure, that is currently being built at Utrecht University and Wageningen University and Research, to provide the Plant Science community with a state-of-the-art research facility for Plant Phenomics.

New approaches to gene targeting in plants by exploiting the unique characteristics of CRISPR-Cas12a.

William de Martines MSc., Richard GF Visser, Jan G Schaart – Department of Plant Breeding, Wageningen University & Research

One of the promises of genome editing has been to efficiently make directed changes in a plant's DNA through gene targeting. Although, with few exceptions this has remained largely out of reach, current attempts at gene targeting commonly use a DNA repair process known as Homology Directed Repair (HDR) to incorporate changes. HDR is a high-fidelity recombination-based DNA repair mechanism, however, it remains inefficient in plants. Therefore, it is of interest to look at other repair mechanisms, that have previously been thought to be error-prone, as a tool for gene targeting. Specifically, alternative nonhomologous end-joining (aNHEJ) is a good candidate for a high-fidelity repair mechanism to exploit. aNHEJ relies on microhomologies to direct DNA repair, this is where the unique characteristics of the Cas12a enzyme come in. Cas12a cuts DNA and leaves overhangs that seem to stimulate a high-fidelity repair that resembles either aNHEJ or a simple ligation in plants. In our research we show for the first time in plants this new type of repair using Cas12a. By designing target sites to leave complementary microhomologies it was possible to direct precise DNA repair and make large DNA deletions in a test construct in agroinfiltrated *Nicotiana benthamiana* without any trace of undesirable sequences. Further we show strong evidence for the integration or replacement of large stretches of DNA using this new type of repair. It is therefore important to follow up these studies to further understand this repair process and to optimize future use for genome editing.

Doubled Haploids: methods and applications.

Dr. Rob Dirks – Managerial Genetics Consulting BVBA

Although the first doubled haploid plants derived from anther culture were discovered almost 50 years ago, and many breeding companies are using large numbers every year, the full scope of possibilities has not been appreciated yet.
In addition, some persistent misunderstandings particularly related to recombination are still present today.
New technologies for making DH lines such as inducer lines appeared the last 5 to 10 years, but are they a valid alternative to "classic" microspore cultures?

Glycoalkaloids from wild *Solanum* provide broad-spectrum disease resistance

Dr. ir. Jaap Wolters, Doret Wouters, Richard GF Visser, Vivianne GAA Vleeshouwers - Wageningen UR, Department of Plant Breeding

Research on disease resistance in plants is generally focussed on immune receptors. These receptors often provide a strong resistance, but they typically have a narrow spectrum. While it is recognized that secondary metabolites also play a role in plant defence, a detailed knowledge of the biosynthesis pathways and the contribution of these compounds to plant immunity is lacking. In our search for resistance against early blight of potato, we found a wild potato relative in which resistance is based on glycoalkaloids. We show that these glycoalkaloids are active against a wide variety of pathogens. As a result, plants producing the compounds have a broad-spectrum disease resistance. This research highlights the potential of secondary metabolites from plants to control disease.

Compact ornamentals by means of Rhizobium rhizogenes transformation and regeneration of Ri lines

Dr. Siel Desmet, Ellen De Keyser, Danny Geelen, Emmy Dhooghe – Ghent University, Department of Plants and Crops; ILVO, Plant Sciences Unit

Plant quality is a primary concern for plant growers. One of the main aspects of plant quality is the plant growth habit, which should be compact and densely branched. For many horticultural crops, compactness is usually obtained through the application of chemical growth retardants. However, plant growers today are faced with increased restrictions on the use of plant growth regulators. Increased independence of growth retardants would offer substantial economic and ecological benefits, and would contribute towards a more environmental friendly plant production. Here, we report on the viability and scope of the genetic transformation strategy using wild type Rhizobium rhizogenes, coined Ri technology, that was implemented to obtain compact growing varieties. Indeed, our results show that more compact genotypes (Ri lines) can be obtained in a broad range of ornamentals crops (e.g. *Sinningia speciosa*, *Viola x wittrockiana* and *Osteospermum fruticosum*). The resulting plants, not classified as genetically modified, will prove useful in future breeding towards compact varieties that require little to no chemical growth regulation.

Identification of bitterness related biosynthesis genes in *Cichorium* using CRISPR/Cas9 genome editing

Dr. Katrijn van Laere, Charlotte De Bruyn, Tom Eeckhaut, Tom Ruttink, Thomas Jacobs, Alain Goossens – VIB, Ugent Center for Plant Systems Biology; ILVO, Plant Sciences Unit

Cichorium intybus var. sativum (chicory) and *var. foliosum* (witloof) are economically important crops with a high nutritional value thanks to many specialized metabolites, including sesquiterpene lactones (SLs). However, SLs are responsible for a bitter taste, limiting the use of *Cichorium* for industrial purposes. Therefore, targeting genes from the SL biosynthetic pathway using CRISPR/Cas9 is used to alter SL metabolite production and thus to change the bitterness. The genes *germacrene A synthase (GAS), germacrene A oxidase (GAO)*, *costunolide synthase (COS)* and *kauniolide* synthase (*KLS*) are already known to control production of SLs. A comprehensive genome-wide screen was executed and enabled to identify new *CiGAS*, *CiGAO*, *CiCOS* and *CiKLS* candidate genes active in the SL biosynthetic pathway. CRISPR/Cas9 targets were designed and knock-outs were induced in these selected SL candidate genes. SL metabolite profiling of the mutant plants using UHPLC-HRMS provided insights in the function of the SL candidate genes, the formation of the SL metabolites and hence the bitterness in *Cichorium*.

Combining genomic and molecular cytogenetic tools to unlock triticale's potential for wheat breeding

Maria Chiara Piro MSc., Katrijn van Laere, Hilde Muylle, Geert Haesaert - Ghent University, Department of Plants and Crops; ILVO, Plant Sciences Unit

Triticale (× *Triticosecale* Wittm.) is an amphidiploid crop resulting from the pollination of wheat (*Triticum aestivum*) with rye (*Secale cereale*). As such, it can represent a readily accessible source of rye chromatin to widen wheat genetic diversity. Rye chromatin has long been exploited in wheat breeding to improve pest and disease resistance. Nevertheless, it could also constitute a valuable resource to improve wheat nutritional quality, in particular the dietary fibre (DF) content of white flour. With the goal of increasing the DF content of wheat white flour, the FIBRAXFUN consortium assembled a triticale diversity panel, to unlock triticale's potential for wheat breeding. Using a genome-wide association study approach, we aim at uncovering arabinoxylan-associated loci on the rye genome of triticale and therefore select parental lines to include in a wheat-triticale crossing platform. At the same time, we will exploit FISH karyotyping to describe the parental material and follow the rye chromatin introgression in the crossing progeny.

How to turn late blight susceptible potato varieties resistant; Insertion and repair of native resistance genes through novel plant breeding techniques

Dr. Jack H Vossen, Daniel Monino-Lopez, Evert Jacobsen, Richard GF Visser - Wageningen UR, Department of Plant Breeding

Till this day, in cross-pollinating crops like potato, it is almost impossible to retain established varieties which have one or few traits added. In contrast to self-pollinating crops, like tomato, the original variety is lost when cross-breeding is pursued. Novel plant breeding techniques (NPBT) are ideally suited to improve established varieties. As plants that are generated using NPBTs are considered GM, there are currently no NPBT crops grown in Europe. Even while it is generally acknowledged that NPBT plants in which native genes from the species itself or from crossable species are used, are equally safe as classically bred material. In this report we provide examples by which NPBTs can be deployed to produce late blight resistant potato plants.

In the first example we produced cisgenic potato plants by adding multiple late blight resistance (*R*) genes from crossable species through marker-free agrobacterium mediated transformation. Using *R* gene and vector backbone specific PCR selection, 17 late blight resistant cisgenic events, without vector backbone and with only cisgenic *R* genes were selected. Whole genome sequencing was deployed to analyse how these 17 events differed from their untransformed parents. In none of the events insertions of vector sequences were detected at locations that were not linked to the cisgene insertion sites. In 12 events we could completely resolve how and where the different T-DNA's had integrated in the genome. Four events contained short (18-27 bp) vector remnants flanking the inserted cisgenes. In three events the flanking sequences were too small (8-15 bp) to make a distinction whether they were vector remnants or native potato sequences. In the remaining five events, the inserted cisgenes were directly flanked by DNA from the recipient genomic location without any other DNA. It is concluded that through the described selection process, we can select true-cisgenic events whose genomes consist purely of potato DNA. As the T-DNA insertions took place at random positions in the genome, the cisgenic events can be compared to *R* gene translocations. Interestingly, translocations of *R* genes have occurred frequently during natural *R* gene evolution.

In a second example we deploy inactive copies of R genes which are present in the genomes of susceptible potato varieties. Through a series of domain exchange experiments we showed that these inactive r genes could be activated by exchanging only a small part of the inactive gene with the homologous part of *R* genes from crossable species. Currently we pursue the repair of an inactive allele rpi-tub1 in a susceptible potato using a small patch of *Rpi-chc1* from *Solanum chacoense*. Therefore, we used CRISPR-Cas induced double strand breaks and homology directed repair. Preliminary results of successful repair will be shown.