Plant Physiology and Flower Bulbs 2.0

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Inaugural lecture upon taking up the post of Special Professor in Physiology of Flower Bulbs at Wageningen University on 13 November 2014

WAGENINGEN UNIVERSITY
WAGENINGEN UR

ISBN 978-94-6257-185-3

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Rector Magnificus, ladies and gentlemen,

Welcome! It is my great pleasure to take you on a tour today along tulip fields and discuss the latest developments and challenges in plant physiology and flower bulbs research and education. Flower bulbs are big business in the Netherlands. Almost 80% of all trading in flower bulbs world-wide is done by the Netherlands and around two-third of all flower bulb fields world-wide are situated in our country. Therefore, it is a great honor for me to be accepted for the position as special Professor of 'Physiology of Flower Bulbs'.

Realizing the importance of the flower bulb sector for the Netherlands and our number one position, you might wonder why it is needed to appoint a professor on this particular topic. And I am not the first! In 1925, Egbertus van Slogteren was appointed as special Professor at the 'Landbouwhoogeschool' in Wageningen and in 1953 his position was converted into a full professor position (Fig. 1). At the moment he got appointed the bulbous plant sector was facing severe setbacks due to bacterial and virus infections. Remarkably, his academic work was characterized by very practical approaches and soon after his appointment he implemented warm water treatments to treat pathogens and serological methods to detect viruses, which were breakthroughs that probably saved the sector. Furthermore, he played with temperature treatments in order to steer flowering time of bulbs, a complicated topic and research line that I will follow up but from a different point of view, as will be discussed later. After van Slogteren, who was not specifically

Figure 1. Special Professor at the 'Landbouwhoogeschool' in Wageningen, Egbertus van Slogeren.

appointed to work on bulbs, Wageningen University has had two additional professors studying bulbs. So, I am not the second Dutch professor in Flower bulb research as some people think and hence, this is not the reason why I am using 2.0 in my title. I hope that the reasoning behind this will become clear during the rest of my lecture.

Let's first come back to my earlier question whether it is needed to invest in research and education for such a famous and successful agronomical sector? The answer is simple: YES, it is! Currently, the flower bulb sector is facing serious threats (FD/AgriHolland, 2014), of which I will summarize the four that in my opinion are the most important:

1. **The consumer.** The image of flower bulbs as product, but also of the flower bulb sector in Western Europe, is not good. Marketing efforts are limited and despite enormous efforts of bulb growers to make the production of bulbs sustainable, it is still one of the agronomic sectors using the largest amounts of pesticides (StatLine, 2010).
2. **Corporate structure and conservatism**. Almost all bulb growing companies are small family businesses. Consequently, the sector is fragmented and not able to act as strong negotiation partner. Due to the small scale of companies it is also difficult to allocate budget for novel innovations. Furthermore, traditions are copied from generation to generation, often without critical evaluation of their relevance in our fast changing world.
3. **Upcoming 'new' economies.** On one hand this is a positive development creating growth markets, such as China. Though, these new markets have their own import requirements and demand that bulbs are free of selected viruses and other pathogens, which often is difficult or expensive to prove, and provides them with ways to manipulate the Dutch producers. Furthermore, these new economies become producers of bulbs as well and start to compete with Dutch bulb growers.
4. **Profitability**. Due to the four before mentioned reasons, profitability is currently under high pressure with the risk that the sector will enter a negative spiral, meaning that due to the low profitability, investments will be limited, resulting in less innovation, etc. etc.

How to deal with all these threads? First of all I suggest to call them not threads, but to regard them as interesting challenges.

Let us make a comparison with developments in computer sciences in order to address these challenges. In case a computer is running less well, we can approach this either by changing the hardware, or by improving the software. Over the last decade we have seen that bulb growers mainly worked on changing the 'software' and regarded the bulb — which can be seen as the hardware — as a fixed input. This seems to be logical because growers can easily change the way they handle and treat the bulbs — 'the software' — but do not have the capacity to change the intrinsic characteristics of the bulb laid down in its DNA. In contrast, bulb breeders have the possibility to change 'the hardware' and do so; but realize that it takes around 20 years to generate and introduce a new bulb variety on the market with the current state-of-the-art and classical breeding approach (Leeggangers et al, 2012). I see it as my duty to change this! However, I cannot do this on my own and improvements of the 'hardware' will be beneficial only — and useful as a tool to address the challenges the sector is facing — when the 'software' evolves at the same time and with a similar speed. To stay in the field of computers and informatics; hardware is improving continuously in this sector and demands updates in operating systems and software as well. Some of you might remember that we went from Windows 3.1 to Windows 3.2. This was a relative small step forward, despite that some frustrating bugs were solved and some highly appreciated improvements were made. With the challenges we are facing today in the bulbous plants sector, this small steps forward will not be sufficient. My predecessor, Prof. Van Slogteren established a solid basis for fundamental research in flower bulbs, which I would like to designate *'Flower bulbs 1.0'*. I see it as my task not to aim for *'Flower bulbs 1.1'*, but to break with the classical way of performing physiological research and to make a large step forward and introduce *'Flower bulbs 2.0'*, which will be mainly based on improvements of the 'hardware'. How to do so will be explained in the coming half hour.

The tulip life cycle

To understand the bottlenecks in bulbous crop improvement and the reasoning for my selected research direction it is first important to have a basic understanding of the life cycle of bulbous plants. Although I am aware of variation in the physiology and behavior of different bulbous plant species, I decided to explain the tulip life cycle only, whereas it is the most grown and probably best known example of a bulbous plant, and furthermore, it has been selected as the plant model system for the special chair on flower bulb research.

The tulip life cycle starts with the formation of seeds after self-pollination or crossing two different tulip varieties (Fig. 2). One seedpod contains hundreds of seeds that all represent unique products of sexual reproduction. In one year time a first bulb with the size of a pinhead is formed from an individual seed. The plant that grows from this small bulb will produce leaves only and cannot be induced to flower. After three to five vegetative growth cycles (3-5 years), the bulb reaches a size of about six to seven centimeters in perimeter and will have made the switch from juvenile-vegetative to the adult-vegetative stage. At this moment the bulb is sensitive for flowering inducing signals and can produce its first flower, enabling performing a next cross. From this moment onwards the tulip follows a yearly cycle (Fig. 3). This cycle starts with the formation of a replacement bulb and a few daughter bulbs after blooming in spring. Around the moment of harvest or during the first weeks in storage, a new floral bud is initiated by heat inside bulbs of sufficient size. Subsequently, these bulbs go into dormancy. The bulbs will be planted in autumn and dormancy will be broken by winter cold. The following period is featured by fast growth, the outgrowth of a small number of leaves and stretching of the stem, ending with the full development of a flower in spring. So to conclude, it takes around seven years to go from a seed to a flowering bulb that can produce yearly around two to three offspring bulbs of sufficient size to give a good quality flower.

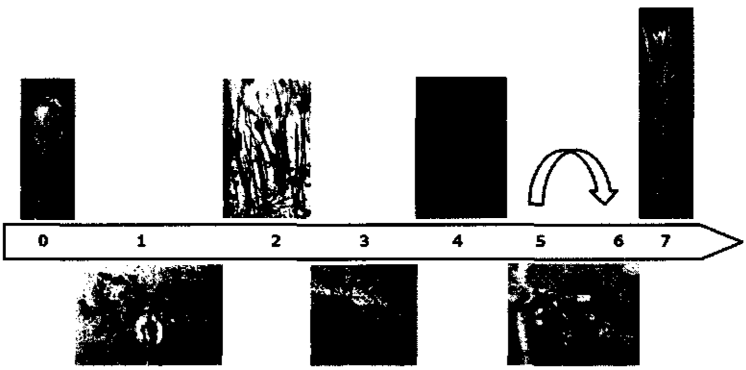

Figure 2. From seeds to a flowering tulip. After sexual reproduction and the formation of seeds, it takes five to seven years before a bulb of sufficient size is formed that is able to flower.

As a consequence of this prolonged life-cycle, the breeding process is tremendously slow and generating sufficient offspring of a promising new tulip variety might takes up-to ten additional years. Because of this and the low investment power of the small family businesses, breeding in the bulbous sector is in general performed in a classical manner with low input, based on selection for spontaneous mutations, and focusing on a limited number of ornamental traits, such as flower shape, size, and color. The added value of the final product is rather limited and profit can only be made by selling large numbers of bulbs.

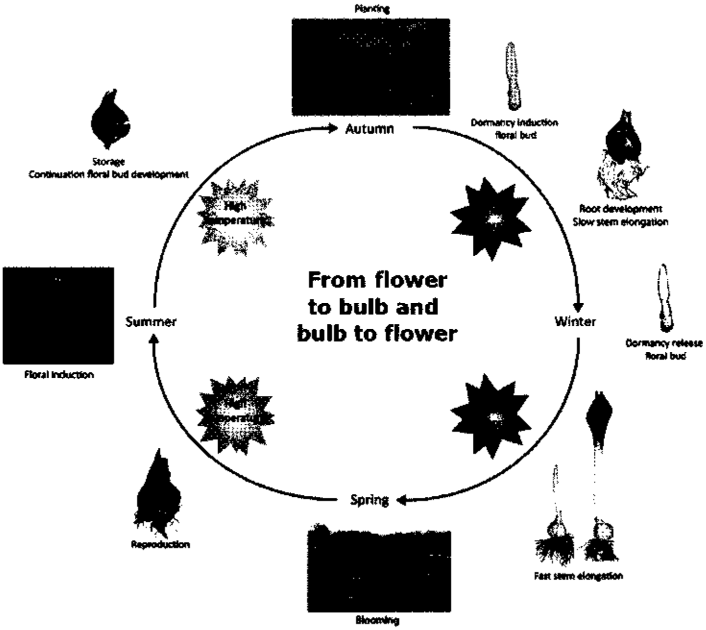

Figure 3. The annual growth cycle of an adult tulip bulb.

Towards a bulbous breeding paradise

How does this situation relate to the high-tech breeding process for various vegetables? Let's take tomato as an example. The complete tomato genome became available in 2012 (The Tomato Genome Consortium 2012) and nowadays a large number of varieties, wild relative, and breeding lines have been re-sequenced (Lin et al, 2014; The 100 Tomato Genome Sequencing Consortium, 2014). Already long before that a detailed genetic map was generated and molecular markers were developed paving the way towards marker-assisted breeding. Nowadays, robotized DNA marker platforms and high-throughput phenotyping technologies are implemented, enabling to breed for a plethora of traits and to produce new tomato varieties in a short time, based on consumer requests. A nice example is the introduction of *'snack tomatoes'*. Within a period of five years, large numbers of new varieties were introduced that took over already more than one-third of the tomato fresh market. In this way, breeders create flexibility and make breeding on demand possible. Consequently, the added value increases continuously, and nowadays a kilogram of good quality tomato seeds has more value than a kilogram of gold!

Is it possible for bulbous plant breeders to enter this '*paradise*'? The answer is simple, '*YES*'; and in my opinion it is essential to maintain our number one position in the world! Nevertheless, we are currently far from this situation and it will need time. Though, I foresee that making this step in breeding allows big improvements in physiological research as well, which I will discuss in more detail later. Let us first discuss what conditions should be met to reach this next level?

1. **A drastically shortened life-cycle for bulbous plants during the breeding process**. In the case of tulip this means a strong reduction of the juvenile vegetative phase and the possibility to grow more than one generation yearly.
2. **Availability of a good quality genetic map** (and ultimately a full-genome sequence) and reliable molecular marker-based selection methods.
3. **Availability of high-throughput and reliable phenotyping methods** for traits of interest, including non-visible characteristics, such as metabolic content and disease resistant levels.
4. **Strongly increased propagation rates.** This is especially of importance towards the end of the breeding process in order to obtain sufficient material for commercial purposes in a reasonable time.

Addressing all four issues at once is impossible for a small research group. Therefore, I decided to study how flowering is regulated in tulip — aiming to influence and shorten the tulip life cycle — and to identify why vegetative propagation rates are so low in tulip in comparison to other bulbous plant species, such as lily?

Both flowering and propagation have been studied in detail in the plant model species *Arabidopsis thaliana* (thale cress) and in important agricultural crops. Hence, we do not have to start our research completely from scratch. However, the challenge in this so-called translational research is to identify which processes and components within the process are conserved? The classical way of doing translational research comprises the identification of causal genes in the plant of interest based on a high level of sequence homology with identified key genes involved in the process in the plant model species. Many success stories exist that are based on this methodology; however, probably a same number of attempts have been done that didn't result in the identification of the right genes and in understanding how the process is controlled in the crop of interest. We may expect the same for translational research from Arabidopsis — a dicot — to bulbous plant species, which are monocots and evolutionary distantly related to Arabidopsis. However, a careful investigation of important biological processes that have been studied in large numbers of species teaches us that when no conservation is present at the gene level, we might see conservation at the underlying molecular mechanistic level. It is good to realize that this is of great help as well in understanding the biological process, because it will teach us how these characteristics evolved and which strategies are used by plants to optimize their survival rate. Let me pick an example to illustrate this: Arabidopsis is a plant that needs a prolonged period of cold during the winter in order to flower, a process known as the vernalization response. For the survival of the species this is of importance, because without this pre-requisite the plants probably would flower in winter and the seeds will fall on the frozen soil and have hardly any change to survive. For humans, the vernalization response is also of interest because plants with a vernalization requirement give in general higher yields. Because of the importance of vernalization, this process has been studied in detail and in Arabidopsis the gene *FLOWERING LOCUS C (FLC)* was identified as key regulatory component (Michaels and Amasino, 1999; Sheldon et al, 1999). FLC acts as a repressor of flowering and its concentration is reduced by cold due to an epigenetic molecular mechanism (Fig. 4).

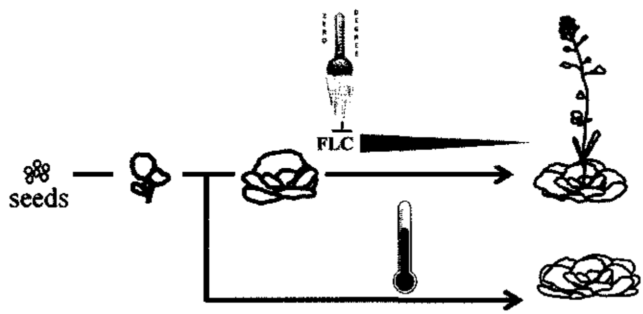

Figure 4. The vernalization response in Arabidopsis thaliana. A prolonged period of winter cold is essential to reduce the expression of the flowering repressor FLC and to enable the induction of flowering by long day conditions.

Researchers started a hunt for the *FLC* gene in a large number of crops, assuming conservation of this gene. Despite an enormous input, these efforts remained without success outside the brassicaceae family. Actually, another gene was found in various monocot species as key regulator of the vernalization process, named *VRN1*. In contrast to *FLC*, this gene acts as an inducer of flowering and its concentration is slowly induced by cold temperatures. This at first sight lack of conservation puzzled researchers for years. Though, recent in-depth analyses revealed that the induction of *VRN1* is regulated epigenetically in a very similar way as the regulation of *FLC*, showing conservation of the process at the level of the molecular mechanism.
I believe that we can learn from the studies in model species in many other cases

— in a similar way as just exemplified for *FLC* — but that we have to keep our mind open for different levels of conservation in order to let the findings make sense. In line with this statement, I would like to stress that — on the other hand — conservation of genes doesn't automatically mean a similar regulation and physiological outcome. A nice example of the latter is strong conservation of the key regulatory genes in flowering time control under influence of photoperiod. The dicot Arabidopsis is induced to flower by long days and the monocot rice by short days. Nevertheless, genes with very similar DNA sequence could be identified in the genomes of these two species and appeared to be key regulators in the photoperiod flowering pathway (Fig. 5). Detailed investigations of the underlying mechanisms showed in this case that the players, i.e. the key regulatory genes, are the same and conserved, but that their interaction is different resulting in completely opposite responses! It is exact these types of insight that are of importance and that will be taken into account when addressing our research questions in bulbs.

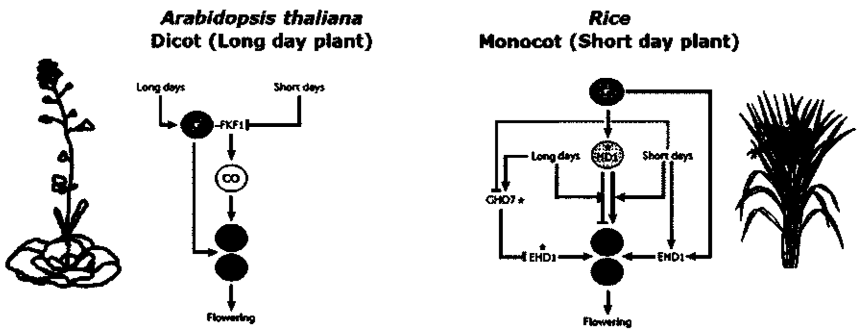

Figure 5. Conservation versus diversification of the gene regulatory network underlying photoperiodic flowering time control in Arabidopsis thaliana and rice. Although, the same genes have been identified in these two species (indicated by circles with the same gray scales), the interactions between the genes are different. As a consequence of this re-wiring of the network, Arabidopsis is induced to flower by long day conditions, whereas rice responds to short day conditions and is repressed by long days.

Let's proceed now with discussing the two major research questions that I introduced and that need to be addressed in order to obtain a solid basis for '*Flower bulbs 2.0*' research.

Life cycle shortening in bulbs: it's all about speeding up flowering!

I like to address three processes related to flowering that all are of importance to obtain life cycle shortening: (I) The switch from juvenile-vegetative to adult-vegetative that is known as the vegetative phase change; (II) The switch from vegetative to reproductive development known as flowering initiation; (III) The need for winter cold to come into full bloom, a process referred to as winter dormancy.

The vegetative phase change in tulips

Shortening the juvenile-vegetative phase is essential in reducing time from seeds to a flowering bulb, which can take around five years. For a long time it is known that in the model plant Arabidopsis this phase change is regulated by a conserved microRNA, *miR156*, and that reduction of *miR156* expression is essential in this process. A great breakthrough came about a year ago when two groups reported simultaneously on their finding that metabolic active sugars are the trigger for *miR156* repression (Yang et al, 2013; Yu et al, 2013). For bulbous plants, it long has been hypothesized that the size of the young bulb and its sugar content determines the moment of the vegetative phase change and that due to the relative slow growth the juvenile-vegetative phase takes long. Though, work in the group of Dr. Geert-Jan de Klerk showed that physiological age is a more important parameter than bulb size (Langens-Gerrits et al, 2003). Furthermore, it has been calculated that the absolute amount of sugars in a much smaller and younger bulb than the first one flowering, should already be sufficient for flower initiation. But why is it then not flowering? Based on these observations, I hypothesize that also in tulip metabolic active sugars are the trigger of the vegetative phase change and that this process is conserved in bulbous plant species. However, it is not the absolute sugar concentration that counts, but it's availability for the shoot apical meristem in which the phase change occurs. Bulbs consist of scales, which are modified leaves serving as storage organs for assimilates. Consequently, the overall sugar content is high, but availability limited. Currently, we are generating seeds, and bulbs of different age until the moment that they reach competence to flower. We will analyze both concentrations and localization of metabolic active sugars in this material and the behavior of conserved and potential key regulatory genes. Altogether, this should show whether control of the vegetative phase change is indeed conserved in tulip.

Flowering initiation

Tulip is a plant that hardly responds to changes in photoperiod and therefore, is very different from Arabidopsis and rice that I just used as example. In contrast, ambient temperature is the pivotal environmental cue regulating flowering initiation in this species. In the fifties of last century the morphological changes of the shoot apical meristem inside a bulb were already investigated (Beijer, 1952). What most people do not know, is that flower initiation in bulbs is induced by heat and that in general, this occurs just after harvesting of bulbs around June. During the first weeks of storage, nothing seems to happen to the bulbs from the outside; however, a look inside shows that at this moment flowering is initiated. Studying this process using tulips grown in the field is complicated, because temperature conditions fluctuate yearly and cannot be controlled. For this reason we are growing tulips in the field inside crates. This way of growing allows transferring the tulips in spring to controlled temperature conditions without plant damage and too much stress. Subsequent incubation at 8 degrees Celsius results in the formation of leaf primordia only, whereas incubation at 19 degrees induces the fast transition to flowering and the formation of a floral meristem inside bulbs. During this time course at the two different temperature regimes, samples have been taken from the central region of the bulb where the switch occurs under influence of heat. It is at this moment that my experience in flowering time research in different plant species — including monocots and dicots — becomes of value. *APETALLA1* (*AP1*) is known as one of the first genes in Arabidopsis being up-regulated when flowering is induced and its expression marks the moment that the process cannot be reversed. Assuming conservation of this function in tulip, a potential functional homolog of this gene could be identified. Although *AP1* expression and function is e.g. not conserved in the monocot grass, its expression appears to mark perfectly the moment that flowering is induced at the molecular level inside a tulip bulb (Fig. 6). We see no expression of this gene in the vegetative meristem and no induction of expression when bulbs are incubated at non-inductive cold conditions. Though, incubation at 19 degrees results in a steep up-regulation about one week before flowering can be scored visually, making it a perfect molecular marker for our flowering initiation research.

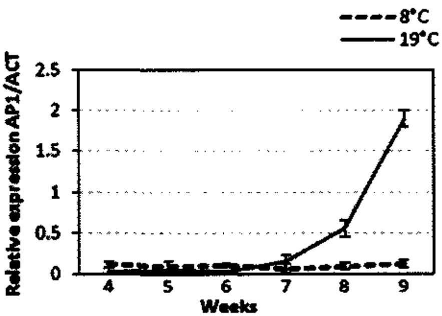

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Figure 6. High temperatures induce flowering in tulip bulbs. The tulip AP1 gene is one of the first upregulated genes upon the switch from vegetative to reproductive development.

Based on this finding we have been able to select the time points just prior to flower initiation by heat and are using the samples from these time points from the cold- and warm-grown bulbs to study global gene expression changes by a Next Generation Sequencing (NGS) approach. The current challenge is to identify from these data-sets those genes, whose expression correlates to the flowering initiation response and that are essential for this process. It is good to realize that this is not an easy task. The transcriptome (all expressed genes) of tulip is complex and not easy to decipher, while we lack a full-genome sequence. It is for this reason that I decided to use part of the budget to appoint an experienced bioinformatician supporting us in data mining.

Once more, I expect that my knowledge about flowering time control in other plant species will be of value to pinpoint to the key regulatory genes and to elucidate the molecular mechanisms underlying this temperature-dependent developmental switch. We made recently the surprising discovery that no specific temperature sensor is needed in Arabidopsis plants in order to respond to different ambient temperatures (Pose et al, 2013). In Arabidopsis, the initiation of flowering by heat appears to depend on the physical characteristics of the regulatory gene *FLOWERING LOCUS M* (*FLM*). High ambient temperatures affect the splicing of the *FLM* gene resulting in the preferential accumulation of an isoform encoding for a flowering activator instead of an isoform encoding for a flowering repressor. Hence, the intrinsic physical characteristics of *FLM* appear to act as a kind of direct sensor responding to changes in ambient temperatures and consequently, resulting in a different flowering behavior (Verhage et al, 2014). Will this be the same in tulip? Most likely this is not the case, because we couldn't identify a gene sharing high sequence homology with FLM in the tulip transcriptome. Though, as already emphasized, conservation might not occur at the level of the gene, but at the mechanistic level. It is exactly with this knowledge in mind that we are investigating this biological process.

Winter dormancy

The last aspect that I like to discuss in relation to flowering or I better should call it blooming, is breaking of dormancy by winter cold. After the initiation of a floral bud inside the bulb in early summer by heat, the bulb goes in a period of apparent rest. This dormancy can be broken by winter cold, which ultimately leads to stretching of the stem, full development of the floral organs and hence, blooming. The question that we would like to answer is: What is exactly happening during this period and whether it can be shortened? Physiological work done in the past revealed that sugars and the plant hormone auxin play important roles (Hobson and Davies, 1977; Rietveld et al, 2000). I am reasoning that for a full understanding of this complex process, it is essential studying it at the morphological, physiological, molecular and metabolic level. Only in this way cause and consequences of observations done in the past can probably be separated. A surprising first observation of our research is that full dormancy of tulip bulbs doesn't exist. We observed growth and activity of the complete sprout inside the bulb throughout the autumn and winter period and noticed that only the floral bud itself is not growing for about one-and-half month. In this respect winter dormancy in tulips resembles bud dormancy that occurs in fruit trees and this provides us with a direction to go in research. Currently, we are generating gene expression and metabolic content data of material sampled from shoots and scales during autumn and winter, which we hope to analyse and integrate in the near future.

Although, we are addressing fundamental research questions here, a better understanding how temperature influences flowering time and blooming in tulip is of direct interest for practical applications. First of all, growers have had problems with degradation of floral buds inside bulbs in the last years, because of a too early transition to flowering due to warm springs. A better understanding how temperature influences flowering initiation will be of help to select tulip cultivars that are less sensitive for this phenomenon. Secondly, our work on winter dormancy is relevant, because nowadays, almost all fresh tulips on the market are the product of so-called forcing practices. Tulip growers don't wait for the winter, but apply controlled temperature regimes to their bulbs, in order to get the bulbs blooming earlier or later in the season and to create a long period that fresh flowers can be provided to the market. We are not directly working on shortening e.g. the need for cold, but knowledge about the underlying signalling network will give clues how forcing programs can be optimized.

From faster breeding to more production: improving vegetative propagation

Our second main research topic deals with the vegetative propagation process. Towards the end of the breeding process, fast multiplication of promising new lines is of utmost importance for breeders and bulb growers to make their large and long term investments profitable. In numerous bulbous crops, vegetative propagation by in vitro culture is common practice. Unfortunately, tulip is an exception because its natural vegetative propagation rate is low — with a factor of two to three fold a year — and *in vitro* culturing is laborious and tremendously slow. Lily is at the other side of the spectrum and for this species very high propagation rates are obtained *in vitro* by a process known as scaling. Most remarkably, *in vitro* bulblet formation from a lily scale initiates spontaneously without the addition of any exogenous substance, such as sugars or hormones (Fig. 7).

Figure 7. Subsequent stages in the de-novo bulblet regeneration process. Bulblets regenerate at the basal end of a detached lily bulb scale explant without the addition of any exogenous hormones or sugars.

Various attempts have been undertaken to get an efficient *in vitro* propagation in tulip as well, but despite intensive research and input, this remained without success. With respect for the research that has been done on this topic so far, the only option I see to get a breakthrough is to distance from the current black-box approaches and start with a thorough investigation of the propagation process at molecular level. Almost all tissue culture work done in the past was based on knowledge from other species and creating comparative circumstances for tulip. Examples are particular hormone treatments and applying well-defined temperature regimes. In this work, the tulip material itself has always been regarded as a fait accompli. We aim to identify the underlying molecular causes of the strong difference in vegetative reproduction capacity between tulip and lily making use of 'omics'-approaches. We decided to focus our work first on lily, because of the difficult and slow propagation in tulip. The lily material is used to follow the entire process of bulblet formation, starting from the first cell divisions till the moment that a complete new young bulb is formed. Currently, various state-of-the-art techniques are implemented to identify differential gene expression, changes in metabolites, and hormone levels during this initial step of propagation. Furthermore, we use latest cell biology tools to identify the origin of a newly formed meristem on the lily scales at single cell resolution. To our surprise, first pilot experiments showed that the initiation starts from somewhere inside the scale and not from the epidermal cells where the first visible changes appear (Fig. 8).

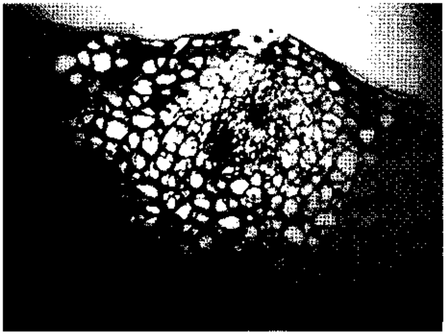

Figure 8. De-novo bulblet regeneration from a lily scale initiates from inside the scale.

Based on these observations, we hypothesise that not each cell has the capacity to de-differentiate and to become totipotent, which is needed for *de-novo* organ formation. I believe that some cells are present inside the lily scales that maintained a kind of meristematic character or that are very sensitive for inducing stimuli. Whether these are cells having pericycle-like identity and sitting close to the vasculature, as has been observed in the dicot Arabidopsis (Atta et al, 2009; Sugimoto et al, 2010), need to be seen.

Initiation without activation is of no use
For propagation two characteristics are of importance: First the initiation rate, which determines the number of new bulblets, and secondly the speed of bulblet outgrowth. For this second aspect of successful propagation, lily is not different from tulip and in both bulbous species young formed bulblets become dormant shortly after their formation, meaning that outgrowth stops. This dormancy is from a physiological point of view comparable to seed dormancy, but it also shows characteristics of bud dormancy in tulips and fruit trees, which have already been discussed. This brings me to a point that continuously comes back, which is conservation of particular traits or biological characteristics at different aggregation levels.

From Bulbs 2.0 to Bulbs 2.1

After discussing my current research in depth it is now time for some reflection. What will be delivered by our research initiatives? In this respect we have to be realistic and accept that with the current approaches, we will obtain at most a detailed description of the biological processes under study at the morphological, physiological, molecular, and metabolic level. I foresee that integration of these datasets — which is challenging — will result in the identification of candidate key controlling factors and associations between gene expression patterns and e.g. propagation rate. But what should be done next? Transgenic approaches aiming to confirm gene functions and the involvement of particular genes in important biological processes, is currently out-of-question. However, in my opinion, exploitation of natural variation is under-represented and would be a good direction to go. Let's go once more back to the vegetable sector for a good understanding. Recently, the genome of hundreds of tomato varieties have been re-sequences (Lin et al, 2014; The 100 Tomato Genome Sequencing Consortium, 2014), showing that due to domestication and breeding only a small part of the 'genomic pond' has been fished. When we take into account current breeding practice in the bulbous plant sector and the domination of e.g. the tulip variety 'Apeldoorn' in the past and 'Strong Gold' today, I dear to conclude that the situation is the same for bulbous plant species and even worse for tulips. We analysed a wild variant of tulip originating from Turkey in a pilot experiment and this showed already the presence of a broad phenotypic plasticity related to winter dormancy. Hence, the introduction of new breeding tools and accomplished exploitation of existing natural variation will open up complete new sources to identify desired characteristics and traits that society, but also the bulbous sector itself, is screaming for. This will go far beyond bringing new varieties on the market with improved ornamental quality, but will pave the road towards breeding for disease resistances or e.g. strongly increased saffron production, which is one of the most expensive spices and a bulbous product. I started off discussing my research direction with indicating four research lines that according to me should be followed to establish a solid fundamental basis for bulbous research. I have presented my thoughts about the research that need to be done to obtain life-cycle shortening, a better control over flowering, and more efficient propagation. Nevertheless, I didn't directly touch the other two important issues. Although I realize that I have to focus my work, we cannot wait with researching these other topics till the currently addressed questions have been answered. In this respect, it is a pleasure to see initiatives in this direction from colleagues at Wageningen University and other universities. Professor Joost Keurentjes and researchers from the University of Amsterdam and Leiden are employing activities to get financing for sequencing of a tulip genome and colleagues from the Plant Breeding department are working hard on the development of molecular markers and the creation of dense and good quality genetic maps for lily, union, and tulip. All these are national initiatives, but together with a number of research groups from all over Europe, we are currently also working hard to get bulbous plants research on the agenda of European Union research programs in the near future.

Is this physiology of flower bulbs 2.0?

You got now a glance of what we are currently doing and in which direction I will develop my research. It may be that this overview has given you the impression that this is not physiological research, but genetics, molecular biology, and bioinformatics. If so, you are not on your own! When I presented my plans for the first time to experts in the field I got the comment that what I am planning to do is not 'Physiology of Flower Bulbs', but 'Molecular Biology of Flower Bulbs'. Well, I cannot refute that molecular biology tools are predominantly applied in order to answer our research questions. Knowing my background and education in molecular biology, this could have been expected. Nevertheless, I cannot stress enough that it is exactly this that I think is how Physiology of Flower Bulbs 2.0 should be designed and performed, first generating a solid basis and getting rid of the current black box approach. When I started my studies at Wageningen University about 17 years ago we had strictly separated departments of Plant Physiology, Plant Breeding, Cell Biology and Molecular Biology that all studied plants using their own experiences. In addition, we had even more distant departments, such as Mathematics, Biochemistry and Biosystematics. Especially, over the last five years the borders between these departments blurred and it is nowadays almost impossible and out of the question to do physiological work without analyzing what is ongoing at e.g. the cellular and molecular level. Consequently, we need to analyze our systems from various angles and integrate the outcomes. In line with these developments 'Systems Biology' emerged as a rediscovered science and became one of the main focal points of our university and this is also fully integrated in my research line. But how is the actual situation in education?

Paradigm shift in plant sciences education

I indicated already that when I studied at Wageningen University, departments where far more autonomously operating. Over the last decade, this situation has changed in a positive direction and plant science students are stimulated to follow courses in e.g. Bioinformatics. Hence, we see integrated study programs emerging, but I noticed that the individual courses remain separate entities that often lack essential coherence. A good example is the way how Bioinformatics is in general taught to plant biologists. Due to the different way of reasoning and thinking between biologists and bioinformaticians, this often doesn't fully work out, with all respect for the involved lecturers. Furthermore, we have to realize that it is impossible to become an expert in all fields essential for performing fully integrated research at systems biology level. Therefore, I see it more relevant to get acquainted with each other's language. I suggest reaching this situation by paying more attention to differences in e.g. attitude and philosophy in the various disciplines. A good way to do this is by organizing fully integrated courses in which teachers from the different disciplines jointly generate the study material. I experienced the added value of this myself and think that it will pave the road towards a fully integrated education system.

In conclusion

I hope that I convinced you that research of bulbous plants is challenging and essential because of the continuous changing markets and demands by society. Despite the fact that much less research has been performed on these plants in comparison to e.g. vegetables, we do not have to start from scratch and can learn from work done in model plant species and other crops. However, in my opinion this so-called translational research will be successful only, when generating first a solid knowledge base and by addressing conservation of biological processes and systems at all possible levels going beyond just physiology. In other words: *'Plant Physiology and Flower Bulbs 2.0'!*

Acknowledgements

This brings me to the end of my inaugural address and the moment to thank a number of people. First of all, I like to thank Sjaak Langerslag, who initiated this special chair and organized to a large extent the essential finances. However, more important, I want to thank you for your open mind, your understanding of the importance of basic research, and last but not least, your inexhaustible enthusiasm. I could never have shown you today all examples from research already performed on this topic, without the input from Melissa Leeggangers and Natalia Moreno. It is a great pleasure to work with the two of you and your scientific insights, dedicated work, and motivation are highly appreciated. Thanks also to Harm Nijveen and Edouard Severing, the two bioinformaticians, and Maarten Holdinga, who perfectly takes care of our plants. A word of thanks goes also to Professor Harro Bouwmeester and all members of the Plant Physiology department for welcoming me in the group. In this respect, a special mention goes to Henk Hilhorst and the Wageningen Seed Lab (WSL). We are feeling at home and accepted as part of the great and stimulating 'WSL-family'.

I like to express also my great thanks to Prof. Gerco Angenent. I am working with you from the start of my career — when I did my PhD — and our discussions and brain storm sessions are always fruitful and you still manage to inspire me. You created another 'family' that I enjoy a lot; the Plant Developmental Systems cluster from Bioscience. A big thanks goes to all members! I am grateful to many more persons that supported me and helped me during my scientific career, but unfortunately, I cannot mention all of you by name.

I reserved the last words of thanks for my family and hope that you allow me to switch to Dutch. Allereerst wil ik mijn ouders bedanken. Paps en mams, jullie hebben mij een heerlijke onbezorgde jeugd gegeven en mij altijd ondersteund: Super! Dank gaat ook naar mijn broer Andre, waarmee ik samen uren over wetenschap kan discuseren en mijn zusje Monique waarmee ik de passie voor bloemen en voor muziek deel. De vele festival en concert bezoeken die wij samen hebben gedaan zijn een heerlijke ontspanning voor mij! Tot slot: Dianne, Rosa, en Emile bedankt voor jullie geduld, ondersteuning, de passie die we samen delen voor reizen en de natuur, en boven alles: jullie liefde!

Thanks for your attention.

Ik heb gezegd

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'The Dutch flower bulb industry is flourishing but facing serious threats. In order to win the battle against pathogens and to fulfil consumer demands, efficient breeding tools are essential. To speed-up the breeding process, life cycles of bulbous species have to be shortened and propagation rates should rise. Therefore, the special chair 'Physiology of flower bulbs' is aiming to understand the molecular and physiological mechanisms underlying flowering control and vegetative propagation. An integrated and 'omics-based' approach is followed to understand how environmental signals are translated by bulbous plants into defined reproductive decisions.'

ISBN 978-94-6257-185-3