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Genetic Manipulation of Gibberellin (GA) Oxidase Genes in *Nicotiana sylvestris* using constitutive promoter to modify Plant Architecture

Bhattacharya Anjanabha [1,2*], Power John B[1] and Davey Micheal R[1]
[1]Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[2]BBIO, Jai Research Foundation, Vapi, Gujarat, INDIA

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Abstract

Gas is a large group of tetracyclic diterprenoid carboxylic acids. Gibberellins (GAs) control many aspects of plant development, including plant development, flowering, leaf expansion and growth. Leaf explants of Nicotiana sylvestris (Solanaceae) were used for Agrobacterium-mediated delivery of a range of GA-biosynthetic genes to determine the influence of their encoded enzymes on the production of bioactive GAs and plant stature in this species. Constructs were prepared containing the nptII gene for kanamycin resistance as a selectable marker, and the GA-biosynthetic genes, their expression under the control of the CaMV 35S promoter. The GA-biosynthetic genes comprised of PcGA2ox1 isolated from Phaseolus coccineus, and, is specific for C_{19}-GAs and 2β-hydroxylates the bioactive GAs i.e. GA_1 and GA_4 and their immediate precursors GA_{20} and GA_9, respectively. AtGA20ox1, isolated from Arabidopsis thaliana, the product from which catalyses the formation of C_{19}-GAs, and MmGA3ox1 and MmGA3ox2, isolated from Marah macrocarpus, which encode functionally different GA 3-oxidases that convert C_{19}-GAs to biologically active forms. Increase in stature was observed in plants transformed with AtGA20ox1, MmGA3ox1, MmGA3ox2 and MmGA3ox1 + MmGA3ox2, their presence and expression being confirmed by PCR and RT-PCR, respectively, accompanied by an increase in GA_1 content, while PcGA2ox1 resulted in dwarf plant with four fold reduction of height and early flowering. The results are discussed in the context of regulating plant stature. Since this strategy would decrease the use of chemicals to promote plant growth and will result in value addition in ornamental industry, in an era of increasing demand, and ever changing consumer appetite.

Keywords: Agrobacterium transformation, gibberellin 2-oxidase, gibberellin 3-oxidase, gibberellin 20-oxidase, Arabidopsis thaliana, Marah macrocarpus, Nicotiana sylvestris.

Introduction

The demand for ornamentals has increased steadily in the international market for the past few decades, which has been influenced, in turn, by the rising disposable income of persons in the developed countries. Emphasis is on quality of ornamentals plants [1] has been also increasing this decade with fierce competition. These further emphasize the importance of floriculture products. The role of gibberellin (GA) has been linked to the control of plant stature [2]. GAs are generally used commercially in poinsettia (*Euphorbia pulcherrima*), carnation (*Dianthus caryophyllus*) and other flowering species to promote flowering, and to break the dormancy of seeds, bulbs and corms. GAs are also important in regulating agronomically important traits, like plant height and flowering, by increasing cell division and elongation. Altering the amount of endogenous plant growth regulators (in this case GA) in plants can potentially modify their growth and development[3, 4,5]. Therefore, regulation of GA biosynthesis introduced by a transgenic plant approach may lead to greater returns than the conventional method of plant improvement in the ornamental industry. Chemical growth substances are being used extensively for the modification of stature[6]. Unfortunately, their usage has added to the cost of crop production, increased manpower requirements to spray the chemicals and contributed to environmental risks by releasing toxic substances upon degradation in the soil. Further, repeated applications of these chemicals are needed in order to achieve the desired results which are highly variable. These chemical growth substances are classified as pesticides and rigorous plant protection regulations, which apply to hazardous chemicals, also apply to their use [chemical forms of GAs, such as commercial preparation of fungal GA_3 and, anti-GA compounds like paclobutrazol, ibenfide, tetracyclis and prohexadione which interferes with anthryocyanin synthesis (involved in floral colour)[6]. Plant growth regulators like GAs are not generally used for field crops as they may cause undesirable effects, such as lodging. The use of photoselective filters can also be used to modify plant stature[7,8], but is not practical. Therefore, it can be argued that the use of genetic manipulation techniques for altered growth could help preserve the environment and, in turn, benefit human health. The public acceptance of GM technology is low especially in Europe, as it is considered to generate unacceptable risks [9]. This is not the case for other developed and developing countries. This concern is primarily seen as a potential risk of the flow of transgenes to wild species. The application of growth retardants is a common commercial practice to inhibit stem elongation of ornamental species to produce compact plants, suitable for growing in pots. The

effects of growth retardants are similar to those found in gibberellins-deficient mutants. However, the cost of chemical growth retardants and also regulators, the general concern regarding applications of agrochemicals, leads to the search for alternative ways of modifying plant stature. The GA biosynthesis pathway has been established and most genes encoding GA-biosynthetic enzymes, including the GA 2-oxidase (*GA2ox*), GA 20-ox and GA 3-ox genes, which encode GA-deactivating enzymes, have been identified [10].

Material and methods

Plant material: Plants of Nicotina sylvestris were grown from seed and maintained under glasshouse conditions in 6:6:1:1 by volume of a mixture of Levington M3 compost (Scotts UK, Ipswich, UK), John Innes No. 3 compost (J. Bentley, Barrow-on-Humber, UK), Perlite (Silvaperl, Gainsborough, UK) and Vermiculite (Silvaperl). Natural daylight in the glasshouse was supplemented with 16h of fluorescent illumination (195 µmol m^{-2} s^{-1}; TLD/58W 35V "Daylight" fluorescent tubes; Phillips, Croydon, UK) with day and night temperatures of 25 ± 1°C. Material for transformation was obtained from the uppermost fully expanded leaves excised from 4-10 weeks-old plants and surface sterilised by immersion in 10% (v/v) "Domestos" bleach (Diversey Lever Ltd., Northampton, UK) for 10 min, followed by 3 washes with sterile, reverse osmosis water. The midribs were removed from each leaf and the laminae were cut into 1cm^2 explants under axenic conditions.

Constructs for *Agrobacterium* transformation of leaf explants *PcGA2ox1*: The details of the construct, is explained by Coles and Cowerkers[20].

MmGA3ox1 and MmGA3ox2: The coding regions were amplified from *Marah macrocarpus* by Polymerase Chain Reaction (PCR) using gene-specific primers *i.e. MmGA3ox1* forward 5'-CCCGATATCATGGCAGATCAGGAGATTACT-3' and reverse 5'-GGGCTCGAGCTAAATTAAAGATGATATTTTTACGG-3' (1083bp product), *MmGA3ox2* forward 5'-CCCGATATCATGGCCACCAAAATAACCGAC-3' and reverse 5'- GGGCTCGAGTTAGCCTACTTTGACCTGACT-3'(1131bp product). After sub-cloning into pCR2.1 (Invitrogen, Groningen, The Netherlands), genes were inserted separately into the *Sac*I site of the binary vector pLARS120 adjacent to the CaMV 35S promoter. The T-DNA of pLARS120 also contained the neomycin phosphotransferase (*npt*II) gene with the *nos* promoter, located next to the left border of the T-DNA.

AtGA20ox1: The cDNA clone was amplified by PCR using the forward primer 5'-CGGTTTCTTCCTCGTGGTCA-3' and the reverse primer 5'-GTGACTTCCTCTCGCTCTTG-3' (677 bp product). The fragment was inserted into the *Xba*I site of the binary vector pCIB200, which contained the *npt*II gene as a selectable marker [21].

Agrobacterium tumefaciens-mediated plant transformation: *A. tumefaciens* strain LBA4404 was transformed as described [22], the binary vectors carrying *MmGA3ox1*, *MmGA3ox2* or *AtGA20ox1*, and cultured in 100ml aliquots of Luria Broth with 40-50 mgl^{-1} kanamycin sulphate and 100 mgl^{-1} streptomycin (50 mg l^{-1}) in 500 ml Erlenmeyer flasks. Cultures were maintained on a rotary shaker (150 rpm) at 27 ± 1°C for 16 h in the dark; those with an OD_{600nm} of 0.7 - 1.2 were used to inoculate leaf explants.

Transformation of leaf explants: Explants were immersed for 5 min in suspensions of *A. tumefaciens*, the latter being diluted immediately before use 1:10 (v:v) with liquid Murashige and Skoog-based culture medium (containing 30 gl^{-1} sucrose, but lacking growth regulators; designated MS0) at pH 5.8. Control explants were treated similarly, except that agrobacteria were omitted from the inoculation medium. Following inoculation, explants were blotted dry on sterile filter paper and transferred to the surface of 25 ml aliquots of MS-based medium containing 1.0 mgl^{-1} zeatin and semi-solidified with 8 gl^{-1} agar (designated MSZ; 8 explants/9 cm Petri dish).

Inoculated explants were maintained at day/night temperatures of 22 ± 1°C and 20 ± 1°C, respectively, with a 16 h photoperiod and a light intensity of 19.5 µmol m^{-2} s^{-1} "Daylight" fluorescent tubes (Thorn EMI Ltd, Hayes, UK). After 2d, leaf explants were transferred to semi-solid MSZ medium supplemented with cefotaxime (500 mgl^{-1}) and kanamycin sulphate (50 mgl^{-1}). Uninoculated explants were transferred to medium either lacking antibiotics to regenerate non-transformed plants for comparison with transgenic plants, or to medium with antibiotics, as used for explants inoculated with *A. tumefaciens.* Cultured leaf explants, which formed callus after 8 - 10 weeks were transferred to 175 ml screw-capped jars (Beatson Clarke and Co., Rotherham, UK), each containing 50 ml of semi-solid MSZ medium, supplemented with the same antibiotics as used previously.

Regenerated shoots were excised from leaf-derived calli and rooted on semi-solid (0.8% w/v agar) MS0 medium with kanamycin at 50 mgl^{-1}. Rooted plants were potted in the same mixture as used for seedlings. Potted plants were covered with 17 cm x 15 cm plastic bags and transferred to the glasshouse under natural daylight. The tops of the bags were opened progressively over a 14 d period.

Phenotypic analyses: The phenotypic characteristics of each plant (height, internode length, leaf length and width) were recorded 12weeks after acclimation of the plants to glasshouse conditions. Transgenic (n=9) and control (n=6) plants were allowed to flower and self-pollinate.

PCR analysis: Primers were manufactured and sequenced by MWG Biotech, Ebersberg, Germany. The sequences used were forward 5'-GCTCTTCGCTCTTCCAAC-3' and reverse 5'-ACCTGTCTGCTAAACCCTTC-3' (*MmGA3ox1*), forward 5'-CCCGATATCATGGCCACCAAAATAACCGAC-3', reverse 5'-GGGCTCGAGTTAGCCTAC TTTGACCTGACT3' (*MmGA3ox2*), forward 5'-GAGAATTCAAAATGGCCGTAAAGTTTCG-3', reverse 5'CGCTCTAGAACTAGTGGATC-3' (*AtGA20ox1*), forward 5'-AGACAATCGGCTGCTCTGAT-3', and reverse 5'-ATACTTTCTCGGCAGGAGCA-3' (*npt*II). Template genomic DNA was extracted from plants using a GenElute Plant Miniprep kit (Sigma-Aldrich, Missouri, USA). PCR was performed using RED Taq Ready Mix (Sigma-Aldrich) according to the manufacturer's instructions.

Amplification was performed in a DNA Thermal Cycler 480 (Perkin Elmer Applied Biosystems Division, Warrington, UK), with initial denaturing (1 cycle, 94ºC, 3 min), denaturating (35 cycles, 94ºC, 1 min), primer annealing [35 cycles; 57ºC (*npt*II), 53ºC (*AtGA20ox1*) or 61ºC (*MmGA3ox1* and *MmGA3ox2*), 1 min], primer extension (35 cycles, 72ºC, 90 sec), final extension (1 cycle, 72ºC, 10 min) and holding at 4ºC (5 min to ∞).

For Reverse Trasnscriptase-PCR (RT-PCR) analysis, RNA was extracted from 100 mg leaf samples of putatively transformed and non-transformed plants using an RNeasy® Plant Mini Kit (Qiagen, Crawley, UK). RT-PCR employed a One Step RT-PCR Kit (Qiagen) according to the manufacturer's instructions. The amplification programme involved reverse transcription (1 cycle, 50ºC, 30 min), polymerase activation (1 cycle, 94ºC, 15 min), denaturation (35 cycles, 94ºC, 1 min) and primer annealing (35 cycles, 55ºC, 1 min). Subsequent conditions were as for PCR analysis.

GA Purification using HPLC (High Pressure Liquid Chromatography) And Quantification Using GC-MS (Gas Chromatography-Mass Spectrometry) Technique.

Freeze dried leaf tissues (500mg) were ground in a ball mill and then resuspended in 100ml of 80% (v/v) aqueous methanol. Internal standards (a mixture of ^{2}H- and ^{3}H- labelled GAs) were added to the resultant mixture GA extraction and purification was done following steps as described in [20]. 500mg of freeze dried leaf samples were taken and replicated twice.

Statistical Analysis: All phenotypic data were subjected to Student's t-test. The F test was used with ANOVA when comparison was made of multiple distributed populations. Standard MINITAB version 15, statistical software (Minitab Inc., PA, USA) was employed in all t and F tests.

Results and Discussion

Uninoculated leaf explants cultured on semi-solid MSZ medium with kanamycin, became necrotic and failed to regenerate shoots. Those on medium without kanamycin developed shoots that were used as controls. Negative segregants were also included as controls wherever feasible. However, no significant difference was found in growth and development parameters between control and negative segregant plants (p=0.05). Dot blot analysis for the presence of transgenes was also carried out wherever feasible (data not shown). Transgenic study was restricted to primary transformants only.

PcGA2ox1: Twenty putative transformed shoots were obtained, of which 14 were randomly transferred to jars for rooting and numbered 1-14. The transformation efficiency was 7%. A total of 11 putative transgenics were analysed for the presence of the *PcGA2ox1* and *npt*II genes. Five plants showed the presence of both genes as confirmed by RT-PCR, and were selected and transferred to glasshouse with 3 controls for phenotypic characterization. RT-PCR was conducted to ascertain the expression of the transgene (s). The Student's t-test results at 0.01 probability revealed that transgenic plants expressing *PcGA2ox1* had significantly reduced height (4 fold reduction), internode lengths (4 fold reduction), although a few plants failed to show transgene expression and were semi-dwarf, which may be related to the time of onset of such silencing and plant height. Chlorophyll a (18%), chlorophyll b (43%), total chlorophyll (30%) and carotenoid content [24%] (mg g^{-1}) were reduced compared to the wild - type control plants. Seed development was inhibited in *PcGA2ox1* transgenic plants due to a reduced style length. The transgenic plants had a compact floral stalk compared with the wild-type (control) plants. The efficacy of GA_1, GA_3, GA_4 and GA_7 application (50µg ml^{-1}, applied twice at weekly intervals, first application being one week after post acclimation) was measured on restoration of plant growth / responsiveness of transformed *PcGA2ox1* plants to applied GAs. In nature, GA_3 and GA_7 are more stable than GA_1 and GA_4 due to their double bond between carbon, C_2 and C_3, and therefore are not inactivated by GA 2-oxidase (*PcGA2ox1*) enzymes. The results confirmed that GA_3 and GA_7 applications to *PcGA2ox1* transformants resulted in rapid restoration of plants growth compared to GA_1 and GA_4 which recovered marginally compared to the control (*PcGA2ox1* plants with water spray), presumably because the applied GAs were mostly deactivated by the GA 2-oxidase enzyme. The GA analysis (ng g^{-1} dry weight) confirmed that GA_1 content was less in transgenic lines compared to the control plant(s) under investigation.

MmGA3ox1: Transformation efficiency was 3%. Phenotypic data were recorded when plants were 12 weeks old, post-acclimation. The Student's t-test was conducted at the 0.01 level of probability. The results of the student's t-test showed that plants carrying *MmGA3ox1* had statistically significant plant height, which varied from 140.4cm (plant No. 42) to 164.8cm (plant No. 11) *i.e.* an 18% increase over the control (mean plant height of 134.6cm). Similarly, the internode length in the transgenic plants (mean 13.9cm) was 17.6% greater than control (mean value 11.8cm). RT-PCR confirmed the expression of the

transgene. The transgenic plants responded to applied GA_9, which demonstrated the greater ability of the transgene to transform prebioactive GA_9 to bioactive GAs (GA_1 and GA_4).

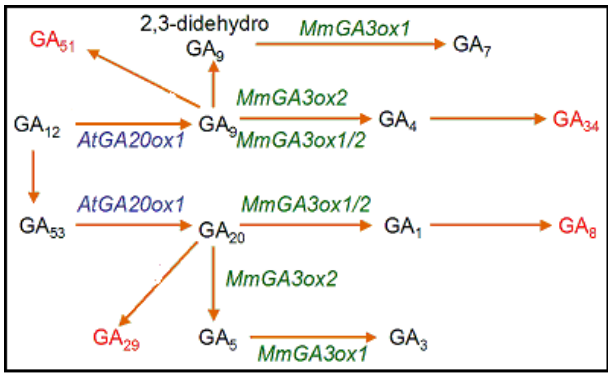
MmGA3ox2: Transformation efficiency was 3.75%. The plants expressing the *MmGA3ox2* gene (n = 11) were significantly taller (33%) than the controls (n = 6) at 0.01 probability (Student's unpaired t-test). The presence and expression of the transgenes were confirmed by PCR and RT-PCR. The height of the transformants expressing the *MmGA3ox2* gene varied from 172.4cm (plant No. 29) to 186.2cm (plant No. 28) with a mean height of 177.1cm, while those of the controls showed an average height of 134.6cm The transgenic plants grew more vigorously than the controls producing more leaves. The internode lengths of the transgenic plants increased by 8.4%, the average being 13.5cm for the transgenic as against 12.4cm for the wild-type control. However, flowering and pollen viability were not affected in the transgenic plants.

MmGA3ox1 + MmGA3ox2: Transformation efficiency was 1%. Very few transgenic shoots expressing *MmGA3ox1* + *MmGA3ox2* were obtained. The callus obtained turned brown and died which may be due to high concentration of GAs in the callus which may have inhibited shoot differentiation. The use of different combination of PGRs and also the use of activated charcoal failed to initiate differentiation in the transgenic callus (data not shown). The plants expressing *MmGA3ox1* + *MmGA3ox2* genes (n = 3) were significantly taller than the controls (n = 3) at 0.01 probability (Student's unpaired t-test). The height of the transformants expressing the *MmGA3ox1* + *MmGA3ox2* genes varied from 178.6cm (plant No. 3) to 182.1cm (plant No. 2) with a mean height of 177.4cm. Those of the controls showed an average height of 134.6cm. The internode lengths of the transgenic plants had increased by 8.5%, the average being 13.5cm for the transgenic as against 12.4cm for the wild-type control. However, flowering and pollen viability were not affected in the transgenic plants.

AtGA20ox1: Transformation efficiency was 3.3%. The plants expressing the *AtGA20ox1* gene (n = 5) showed an increase in plant height compared to the controls (n = 3) at 0.05 probability (Student's unpaired t-test). The presence and expression of the transgenes were confirmed by PCR and RT-PCR. The height of the transformants expressing the *AtGA20ox1* gene varied from 179.2cm (plant No. 2) to 167.2cm (plant No. 5) while those of the controls showed an average height of 160.2cm. Therefore, the average increase in height in transformed plants over the wild type control plants was by 7% *i.e.* 16.7cm while plant No. 2 expressing *AtGA20ox1* had 12% increase *i.e.* 25.7cm increase in height. The internode lengths of the transgenic plants had increased by 4%, the average being 10.8cm for the transgenic as against 10.4cm for the wild-type control, thus showing that number of internodes must have increased rather than the length of internodes. However, flowering and pollen viability were not affected in the transgenic plants.

The present study illustrates that modification of plant stature can be achieved by modifying GA biosynthesis. It is also possible that manipulating GA metabolism and signalling pathways by adopting molecular breeding approaches certain morphological and physiological traits in both horticultural and floricultural crops can be altered to increase the productivity [9, 10]. In this study, GA encoding genes acting late in the pathway belonging to GA 2-, 3-, 20- oxidases were targeted, as genes acting early in the GA biosynthesis have been shown to have minimal effect on GA biosynthetic flux. It is well accepted that GA genes acting later in the pathway are under the action of feedback regulation and may not be entirely suitable for increasing the flux of GA, but they can be effectively used to increase the relative concentration of their precursors and, therefore, are widely used. The ectopic expression of the GA 2-oxidase, *PcGA2ox1* from *Phaseolus coccineus*, resulted in a dwarf phenotype *Nicotiana sylvestris*. Plants expressing *PcGA2ox1* also contained low concentrations of GA_1 and GA_{20}, the transformed plants showing significant reduction ($P \leq 0.01$) in stature with a compact architecture. Some of the severely dwarf plants showed prostrate growth with complete loss of apical dominance and had increased outgrowth of lateral shoots, as was also reported in transgenic wheat[10]. The latter workers also found that transgenic lines carrying a different number of copies of the transgene showed similar expression as detected by QRT-PCR which could be due to selective transcriptional silencing of the transgene. However, the transgenic plants responded by restorating their growth when bioactive GA was applied. This confirmed that the dwarf phenotype is due to GA deficiency. The other strategy adopted was to increase the concentration of GA in plant tissues to mimic elevated concentrations of GA in plants, the latter being obtained by applying GAs. Previous studies have shown that the levels of bioactive GAs are most sensitive to the activity of the dioxygenase enzymes acting late in the pathway and that the genes encoding these enzymes are very highly regulated. it is accepted that the GA 3-oxidase class of enzymes converts immediate precursors of GA to bioactive forms[11,12,13]. Thus, it was speculative that over-expressing this class of enzymes would result in an increase in the concentration of bioactive GAs in plants. The GA 3-oxidase genes *MmGA3ox1* and *MmGA3ox2* used in the present study were isolated from the embryos of *Marah macrocarpus* and were chosen because they encode functionally different enzymes. They both catalyse the formation of GA_1 and GA_4, while in combination they catalyse the formation of GA_3 and GA_7. *In vitro* studies [14] showed that *MmGA3ox1* has a strong preference for 13-non-hydroxylation substrates, which is more relevant to *Arabidopsis* rather than *N. sylvestris*, which mainly follow the early 13-hydroxylation pathway. Expression of *MmGA3ox2* resulted in significant height increases of 32% in *N. sylvestris*, respectively. While *MmGA3ox1* activity may not limit GA_1 or GA_4 production, *MmGA3ox2*, in combination with *MmGA3ox1* or the native GA3ox enzymes, may increase production of GA_3 or GA_7 resulting in a growth increase. GA 20-oxidase is the rate-limiting reaction for GA biosynthesis in most species that have

been examined and indeed, when *AtGA20ox1* was over-expressed in *N. sylvestris*, it resulted in a statistically significant increase in stature. Plants expressing either of the two GA 3-oxidases contained elevated concentrations of GA_1. The greatest increase in plant height by over-expressing GA 20-oxidase (*AtGA20ox1*), with over-expression of the 3-oxidase (*MmGA3ox1*) having less effect. The ectopic expression of transgenes must be strong enough to over-come the internal homeostatic mechanism of GA biosynthesis in higher plants, which is closely regulated, particularly through the action of endogenous GA 2-, 3- and 20- oxidase genes. The observed phenotypes, as found in the present study, were distinctly different from those of controls and were confirmed at the molecular, biochemical and metabolomic levels [15].



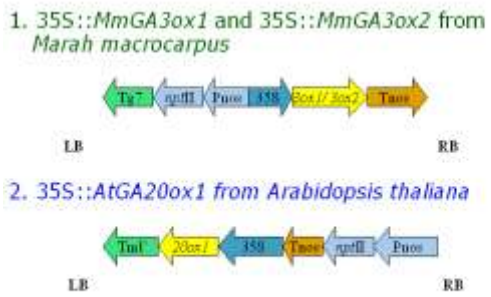


Figure - 1
A. Mode of action of GA genes employed in the present investigation. B. Constructs used in the present study

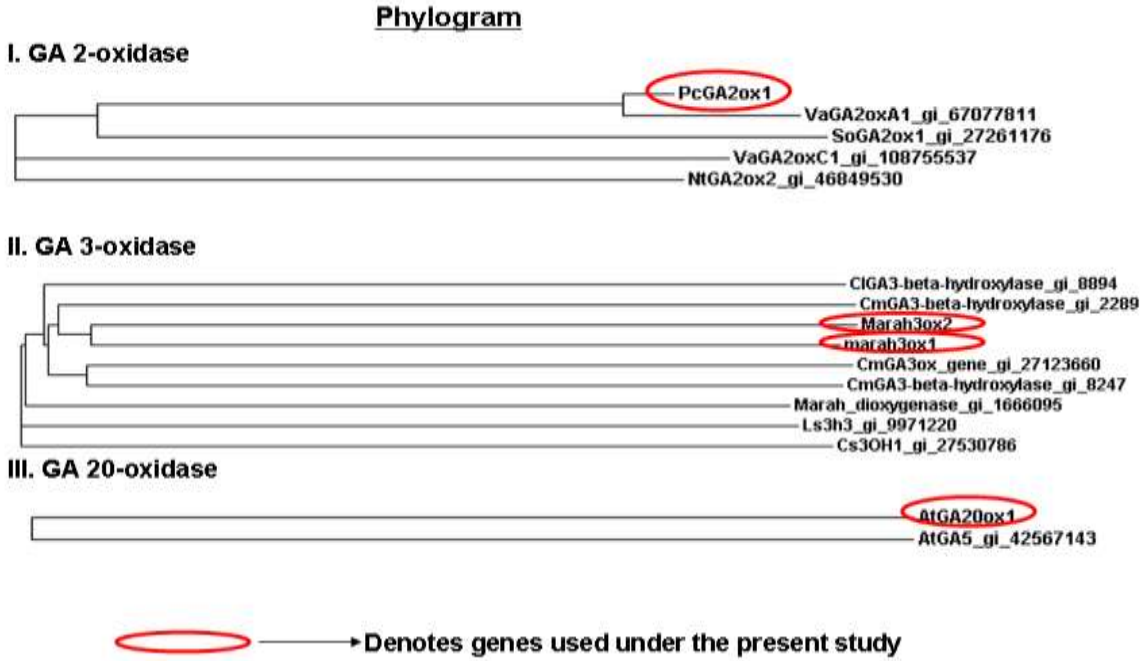


Figure - 2
Phylogram of GA genes used in the present study

Table – 1
Limited GA analysis of *PcGA2ox1* plants

Plant number	Plant Height (cm)	**GA_1**	GA_3	GA_8	GA_{19}	GA_{20}	GA_{29}	GA_{34}
Control (4)	134.6	**5.49**	4.5	2.8	4.8	0	0	0
1	18.4	**0.22**	0	0	8.5	0	0	0.19
2	12.6	**2.25**	4.5	0	0.5	0.083	0	0

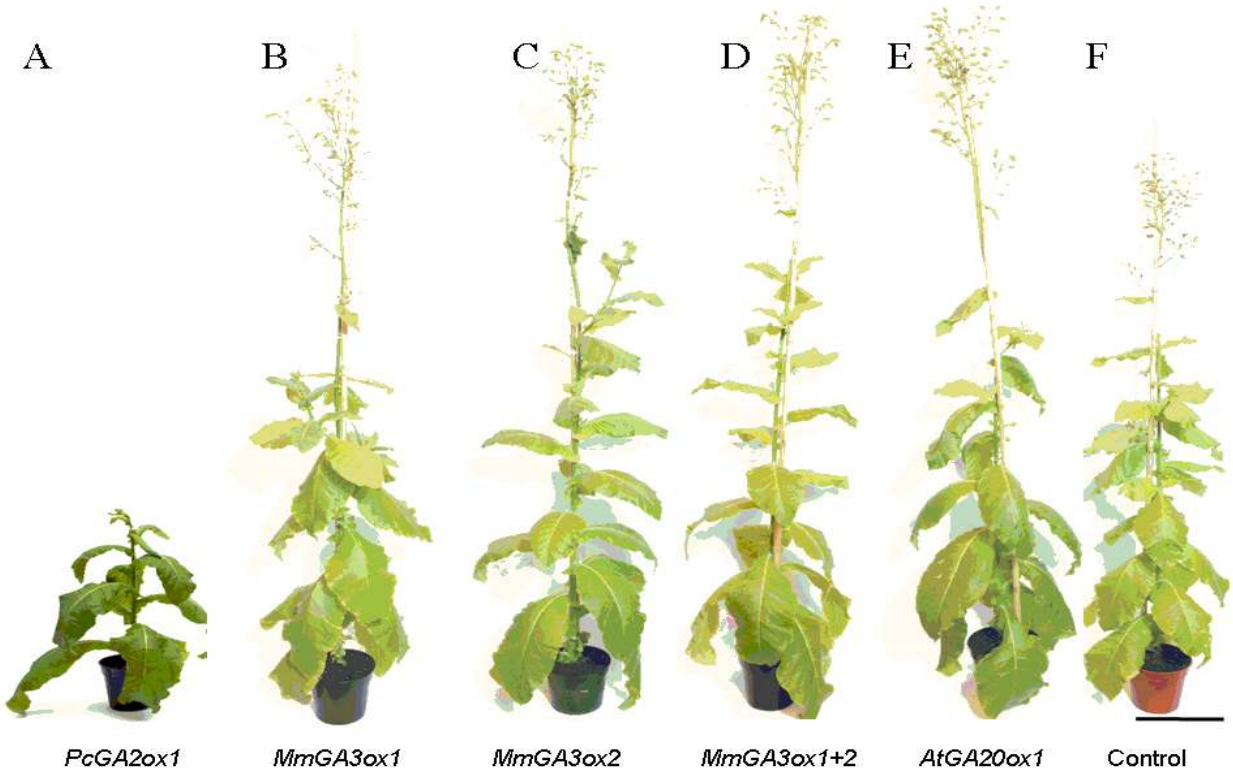


Figure – 3
Comparison of the heights of *PcGA2ox1, MmGA3ox1, MmGA3ox2* and *MmGA3ox1 + MmGA3ox2, AtGA20ox1* transformed plants with a non-transformed plant at 12weeks after potting. Pot size = 13 cm diam

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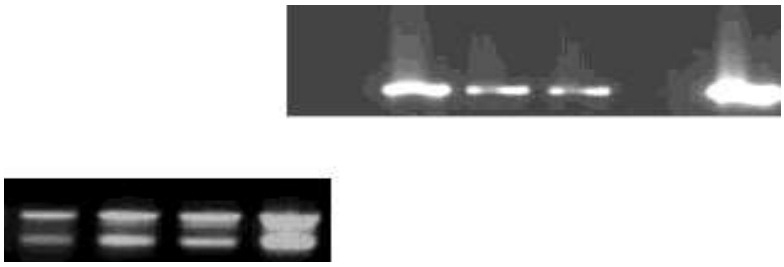

Figure – 4
Typical RT-PCR analysis of selected, kanamycin resistant plants of *N. sylvestris* transformed with *MmGA3ox1* confirming expression of the transgene (Lanes 2 - 4). Non-transformed (control) plants did not express the transgene (Lanes 1), PCR water-Control (Lane 5). Lane 6 = positive control, PCR amplified *MmGA3ox1* construct.

Conclusion

Commercialization and application of genetic modification is limited in ornamentals[16] due to lack of efficient transformation and regeneration systems[17], and a limited market for individual ornamental species compared to major food crops. The development of efficient, reproducible and cultivar-independent transformation techniques is vital to the growth of the floriculture industry. Many more plants species such as rose, carnation and poinsettia are being treated regularly with growth retardants, which block GA biosynthesis to create dwarfism. Other ornamental plants can be genetically modified with increased height to create novelty. Growth regulators (including retardants) are costly; need repeated application and lack uniformity in inducing desired results in plants, besides being environmentally unfriendly[18,19]. There has been a growing public concern against the use of these chemicals. However since, genetically modified ornamentals are used for their

aesthetic value and not as a food crop, they may be more acceptable to the general public than GM food crops.

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References

1. Bushman J.C.M. Globalisation – flower – flower bulbs – bulb flowers. *Acta Horticulturae* **673,** 27-33 (2005)

2. Spielmeyer W., Ellis M.H. andChandler P.M. Semidwarf (sd-1), green revolution rice, contains a defective gibberellin 20-oxidase gene, *Proceedings Nat Acad Sci*, USA **99,** 9043–9048 **(2002)**

3. Hedden P. and Kamiya Y., Gibberellin biosynthesis: enzymes, genes and their regulation, *Plant Mol Biol* **48,** 431-460 **(1997)**

4. Lange T. Molecular biology of gibberellin synthesis, *Planta* **204,** 409-419 **(1998)**

5. Phillips A.L. Genetic and transgenic approaches to improving crop performance. In: Plant Hormones: Biosynthesis, Signal Transduction, Action! (Davies PJ, ed) Kluwer Academic Publishers, Dordrecht, The Netherlands, 582-609 **(2004)**

6. Rademacher R. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways, *Ann Rev Plant Phy Mol Biol* **51,** 501-531 **(2000)**

7. Rajapakse N.C., Young R.E., McMohon M.J. and Oi R. Plant height control by photoselective filters: current status and future prospects, *Hort Tech* **9,** 616-624 **(1999)**

8. Uzogara S.G., The impact of genetic modification of human foods in the 21st Century, *Biotech Adv* **18**, 179-206 **(2000)**

9. Mino M., Oka M., Tasaka Y. and Iwabuchi M. Molecular biology of the metabolism and signal transduction of gibberellins, and possible application to crop improvement. *J Cr Impv* **18**, 365-390 **(2006)**

10. Appleford N.E.J., Wilkinson M.D., Ma Q., Evans D.J., Stone M.C., Pearce P.S., Powers S.J., Thomas S.G., Jones H.D., Phillips A.L., Hedden P. and Lenton J.R., Decreased shoot stature and grain amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat, *J Exp Bot* **56,** 112-120 **(2008)**

11. Martin D.N., Proebsting W.M. and Hedden P. Mendel's dwarfing gene: cDNAs from the Le alleles and function of the expressed proteins. *Proc of Nat Sci*, USA, **9**4, 8907–8911 **(1997)**

12. Itoh H., Ueguchi M.T., Kawaide H., Chen X., Kamiya Y. and Matsuoka M. The gene encoding tobacco gibberellin 3β-hydroxylase is expressed at the site of GA action during stem elongation and flower organ development, *Plant J* **20,** 15-24 **(1999)**

13. Yamaguchi S. Gibberellin biosynthesis in *Arabidopsis*, *Phytochem Rev* **5**, 39-47 **(2006)**

14. Hedden P. and Phillips A.L. Gibberellin metabolism: new insights revealed by the genes, *Trends Plant Sci* **5,** 523-530 **(2000)**

15. Mol J.N., Holton T.A. and Koes R.E. Floriculture: genetic engineering of commercial traits, *Trends Biotech* **13,** 350-355 **(1995)**

16. Newell C.A., Plant transformation technology, *Mol Biotech* **16,** 53-65 **(2000)**

17. Rani C.R., Reema C., Singh A. and Singh P.K., Salt tolerance of Sorghum bicolor cultivars during germination and seedling growth *Res J Recent Sci*., **1(3),** 1-10 **(2012)**

18. Bora A., Science Commmunication through Mass media Res J Recent Sci., **1(1),** 10-15 **(2012)**

19. Bhattacharya A., Ward D.A., Hedden A., Power J.B. and Davey M.R., Engineering gibberellin metabolism in *Solanum nigrum* L. by ectopic expression of gibberellin oxidase gene, *Plant Cell Rep*, 1214-8 **(2012)**

20. Coles J.P., Phillips A.L., Croker S.J., Garcia-Lepe R., Lewis M.J. and Hedden P., Modification of gibberellin production and plant development in *Arabidopsis* by sense and antisense expression of gibberellin 20-oxidase genes, *Plant J* **17,** 547-556 **(1999)**

21. Sambrook J., Fritsch E. and Maniatis T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY **(1989)**