plant reproduction

recent achievements and technologies

WAGENINGEN UR
For quality of life

plant reproduction

from flower to seed and fruit

In this brochure we highlight some recent achievements in our research on plant development and show how your breeding program or product development can benefit from our expertise and technologies.

Plant reproduction starts with the formation of the flower, which harbours the reproductive organs and cells. After fertilisation, the new offspring resides in the seed and a new generation is born.
Our food consists primarily of fruits and seeds - products of the reproduction process. For plant breeding and crop propagation the sexual reproduction process is of utmost importance.

our expertise

Our research group at Wageningen UR studies several aspects of the plant reproduction process and how the various steps in reproduction, from flower to seed and fruit, are regulated at the genetic, physiological and molecular level.
What do we offer:

- Genes that regulate important traits related to reproduction,
- Knowledge about the regulation of these genes and the influence of the environment,
- Protocols and screening methods to improve in vitro propagation and doubled-haploid (DH) production systems,
- Approaches for gene function determination,
- Methods for determining quality and storability of seeds.

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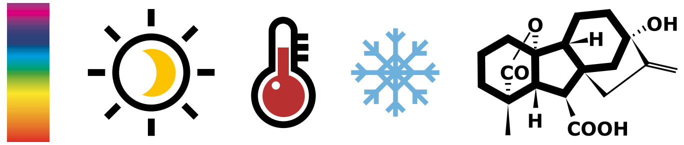
flowers for food and breeding

Flower formation is key for seed and fruit production. But how does a plant know when it is time to bloom? Plants sense their environment and measure factors such as day length and temperature to determine the right time for reproduction. The environmental dependence of flowering is not always desirable for plant growers, because it hampers the delivery of their products to retailers and consumers year round.



Flowering under control

Environmental signals trigger several signalling pathways that either promote or delay flowering. The main pathways are day length, light quality, temperature, age, and hormone-dependent. The challenge is to identify the genes underlying these pathways in crops and to determine their natural variation. This knowledge can be used to:

• predict flowering time
• select genotypes with a desired environment-independent flowering response
• modify flowering time for life-cycle shortening or flowering delay.

the thermometer of flowering

A change in ambient temperature can delay or promote flowering dramatically. For instance, cauliflower blooms later when temperatures rise. A few hot days in spring delays the formation of curds by a few weeks. We aim to determine how a plant senses ambient temperature and to identify the responsible genes.

From model to crop

Previous studies have shown that more than one hundred genes are involved in flowering time regulation. We have identified a small number of genes that regulate the response to temperature in the model species *Arabidopsis thaliana*. Now, the search for similar genes in cauliflower and other crops can start.

Key publications:

• Verhage et al. (2014). Research on floral timing by ambient temperature comes into blossom. Trends in Plant Sciences, 19

• Pose et al. (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature, 503 (7476), 414 - 417

• Immink et al. (2012). Characterisation of SOC1's central role in flowering by the identification of its up- and downstream regulators. Plant Phys. 159 (4), 1511 - 1523

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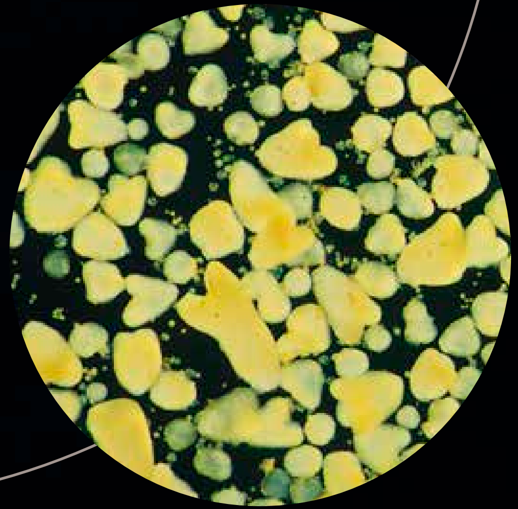
high throughput plant propagation

Commercial plant breeding relies heavily on tissue culture techniques to mass propagate important breeding lines or to produce doubled-haploid (DH) lines. Conventional tissue culture can be a tedious approach that requires many years of optimisation before an efficient protocol can be developed. Still, successes are few and many crops cannot be commercially propagated. We are using a high-throughput approach to accelerate the development of new tissue culture protocols.



Custom chemicals

Plant breeders traditionally rely on a handful of growth regulators to propagate cultured plant tissues. They face a dead-end when none of these delivers the desired effect. A faster and more saturating approach is to screen libraries of tens of thousands of commercially-available chemicals to identify new growth regulators. We develop and apply methods to perform these screens in a high throughput manner. A number of broad-acting compounds that enhance plant propagation processes have already been identified using this approach.

Efficient regeneration with BABYBOOM

In vitro regeneration through tissue culture is the cornerstone of plant breeding, whether it be for clonal propagation, DH production, embryo rescue or transformation. Unfortunately, many crops and crop genotypes are recalcitrant to *in vitro* regeneration, making it difficult to develop new genotypes that are optimised for grower and consumer-based traits. We have shown that the BABY BOOM transcription factor is a powerful tool to enhance a broad range of regeneration processes in different species.

New traits in pepper

Pepper (*Capsicum*) is an economically important crop that is cultivated throughout the world. Pepper is highly recalcitrant for transformation, and current transformation protocols are either inefficient, cumbersome or highly genotype dependent. We have shown that ectopic expression of the BABY BOOM gene can be used to efficiently regenerate transgenic plants and produce somatic embryos from recalcitrant sweet pepper varieties. This development opens the door for testing and introduction of new traits in pepper.

Key publications:

- *Li et al., (2014). The Histone Deacetylase Inhibitor Trichostatin A Promotes Totipotency in the Male Gametophyte. The Plant Cell, 26 (1), 195 - 209*
- *Horstman et al. (2013) AINTEGUMENTA-LIKE proteins: hubs in a plethora of networks. Trends in Plant Sciences 19, (3), 146 - 157*
- *Heidmann et al., (2011). Efficient sweet pepper transformation mediated by the BABY BOOM transcription factor. Plant Cell Rep. 30: 1107-1115*

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fruit quality and ripening

Fruits and their products are an indispensable part of the human diet. They provide us with calories, as well as with many essential nutrients, vitamins and other health-sustaining compounds and, not the least important, with many pleasant taste sensations. The currently available genomes of many tomato varieties, land races and wild relatives can help us to identify genetic variation for a large number of important fruit related traits.

Ripening and shelf life

We have identified and functionally characterised a number of genes involved in tomato fruit ripening. Our growing understanding of the regulation of tomato fruit development and ripening allows us to translate our findings to other fleshy fruits with similar requirements for yield and quality. To find markers and develop methods to asses fruit quality aspects we are deploying a range of techniques such as transgenic plants, virus-induced gene silencing, gene expression analysis, proteomics, and metabolomics.

gene functions in tomato and pepper

The genome sequences of important crops uncovered thousands of genes for which the function is not known. To study the role of candidate genes in the establishment of a trait, fast tools should be available for gene function discovery. We have established efficient gene inhibition methods in tomato and pepper based on Virus-induced gene silencing (VIGS).

Functional assay for ripening genes

Virus-induced gene silencing (VIGS) offers an attractive alternative for knocking out a gene without the need to genetically transform the plant.
Other benefits include:

- Short time between application and phenotype
- Fast construct preparation
- Simple plant applications
- Universal technique (multiple crops)
- No populations are needed (in contrast to TILLING)

Key publications:

- *Karlova, R. (2013). Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis. J. Exp. Bot. 64 (7), 1863 - 1878*
- *Bemer, M. et al. (2012). The tomato FRUITFULL homologs SlFUL1 and SlFUL2 regulate fruit ripening characteristics. The Plant Cell 24, 4437 - 4451*
- *Karlova, R. et al. (2011). Transcriptome and metabolite profiling show that SlAP2a is a major regulator of tomato fruit ripening. The Plant Cell 23 (3), 923 - 941*

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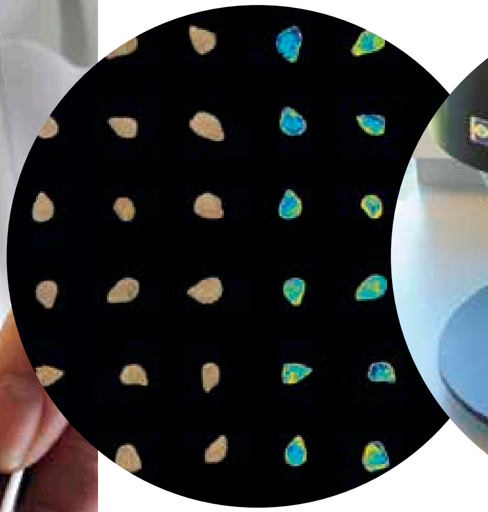
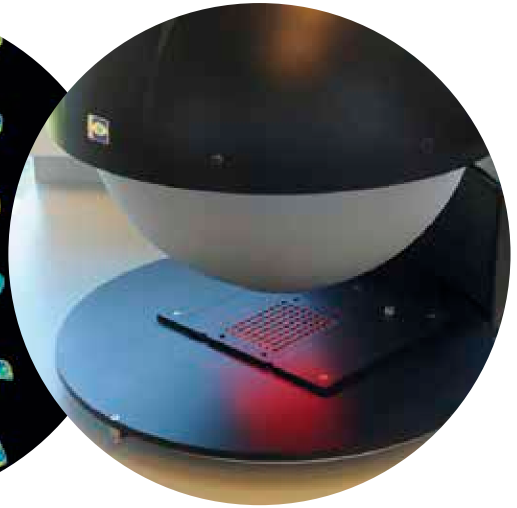
seed quality and storability



spectral image



videometer

breath analyser

oxygen tank

Seeds are a key food component and also the basis of most crop propagation systems. High seed quality, uniformity, viability and storability are key requirements for seed companies and their clients. Based on our expertise in seed physiology, we developed innovative methods for upgrading seed lots and for improving seed treatment and seed storage.

Assays for seed quality

Fast, reliable and non-destructive assays to determine seed quality aspects are valuable tools for selecting high-performing seed lots and for the evaluation of seed treatment procedures. As an alternative for time consuming germination tests, we developed two innovative and non-destructive seed quality assays:

- a modified breath analyser measures ethanol levels, which are inversely correlated with seed quality,
- a Videometer, which makes spectral images of seeds and correlates them to seed quality.

How to keep your seeds alive

For seed producers, seed banks and farmers it is of utmost importance to store seeds properly to guarantee viability and quality during storage.
We studied the role of oxygen in seed ageing and showed that dry and anoxia storage extend seed longevity.

Assay for seed storability

Waiting several years before you can evaluate the storability of a seed batch is not an option. We developed a treatment that mimics a long storage period. Seeds stored under Elevated Partial Pressure of Oxygen (EPPO) conditions deteriorate much faster and the symptoms resemble those observed with seeds stored for longer periods. This assay allows seed companies to study storability of (treated) seeds in a relatively short time frame (weeks to months vs years).

Key publications:

- *Groot et al. (2012). Seed storage at elevated partial pressure of oxygen, a fast method for analysing seed ageing under dry conditions. Ann. of Bot., 110: 1149 - 1159*
- *Kodde et al. (2011). A fast ethanol assay to detect seed deterioration. Seed Sc. Res, 22: 55 - 62*

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