Stichting voor Plantenbiotechnologie en -Weefselkweek (SVPW)
Society for Plant Biotechnology and Tissue Culture
KvK nr. 89069528 NL42INGB0004240007 www.svpw.nl info@svpw.nl

SVPW spring symposium

Friday, June 9th, 2023

Hotel de Nieuwe Wereld, Marijkeweg 5, 6709 PE, Wageningen

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

09:55 **Opening by prof. dr. Remko Offringa**

10:00 **Prof. dr. Holger Puchta - Karlsruhe Institute of Technology**
Heaving CRISPR/Cas applications in plants to new levels: chromosome and tissue engineering

10:45 **Dr. Ruud A. de Maagd - Wageningen UR, cluster Plant Developmental Systems, Bioscience,**
Gene editing in tomato for insights into fruit development, crop improvement, and biosafety research

11:20 **Elevator pitch by exhibitors**

11:25 **Coffee / tea break**

11:40 **Dr. Jan Schaart and dr. Martina Juranić - Wageningen UR, Department of Plant Breeding**
Gene editing to support breeding in ornamental species

12:15 **Jillis Grubben MSc. - Wageningen UR, Department of Plant Breeding**
Inducing kilobase to mega base-sized inversions in tomato using CRISPR/Cas9: The larger, the rarer?

12:50 **Lunch**

13:20 **General meeting NVPW**

13:50 **Kitty Huijben BSc. – Hudson River Biotechnology B.V.**
TiGER; A Transgene-free CRISPR Workflow for Genome-edited Plants in a Single Generation; Examples in *Solanaceae*

14:25 **Dr. Frans Krens – Wageningen UR, Department of Plant Breeding**
Hyperhydricity and nutrient flow, two important factors determining quality of in vitro cultured plants

15:00 **Coffee / tea break**

15.15 **Prof. dr. Remko Offringa – Leiden University, Plant Developmental Genetics, Institute of Biology**
Structure-activity relationship of 2,4-D analogues for the induction of somatic embryogenesis in *Arabidopsis thaliana*

15:50 **Lucas van der Zee MSc. – Wageningen UR, Department of Plant Sciences**
Sugar-based agriculture - mixotrophic lettuce and tomato fruit production

16:25 **Closing drinks**

Attendees: contribution to the symposium @ **€ 30**, may be in cash at the entrance but would be nice, if possible 3 days in advance via: https://www.ing.nl/particulier/betaalverzoek/index.html?trxid=p7rH0BLETicFb3TFpBAWUWn0G7uEIY52 or at IBAN of the NVPW (NL42 INGB 0004 2400 07) mentioning the names concerned and "tbv van SVPW symposium".
The printed day programme and abstracts will be available at the symposium.Lunch, coffee/tea and closing drinks included.

Please subscribe before Monday, June 5th via info@svpw.nl

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Summaries of the lectures on the SVPW spring symposium,
Friday, June 9th, 2023
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Heaving CRISPR/Cas applications in plants to new levels: chromosome and tissue engineering

Prof. dr. Holger Puchta, Karlsruhe Institute of Technology

The CRISPR/Cas technology has been applied in plants mainly on genes for the improvement of traits. However, breeding also requires the breaking or establishing genetic linkages. We were able to change genetic linkages by inducing heritable translocations in the Mb range between heterologous chromosomes in Arabidopsis thaliana. Recent improvements in sequence analysis of crop plants reveal that multi Mb long inversions occur with high frequency between different genotypes, leading to crossover suppression. We were not only able to demonstrate that inversions of up to almost chromosome size can be achieved, but also that meiotic recombination can be redirected this way. Finally, we developed a new technology for tissue engineering named CRISPR-Kill, allowing us to induce targeted cell death at defined time points in different organs at select developmental stages.

Gene editing in tomato for insights into fruit development, crop improvement, and biosafety research

Dr. Ruud A. de Maagd, cluster Plant Developmental Systems, Bioscience, Wageningen University & Research

New Breeding Techniques (NBTs), specifically gene editing using CRISPR/Cas, have revolutionized molecular biology research, including plant research. They also hold a promise for developing or improving crops. In this presentation, I will give an overview of what this revolution has done for our research and global research in tomatoes using the three examples mentioned in the title. Tomato is a model species for studying fleshy fruit development due to its autogamy, relatively short life cycle, transformability, genetic resources, and sequenced genomes. It is also an economically important crop in the Netherlands. Tomato is also very amenable to mutagenesis by CRISPR/Cas, and not coincidentally, tomato delivered the first commercial CRISPR/Cas-derived variety for consumers in Japan. Still, sufficient challenges remain.

Gene editing to support breeding in ornamental species

Dr. Jan Schaart and dr. Marina Juranić, Department of Plant Breeding, Wageningen University & Research

Breeding of ornamental species is primarily focused on creating new varieties with unique ornamental value, and in recent years, developing resistance to pests and diseases has become increasingly important. Traditional breeding approaches, based on crossing and selection, can be made more efficient using marker-assisted selection. However, for many ornamental species, breeding remains challenging. The emergence of New Plant Breeding Techniques, such as gene editing using CRISPR-Cas, holds great promise for supporting the breeding of ornamental crops. In our presentation we will explore the possibilities and challenges of applying gene editing in ornamental species. It will provide examples of gene editing applications in some ornamental crops and discuss the opportunities offered by this technique for breeding new ornamental varieties with improved resistance to pests and diseases.

Inducing kilobase to mega base-sized inversions in tomato using CRISPR/Cas9: The larger, the rarer?

Jillis Grubben MSc. / dr. ir. Henk J. Schouten, Department of Plant Breeding, Wageningen University & Research

The CRISPR/Cas system has revolutionized plant biotechnology by enabling targeted genetic modifications, including the induction of inversions. In many crops, vital resistance genes are located within inversions. Inversions do not allow meiotic recombination, leading to linkage drag. This phenomenon occurs when undesirable traits are inherited alongside the desired gene and cannot be separated through recombination during backcrossing. For instance, tomatoes we consume today contain inversions and considerable linkage drag, as researchers prioritize the essential resistance gene.

This presentation delves into the factors affecting the efficiency of CRISPR/Cas-induced inversions in tomato, focusing on the relationship between inversion length and induction frequency. By generating a range of inversions from 1 kb to 37.5 Mb in size, we aim to uncover the determinants of inversion induction efficiency. Intriguingly, our findings revealed unexpected results, hinting at the possibility of an unknown mechanism at play. Additionally, two screening methods for genetic inversion events will be discussed, providing valuable insights for plant biotechnology experts working to overcome linkage drag and enhance crop quality.

Join us as we decode inversion efficiency, paving the way for innovative advancements in plant biotechnology and tissue culture, and opening new doors for creating resilient and sustainable crops.

TiGER; A Transgene-free CRISPR Workflow for Genome-edited Plants in a Single Generation; Examples in Solanaceae

Kitty Huijben BSc., Hudson River Biotechnology B.V.

For a wide range of species, our TiGER workflow overcomes CRISPR bottlenecks to rapidly deliver top-quality products that can be directly introduced into the market development pipeline. In this way, we tackle the most common challenges associated with CRISPR gene editing. Our proprietary TiGER workflow yields a genetically uniform edited organism in 6-18 months, offering benefits compared to traditional approaches:

* Transgene-free delivery protocols without the use of foreign DNA, which limits regulatory issues;
* MAD7 nuclease, a commercially more attractive option than Cas9, with high editing efficiencies to minimize costs for edit screening;
* Proprietary guide design software to target single or multiple genes in one editing round while minimizing chances of off-target edits;
* Single-cell protoplast regeneration to avoid edited chimeras. Even for recalcitrant species difficult to handle in vitro, we have achieved CRISPR active delivery in intact cells using nanoparticle-based transfection methods.

Hyperhydricity and nutrient flow, two important factors determining quality of *in vitro* cultured plants.

Dr. Frans Krens, Department of Plant Breeding, Wageningen University & Research

Hyperhydricity (HH) is a physiological disorder affecting severely plant production and quality in some cultivars of some species of *in vitro* cultured plants. The main cause is a defective water balance due to the high relative humidity in the growth containers. We have studied the phenomenon in order to increase our knowledge on the underlying mechanisms and find measures to prevent development of HH. We looked at stomatal functioning, the role of cell-wall components such as lignin, and the role of Ca^{2+} in *in vitro* grown *Arabidopsis* plants. Results were validated in a commercial crop, Statice.
Another important determinant of *in vitro* plant quality is nutrient flow. Plants *ex vitro* acquire their carbohydrates from photosynthesis and although there is photosynthesis under *in vitro* conditions, it is not sufficient and, therefore, carbohydrates are added to the medium. This, e.g., sucrose has to go from the lower parts of the plants to the top, opposite to the usual situation *ex vitro*. Driving force is a.o. transpiration. Critical for sucrose transport are membrane sucrose translocators. Water transport is also vital for nutrient flow and, here, water translocators or aquaporins play a central role. We started a unique project studying gene expression of sucrose and water translocators *in vitro* in an attempt to understand the processes involved and to link it to growth. The important role also here for transpiration was validated in *in vitro* grown apple shoots.

Structure-activity relationship of 2,4-D analogues for the induction of somatic embryogenesis in *Arabidopsis thaliana*

Prof. dr. Remko Offringa, Plant Developmental Genetics, Institute of Biology Leiden, Leiden University

2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic analogue of the plant hormone auxin that is commonly used in many *in vitro* plant regeneration systems, such as somatic embryogenesis (SE). Its effectiveness in inducing SE, compared to the natural auxin indole-3-acetic acid (IAA), has been attributed to the stress triggered by this compound rather than its auxinic activity. However, this hypothesis has never been thoroughly tested. I will present how we used a library of 40 2,4-D analogues to test the structure-activity relationship with respect to the capacity to induce SE and auxinic activity in *Arabidopsis thaliana*.

Sugar-based agriculture - mixotrophic lettuce and tomato fruit production.

Lucas van der Zee MSc., Department of Plant Breeding, Wageningen University & Research

Sugar-based agriculture is a novel concept that proposes growing crops using carbohydrates instead of light. If done using synthetic or circular sources of carbohydrates, it could open a radically sustainable way to produce food. While many papers describe the use of sugars for tissue culture, very few investigate the use of sugar to grow crops for food. I will discuss our research on two methods of growing crops using sugar. First, we've investigated growing whole lettuce plants on a liquid medium of sugars and nutrients. The plants seem to take up and incorporate the sugars. However, our current data suggests that growth rate is limited by light intensity independent of assimilate availability. Secondly, we're exploring the production of tomato fruits directly from tissue. We will present literature and early results that show that it is possible to grow "lab-fruit" and discuss key challenges around flower induction, fruit growth and scalability. I hope to inspire others to explore sugar-based agriculture with this presentation.

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