



NVPW fall symposium

Friday, December 9th, 2022

Telders Auditorium, Academy Building, Rapenburg 73, Leiden

- 09:30 **Registration and coffee / tea**
- 09:55 **Opening by Remko Offringa**
- 10:00 **Charles Underwood – MPI for Plant Breeding, Cologne**
Engineering apomixis: clonal seeds approaching the fields
- 10:35 **Martina Juranic – Wageningen UR, Plant Breeding**
Cracking apomixis using Mendel's hawkweeds as a research model
- 11:10 **Elevator pitch by exhibitors *)**
11:20 **Coffee / tea break**
- 11:40 **Kim Boutilier – Wageningen UR, Bioscience**
New morphogenic gene alleles for plant regeneration
- 12:15 **Wessel Holtman – Fytagoras BV, Leiden**
Doubled haploid technology: from academic knowledge towards a practical tool for breeders
- 12:40 **Lunch**
- 13:10 **General members meeting**
- 13:50 **Danny Geelen – HortiCell, Ghent University, Belgium**
Adventitious root formation in hypocotyls is controlled by a conserved cotyledon hypocotyl signaling pathway
- 14:25 **Coffee / tea break**
- 14:55 **Sander Hogewoning – Plant Lighting BV, Bunnik**
LED-lighting in greenhouses: consequences for photosynthesis, plant morphology and transpiration.
- 15:30 **Marten Elsinga – Iribov BV, Heerhugowaard**
Flow cytometry and its possibilities for tissue culture
- 16:05 **Closing drinks**

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

*) **exhibitors:** www.bronsonclimate.nl www.bioclimatic.nl www.plantcytometry.nl

Summaries of the lectures on the NVPW fall symposium,
Friday, December 9th, 2022
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Engineering apomixis: clonal seeds approaching the fields

Charles Underwood – MPI for Plant Breeding, Cologne

Apomixis is a form of reproduction leading to clonal seeds and offspring that are genetically identical to the maternal plant. While apomixis naturally occurs in hundreds of plant species distributed across diverse plant families, it is absent in major crop species. Apomixis has a revolutionary potential in plant breeding, as it could allow the instant fixation and propagation through seeds of any plant genotype, most notably F1 hybrids. Mastering and implementing apomixis would reduce the cost of hybrid seed production, facilitate new types of hybrid breeding, and make it possible to harness hybrid vigor in crops that are not presently cultivated as hybrids. Synthetic apomixis can be engineered by combining modifications of meiosis and fertilization. I will review the current knowledge and highlight a major achievement toward the development of efficient apomictic systems usable in agriculture.

Cracking apomixis using Mendel's hawkweeds as a research model

Martina Juranić - Wageningen UR, Plant Breeding

It's a lesser-known fact that after years of working with peas, Gregor Mendel started experimenting with "constant hybrids" in hawkweeds (*Hieracium* spp., syn. *Pilosella*) which, unlike peas, breed true through seeds as clones. Today we know that hawkweeds disobey the basic laws of inheritance by bypassing meiosis and fertilization via apomictic reproduction. The promise of apomixis in the fixation and indefinite propagation of a desired genotype has long been recognized, however, only recently highly sophisticated techniques have been developed to unravel the genetic basis of apomixis. Research efforts using the hawkweed as an apomictic model will be discussed during the talk.

New morphogenic gene alleles for plant regeneration

Kim Boutilier, Wageningen UR, Bioscience

Plants are developmentally flexible and can be induced to regenerate new tissues, organs and even embryos in vitro. This plasticity forms the basis for numerous applications in plant breeding and propagation, including in vitro embryogenesis and organogenesis. Improvements in plant regeneration protocols have largely been made using empirical modification of tissue culture parameters. More recently, a number of transcription factors have been identified that can be used to overcome plant regeneration barriers. However, use of these so-called morphogenic genes relies on stable or transient expression of gene constructs, which is subject to GMO regulations in many countries. We have used CRISPR-Cas9 mutagenesis to generate novel alleles of two morphogenic genes, *BABY BOOM* and *LEAFY COTYLEDON1*, that promote plant regeneration in vitro. The mutated regions are found in evolutionarily conserved non-coding nucleotide sequences, opening up the possibility for finding similar mutations in crop species.

Doubled haploid technology: from academic knowledge towards a practical tool for breeders

Wessel Holtman, Fytagoras BV, Leiden

Protocols for production of doubled haploid plants have been developed for many plant species last decades. However, DH protocols published from an academic perspective often lack possibilities for practical application. A practical DH protocol for use in breeding also includes production of sufficient, unique, high-quality DH plants in an efficient way

and at reasonable costs. Some hurdles, which Fyttagoras solved in this respect will be discussed in the presentation.

Adventitious root formation in hypocotyls is controlled by a conserved cotyledon hypocotyl signalling pathway

Danny Geelen, HortiCell, Ghent University, Belgium

Adventitious root (AR) formation is an adaptive developmental response that can be triggered by exogenous auxin application. We are using Arabidopsis etiolated hypocotyls and inflorescence stem segments to study the regulatory processes and role of auxin in de novo root organogenesis. To address underlying processes of AR induction, we screened a collection of auxin-like molecules for AR specific induction. A molecule was identified that massively induces AR in etiolated Arabidopsis seedlings without pronounced effects on primary root growth and lateral root branching. The novel compound hysparin, for hypocotyl specific adventitious root inducer, does not trigger a typical molecular response of DR5-reporter activation or DII-Venus degradation. In fact, auxin response genes are not activated within the first 8 hours. Instead, hysparin activates the cotyledons in producing a signal that induces AR formation on the elongated hypocotyl. Formation of hypocotyl AR depends on the presence of cotyledons and hysparin does not induce AR locally. As hysparin induces AR on elongated hypocotyls of several other species including tomato and rapeseed, its bioactivity impinges on a conserved cotyledon hypocotyl communication leading to adventitious rooting. In an approach to reveal what genetic elements are involved in this communication, we found only a few hormone signalling elements contributing to hysparin mediated AR induction. The findings support the presence of a specific cotyledon signalling route controlling hypocotyl adventitious root induction.

LED-lighting in greenhouses: consequences for photosynthesis, plant morphology and transpiration.

Sander Hogewoning, Plant Lighting BV, Bunnik

High energy prices drive a rapid transition from HPS lighting to full-LED lighting in greenhouses. LEDs do not only save electricity compared with HPS. There are also consequences for crop photosynthesis, morphology, and transpiration, dependent on the spectral composition of LED-lighting fixture. This imposes both opportunities and risks for growers. Opportunities, as the flexibility in light spectrum allows manipulation of the crop towards the desired response (e.g. rooting, branching, flowering). Risks, as insufficient knowledge may result in the wrong choices. The consequences of the transition from HPS to LED will be discussed.

Flow cytometry and its possibilities for tissue culture

Marten Elsinga – Iribov BV, Heerhugowaard

Flow cytometry (FCM) is a well-established method for the analysis of single cells, and finds many applications in research and production in an array of research subjects ranging from environmental to medical research. At Iribov the most used application is the determination of DNA content and ploidy levels of plants. Overall the use of FCM is, however, not conventional and widespread in plant breeding and tissue culture. The focus of our presentation will be on the added value of FCM in tissue culture applications to support researchers by reducing cost and time. Iribov is a large scale propagator through tissue culture and uses FCM as a routine analyses for a variety of tissue culture techniques. Our FCM researcher, Marten Elsinga, will present the use of FCM for applications such as quality control of microspores for dihaploid production, determination of transformation efficiency of protoplasts, and the control of microbial contamination in tissue culture.