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Transformation with *rol*-genes of *Agrobacterium rhizogenes* as a strategy to breed compact ornamental plants with improved postharvest quality

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Introduction

In production volume *Kalanchoe blossfeldiana* represents the most produced flowering potted plant. Denmark is one of the leading countries in the production of potted ornamental plants. Around 85% of the production is exported, valued at EUR 467 millions. Exporting potted plants imposes a great demand on plant quality. One important qualitative criterion is that the plants should be compact; thus in commercial production growth is controlled by the application of chemical growth retardants. Chemical growth retardants are hazardous to human health and the environment and therefore in many countries some commonly used compounds can no longer be used. In the near future more growth regulators are likely to be banned.

Methods

An alternate strategy to the use of chemical growth retardants is to produce compact genotypes by inserting the *rol*-genes from the naturally occurring bacterium *Agrobacterium rhizogenes* into the plants. In various plant species *A. rhizogenes* causes the growth of hairy-roots by a natural transformation phenomenon due to the insertion of *rol*-genes. Plants regenerated from these transformed roots exhibit changes in morphology. Here, we present the transformation of *Kalanchoe blossfeldiana* with *rol*-genes with the aim to improve plant quality, especially compactness. We evaluate this method for future breeding programs and analyse T1, F1 and F2.

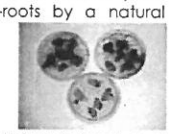
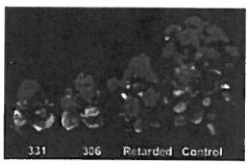


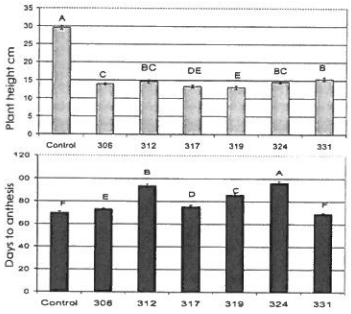
Fig.1 Hairy root phenotype of *Kalanchoe blossfeldiana* transformed with *Agrobacterium rhizogenes*

Characterisation of T1



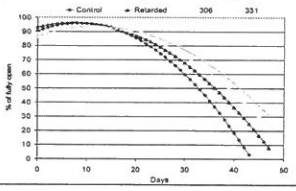
The plant height was reduced significantly in all Ri-lines compared to the control and there were significant differences among the Ri-lines. Time to anthesis was the same in Ri-line 331 as in control plants and delayed by only 3 days in the Ri-line 306. Other lines flowered later.

Plant morphology T1



Longevity

The transformants had a clearly improved postharvest life. The longevity of detached single flowers was defined as the stage where flowers were 70% open. The longevity of the two Ri-lines, 306 and 331, was 32 and 34 days, chemical growth retarded and control plants lasted 28 days and 26 days.



Ethylene tolerance

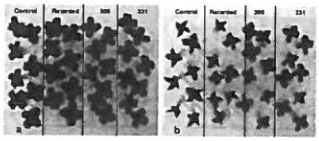


Fig. 2 Effect of ethylene exposure on flowers of *Kalanchoe blossfeldiana* control and Ri-lines.
a. Control treatment without ethylene.
b. Flowers treated with 1µl l-1 ethylene for 72 h

In response to ethylene exposure, the flowers of the plants transformed with *rol*-genes exhibited tolerance while control and chemical growth regulated plants were sensitive.

Characterisation of F1

T1 X Sarah
↓
F1

Plant morphology F1

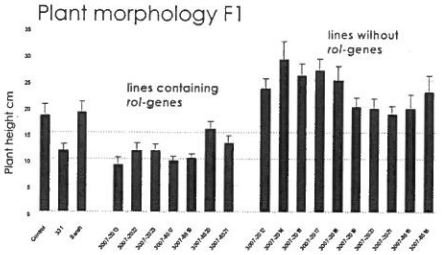
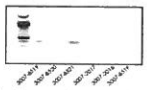


Fig. 3
Example of PCR analysis using a primer set amplifying the *rolD* gene



PCR confirmed inheritance of the *rol*-genes in several lines, *rol* A, B, C, and D were transferred together to the progeny. The plant height was reduced in the progeny containing the *rol*-genes. All plants, which did not inherit the *rol*-genes, were taller than plant lines containing *rol*-genes.
The F1 progeny produced fertile pollen and selfing resulted in vital seeds for many crossings. Per crossing 1-300 seeds were harvested and sown. About a third of the crossings did not give any seed.

Characterisation of F2

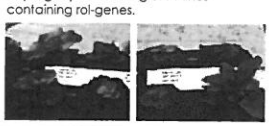
F1 X F1
↓
F2

Presently, F2 plants are growing under greenhouse conditions. Lines with interesting traits will be selected and genotypically analysed. Especially of interest is the fact whether the *rol*-genes always are linked or whether we can find genotypes with only single *rol*-genes. Genotypes of interest will be vegetatively propagated and phenotypcally characterised in a block experiment.

Phenotypically analysis of the F2 progeny will comprise:

- Height total
- Height without flowers
- Days until first open flower
- Days until first wilting flower
- Number of shoots (main shoot, side shoots)
- Plant diameter

Fig. 4
F2 progeny from selfing of F1 lines containing rol-genes.



Conclusion

- *In Kalanchoe blossfeldiana* dwarfed Ri-lines were created through the insertion of the *rol*-genes from *Agrobacterium rhizogenes*.
- Transformants were selected solely based on the hairy root phenotype without the use of antibiotic resistance genes as marker.
- Ri-lines exhibited various degrees of the Ri-phenotypic characteristics.
- Plant height was reduced and the internodes of the Ri-lines were clearly shorter giving a compact growth habit.
- Transformants had improved postharvest quality and ethylene tolerance.
- Transformants are fertile and produce fertile progeny.
- F1-lines containing *rol*-genes are compact.
- Transformation with *rol*-genes of *A. rhizogenes* is an interesting approach to create dwarfism and to improve postharvest quality in ornamental plants.

References

Christensen B., Sriskandarajah S., Serek M. and Müller, R. (2008). Transformation of *Kalanchoe blossfeldiana* with *rol*-genes of *Agrobacterium rhizogenes* is useful in molecular breeding towards compact growth. Plant Cell Reports 27: 1485-1495

Christensen B. and Müller R. (2009) *Kalanchoe blossfeldiana* transformed with rol-genes exhibits improved postharvest performance and increased ethylene tolerance. Postharvest Biology and Technology 51: 399-406.

Christensen B., Sriskandarajah S. and Müller R. (2009) Transformation of *Hibiscus rosa-sinensis* L. by *Agrobacterium rhizogenes*. J. Hort. Sci & Biotech 84: 204-208.

Christensen B, Sriskandarajah S, Müller R. (2009). Biomass distribution in *Kalanchoe blossfeldiana* transformed with *rol*-genes from *Agrobacterium rhizogenes*. HortScience 44(5): 1233-1237.

Christensen B. and Müller R. (2009) *Agrobacterium rhizogenes* and its *rol*-genes in ornamentals. Journal of European Horticulture. In press.