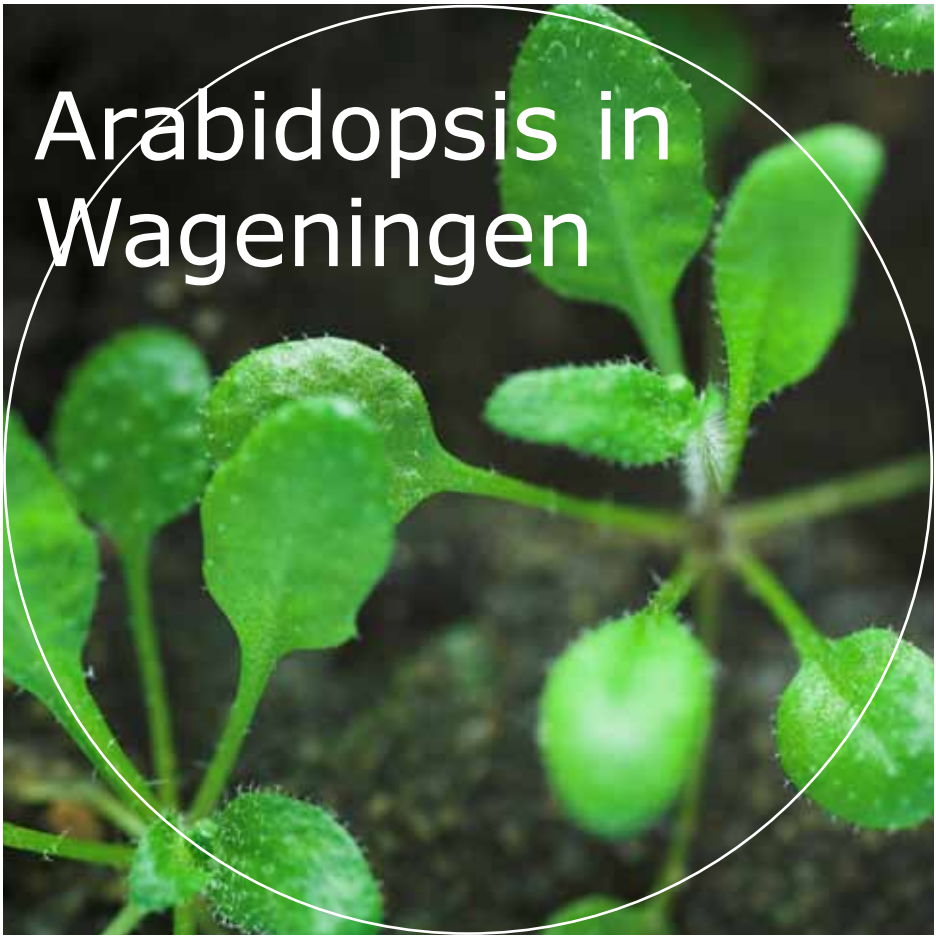
Arabidopsis in Wageningen



Prof. dr *ir.* Maarten Koornneef

Farewell address upon retiring as Personal Professor at the Laboratory of Genetics at Wageningen University on 11 April 2013

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How genetics became an integral tool in plant biology and the role Wageningen played in this development

Rector Magnificus, ladies and gentlemen,

For my farewell address, I would like to reminisce on my time as a scientist at Wageningen University. Over the years I have witnessed important developments in the approach to plant science, both here and internationally.

The integration of genetics and the model plant species *Arabidopsis thaliana* into many disciplines led to a more multidisciplinary approach. I have the feeling that I was able to contribute substantially to this development, because I started my research at the right time and because I could build on the experience of the Genetics department and of my supervisor Prof. Jaap van der Veen. I would like to show how our Arabidopsis research developed and how it was perceived by the wider scientific community.

Arabidopsis research in Wageningen around 1980

When I joined the Genetics department of Wageningen University (then still 'De Landbouwhogeschool') in 1976, Arabidopsis was Prof. Jaap van der Veen's main research plant. The department had worked with this species since 1962 when it was introduced into the laboratory by Dr. Will Feenstra, who continued his research with Arabidopsis when he later became professor of Genetics at Groningen in 1967.

When I started my studies in the 1970's Arabidopsis research was at a low point internationally. After a slow start in the 1950's there had been great expectations in the 1960's, with the foundation of an Arabidopsis research community, establishment of a newsletter, an international conference in 1965 and a stock centre. Van der Veen dealt with a number of research topics which included the study of chromosome number variants (trisomics and tetraploids) and mutagenesis. In 1976 he identified two mutants that failed to germinate unless you provided their seeds with the plant hormone gibberellin (GA). These mutants were short in stature, the dwarf habit of which could be restored to wild-type height by spraying them with the hormone. This suggested that they could be GA-biosynthesis mutants similar to those already described for maize, rice and pea. I continued the study of these Arabidopsis mutants as part of my PhD research. The available mutants, combined with many new mutants that I isolated together with my fellow PhD student Lidwine Dellaert were a rich source for genetic and physiological studies. Abscisic acid (ABA)-deficient mutants were selected as germinating GA mutants and together with the GA mutants themselves, they were analysed in physiological experiments, in collaboration with the two Plant Physiology departments. Looking at the available mutants in our collection, the so-called long-hypocotyl mutants attracted my attention, since they seemed to be the opposite of GA dwarfs. Prof. Bruinsma from the Plant Physiology department suggested that they looked like plants growing in darkness, which implied a direct link with light perception. To investigate this further we carried out experiments in the Plant Physiological Research department, where Dr. Carl Spruit had a number of light cabinets, with different light colours, in the cellar of the laboratory. It quickly became apparent that in a locus specific way, these mutants responded differently to the various light wavelengths. Some of them were insensitive (blind) to blue light and others to red and/or far-red light. Dr. Spruit immediately saw the importance of these observations and suggested we measured the best known photoreceptor phytochrome, which was one of his research specialities. These measurements revealed that phytochrome could not be detected in two of the mutants. When we were considering publication of these results we were afraid that the specialists in the field would simply not believe that plants without this essential photoreceptor pigment could be viable. To make our life easy we

decided not to submit our paper to a high impact journal, which probably partially explains why it took several years before the importance of this paper was recognised.

Collaboration with Dr. Cees Karssen, in the Plant Physiology department, was essential for the GA- and especially the ABA- mutants, since his group showed that these mutants lacked ABA and could confirm the important role of this hormone in stomatal closure and seed dormancy (Karssen 2002). These hormone mutants, for which we identified a large number of loci, were also important later, when mutants became the basis of the isolation of a mutated gene, using so-called map-based cloning or other techniques including T-DNA tagging.

In addition to the mutant studies I continued working on the trisomics that van der Veen had isolated. I used these to map genes, via their mutants, to specific chromosomes. This was followed by classical linkage analysis in mapping populations derived from intercrosses of the mutants within our collection. Surprisingly, there was no good genetic map of Arabidopsis until we published such a map with 76 loci in 1983. At that time mapping was waiting for the breakthrough of molecular markers, and as map making was considered to be old-fashioned genetics, it was difficult to publish it. However, a revival of Arabidopsis research in the early 1980's helped to get the publication accepted. To construct maps it was important to combine the data from many crosses in a statistically justified way. My colleague Dr. Piet Stam was a great help in this and our collaboration was the start of Piet Stam and Wageningen becoming leaders in the development of mapping software such as JOINMAP and MapQTL. From this time on genetic maps became an essential basic source of information for both fundamental and applied sciences.

My involvement with these projects resulted in 8 publications over a 6-year period and they were integrated into my PhD thesis in 1982.

Changing the focus to tomato

Although we were convinced that our Arabidopsis research was very relevant and in many aspects also novel, it still did not to attract much attention. This was one of the reasons, why I decided to think about other species such as tomato, in which one could use genetics, but also haploids and tissue culture as research methods. At that time these were emerging technologies that seemed essential for several novel methods to transfer genetic information between species, using protoplast fusion or transformation.

In the mean time we had identified some hormone and photoreceptor mutants in tomato, similar to those in Arabidopsis. Being larger these were more attractive for physiological research than Arabidopsis. This led to further collaboration on plant hormones with Cees Karssen and photoreceptor work with Dr. Dick Kendrick, the successor to Dr. Spruit. The collaboration with Kendrick and his PhD students, and PhD student Ageeth van Tuinen from Genetics, lasted for many years and showed nicely how genetic and physiological expertise can complement each other. When Kendrick became head of a RIKEN laboratory in Japan, this also widened my interactions with Japanese scientists, since during my yearly visits as scientific advisor I could take the opportunity to visit other Japanese groups.

Tomato was also the species of choice of Dr. Pim Zabel in the Molecular Biology department, who had changed his research from the topic of viruses to the study of tomato. It was my task to provide genetic input into his research projects, which were aimed at the map-based cloning of a nematode-resistance gene. This was very fruitful and enabled me to become familiar with molecular biology techniques, before we applied this approach in my own group, including the use of PCR based markers for genetics. In particular PhD students Ellen Wisman and Tsveta Liharska participated in projects where genetics was integrated with relevant topics of the Molecular Biology department. Later, a fruitful collaboration between Dr. Hans de Jong and Pim Zabel developed molecular cytogenetics in tomato, which is still one of the specialities in Hans de Jong's group, and which is nicely integrated into the genome research of tomato and potato.

However, I also wanted to set up a new line of research in tomato using my new tissue-culture experience obtained during my sabbatical/postdoc time with Dr. Pat King at the Friedrich Miescher Institute in Basel, Switzerland in 1983. This is where, for the first time, I saw how international top science operates and noticed how important it is to know your colleagues, and competitors, personally.

In 1985 I obtained a NWO grant on protoplast fusion in tomato. This allowed me to employ Jelle Wijbrandi, my first PhD student, who graduated in 1989, and whose work was continued by Annemarie Wolters and Herman Schoenmakers with grants from a biotechnology program. For the tomato projects, in collaboration with Pim Zabel's group I could also add my tissue culture expertise to develop tomato transformation in Wageningen, before most other groups had succeeded in getting this technique working.

What happened in the mean time with Arabidopsis research worldwide?

In Wageningen we finalized several Arabidopsis projects which were not included in my PhD thesis, which resulted in two publications per year. One of them involved a new collaboration with Prof. Jan Zeevaart from the Plant Research laboratory in East Lansing, Michigan, USA. Jan was a Wageningen alumnus who emigrated in 1960 to the USA, where he became one of the leading scientists on plant hormones. After reading our recent papers on plant hormone mutants, he visited us in 1982 in Wageningen and started to make use of them, often in collaboration with us (Zeevaart 2006). Jan Zeevaart was a classical plant physiologist who kept working at the bench until he retired, and who had an impressive knowledge of the physiological literature. In addition, he was open to new developments such as the use of genetics and molecular biology, which also led to the cloning of one of the genes in GA biosynthesis, using one of our mutants and our mapping data. Apart from our collaboration, he was also an important ambassador for me in the USA, especially at his institute where Prof. Chris Somerville was a colleague. Somerville together with Prof. Elliot Meyerowitz put Arabidopsis back on the research agenda in the USA in the early 1980's. Meyerowitz, a former Drosophila (fruit fly) geneticist at Caltech, Pasadena, USA, wrote to me to ask for some of our hormone and floral morphology mutants and explained the terms homeotic genes and mutants to me.

My first visit of a month to the USA in 1985 was an impressive experience. I met many of the plant scientists who had just adopted Arabidopsis as a research subject and I also tasted the scientific atmosphere in the USA. It was clear that I should not put my Arabidopsis expertise in the waste basket and that there was a bright future for the species, especially if classical botany including genetics was to be integrated with molecular biology. The latter approach was paramount in leading to the identification of the genes controlling the specific processes we were studying and opened up a new world of in-depth studies that included cell biology and biochemistry.

The reasons why Arabidopsis became so popular are described in several historical reviews (Meyerowitz 2001, Somerville and Koornneef 2002).

The main requirement was for a species, adaptive for molecular biological approaches that was also amenable to genetics, but in addition one with a small genome. Using Arabidopsis made looking for a needle in a haystack easier, since the haystack was smaller. It was also very important that some well-established scientists saw the importance of Arabidopsis and moved the species away from its image as second-rank biology. During the years Arabidopsis became more popular as important technical developments took place. A very effective non-tissue culture

transformation method was developed, where the name 'floral dip` (in an Agrobacterium culture) more or less explains the protocol in two words. This efficient transformation allowed the massive generation of plants with T-DNA insertions (from the Agrobacterium Ti-plasmid) that were integrated more or less at random in the genome and resulted in tens of thousands of mutants affecting almost all Arabidopsis genes. Because the insertion sites could be identified by sequencing the flanking DNA, the insert positions could be determined. This was only possible after the full genome sequence became available around 2000. This high quality, first sequence of a plant genome, was a landmark in Arabidopsis and plant research. It provided tools for all Arabidopsis researchers, even those with limited resources, to study the genes and to use so called 'knock-out' mutants, because this research and corresponding information was free. Important community resources were stock centres and the TAIR information website. The importance of Arabidopsis research was also visible in the impressive, growing number of papers now appearing on Arabidopsis each year (3500 in 2011).

The return of Arabidopsis to Wageningen

The Arabidopsis boom in the USA made the EU notice that the Arabidopsis star was rising and that it would be good to fund Arabidopsis research. As a result I obtained two EU grants in the so-called 'Bridge' program. This allowed me to appoint Dr. Ton Peeters as a postdoc, who introduced molecular biology in my group and who stayed with me for almost 10 years. The other grant was used to appoint Karen Léon-Kloosterziel, my first Arabidopsis PhD student, to work on ABA and seed germination.

The main emphasis at that time was on the seed work together with flowering time funded by STW, NWO and the EU with several PhD students (after Karen, Wim Soppe, Leonie Bentsink and Emile Clerkx) and Ton Peeters, Carlos Alonso-Blanco, Vered Raz and Isabelle Debeaujon as postdocs. The mutant work of Karen Léon-Kloosterziel and Ton Peeters resulted in interesting reduced-dormancy mutants for which the genes were subsequently cloned by Dr.Wim Soppe's group in the Max Planck Institute, Cologne, Germany and led to the identification of new components in the seed dormancy pathway. The research on seed colour mutants by Dr. Isabelle Debeaujon, who cloned the first flavonoid transporter and provided strong arguments for the vital role of the seed coat in seed germination, was also important. This work is being continued by Dr. Debeaujon in France.
The advantage of EU projects was not only that one obtained financial support, but also that it meant becoming part of an international network of researchers with similar scientific interests and it gave me the opportunity of visiting major centres of plant science research.

Flowering time research

Ton Peeters' project on flowering time was in collaboration with Dr. George Coupland and Dr. Caroline Dean of the John Innes Centre, UK and Dr. Jose Martinez-Zapater, Spain. Flowering time had been the topic studied by Jaap van der Veen. He had collected many mutants that were late flowering, which he used to enable students to gain experience with quantitative genetics studying segregating populations. I had collected additional mutants and I also located many of the genes on the genetic map. When van der Veen retired in 1987, I took over this project and completed the experiments that led to an important publication with him in 1991. This research attracted a lot of attention since it provided the starting material for the research of the scientists mentioned above with whom we received the first EU Arabidopsis flowering-time grant. In this project we had chosen to clone the gene mutated in the *fwa* (Flowering Wageningen) mutant. Ton Peeters started this together with other projects and further momentum occurred when Wim Soppe joined the group as a PhD student in 1993, followed by Dr. Carlos Alonso-Blanco. Map-based cloning was a laborious effort in those days since all the genome information, now accessible via internet, was not available. For mapping we had to cross our mutant with another Arabidopsis accession which complicated the analysis of the mutant phenotype, since the parents also differed in other genes affecting the same trait.This gave rise to many problems and required a lot of perseverance by Wim Soppe. Just as we started to believe that we would not get a hand on the gene, a breakthrough occurred. This was achieved by bringing together observations that were difficult to explain and by looking at the expression of the gene that could be the candidate. It appeared we were dealing with one of the rare mutations where a difference in the methylation of the DNA leads to differences in gene expression, and not a difference in DNA sequence, which is usually the case in mutants. This so-called 'epi-allele' is now used extensively in Arabidopsis research where epigenetic changes are a hot topic.

Arabidopsis natural variation

Up to this time we had worked mainly with one lab-strain (called accession or ecotype) and its mutants. This was Landsberg *erecta* (L*er*) brought to Wageningen by Dr. Feenstra in 1962. Other laboratories worked with Col and Ws. Gradually, we also introduced other accessions and Dr. George Coupland provided us with some seeds of the Cape Verde Island (Cvi) accession. It attracted my attention that the seeds of this Arabidopsis genotype were relatively large, which I found of interest because of our work on seed biology. We made a cross between Ler and Cvi and developed segregating populations aimed at the isolation of Recombinant Inbred Lines (RILs), which would be useful for Quantitative Trait Locus (QTL) mapping. RIL populations had already been constructed by other groups among which the Ler x Col population developed by Dr. Caroline Dean's group had been very useful in providing the

genetic backbone of the physical map of Arabidopsis, which was essential for the international sequencing effort that was taking place at that time. We had used this Ler x Col population to look at seed dormancy genetics and could also establish the contact between the Dean group and Dr. Ritsert Jansen to test the Wageningen QTL mapping procedures on their data.

When Carlos Alonso-Blanco, who had worked on the flowering-time project, told me that he wanted to stay longer and would try to get a personal EU grant, I suggested that he studied natural variation using our Ler x Cvi RIL population that was in development. It was clear that with the Ler x Cvi material we had hit a gold mine, as these two accessions differed in many traits that could be mapped using the QTL approach and confirmation of QTL by Near Isogenic Lines (NILs). We quickly identified several QTLs with large effects on flowering time and seed dormancy. This led to the expectation, that cloning such a QTL would be possible by using the similar map-based cloning strategies as used for mutants. This technology became more effective and easier because of the availability of the genome sequence in 2000 and the efficient transformation technologies. With the support of the government of Egypt, for PhD student Salah El-Assal we were able to clone our first QTL. The identification of this flowering time QTL and the single amino-acid change that caused a large difference in flowering resulted in our first Nature Genetics paper with Salah El-Assal as first author. Dr. Leonie Bentsink worked on the dormancy QTL for her PhD and continued as postdoc eventually cloning the *DOG1* gene, that was in contrast to the flowering time gene an unknown player in seed dormancy. DOG1 now appears to be a major factor in this process.

Gradually natural variation became the main topic of the laboratory in close collaboration with Dr. Dick Vreugdenhil at the Plant Physiology department, with whom I shared Mohamed El-Lithy as a PhD student. The emphasis was on growth and biochemical traits, as well as mineral accumulation, in which we also collaborated with Dr. Mark Aarts and the Free University of Amsterdam. An important new development was the QTL Express programme together with groups from the universities of Utrecht and Groningen, which was funded by the NWO Genome program in 2002. In this project Joost Keurentjes and collaborators tested the concept of Genetical Genomics where the traits were genome-wide, gene expression and and a large number of metabolites. This led to the papers that pioneered these concepts and which were published in high-ranking journals including two in Nature Genetics. Both in Wageningen and Cologne this line of research is continued and new genetic tools have been added, such as Genome Wide Association (GWA) Mapping and Next Generation Sequencing (NGS).

The follow up

When programs between Wageningen University and China were initiated, we were able to participate and we decided to work on *Brassica rapa*, a favourite crop species of the Chinese. We tried to translate our experiences with Arabidopsis to Brassica. In Wageningen this resulted in a productive collaboration with Dr. Guusje Bonnema from Plant Breeding, who coordinated the China project with the group of Prof. Xiaowu Wang, with whom we shared the PhD students Jianjun Zhao and Jian Wu.

Our natural variation work was greatly appreciated by plant breeders who were applying this technology to their crops, although doing molecular biology of their QTLs was difficult 10 years ago. My experience with this topic with its link to plant breeding was probably paramount in my appointment as Max Planck director in Cologne in 2004. This appointment meant that, after 28 years, I began to work outside the Genetics department. By mutual agreement, I continued to work for one day per week in Wageningen, in the Botanical Genetics section within the Genetics department. In Cologne I continued working on natural variation while trying to finalize some of the PhD student projects for which it was hard to get additional funding in The Netherlands. In Cologne, especially the groups of Dr. Wim Soppe (seed dormancy research) and Dr. Matthieu Reymond (quantitative and adaptive genetics) continued with research projects that originated in Wageningen, but they also provided new angles to this research. In addition, a group started to work on population aspects of natural variation headed by Prof. Juliette de Meaux. I, myself focussed on the development and use of new types of mapping populations in Arabidopsis and barley. This project needed computational genetics, which was provided by the group of Prof. Fred van Eeuwijk and his co-workers of whom Dr. Joao Paulo, a shared post doc, contributed a great deal. Also in Cologne I appreciated the collegial attitude and the possibilities to collaborate. Being familiar with the knowledge and expertise both in Wageningen and Cologne it allowed me to initiate a number of collaborations which I hope will continue after my retirement from both places.

What was special in Wageningen

When looking back at my scientific career in Wageningen, I conclude it was a success because I was lucky to work on the right topics in the right plant species at the right time. This coincidence of favourable factors was not due to me *per se*, but due to my PhD supervisor Prof. Jaap van der Veen and the scientific environment in Wageningen. Prof. Jaap van der Veen quickly trusted me to work as an independent researcher, interacting and establishing contacts with colleagues within and outside Wageningen. It meant that I received more credit than van der Veen did, especially since he only wanted to be co-author on papers where his own data were included.

This attitude is no longer normal today where scientific performance, also of senior scientists is measured by publication lists, by number of first and last author papers and increasingly by citation numbers. We could work on Arabidopsis in Wageningen despite this being considered far away from applications aimed at helping to feed the world. We could do this fundamental research because there was some basic funding, support from excellent technicians and because many motivated Msc students performed their research projects with us and produced data that could often be included in scientific papers. The international funding became especially important after 1990 and this, complemented with NWO support, expanded the group and made it much more international. The interaction and collaboration with other groups has always been essential. The interactive research environment of the graduate school Experimental Plant Sciences (EPS), initially with Prof. Evert Jacobsen and later with Prof. Pierre de Wit and Prof. Ton Bisseling as directors, contributed a great deal in this respect. Much of the added value of collaboration was based on using each others biological materials and expertise, mostly without formal agreements and contracts, fully based on mutual trust and appreciation of everyone's specific expertise.

Thanks

From what I have described above it is obvious that when I have referred to my work and my research, it resulted from and still is a group effort that was impossible without the help of many people. I would especially like to thank my assistants, of whom Corrie Hanhart and Hetty Blankestijn worked with me for most of the time that I was in Wageningen. They were always in a good mood and highly motivated. I mentioned the PhD students and postdocs whom I thank not only for the quantity of data they generated, but also for their intellectual input and for keeping an excellent atmosphere in the laboratory. In addition to them I should thank the many Msc students who carried out projects with us. They were much better at socializing than me. I already emphasized the collaborators in the other departments and those outside Wageningen who not only increased the productivity of the group, but have also provided many friendships for life. I also want to mention the colleagues at the Genetics department. Although we had genetics as common theme, the research lines were often rather different, meaning that my internal collaborations were restricted to the cytogenetics group of Prof. Jaap Sybenga and Prof. Hans de Jong, and to Prof. Christa Heyting as co-supervisor of my first PhD students, and to the mathematical support from Prof. Piet Stam. However, this diversity also had its attractive sides as it taught me many aspects of genetics. The Genetics laboratory was a house where I felt at home. The latter was also very much due to the atmosphere and type of leadership of the department heads: Jaap van der Veen, Rolf Hoekstra and in the recent years Bas Zwaan. He managed to convince us of our common mission in increasing the

interaction between our subdisciplines. I would like to give a special thanks to the support group: secretaries, technical and IT services and very important the gardeners, especially Gerrit van IJmeren, who took care of tens of thousands of Arabidopsis plants.

I thank my wife Elly and children Wietse and Annemart who hopefully were happy that I enjoyed my job, although it often meant that I was travelling a lot and was not always the centre of our family and social life.

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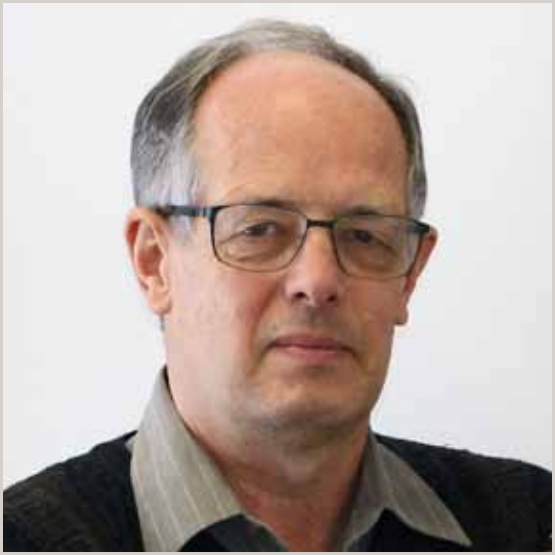

Prof. dr *ir*. Maarten Koornneef

'Arabidopsis thaliana is the plant species that in the past 25 years has developed into the major model species in plant biology research. This was due to its properties such as short generation time, its small genome and its easiness to be transformed. Wageningen University has played an important role in the development of this model, based on interdisciplinary collaborations using genetics as a major tool to investigate aspects of physiology, development, plant-microbe interactions and evolution.'

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