plant reproduction
recent achievements and technologies
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 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.

our expertise

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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.

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.
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 BABYBOOM 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 BABYBOOM 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 Tetrapotency in the Male Gametophyte. The Plant Cell, 26 (3), 195 - 209

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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 assess fruit quality aspects we are deploying a range of techniques such as transgenic plants, virus-induced gene silencing, gene expression analysis, proteomics, and metabolomics.

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:**
- 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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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).

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.

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.

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

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