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REVIEW

Genetic engineering and sustainable production of ornamentals: current status and future directions

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Abstract Through the last decades, environmentally and health-friendly production methods and conscientious use of resources have become crucial for reaching the goal of a more sustainable plant production. Protection of the environment requires careful consumption of limited resources and reduction of chemicals applied during production of ornamental plants. Numerous chemicals used in modern plant production have negative impacts on human health and are hazardous to the environment. In Europe, several compounds have lost their approval and further legal restrictions can be expected. This review presents the more recent progress of genetic engineering in ornamental breeding, delivers an overview of the biological background of the used technologies and critically evaluates the usefulness of the strategies to obtain improved ornamental plants. First, genetic engineering is addressed as alternative to growth retardants, comprising recombinant DNA approaches targeting relevant hormone pathways, e.g. the gibberellic acid (GA) pathway. A reduced content of active GAs causes compact growth and can be facilitated by either decreased anabolism, increased catabolism or altered perception. Moreover, compactness can be accomplished by using a natural transformation approach without recombinant DNA technology. Secondly, metabolic engineering approaches targeting elements of the ethylene signal transduction pathway are summarized as a possible alternative to avoid the use of chemical ethylene inhibitors. In conclusion, molecular breeding approaches are dealt with in a way allowing a critical biological assessment and enabling the scientific community and public to put genetic engineering of ornamental plants into a perspective regarding their usefulness in plant breeding.

Keywords Biotechnology · Compactness · Ethylene · Growth regulator · Molecular breeding · Transformation

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Introduction

Ornamental plant production

The worldwide production value of ornamental potted plants and cut flowers comprises about 50 billion €, corresponding to an estimated global consumer consumption between 100 and 150 billion € (Chandler and Tanaka 2007; Chandler and Brugliera 2011). The main areas of both production and consumption of ornamental plants are Europe and the United States, with a fast growing ornamental plant industry in Japan and China. For a long time, Europeans have had the strongest culture of utilising cut flowers and ornamental potted plants and the consumption per capita is higher than the purchase of floricultural products in the United States (Chandler and Tanaka 2007). The economical importance of ornamental plant industries can be seen clearly in several countries in Europe as well as USA. The production of ornamental potted plants and cut flowers within Danish horticulture reached a value of approximately 300 million € in 2010 (Dansk Gartneri 2011).

Around 85 % of the production is exported to the other European countries, which consequently imposes a great demand on plant quality. With an export value of 250 million €, Denmark takes the position as number two of the worlds' leading export countries for ornamental potted plants, only surpassed by The Netherlands (Dansk Gartneri 2011). In production, volume *Kalanchoë* represents the most important potted flowering plant with 45 million plants sold in Denmark in 2010. With 24 million sold plants, *Rosa hybrida* ranks as number two on the list of the most sold ornamental potted plants in Denmark (Floradania Marketing 2011). Poinsettia (*Euphorbia pulcherrima*) is a contemporary symbol of Christmas in most parts of the world. In Norway, poinsettia is one of the most important pot plants with about six million plants sold yearly with a market value of over 200 million NOK (Norsk Gartnerforbund 2010). The annual production in the USA and the EU is 50 million and 100 million plants, respectively, and the yearly production in 2008 was estimated to comprise a value of around 155 million US$ (USDA [NASS] 2009), hence, the innovation potential of poinsettia is considerable.

The market for cut flowers and potted ornamental plants is not only determined by producers' choices and distributors' needs, but also by consumer wishes and driven by a continuously growing demand for novelties and high quality (Ascough et al. 2008). Research and product development sections have gained increasing importance in competitive companies. According to the company Florigene, "novelties sustain the industry" (Tanaka et al. 2010). Excellent quality of the product, outstanding postharvest performance of ornamental potted plants, long vase life of cut flowers and stress-tolerant plant species and cultivars are essential criteria in a competitive floriculture market (Müller 2011). Apart from internal quality, such as longevity, stress tolerance and resistance against pest and diseases, external characteristics are significant for competitive products (Clarke et al. 2008). Morphology and plant architecture, e.g. compactness (Christensen et al. 2008; Lütken et al. 2010, 2011), and flower shape, e.g. filled flowers and colour, e.g. blue carnation (Nishihara and Nakatsuka 2011; Tanaka et al. 2010) have gained increasing importance in breeding programmes of ornamental plants. Furthermore, environmentally friendly production of ornamentals has also become an important issue for the flower industry worldwide.

Ornamental breeding

In the past, the introduction of new traits into ornamental plants mainly depended on classical breeding, a combination of crossing and selection, as well as mutational breeding. However, classical breeding has been difficult in many ornamentals due to their high ploidy levels high chromosome numbers and incompatibility (Müller 2011; Petty et al. 2003). Interspecific and intergeneric hybridization and subsequent tissue culture of obtained embryos are applied techniques to further to increase gene pools. Nevertheless, it seems that conventional breeding and plant improvement techniques have become inadequate to keep up with progression and quality demands, hence biotechnological breeding strategies are increasingly adopted (Chandler and Tanaka 2007; Potera 2007). Fortunately, the last decades have enabled specific alterations of single traits through the exploitation of genetic engineering. Hereby, the available gene pool can be extended as desirable genes from unrelated species or even organisms can be used for crop improvement. Moreover, as the genes of interest can be both overexpressed and downregulated, a plethora of phenotypes can potentially be produced. This technology has further enhanced the quality of ornamental plant cultivars that were already superior by alteration of traits that influence the overall plant morphology, e.g. plant height, rooting potential, colour, shape or even fragrance of flower as well as the vase life in cut flowers. Plant growers have for centuries pursued to develop a blue rose, but it has only been possible to achieve with the help of genetic engineering (Tanaka et al. 2010). To date, transgenic ornamentals from over 30 genera have been produced by different transformation approaches (Nishihara and Nakatsuka 2011).

Potentials and limitations of genetic modification

Genetic engineering has demonstrated its great potential and a number of genetically modified (GM) crops, e.g. maize, soybean, cotton, canola, papaya, have been commercialized during the last 16 years (James 2010). In 2010, 15.4 million farmers in 29 countries cultivated 148 million hectares genetically engineered crops, a 10 % increase compared with that of 2009 (James 2010). However, those encouraging successes have been restricted to a limited number of major food crops with significant economical impacts. A relatively small market for ornamental crops makes many costly breeding strategies less profitable for breeders within floricultural industries. High expenses for patents and licenses for patented methods and gene applications in combination with high-priced approval and registration procedures of GM plants hold back the use of gene technology and make GM plants less profitable for breeding companies (Müller 2011). Another factor restricting manifestation of GM ornamental plant species may be the limited acceptance of GMOs by the public and retailers. However, the observations from about 15 years with GM carnation flowers on the market does not support this concern (Chandler and Tanaka 2007), but no comparable conclusion can be drawn for the European market due to restrictions on the European market (Tanaka et al. 2005).

Furthermore, the history of "Golden rice" has clearly showed the potential complication and Marathon-like time race before it can be released eventually (Mayer and Potrykus 2011), although this example not necessarily is the case for all GM plants. Despite of the strong evidence of advantages and proved benefits, many GM crops are still not commercially released. Undesirable and unpredictable impacts on the environment are one reason of concern, another aspect hampering success of recombinant technologies is the fact that transgenic food meets scepticism among consumers. On a global scale, the permissions for producing GM ornamentals vary substantially. In general, USA and China are relatively permissive, whereas Europe is quite restrictive. Countries like Australia are somewhat in between (Auer 2008). There are still many countries, e.g. in Europe, which have restrictions on GM crops (Chandler and Tanaka 2007). However, as GM ornamentals are non-food crops their consumer acceptance seems relatively uncomplicated compared to edible crops.

To overcome the aforementioned difficulties and to sustainably produce ornamentals, an alternative to genetic modification is the use of biotechnological methods based on natural transformations without recombinant DNA techniques. In Denmark, plants derived from natural transformations using unmodified bacterial strains without using recombinant nucleic acids are according to present regulations in the European Union not classified as GMO (Christensen et al. 2008, 2010; European Union 2001). Similarly, in Japan, transformants derived from wild-type *Agrobacterium rhizogenes*-mediated transformation (i.e. natural genetic transformation) are free from the legal controls of GMOs (Mishiba et al. 2006). This makes natural transformation with, e.g. wild type *A. rhizogenes* and its *rol*-genes to a promising tool in molecular plant breeding for increasing genetic diversity, and it appears particularly useful for breeding towards compact ornamental plants (see below). However, although the legislation on natural transformation appear relatively straightforward, it is still important to bear in mind that the plants created using this strategy are developed using biotechnological methods. It can be taken into consideration that mutational breeding also is based application of molecular tools. How plants produced using these methods are perceived by consumers and producers is based on matters of individual views.

Environmentally and health-friendly production of ornamentals

During the last decades, environmentally and health-friendly production methods and conscientious use of resources as input to plant production have become a crucial sustainability goal. Protection of the environment requires adequate consumption of limited resources used in horticulture as well as agriculture, which necessitates the development and implementation of sustainable energy saving strategies and alternative production methods with reduced water and fertilizer needs. Additionally, there is an indispensable demand of developing methods and technologies to reduce application of pesticides and other chemicals; this includes finding better alternatives for plant growth regulators and postharvest treatments without the use of harmful substances. In this review, we describe the more recent progress in improving the quality of ornamentals through genetic engineering with focus on improvement in respect to environment and human health. The two main topics discussed in this review are biotechnological alternatives to growth retardants and chemical ethylene inhibitors with the aim of developing stable alternatives to replace agro-chemicals in ornamental plant production.

Alternatives to growth retardants

Plant height is a quality parameter of primary importance in a large number of economically important ornamental crops. Consumers prefer short, compact plants with good keeping quality and ornamental value. In addition, compact plants tolerate mechanical handling and transport far better than more elongated plants. Furthermore, smaller plants require less space in expensive production facilities, are easier to handle, have reduced transportation costs and advantages for retailers (Müller 2011). Thus, compact plants are preferred throughout the whole production and postharvest chain increasing profit in production and distribution. Currently, the feature compactness is obtained through the extensive utilization of chemical growth retardation, e.g. chlormequat, daminozide or paclobutrazol. The number of approved growth retardation compounds is highly dependent on the country of production. For example, application of two commonly used chemicals, paclobutrazol and daminozide, is no longer approved in many European countries. Even though there are differences between plant genera and species many of the important ornamental plants in the world (e.g. potted roses, *Hibiscus*, *Kalanchoë*) are presently produced by the use of chemical growth retardants (Grøn Plantebeskyttelse ApS 2008). During recent years, other methods of growth regulation are increasingly being developed and implemented, e.g. the use of a temperature drop and manipulation with light quality can contribute to a certain extent in control of shoot elongation (Clifford et al. 2004; Oerum and Christensen 2001). Concern about the negative impact of chemical compounds on human health is increasing due to potential toxicity (Mullins 1989) and possible carcinogenic

effects (Yamada et al. 2001) as well as negative effects on the environment has limited their availability and utilization (De Castro et al. 2004; Sørensen and Danielsen 2006; US Environmental Protection Agency 1993). One solution to this problem is the development of plants with inherited traits that renders the use of hazardous growth-retarding chemicals unnecessary through the use of genetic engineering. Similarly, conventional breeding and selection has been pursued as an option. However, conventional breeding alone has in many plant species not resulted in compact phenotypes, which can be produced in an appropriate quality and/or quantity without the use of chemical growth retardants. Key strategies for developing compact plants by genetic engineering are indicated in Fig. 1 and Table 1, functionally described in Fig. 2 and are presented and discussed below.

Genetic engineering of GA metabolism for induction of dwarfism

As growth-retarding chemicals often targets inhibition of the synthesis of the gibberellic acid (GA) hormones (Rademacher 2000), modulation of this pathway is an obvious target towards the development of compact plants. GAs are diterpenoids consisting of 19 or 20 carbon atoms and they are all formed from the C5 compound isopentenyl diphosphate (Rademacher 2000). GA biosynthesis can be divided into three major stages according to the localization and the nature of the enzymes involved (Graebe 1987; Hedden 1997; Hedden and Kamiya 1997; Hedden and Phillips 2000). In the last stages, the active GAs are formed by the enzymes GA20 oxidase (GA20ox) and GA 3β hydroxylase (Rademacher 2000). Subsequently, the active GAs are degraded by GA2 oxidase (GA2ox), the major GA catabolic enzymes in plants (Rademacher 2000). In recent years, the elucidation of GA biosynthetic pathways and the cloning of most genes coding for enzymes of GA biosynthetic pathway offered the opportunity to control plant height by genetic modification of genes related to GA biosynthesis (Biemelt et al. 2004; Coles et al. 1999; Dijkstra et al. 2008; Hedden and Phillips 2000; Qiao et al. 2007; Sakamoto and Matsuoka 2004). Up to now, two molecular engineering approaches to reduce the endogenous biologically active GA content in order to modulate plant height have been demonstrated. The first is increasing expression of *GA2ox* genes, causing increased degradation of GA and the second is reducing expression of the *GA20ox* genes, thereby suppressing GA biosynthesis. Furthermore, modulation of regulatory genes involved in GA perception and signal transduction, (e.g. *gai* *GA INSENSITIVE*) approaches have led to dwarf or semi-dwarf phenotypes. The strategies are discussed below in detail.

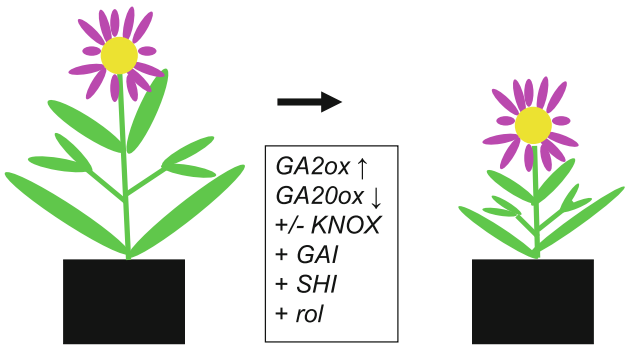

Fig. 1 Key strategies for developing compact ornamentals. Compact ornamental plants can be developed by modulation of the gibberellin (GA) biosynthesis pathway. *GA2ox* genes can be overexpressed or alternatively the expression of *GA20ox* genes can be downregulated. Similarly, the hormone homeostasis can be changed by alteration of homeotic genes (e.g. *KNOX*). Furthermore, the GA perception can be altered by overexpression of the transcription factors *gibberellin insensitive* (*GAI*) and *short internodes* (*SHI*). Another method to breed for compactness involves the *root loci* (*rol*)-genes from *Agrobacterium rhizogenes*

Overexpression of GA inactivation genes

In respect to the first approach, overexpression of genes encoding GA2-oxidases has been carried out in a number of plants such as *Arabidopsis*, tobacco, wheat, rice and plum as well as species from the *Citrus*, *Populus* and *Solanum* genera (Appleford et al. 2007; Biemelt et al. 2004; Busov et al. 2003; Coles et al. 1999; Curtis et al. 2000; Dijkstra et al. 2008; El-Sharkawy et al. 2012; Eriksson et al. 2000; Fagoaga et al. 2007; Gou et al. 2011; Huang et al. 1998; Israelsson et al. 2004; Qiao et al. 2007; Radi et al. 2006; Sakamoto et al. 2003; Schomburg et al. 2003). In ornamental plants, this approach has been utilized in induction of dwarfism in poinsettia aiming to develop a cost-effective, environmentally friendly alternative by genetic engineering of the GA biosynthesis pathway (Clarke et al., unpublished data). The *PcGA2ox* gene from runner bean *Phaseolus coccineus* (Thomas et al. 1999) has been overexpressed in three poinsettia cultivars. The transgenic plants are currently being characterized (Clarke et al., unpublished data) and will provide valuable information regarding the feasibility of overexpressing GA inactivation genes in ornamental plants aiming at increasing the GA catabolism. Although application of the constitutive 35S promoter to alter expression of GA-metabolism genes in some cases have resulted in loss of fertility, it has also been used with success to produce plants with reduced stem height without affecting reproductive development (Dijkstra et al. 2008). As an alternative, an inducible promoter might be used with advantage (Topp et al. 2008). However, such a strategy might not be straight forward due to a continuous demand for regulation of shoot elongation

Table 1 Major approaches towards compactness in ornamentals

Approach	Gene and protein function	Effect	Origin	Target plant	Results	Side effects	References
Up-regulation of GA2 oxidase	*PcGA2ox* Catabolic enzyme	Reduction of active GAs	*Phaseolus coccineus*	Poinsettia	N/A	N/A	Clarke et al., unpublished data
Down-regulation of GA20 oxidase	*GA20ox's* Anabolic enzyme	Reduction of active GAs	*Kalanchoë blossfeldiana*	*Kalanchoë blossfeldiana*	Reduced plant height	Delayed flowering	Topp et al. (2008)
Modified hormone homeostasis	*KNOXs*	Reduced ability to perceive hormones	*Kalanchoë* × *houghtonii*	*Kalanchoë* × *houghtonii*	Compact plants Darker green leaves	Altered leaf morphology	Laura et al. (2009)
				Kalanchoë blossfeldiana	Compact plants Darker green leaves	Altered leaf morphology	Lütken et al. (2011)
	Aspartic proteases Catabolic enzyme	Reduction of active hormones	*Arabidopsis thaliana*	*Pelargonium zonale*	Dwarfness Multi-branching	Reduced flower size Reduced number of petals	Chabannes et al. (2009)
	gai Transcription factor	Reduced ability to perceive GA	*Arabidopsis thaliana*	*Chrysanthemum*	Dwarf plants	Delayed flowering, reduced flower size and numbers of flowers	Petty et al. (2001, 2003)
				Petunia	Dwarf plants	N/A	Chandler et al. (2005)
	shi Transcription factor	Reduced ability to perceive GA	*Arabidopsis thaliana*	*Kalanchoë*	Compact plants, a range of degrees of compactness	N/A	Lütken et al. (2010)
				Poinsettia	Compact plants	N/A	Clarke et al. Unpublished data
	rol *rol*B: tyrosine phosphatase activity (Filippini et al. 1996) *rol*D = ornithine cyclodeaminase (Trovato et al. 2001) *rol*A and *rol*C: unknown functions	Altered ability to perceive hormones	*Agrobacterium rhizogenes*	Numerous (see text for further details)	Compact plants Increased rooting Darker green leaves Improved postharvest performance	Wrinkled leaves Delayed flowering Male sterility Abnormal flowers	Casanova et al. (2005) Christensen and Müller (2009c)

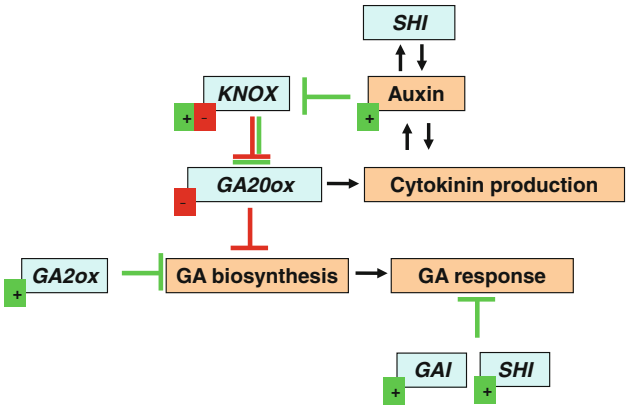

Fig. 2 Targets of genetic engineering in breeding approaches towards compactness. *SHI* gene members are generally involved in the maintenance of auxin homeostasis. Auxin in return downregulates expression of *KNOX* genes during organ emergence. When the expression levels of *KNOX* genes are modified, they potentially can inhibit *GA20ox* genes, thereby inhibiting GA biosynthesis. Similarly, upregulation of *GA2ox* genes causes increased degradation of GA. Finally, overexpression of the transcription factors *GAI* and *SHI* can be used to alter the perception of GA. Genes are highlighted in *pale turquoise boxes* whereas hormones are shown in *tan-coloured boxes*. *Perpendicular symbol* illustrates inhibitions, whereas the *plus and minus symbols* indicate the techniques to achieve compactness in terms of overexpression and downregulation, respectively, of the listed genes

in many plants, for instance, poinsettia until flowering occurs. A tissue-specific promoter might also be used. This should then be restricted to the subapical meristem, which forms the majority of the cells of the stem.

Downregulation of GA20oxidases

The second approach involves downregulation of *GA20oxidases*. In *Arabidopsis*, antisense silencing of the gene *GA20ox,* encoding a rate-limiting enzyme in the GA biosynthesis pathway (Lange 1998), resulted in various phenotypes displaying smaller leaves, delayed flowering time and reduced fertility (Coles et al. 1999). Although GA application rescued the time of flowering, it, however, also resulted in reversion to WT height. These side effects are not unexpected as GA also influences several crucial parameters, e.g. flowering time and fertility as well as morphogenesis in general (Fleet and Sun 2005). Nevertheless, inhibition of *GA20ox* has presented a promising target as seen by the "green revolution rice" that exhibited semi dwarf growth combined with significantly increased crop yield (Hedden 2003). Modulation of *GA20ox* has also been pursued in ornamentals. In *K. blossfeldiana*, downregulation of a gene encoding *GA20ox* has been used to inhibit elongation growth. By this approach, an alcohol-inducible promoter system was applied to silence the *GA20ox* biosynthesis genes. Before activation of the alcohol-induced promoter system, the plants were phenotypcially indistinguishable from control plants. When silencing was induced by low concentrations of alcohol, plants exhibited reduced height and delayed flowering (Topp et al. 2008).

Modification of hormone homeostasis

Modulation of the structural homeotic genes represents a platform for modification of plant architecture. From the 14 classes of plant homeotic genes (Mukherjee et al. 2009), the *BELL* and *KNOX* genes, belonging to the TALE group, have central roles in developmental processes as organ differentiation and meristem establishment. The *KNOX* genes have been divided into three classes (class I–III) (Hamant and Pautot 2010). Class I genes are primarily expressed in the meristematic tissue and contain a HWKPS motif in the homeodomain of the protein. The more widely expressed class II contain a NWHSN motif whereas the class III is lacking a homeodomain and is only represented by a single member (Kerstetter et al. 1994). The TALE members have been shown to be correlated with homeostasis control of cytokinin and GA (Shani et al. 2006). In general, activation of *KNOX* genes leads to increased cytokinin biosynthesis and regulates GA biosynthesis by repressing GA20ox's (Gordon et al. 2009; Leibfried et al. 2005). Furthermore, it has been shown that auxin plays a major role in downregulation of *KNOX* expression during organ differentiation (Hay et al. 2006).

Although several of these genes have been functionally characterized both in *Arabidopsis* and major crop plants, they have only recently been used in molecular breeding towards compactness in ornamentals. Overexpression of the *KxHKN5* KNOX class I gene from the *Kalanchoë* × *houghtonii* hybrid caused compactness and altered leaf morphology when endogenously overexpressed (Laura et al. 2009). Similarly ectopic overexpression of the *KxHKN5* gene caused compact plant with altered leaf morphology in *K. blossfeldiana* 'Molly'. In contrast, silencing of this gene resulted in compact plant with unaltered leaf morphology (Lütken et al. 2011). The class II gene *KxHKN4* has also been overexpressed in *K. blossfeldiana* 'Molly' causing compact plants with a significantly higher number of relative inflorescence branches compared to WT as well as dark green leaves due to elevated chlorophyll content (Lütken et al. 2011).

Another method of alteration of hormone homeostasis to obtain compact plants involves aspartic proteases. In general, they act as endopeptidases facilitating proteolysis of internal α-peptide bonds within the target proteins. Aspartic proteases have shown to be involved in the processing of hormones conferring a regulatory effect. Moreover, in *Arabidopsis* overexpression of the constitutive disease

resistance (*CDR1*) aspartic protease gene resulted, besides increased resistance towards bacteria, in dwarfing (Xia et al. 2004). In ornamentals, this strategy has been pursued in *Pelargonium zonale*, where the *Arabidopsis* aspartic protease gene At2g28010, named *CDS10*, was constitutively overexpressed leading to bushy, multibranched dwarf phenotypes. However, in all the three transformed cultivars, the increase in number of branches was correlated with decreased number of petals in the flowers, i.e. the double flowers were reduced to only single flowers (5 petals) (Chabannes et al. 2009).

Alteration of GA perception and response

Lately, regulatory genes involved in GA signalling have been identified, and a complicated regulatory network of proteins with a high degree of functional redundancy have been unraveled (Hirano et al. 2008). Several of the identified genes belong to the DELLA family of transcriptional regulators (Eckardt 2002; Wen and Chang 2002). Generally, DELLA proteins function as negative regulators of the GA response. The most studied *DELLA* gene is *GAI* (*GA INSENSITIVE*), first described in *Arabidopsis* (Koornneef et al. 1985). Genetic analysis has revealed that deletion of the motif DELLA"VLGYKVRSSEMA" results in increased potency due to enhanced resistance to GA-mediated degradation (Peng et al. 1997). As a consequence, the mutated *GAI* gene product constantly suppresses GA responses resulting in a dwarf phenotype, which is insensitive to GA. Alteration of this gene has been pursued in several ornamental plant species; in *Chrysanthemum* ectopic expression of this gene resulted in dwarf plants, unfortunately, both the number and size of the flowers were reduced and the transgenic plants had delayed flowering time (Petty et al. 2001). Growth, chlorophyll content and flower characteristics including time to first open flower correlated with the level of expression of the transgene (Petty et al. 2001, 2003). In the transgenic lines, the extent of dwarfing was related to the reduction in the response to exogenous GA (Petty et al. 2001, 2003). In petunia, dwarfing was also induced by introducing the genomic *GAI* sequence leading to decreased internode length and increased compactness (Tanaka et al. 2005). Similarly, overexpression of *AtGAI* in apple resulted in reduced plant size, although the transgenic lines exhibited reduced rooting ability (Zhu et al. 2008).

The *SHORT INTERNODES* (*SHI*) transcription factor isolated from *Arabidopsis* by Fridborg et al. (1999) has also been demonstrated to be involved in GA response. *AtSHI* belongs to the *SHI* family of putative transcription factors where nine members are found in *Arabidopsis* (Fridborg et al. 1999, 2001; Kuusk et al. 2006). The SHI proteins are characterized by the presence of the conserved IGGH domain and the RING finger-like zinc finger motif (Fridborg et al. 2001; Kuusk et al. 2006). The SHI members act as DNA-binding transcriptional activators and their function is primarily to maintain auxin homeostasis (Sohlberg et al. 2006; Staldal et al. 2008) and they are partially redundant, e.g. they can compensate for each other and they probably function as multimers (Kuusk et al. 2006). The phenotype of *SHI* overexpression is stunted plant growth, elevated levels of GA, reduced apical dominance and an increased number of flowers. Interestingly, normal flowering time could be restored by GA application whilst the mutants remained dwarf (Fridborg et al. 1999). Due to the separation of flowering time and elongation, the *AtSHI* gene represents a key target for a biotechnological approach to retard ornamental plants to produce compact cultivars in horticulture. Ectopic expression of *AtSHI* in *Kalanchoë* resulted in dwarf plants with compact inflorescences (Lütken et al. 2010). Compared to endogenous expression in *Arabidopsis* several parameters differed; transgenic *Kalanchoë* lines did not show altered leaf morphology. However, transgenic *Kalanchoë* exhibited a lower number of branches. Most importantly, the transgenic lines were not delayed in flowering and application of exogenous GA_3 in low concentration completely restored flowering. Moreover, when expression was driven by the endogenous *SHI* promoter from *Arabidopsis*, a longer flowering period was observed. Furthermore, two *AtSHI*-like genes were found in *Kalanchoë* indicating a widespread presence of this transcription factor (Lütken et al. 2010). Recently, *AtSHI* was overexpressed in poplar, where it also caused compactness in terms of reduced internode and petiole length. Similarly, RNAi suppression of two cloned *Populus SHI RELATED SEQUENCES* (*SRS*) genes were found to enhance shoot and root growth (Zawaski et al. 2011) adding further support to an important role of the *SHI* gene family members in the regulation of vegetative growth. The *AtSHI* gene has also been successfully overexpressed in three poinsettia cultivars 'Polar Bear', 'Early Prestige' and 'Millenium' to generate transgenic poinsettia with compact growth. The molecular analyses verified poinsettia *SHI* transformants, and morphological analysis showed reduced plant height when compared with the non-transformed controls. GA analyses are in progress and will improve our understanding about the effectiveness of expression *AtSHI* in poinsettia for the induction of dwarfism (Clarke et al., unpublished data).

Application of *rol*-genes for the generation of compact growth in ornamentals

Agrobacterium rhizogenes has for a long time been known to cause the hairy-root disease (Riker et al. 1930). The soil born bacterium *A. rhizogenes* is plant pathogenic and gives

rise to the development of the characteristic "hairy roots" at the site of infection. This characteristic symptom of hairy root disease is the formation of a mass of roots. Morphologically, a large number of small roots protrude as fine "hairs" directly from the infection site in response to *A. rhizogenes* infection in a range of plant species, a phenomenon that gave rise to the term "hairy root" (Tepfer 1990). After infection with *A. rhizogenes,* usually the whole T-DNA, located on a large root-inducing (Ri) plasmid, is inserted and integrated in the plant host DNA (Chilton et al. 1982). Several genes are present on the Ri plasmid and at least 18 open reading frames (ORFs) have been described. The most well-characterized genes are the root loci (*rol*) genes termed *rol*A, *rol*B, *rol*C and *rol*D, which coincides with ORFs 10, 11, 12 and 15, respectively (Slightom et al. 1986; White et al. 1985). Moreover, it has been shown that the ability of *rol*A, *rol*B and *rol*C is sufficient for the hairy root phenotype (Mariotti et al. 1989; Schmulling et al. 1988). Analysis of the other ORFs has indicated that several of these genes also influence plant morphology and hormone sensitivity (Lemcke and Schmulling 1998).

Bacterial *A. rhizogenes* strains are classified according to the type of opines they are causing the infected host plant to produce. The Ri plasmid of the cucumopine and mannopine type strains contain one T-DNA region (Hansen et al. 1991; Moriguchi et al. 2000). However, Ri plasmids where a second T-DNA is present have also been described in the agropine type strains of *A. rhizogenes*. These types are called split T-DNA with left (T_L) and right (T_R) T-DNA, both are usually in the range 15–20 kb and separated by an approximately 16 kb DNA fragment which is not integrated in the plant genome (White et al. 1985). The *rol*-genes are here located on the T_L-DNA whereas two auxin-homeostasis genes, designated *aux1* and *aux2* are located on T_R-DNA (Jouanin et al. 1987). Moreover, it has been demonstrated that the two T-DNAs are transferred and integrated independently into the host plant genome (Christensen et al. 2008; Jouanin et al. 1987).

Transformation of ornamentals with *rol*-genes typically results in plants with stunted growth, decreased internode length and reduced apical dominance resulting in an increased number of lateral shoots. The leaves are often thicker, wrinkled and reduced in size. However, it should be noticed that usage of individual *rol*-genes or combinations in constructs results in GM plants, while the use of the naturally occurring bacterial strains with recombinant DNA is a non-GMO technique. Several examples of obtained phenotypes of Ri-ornamentals are described in detail in the following paragraphs. Numerous ornamentals have been transformed with *A. rhizogenes*. In several plant genera or species, hairy roots regenerated spontaneously shoots e.g. in *Antirrhinum majus* and *Eustoma grandiflora* (Gennarelli et al. 2009; Handa 1992a, b), while in other plant species different types of cytokinins were essential to promote shoot regeneration, e.g. in *K. blossfeldiana* (Christensen et al. 2008). In some plant species, direct regeneration of shoots from hairy roots was unsuccessful and regeneration was only possible through somatic embryogenesis via callus. In other species, e.g. *Hibiscus rosa-sinensis*, no regeneration was possible to induce from the transformed roots (Christensen et al. 2009b). Typically, the degree of Ri phenotype in the regenerated plants varied among plant species, differs for various strains of *A. rhizogenes* due to dissimilar interaction of bacterial genes and plant genes, and is dependent on the actual transformation event, i.e. copy number of inserted T-DNAs, the insertion site of the T-DNA and transcriptional inactivation of T-DNA genes (Christensen and Müller 2009c). Furthermore, the phenotypic expression might be due to differences in copy number of T-DNA integrated into the plant genome, the integration site of the T-DNA or even the length of the insert (Tepfer 1984, 1990). In *Hypericum perforatum*, the transfer of T_L-DNA to plant explants has been shown to be incomplete with different *rol*A, *rol*B and *rol*C copy numbers. Furthermore, the highest copy number of transferred *rol*-genes resulted in weak expression of transgenic character (compactness) and comparable quantitative parameters compared to the controls (Komarovska et al. 2010). Additionally, it has been assumed that T-DNA gene products do not function in the same way in all host plants (Porter 1991).

In *Pelargonium*, scented species are appreciated for their pleasing odour, but due to long internodes they exhibit an elongated and disorganized growth habit. Ri-plants from scented *Pelargonium* were clearly more compact due to reduced plant height, and an increased number of leaves and side branches. Apart from the improved visual quality, obtained benefits comprised enhanced rooting and higher essential oil production (Pellegrineschi et al. 1994).

Several *Gentiana* species have successfully been transformed with *A. rhizogenes*. Ri-plants of *G. scabra* exhibited elongated internodes compared to the rosette phenotype of control plants. In addition, transformed plants had reduced apical dominance and intense lateral branching, leaves were wrinkled and root growth improved. Moreover, *G. scabra* transformed with *rol*-genes exhibited early flowering (Suginuma and Akihama 1995). Ri-plants of the interspecific gentian hybrid, *G. triflora* × *G. scabra*, exhibited different degrees of compactness, reduced apical dominance and had very branched shoots with smaller and elliptically formed leaves. Furthermore, Ri-plants had increased flowering, were compact with reduced height and intensive branching (Hosokawa et al. 1997; Mishiba et al. 2006). In another gentian hybrid, *G. cruciata* ×

G. purpurea, *rol*-genes resulted in Ri-plants with short internodes and small leaves (Momcilovic et al. 1997).

In *E. grandiflorum*, conventionally used as cut flower, the creation of dwarf phenotype for use as potted plant was attempted. By *A. rhizogenes* transformation Ri-plants of *E. grandiflorum* were produced by applying two different *A. rhizogenes* strains. The approaches resulted in Ri-plants with short internodes and changed flower shape (Giovannini et al. 1996; Handa 1992b). Furthermore, in the study by Handa (1992b), transformed plants had small wrinkled leaves, reduced fertility and an increased rooting. Giovannini et al. (1996) produced Ri-plants that exhibited varying degrees of changed morphology with a decrease in plant height, enhanced number of internodes and better rooting capability. In *Catharanthus roseus*, Ri-plants exhibited abundant rooting and short internodes. Half of the obtained Ri-plants had wrinkled leaves, while the leaves of other half appeared normal (Choi et al. 2004). *Datura sanguinea* transformed with *rol*-genes displayed compactness due to reduced plant height and shorter internodes. The Ri-plants had an increased number of dark and small leaves, additionally rooting was improved (Giovannini et al. 1997). In *Calibrachoa excellens*, transformation with *rol*-genes resulted in both wrinkled leaves and altered flowers, both in terms of colour and size as one line had larger flowers and another line had smaller flowers (Gennarelli et al. 2009). Ri-plants of *Antirrhinum majus* exhibited dwarf growth with short internodes and decreased apical dominance. The leaves were small, fertility was reduced and in some lines sterility occurred. Additionally, number of flowers was increased due to the increased number of side branches (Handa 1992a; Hoshino and Mii 1998). In *Bacopa monnieri*, growth and biomass accumulation was significantly higher in the transformed shoots (twofold) and roots (fourfold) than in the non-transformed (WT) plants. This caused a shift in the biomass accumulation from the shoot to the root (Majumdar et al. 2011). Altered biomass distribution was also observed in *Kalanchoë* where a greater part of dry matter was allocated into leaves (Christensen et al. 2009a). These examples represent only a small selection from the numerous studies, reporting transformation with *rol*-genes to improve quality in ornamental plants.

Kalanchoë is one of the plants where the effect of the *rol*-genes has been studied in detail. Early studies using individual *rol*-genes driven by the constitutive 35S promoter resulted in compact plants (Spena et al. 1987). Similarly, regenerated *K. blossfeldiana* plants from hairy roots, derived from transformation using unmodified *A. rhizogenes* strains exhibited retarded plant growth, such as short internodes, highly branched aerial parts, changes in flower size and increased rooting, but also atypical leaf morphology (Christensen et al. 2008). Moreover, the Ri-plants had improved postharvest quality due to reduced ethylene sensitivity (Christensen and Müller 2009a, b). Further studies and progeny analysis revealed that the *rol*-genes were inherited together in both F1- and F2-lines and the presence of *rol*-genes was confirmed in all F1 and many F2 plant lines exhibiting dwarfism. Besides decreased plant height, several F1 and F2 lines containing *rol*-genes exhibited changes in plant diameter, number of branches, flower diameter and time to first open flower and duration of flowering compared to. Moreover, reduced ethylene sensitivity was documented in several plant lines containing *rol*-genes compared to the WT 'Sarah' (Christensen et al. 2010; Lütken et al., unpublished data).

Reduced fertility or even sterility has been reported in Ri-plants from a number of ornamental plant species. Low fertility does not appear to be a big problem in ornamental plant production since many flowering potted plants are vegetatively propagated (Christensen et al. 2008). Nonetheless, some fertility is needed within ornamental breeding and the possibility to create fertile progeny from Ri-plants has been documented in *Kalanchoë* (Christensen et al. 2010; Lütken et al. unpublished data).

Transformation with *rol*-genes has also resulted in other beneficial properties, e.g. enhanced production of secondary metabolites have been reported (Bulgakov 2008; Pellegrineschi et al. 1994). Moreover, in some species transformation with *A. rhizogenes* has circumvented the need for vernalisation (Sun et al. 1991; Tepfer 1984).

The distinct morphology of the hairy roots can be used directly in the selection process as primary indicators of transformation. Hence, this marker-free selection offers an advantage to the commonly used antibiotic resistance markers used in plant selection. The use of antibiotic resistance marker genes, which are common in the development of transgenic plants, has been banned in the European Union and since 2008 plants containing antibiotic resistance genes are prohibited (European Union 2001).

For a more comprehensive overview about the use of *rol*-genes in floriculture, the research within the field has been reviewed by (Casanova et al. 2005; Christensen and Müller 2009c; Christey and Braun 2005).

Improvement of tolerance towards ethylene

Apart from growth regulators, several ornamental plant species are treated with chemicals to improve postharvest quality and prolong flower longevity. Many of the applied postharvest treatments towards reducing the impact of ethylene contain silver ions and are toxic for humans and harmful to the environment (Nell 1992). A new generation of more environmentally safe chemicals is presently

finding its way from research into the market for fruits and more recently for ornamentals (Watkins 2008). Moreover, these substances can inhibit ethylene responses effectively for a longer time period, although not for all plants species and flower types (Müller 2011). With an increased focus on environmentally safe production, postharvest treatments of flowering plants are a crucial field of interest. Discovering and assessment of save postharvest treatments as well as developing alternative technologies targeting elements of ethylene perception and signalling are of crucial importance.

The role of ethylene in flower senescence

The plant hormone ethylene controls various aspects of plant growth and development. The most important developmental and physiological functions are seed germination, root initiation, flower induction, development and senescence, fruit ripening, as well as biotic and abiotic stress responses. Due to its role as key regulator of fruit ripening and flower senescence, ethylene is of extraordinary economic importance in floriculture and for fruit industries. Gaseous ethylene is biologically active in trace amounts and its effects are of great commercial importance; substantial postharvest losses and quality decrease of perishable plant products are linked to ethylene effects. In floricultural industries, both cut flowers and ornamental potted plants, ethylene has significant importance for quality, flower longevity and postharvest performance of the plant or individual flower. Quality losses of potted flowering plants due to drop of flower buds or abscission of mature flowers as a consequence of ethylene in the environment are a common difficulty in ornamentals, e.g. in potted roses (Müller et al. 1998, 2001a).

Regarding their ethylene response, flowers are classified as being climacteric or non-climacteric. In climacteric flowers, senescence processes are accompanied by a clear, transient rise in ethylene production and respiration. Flowers categorised as climacteric are usually, but not always, sensitive to exogenous ethylene and treatment of non-senescent flowers with ethylene typically hastens flower senescence. Commercially important examples of flowers with climacteric senescence are carnations, orchids, *Kalanchoë*, *Campanula* and roses (Müller et al. 1998, 2000a; Serek et al. 2006; Sun et al. 2009; Woltering and Harkema 1987). In non-climacteric flowers, no increase in ethylene production and respiration accompanies flower senescence processes, and these flowers are typically not or only slightly sensitive to ethylene in the surrounding environment. Economically important plant genera in this category are tulips, gladiolus and iris (Woltering and van Doorn 1988). In plant genera with ethylene-independent flower senescence, other developmental aspects may be regulated by ethylene responsive and other plant parts can exhibit sensitivity to the plant hormone ethylene (Serek et al. 2006). Susceptibility to ethylene is roughly found to be determined at the family level (Woltering and van Doorn 1988). High sensitivity to ethylene has been documented in the dicotyledonous families Campanulaceae, Caryophyllaceae, Geraniaceae, Lamiacae, Malvaceae, Orchidaceae, Primulaceae, Ranunculaceae and Rosaceae, while low sensitivity to the phytohormone was documented in the families Asteraceae, Liliaceae, Iridaceae and Amarylidaceae. Even though the categorization 'ethylene sensitive' or 'ethylene insensitive' in broad sense is fixed at the plant family level, significant differences are found among species and cultivars within a family (Müller et al. 1998, 2001a).

Approaches to inhibit negative effects of ethylene on ornamental plants

The negative effect of endogenous and exogenous ethylene on quality of ornamental plants with respect to their flower senescence and organ abscission can be inhibited, or at least reduced, with different strategies (Fig. 3):

- Inhibition of endogenous ethylene production through changes in temperature or atmospheric composition
- Application of inhibitors of ethylene biosynthesis
- Blocking the ethylene-binding site with an inhibitor of ethylene action (antagonist)

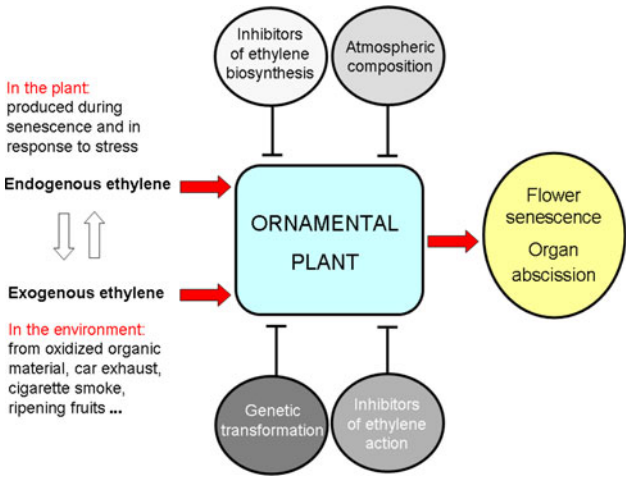

Fig. 3 Effects of ethylene on ornamentals and inhibition of its biosynthesis and action. Plant quality, flower senescence and organ abscission, is determined by endogenous and exogenous ethylene. The plants' endogenous ethylene production can be repressed by temperature, chemicals or atmospheric composition. In contrast, ethylene inhibitors or genetic modification conferring insensitivity can inhibit the impact of both exogenously occurring ethylene as well as the ethylene endogenously produced (with permission from Müller 2011)

- Genetic modification, e.g. insertion of mutated *ETR1* (*ethylene response 1*) or isopentenyl transferase (*IPT*)

The efficiency of the methods used to minimise ethylene effects depends on the specific method, the targeted components in ethylene biosynthesis or signal transduction and is reliant on the specific plant species. However, the overall usefulness of the different approaches to be applicable in the production and postharvest chain differs greatly (Müller 2011). Inhibition of the plants' endogenous ethylene production by temperature or atmospheric composition is mainly used in horticultural practices when fruits and vegetables are stored. The temperature is the key determinant for ethylene responses, and lowering the temperature reduces ethylene damage significantly. However, many of the economically important ornamental plants come from tropical and subtropical regions and thus demand higher temperatures than 12°C (Høyer 1997; Serek et al. 2006). While storage at increased carbon dioxide level and reduced oxygen is commonly used to store fruits in controlled atmosphere storages, this technique is not used for postharvest environment of ornamental plants (Serek et al. 2006). However, it appears difficult to store and transport potted plants in gas tight units with increased carbon dioxide.

Application of inhibitors of ethylene biosynthesis does not protect the plants from ethylene pollution in the environment. Thus, strategies aiming at blocking ethylene biosynthesis have limited practical use because of potential quality reduction of the commodity due to ethylene in the surrounding atmosphere. Blocking the ethylene-binding site with an inhibitor of ethylene action, in contrast, protects climacteric plant species effectively against the negative effects of ethylene, both endogenously produced and from the environment (exogenously). Although numerous chemicals have been assessed for their usefulness as inhibitors of ethylene action (Serek et al. 1994; Sisler et al. 2006; Sisler and Serek 1997, 2003), presently only silver thiosulphate (STS) and 1-methylcyclopropene (1-MCP) have economical importance in floriculture. Silver ions have been documented to be a powerful inhibitor of ethylene action in ornamental plants and the widely used formulation of STS blocks the effects of ethylene-binding to the receptor (Sisler et al. 1986; Veen 1983). However, silver is a heavy metal, toxic for humans and detrimental for the environment (Nell 1992). In many countries, STS is not longer approved as an agent to extend flower life, thus, there is an urgent need for finding alternatives to the use of STS. 1-MCP is a cyclic olefin, which irreversibly binds to the ethylene receptor. 1-MCP was evaluated as being a non-toxic inhibitor of ethylene action and has been reviewed thoroughly by Sisler and Serek (1997, 2003), Sisler et al. (2006) and Blankenship and Dole (2003). Both inhibitors, STS and 1-MCP, improved postharvest characteristics of, e.g. *Campanula carpatica*, *Schlumbergera truncata* (Serek and Sisler 2001), potted roses (Müller et al. 2000b, 2001b) and *Phalaenopsis* hybrids (Sun et al. 2009), suggesting that 1-MCP is an effective and environmentally friendly substitute for STS. When compared to STS, however, 1-MCP is in most cases, however, less effective (Blankenship and Dole 2003; Müller 2011).

Genetic modification has successfully been applied to engineer the ethylene biosynthesis pathway and inhibiting ethylene production or by reducing ethylene sensitivity by targeting perception. Ethylene is synthesised from the precursor amino acid methionine, from which adenosyl methionine (SAM) is catalysed. The last two steps in the ethylene biosynthesis are conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC to ethylene. The rate-limiting enzymes in this pathway are ACC synthase and ACC oxidase. Strategies reducing sensitivity have mainly targeted ethylene receptors, these approaches will be presented more in detail in the following paragraphs. However, other approaches have also generated plants with alterations in ethylene sensitivity even though some of these studies did not have this trait as a main target of quality improvement (e.g. natural transformation with *rol*-genes, see above).

Genetic modification targeting genes of the ethylene biosynthetic pathway

With the identification and characterisation of key enzymes regulating ethylene biosynthesis, ACC synthase and ACC oxidase (Argueso et al. 2007), genetic modification of ethylene production in plants has become possible. A number of physiological responses have been modified in a range of plant species. The effects of decreased ACC oxidase activity in ornamental plants were studied in *Dianthus*, *Begonia* and *Torenia* (Czarny et al. 2006; Müller and Stummann 2005; Stearns and Glick 2003). Using an *ACC oxidase* cDNA from *Dianthus*, transgenic carnation plants expressing an antisense *ACC oxidase* gene were created under a constitutive promoter (Savin et al. 1995). Flowers of the transformed *Dianthus* cultivars 'Scania' and 'White Sim' exhibited a distinct reduction in ethylene production and clearly delayed flower senescence (Savin et al. 1995). In *Begonia* × *cheimantha*, an approach with antisense *ACC oxidase* resulted in transgenic plants with improved longevity similar to that of non-transformed control plants sprayed with STS (Einset and Kopperud 1995; Hvoslef-Eide et al. 1995). Transgenic *Torenia* with an antisense *ACC oxidase* gene had a vase life up to 3.5 times that of wild type plants and also produced more flowers (Aida et al. 1998). However, plants with inhibited ethylene production by genetic engineering are still

sensitive to exogenous ethylene. Hence, it is predictable that a strategy aiming at blocking ethylene perception is more effective for improving flower longevity and postharvest performance in ornamental plants, since ornamentals with blocked ethylene action are protected against exogenous as well as endogenous ethylene (Müller 2011; Müller and Stummann 2005).

Genetic modification targeting ethylene perception

Modification of ethylene perception or signalling seems to be a promising approach to protect ornamental plants from the detrimental effects of ethylene. In case of a successful transformation strategy, these plants will be protected both against their own ethylene production and against ethylene, potentially present in the surrounding environment (Fig. 3) (Müller 2011).

In *Arabidopsis,* the gene *ETR1* (*ethylene response 1*) encodes a two-component histidine kinase-like ethylene receptor that in the mutant *etr1-1* fails to bind ethylene (Bleecker et al. 1988; Chang et al. 1993). The mutant allele *etr1-1* confers ethylene insensitivity. Until now, five members of the putative ethylene receptor family have been cloned from *Arabidopsis*, belonging to two subfamilies. Subfamily 1 comprises ETR1 and ERS1, while subfamily 2 consists of ETR2, EIN4 and ERS2. With the isolation of ethylene receptor genes and identification of dominant mutated genes providing ethylene insensitivity it has become possible to inhibit ethylene responses in plants. Introduction of the mutated gene *etr1-1* isolated from *Arabidopsis* into *Petunia* conferred ethylene insensitivity to this ornamental plant species (Clark et al. 1999; Clevenger et al. 2004; Gubrium et al. 2000; Wilkinson et al. 1997). The obtained ethylene tolerance clearly delayed flower senescence and postponed flower abscission. The postponed flower senescence was documented in the presence and absence of ethylene, and in both pollinated and non-pollinated flowers. In these comprehensive studies, a constitutive promoter was used and subsequently negative effects were also observed in the transgenic plants such as poor rooting, low seed weight, reduced seed germination and decreased pollen viability.

To avoid negative side effects of the gained insensitivity to ethylene during growth and developmental processes, Bovy et al. (1999) used a flower-specific promoter from *Petunia* (fbp1) in their transformation approach with *etr1-1* in *Dianthus caryophyllus*. The resulting carnation flowers exhibited a strong insensitivity to ethylene and an improved postharvest performance in an ethylene-free environment and in response to exogenous ethylene (Bovy et al. 1999). Subsequently, the same gene construct has successfully been used in *C. carpartica* (Sriskandarajah et al. 2007), *K. blossfeldiana* (Sanikhani et al. 2008) and *Odontoglossum* and *Oncidium* (Raffeiner et al. 2009) as a tool for reduced ethylene sensitivity (Mibus et al. 2009).

Shaw et al. (2002) obtained ethylene insensitive *Petunia* using a mutated *ERS* homologue from *Brassica oleracea*. Like *etr1-1*, this gene encodes an ethylene receptor that is not able to bind ethylene. The results from this approach resembled those obtained with the *etr1-1* gene and the flowers were larger and had longer flower longevity than for control flowers. However, analysis of the mutants revealed reduced disease resistance. In another approach, the ornamental plant *Nemesia strumosa* was transformed with a mutated *ETR1* homologue from melon, with comparable results of a longer flower life (Cui et al. 2004).

Downstream in the signalling pathway from the ethylene receptors the CTR1 protein was identified from the mutant *constitutive triple response* (*ctr1*), exhibiting constitutive ethylene response in the absence of the hormone. *CTR1* (and its homologues) encodes a Raf-like serine/threonine kinase that interacts with the receptors (Clark et al. 1998; Kieber et al. 1993). In several plants including *R. hybrida*, more than one *CTR1* homologue has been identified (Müller et al. 2002). Downstream of the receptor- CTR1 complex is EIN2 (ethylene insensitive 2), which is a positive regulator of the ethylene response (Alonso et al. 1999). In *Petunia*, a transgenic approach aiming for reduced expression of the gene *PhEIN2* distinctly delayed flower senescence. Some negative side effects were obtained similar to those in transformants with *etr1-1* including inhibition of adventitious roots. An even more striking reduction in ethylene sensitivity was obtained when reduced *PhETR1* expression and the transformation with *etr1-1* were combined (Shibuya et al. 2004).

Transformation with *IPT*

Another promising strategy towards improved postharvest quality and reduced ethylene sensitivity is the integration of the *IPT* gene from *A. tumefaciens*. This strategy has been pursued in some ornamental plants, like *Petunia* (Chang et al. 2003) and rose (Zakizadeh et al., unpublished data). The IPT protein catalyses the first and rate-limiting step of cytokinin production. Moreover, it is known that cytokinin applications delay senescence and are associated with reduced ethylene biosynthesis (Chang et al. 2003). Hence, when inserted into plants this gene can increase cytokinin concentration and inhibit senescence processes. Due to the fact that a high cytokinin concentration implies negative effects on physiological and morphological processes, a senescence-specific promoter appears to be a suitable strategy to improve postharvest quality. *SAG12* is a highly senescence specific gene, expressed in an age-dependent manner during the onset of senescence (Gan and Amasino 1997). Thus, its promoter can be used to regulate the

expression of the cytokinin biosynthesis gene *IPT* resulting in an autoregulatory senescence inhibition system. In the construct P_{SAG12}:IPT, the *Arabidopsis* SAG12 promoter was fused to the *IPT* gene from *A. tumefaciens*. Consequently, the construct should upregulate the expression of *IPT* at the onset of senescence, leading to elevated cytokinin levels and thus prevent senescence. The retardation of senescence attenuates promoter activity and thus prevents cytokinin overproduction (Gan and Amasino 1995). The cytokinin content rose after stress induction when the *IPT* gene was placed behind the SAG12 promoter and enhanced the ethylene tolerance in *Petunia* and roses significantly. Furthermore, in *Petunia* flower longevity was prolonged (Chang et al. 2003). Approaches using other promoters have been pursued in *Petunia* and *Dendranthema*. Application of a cold-inducible promoter driving the expression of *IPT* improved both dark and cold tolerance (Khodakovskaya et al. 2005). In contrast, the *LEACO1* promoter led to compactness and increased amount of flowers (Khodakovskaya et al. 2005, 2009) illustrating the significance of the specific promoter applied.

Perspectives

Environmentally and health-friendly production methods of ornamental plants have become crucial for reaching the goal of a more sustainable plant production and protection of the environment. Due to their negative impacts on human health and hazardous effects on the environment, numerous chemicals used in modern plant production have lost their approval and further legal restrictions can be expected. Hence, development of methods and technologies to reduce application of chemicals used to control plant growth and to improve postharvest performance are needed. In the form of genetic engineering, several alternatives to growth retardants are currently applicable comprising recombinant DNA approaches targeting involved hormone pathways and a natural transformation approach without recombinant DNA technology. In the recombinant approaches, overexpression of *GA2ox* genes and reducing expression of *GA20ox* genes as well as modulation of regulatory genes involved in GA perception and signal transduction (*GAI, SHI*) and overall hormone homeostasis (*KNOX*) have successfully led to compact plants. In a natural transformation approach, unmodified strains of *A. rhizogenes* can be used as a non-GMO method to develop compactness. Similarly, metabolic engineering approaches targeting elements of the ethylene signal transduction pathway and its perception (e.g. *etr1-1* and *IPT*) serve as possible alternatives to avoid the use of chemical ethylene inhibitors. In conclusion, the molecular breeding approaches represent vast opportunities as alternatives to chemical treatments, however, in several studies unwanted effects (e.g. leaf and flower alterations) have been encountered and the effects typically vary among plant species. Ornamental plants seem to be an ideal product for quality improvement by transformation approaches, production cost are high and classical breeding is difficult because of high ploidy levels. All GM approaches greatly contributed to increase knowledge and understanding of growth and developmental processes in respect to the genes used in the approaches, independent from the usefulness of the approaches in product development. Genetic engineering has potential to create ornamental plants suitable for environmentally friendly production; however, consumers and retailers scepticism against GMO's as well as costly patents and registration procedures have to be taken into consideration to give the full picture.

Conflict of interest The authors declare that they have no conflict of interest.

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