Gene expression profiling of candidate genes for flowering in Belgian pot azalea

Ellen De Keyser[1], Annelies Christiaens[2,3], Els Pauwels[3] , Marie-Christine Van Labeke2, Bruno Gobin[3], Johan Van Huylenbroeck[1] and Jan De Riek[1]

[1] Institute for Agricultural and Fisheries Research (ILVO) – Plant Unit, Caritasstraat 21, 9090 Melle, Belgium

[2] Department for Plant Production, Ghent University, Coupure links 653, 9000 Gent, Belgium

[3] Research Centre for Ornamental Plants, Schaessestraat 18, 9070 Destelbergen, Belgium

Objectives

The past decade, pot azalea growers and their associations have made efforts to improve both the quality of the vegetative as well as the flowering plants; however, suboptimal flowering is often observed. The non-uniform opening of flower buds at anthesis when forcing the plants in the greenhouse or flowers that do not entirely open at the consumers place, even when plants are sold in the ideal candle stage, are often observed. Problems related to flowering are detrimental for the good image of azalea as a quality product. Different potential causes have been quoted. However, a clear cut direct cause is seldom found; interaction between several elements related to the culture conditions should be at the base. Therefore we aimed at identifying the influential factors related to flowering quality by an integrated approach focusing on the induction of processes at the gene expression level (RT-qPCR) and physiological measurements. The growth scheme was split up in 3 major processes: flower initiation and differentiation, dormancy breaking and anthesis (Fig. 1).

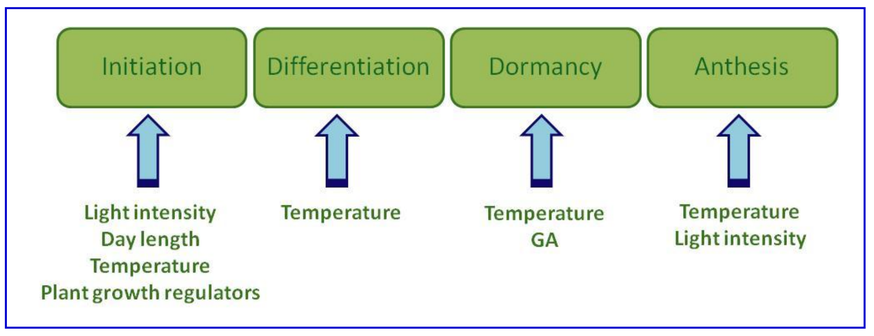

Fig. 1: Growth scheme of Belgian pot azaleas. Factors influencing the different processes are indicated.

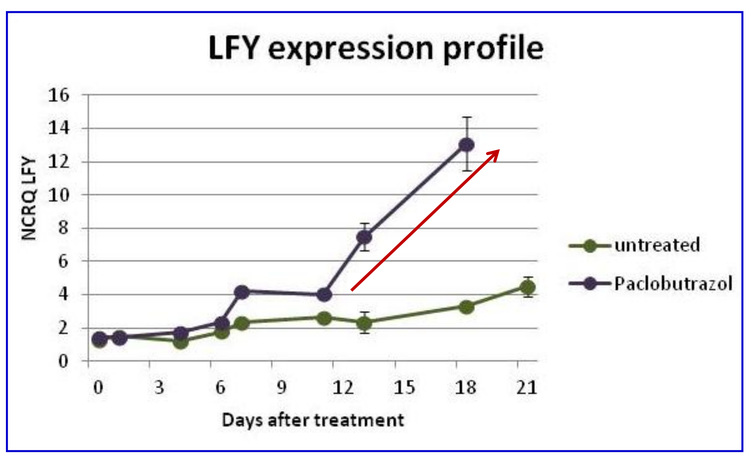

Fig. 3: Gene expression profile (RT-qPCR) of LEAFY in meristems of 'Hellmut Vogel' plants grown under LD conditions.

Flower initiation

'Hellmut Vogel' plants were grown at 20-22˚C and 16h DL. When each new shoot had on average 10 leaves, half of the plants was transferred to short day conditions (8h DL). In both growth chambers plants were split up again: half of the plants was treated twice with paclobutrazol (40 ppm) and the remaining half was left untreated. A bulk sample was taken every 2/3 days on 3 plants per treatment. The shoot apex was harvested on 2 shoots per plant. All sampling was done at the end of the light-period; samples were immersed in liquid nitrogen and stored at -80˚C. Harvesting ended when bud initiation could be determined microscopically (Fig. 2). Day-length was not a determining factor for initiation, but the application of plant growth regulators did have an effect on the initiation. The expression of a *LEAFY*-like gene (that promotes the transition to generative growth) was clearly up-regulated 13 days after treatment with paclobutrazol (Fig. 3), whereas this could only be detected in untreated samples after 21 days. This is in accordance with the later (visual) initiation that was seen. LFY can therefore serve as a good indicator for the start of differentiation in azalea.

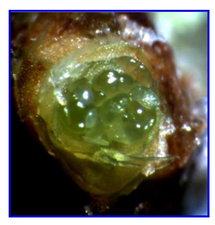

Fig. 2: Microscopic picture of an initiated azalea flower bud

Dormancy

Fully initiated 'Hellmut Vogel' plants were transferred to the cold chamber (7˚C) for 6 weeks. Simultaneously, 'Hellmut Vogel' plants were also kept in the greenhouse at 21˚C. Every week bulked leaf samples were harvested on 3 plants under both conditions. A bulk sample was also made of 2 buds from the same 3 plants used for leaf sampling. Outer bud scales were removed. All samples were immersed in liquid nitrogen and stored at -80˚C. The expression of dehydrin (Fig. 4) is clearly up-regulated under cold conditions and decreases again after 4-5 weeks when dormancy has been released. Also in grapes, the decreased expression of dehydrin is reported as a marker for dormancy release (Keilin et al. 2007). An ABA-based in-vitro bio-assay confirmed dormancy release. When we look at the carbohydrate metabolism, the expression of SUSY appears to be up-regulated in both leaves (Fig. 5) and buds of plants grown at cold temperatures. Plants in cold conditions have a higher need for carbohydrates to protect their tissues against the lower temperatures. Moreover, since these plants are grown in the dark, no photosynthesis can occur and SUSY is needed to provide energy. This was confirmed by a significant decrease in the starch level in the leaves during cold treatment.

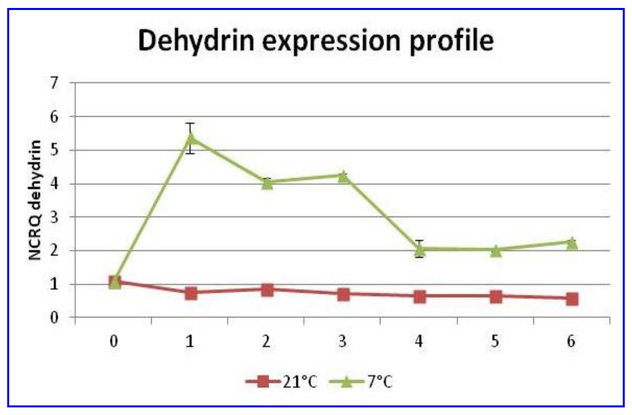

Fig. 4: Gene expression profile (RT-qPCR) of dehydrin in flower buds of 'Hellmut Vogel' plants during treatment for dormancy breaking

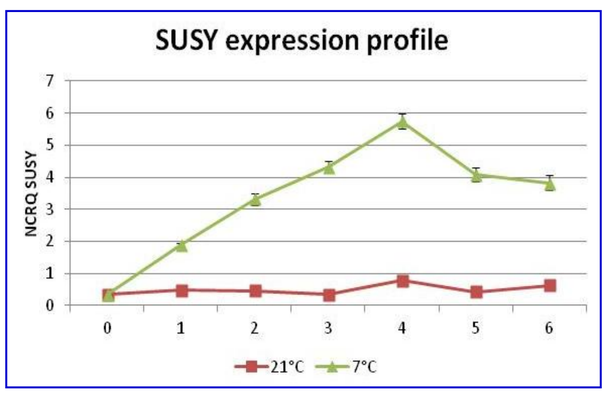

Fig. 5: Gene expression profile (RT-qPCR) of SUSY in leaves of 'Hellmut Vogel' plants during treatment for dormancy breaking

RT-qPCR

All samples were analyzed in duplo, noRTs and NTCs were included as controls. Gene-specific amplification efficiencies were used for calculations in qBase[PLUS] and 2 (meristem/buds) or 3 (leaves) validated reference genes were used for normalization.

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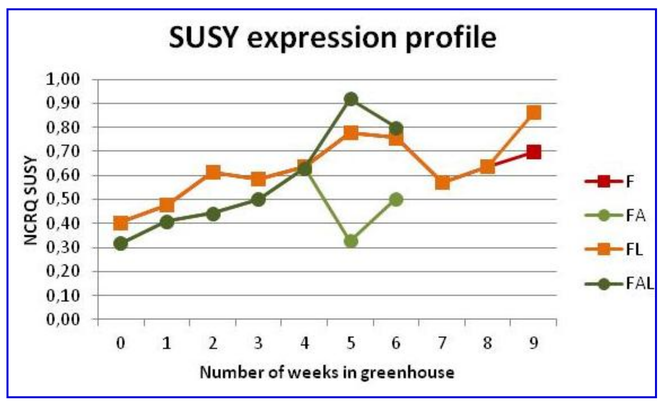

Fig. 6: Gene expression profile (RT-qPCR) of SUSY in flower buds of 'Sachsenstern' during anthesis. F: forced without extra light; FA: forced with assimilation light; L: living room conditions after F or FH

Anthesis

'Sachsenstern' plants were transferred to the greenhouse (21˚C) for forcing after 7 weeks of cold treatment. Assimilation light was supplied to half of the plants. When flower buds showed their color, half of the plants of both groups was transferred to living room conditions (18˚C, low light). Bulked leaf and bud samples were taken every week until complete flowering. The expression profiles of 2 genes of the carbohydrate pathway (SUSY and α-amylase) was determined during anthesis. Plants that were grown under assimilation light flowered up to 4 weeks earlier. The expression of SUSY in flower buds was slightly lower compared to the plants that did not receive additional light. However, when the plants were transferred to living room conditions, the expression of SUSY in leaves and buds (Fig. 6) significantly increased in both groups. Under low light conditions, the photosynthetic capacity of the plants is too low and SUSY and a-amylase (results not shown) are up-regulated to produce soluble carbohydrates. These are needed as an energy source for opening of the flowers.