Plant Cell Rep (2008) 27:463–470
DOI 10.1007/s00299-007-0471-z

GENETIC TRANSFORMATION AND HYBRIDIZATION

Over-expression of a gibberellin 2-oxidase gene from *Phaseolus coccineus* L. enhances gibberellin inactivation and induces dwarfism in *Solanum* species

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Received: 9 July 2007 / Revised: 8 October 2007 / Accepted: 19 October 2007 / Published online: 13 November 2007

Abstract Gibberellins (GAs) are endogenous hormones that play a predominant role in regulating plant stature by increasing cell division and elongation in stem internodes. The product of the GA 2-oxidase gene from *Phaseolus coccineus* (*PcGA2ox1*) inactivates C_{19}-GAs, including the bioactive GAs GA_1 and GA_4, by 2β-hydroxylation, reducing the availability of these GAs in plants. The *PcGA2ox1* gene was introduced into *Solanum melanocerasum* and *S. nigrum* (Solanaceae) by *Agrobacterium*-mediated transformation with the aim of decreasing the amounts of bioactive GA in these plants and thereby reducing their stature. The transgenic plants exhibited a range of dwarf phenotypes associated with a severe reduction in the concentrations of the biologically active GA_1 and GA_4. Flowering and fruit development were unaffected. The transgenic plants contained greater concentrations of chlorophyll *b* (by 88%) and total chlorophyll (11%), although chlorophyll *a* and carotenoid contents were reduced by 8 and 50%, respectively. This approach may provide an alternative to the application of chemical growth retardants for reducing the stature of plants, particularly ornamentals, in view of concerns over the potential environmental and health hazards of such compounds.

Keywords *Agrobacterium* transformation · Dwarfism · Gibberellin · Gibberellin 2-oxidase · *Phaseolus coccineus* · *Solanum melanocerasum* · *S. nigrum*

Communicated by W. Harwood.

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Introduction

Gibberellins (GAs) are endogenous plant hormones that influence several aspects of plant development including seed germination, shoot growth, flower induction and development, and fruit expansion (Thomas and Hedden 2006). Since many of these developmental traits are of importance in agriculture and horticulture, the GA-signalling pathway, including biosynthesis and signal transduction, is a major target for intervention by chemical or genetic manipulation. The use of growth retardants that inhibit GA biosynthesis (Rademacher 2000) to control stem growth is widespread, while mutant alleles that impart semi-dwarfism have been introduced into a number of important crop species. For example, the Green Revolution that has been an important factor in feeding the world's expanding population was enabled by the introduction into wheat of *reduced height* (*Rht*) dwarfing alleles, which reduce response to GA, and of *semi-dwarf 1* (*sd1*), a mutant allele of a GA-biosynthetic gene, into rice (Hedden 2003). The semi-dwarf varieties are more stable (less prone to lodging) in adverse weather and are higher yielding than taller cultivars. Growth retardants, such as chlormequat and daminozide, are applied to ornamental plants to enhance their appearance and manageability by reducing stature. However, the potential risks to health and the environment

of applying such chemicals have limited their use and are likely to be of increasing concern in the future. Although genetic control of height is preferable to the use of chemicals, suitable dwarfing alleles are not available for many species. Therefore, the introduction of genes to manipulate the GA-signalling pathway would seem to be a potentially useful alternative.

The biosynthesis of GAs from their diterpenoid precursor geranylgeneranyl diphosphate (GGDP) requires the activity of terpene cyclases, cytochrome P450 monooxygenases and 2-oxoglutarate-dependent dioxygenases. Growth retardants have been developed that target members of each enzyme type (Rademacher 2000). The dioxygenases, comprising the biosynthetic enzymes GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox) and the inactivating enzymes GA 2-oxidase (GA2ox), are important sites of regulation of the pathway (Hedden and Phillips 2000). As such, they have been major targets for genetic intervention (see Phillips 2004). In most experiments to manipulate GA biosynthesis genetically, the goal has been to control plant stature by reducing GA content. This has been achieved by suppressing expression of biosynthetic genes (Coles et al. 1999; Carrera et al. 2000; Bulley et al. 2005), but the more effective method has been the ectopic expression of *GA2ox* genes, which encode enzymes that 2β-hydroxylate the biologically active C_{19}-GAs, or their immediate precursors, to inactive forms or to products that cannot be converted to active GAs (Thomas et al. 1999). A separate group of GA 2-oxidases target the C_{20}-GA intermediates (Schomburg et al. 2003). Ectopic expression of *GA2ox* genes has been described for a number of species, including *Arabidopsis thaliana*, in which severe dwarfism (Hedden et al. 1999; Radi et al. 2006) or loss of fertility (Singh et al. 2002) has been achieved. Expression of a *GA2ox* gene from a constitutive promoter in rice resulted in dwarfing in a range of severities, presumably related to the level of transgene expression, as well as loss of fertility (Sakamoto et al. 2001, 2003). More targeted expression of a *GA2ox* to the sites of GA biosynthesis in vegetative tissues of rice, by a promoter from the GA-biosynthetic gene *OsGA3ox2*, resulted in mild dwarfism with normal fertility (Sakamoto et al. 2003).

The aim of the work described in the present paper was to examine the feasibility of using *GA2ox* expression to restrict the growth of target plant species, as an alternative to the application of growth retardants. This paper describes the over-expression of a GA 2-oxidase gene (*PcGA2ox1*; accession No. AJ132438 in the NCBI database) from runner bean (*Phaseolus coccineus*; Thomas et al. 1999) in *Solanum melanocerasum* and *S. nigrum* (Solanaceae), these species being used as experimental material to demonstrate proof of concept and because of their ease of transformation. Although the constitutive CaMV 35S promoter was used, it was possible to produce plants with reduced stem height and leaf size without affecting reproductive development.

Materials and methods

Plant material

Plants of *S. melanocerasum* and *S. nigrum* were 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). The uppermost fully expanded leaves were excised from 4–10 week-old plants and surface sterilised by immersion in 10% (v/v) "Domestos" (Johnson Diversey Ltd., Northampton, UK) for 10 min, followed by three washes with sterile, reverse osmosis water. The midrib was removed from each leaf and the laminae were cut into 1 cm^2 explants under aseptic conditions.

Plasmid expression constructs

The *Phaseolus coccineus GA2ox1* coding region was amplified by PCR using specific 5′ (5′-TGAGCTCAAC CATGGTTGTTCTGTCTCAGC-3′) and 3′ (5′-TGAGC TCTTAATCAGCAGCAGATTTCTGG-3′) primers. The amplified product was sub-cloned into pCR2.1 (Invitrogen, Groningen, The Netherlands) and the sequence confirmed by DNA sequence analysis. The *PcGA2ox1* coding region was excised from pCR2.1 by *Sac*I digestion and inserted into the *Sac*I site of the binary vector pLARS120 (Fig. 1). Constructs containing the *PcGA2ox1* coding region in the sense orientation were identified by restriction digest analysis and subsequently designated as pLARS124. The T-DNA also contained the neomycin phosphotransferase (*npt*II) gene with the *nos* promoter, located next to the left border of the T-DNA.

Agrobacterium tumefaciens-mediated plant transformation

The vector was inserted into *A. tumefaciens* strain GV3101. Overnight cultures of *A. tumefaciens* GV3101 carrying 35S::*PcGA2ox1* were grown in Luria Broth (LB; Sambrook et al. 1989) with 40 mg l^{-1} rifampicin, 50 mg l^{-1} kanamycin sulphate, 25 mg l^{-1} gentamycin and 100 mg l^{-1} carbenicillin. Cultures with an $OD_{600\ nm}$ of 0.7–1.2 were used to inoculate leaf explants, which were immersed for 5 min in overnight suspensions of *A. tumefaciens* diluted

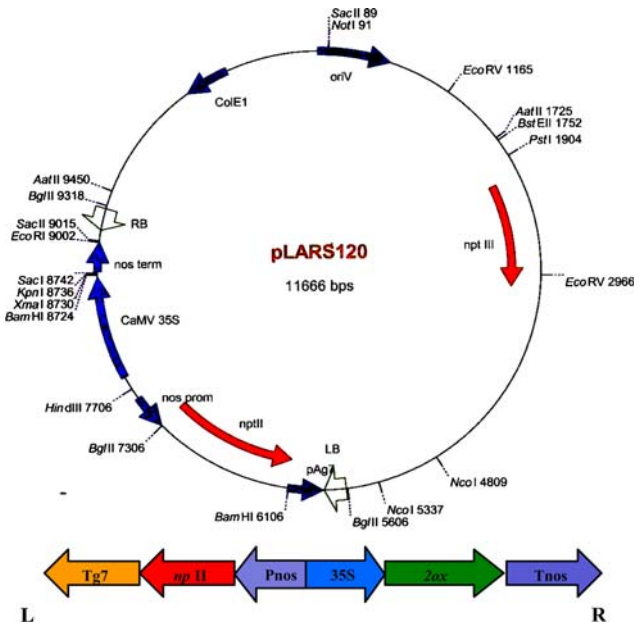

Fig. 1 Binary vector pLARS120 and T-DNA, carrying the *PcGA20x1* and *npt*II genes, used for transformation of *Solanum* species

1:10 (v:v) with liquid MS-based medium (Murashige and Skoog 1962), containing 30 g l^{-1} sucrose, but lacking growth regulators, pH 5.8 (designated MS0). Control explants were treated in the same way, 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 with 1.0 mg l^{-1} zeatin and semi-solidified with 8 g l^{-1} agar (designated MSZ; 8 explants/dish). Inoculated explants were maintained at day/night temperatures of 22 ± 1 and 20 ± 1°C, respectively, with a 16 h photoperiod and a light intensity of 19.5 µMol m^{-2} s^{-1} using cool white "Daylight" fluorescent tubes (Thorn EMI Ltd, Hayes, UK). After 2 days, leaf explants were transferred to semi-solid MSZ medium supplemented with cefotaxime (500 mg l^{-1}) and kanamycin sulphate (50 mg l^{-1}). Uninoculated explants were transferred to medium either lacking antibiotics to regenerate plants for comparison with transgenics, 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, each containing 50 ml of semi-solid MSZ medium supplemented with the same antibiotics as used previously. Selection was maintained by transfer of explants to new medium with antibiotics every 4 weeks. Regenerated shoots were excised from leaf-derived calli and rooted on semi-solid (0.8% w/v agar) MS0 medium with kanamycin at 50 mg l^{-1}. Rooted plants were potted in a mixture (6:6:1:1, by volume) of Levington M3, John Innes No. 3, Perlite and Vermiculite in 9 cm diameter plastic pots, 12–14 weeks after transformation. Potted plants were covered with 17 cm × 15 cm plastic bags and transferred to the glasshouse under natural daylight. The tops of the bags were opened progressively over a 14 days period.

Phenotypic analyses

The phenotypic characteristics of each plant (height, internode length, leaf length and width) were recorded at 21 days (*S. melanocerasum*) and 42 days (*S. nigrum*) post-acclimation. The concentrations of chlorophylls *a* and *b* and total carotenoids were determined spectrophotometrically (Lichtenthaler 1987).

Transgenic (n = 16) and control (n = 10) plants for both species were allowed to flower and self-pollinate. One hundred ripe berries were collected per plant, and the fresh weight of berries recorded. Berries from individual plants were combined, macerated and incubated in 30 ml aliquots of 1 M HCl to remove enveloping tissues. Seeds were stirred in running tap water and collected on a nylon sieve (1 mm^2 mesh), dried overnight on No. 1 filter paper (Whatman International Ltd., Maidstone, UK) at 23 ± 2°C and weighed. The weight of 100 seeds was recorded for transgenic and non-transformed plants.

Polymerase chain reaction (PCR)

Primers were manufactured and sequenced by MWG Biotech, Ebersberg, Germany. The sequences used were 5′ AGA CAA TCG GCT GCT CTG AT 3′ and 5′ ATA CTT TCT CGG CAG GAG CA 3′ (*npt*II) and 5′ TCA TAG TGA ACG CCT GTA GG -3′ and 5′ TGT TCT TCA CTG CTG TAA TG 3′ (*PcGA2ox1*). Template genomic DNA was extracted 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, 94°C, 3 min), denaturating (35 cycles, 94°C, 1 min), primer annealing [35 cycles; 57°C (*npt*II) or 55°C (*PcGA2ox1*), 1 min], primer extension (35 cycles, 72°C, 90 s), final extension (1 cycle, 72°C, 10 min) and holding at 4°C (5 min to ∞).

Reverse transcriptase (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, 50°C, 30 min), polymerase activation (1 cycle, 94°C, 15 min), denaturation (35 cycles, 94°C, 1 min) and primer annealing (35 cycles, 55°C, 1 min). Subsequent conditions were as for PCR analysis.

PcGA2ox1 gene expression studies using Northern blot analysis

Total RNA (15 μg) was isolated from leaves using an RNeasy® Plant Mini Kit. RNA was capillary blotted onto nylon membrane (Roche Diagnostics Ltd., Lewes, UK) and the latter prehybridised in DIG-Easy-Hyb buffer (Roche) at 68°C for 1 h. For hybridisation, 10 ml of DIG-Easy-Hyb, to which had been added DIG-labeled RNA probes (100 ng ml^{-1}; Roche), were used per 100 cm^2 of membrane. Hybridisation was at 68°C for 16 h. Membranes were washed for 2 × 5 min in 2× SSC/0.1% SDS (Roche) and for 2 × 15 min in 0.1× SSC/0.1% SDS at 68°C, preincubated for 45 min in blocking solution (Roche) and incubated for 30 min with anti-DIG-AP conjugate (diluted 1:10,000, v:v). Two 15 min washing steps preceeded equilibration in detection buffer for 5 min. Detection involved CDPStar (Roche) with a 20 min exposure to X-ray film (Kodak).

Quantification of GAs

Whole shoots above the sixth leaf were harvested from individual plants, frozen in liquid N_2, freeze–dried and homogenised to a fine powder in a ball mill. Aliquots (500 mg) of each sample were stirred overnight in 80% (v/v) methanol (100 ml) containing ^2H and ^3H-labelled GAs as internal standards. GAs were extracted and purified before being quantified by gas chromatography-mass spectrometry (Coles et al. 1999).

Results

Solanum melanocerasum

Of 40 plants regenerated in the presence of kanamycin from leaf explants inoculated with *A. tumefaciens* harbouring pLARS124 carrying the *npt*II and *PcGA2ox1* genes, 34 were confirmed by PCR to have integrated both of these genes. Four kanamycin resistant plants had only the *PcGA2ox1* gene; two kanamycin resistant plants were escapes lacking both the *npt*II and *PcGA2ox1* genes. As expected, ten plants regenerated from uninoculated leaf explants were PCR negative. Uninoculated explants cultured on medium with kanamycin, became necrotic and failed to regenerate shoots. Thirty-five transgenic plants that were PCR-positive for *PcGA2ox1* were transferred to the glasshouse and grown alongside 10 non-transformed plants for phenotypic analysis. Of 10 trangenic plants selected randomly from this population of 35 PCR-positive transgenic plants, 8 were shown by RT-PCR to express *PcGA2ox1*.

Transgenic plants varied considerably in height, ranging from severely dwarfed, such as plant 19B (Fig. 2a), which was 1.4 cm in height, to plants such as 1A which at 18.3 cm was more comparable in height to a non-transformed plant (23.0 cm; Fig. 2a, b). Student's *t*-test confirmed that plant height and leaf size were reduced significantly ($P < 0.01$) in transgenic plants compared to these parameters for non-transformed plants. Additionally, transgenic plants had reduced leaf length and width (Fig. 2c) with darker green leaves compared to non-transformed plants. Although chlorophyll *a* content was reduced in the leaves of transgenic plants, chlorophyll *b* and total chlorophyll concentrations were increased in these plants (Fig. 3a–c). Carotenoid content was also reduced in transformed plants (Fig. 3d). Flowers and fruits of transformed and control plants were identical (Fig. 2d–f). The timing of flower and fruit production (30–35 and 35–40 days, respectively) following transfer of plants to the glasshouse, was unaffected; mean fruit weight was 478 mg for both transformed and control plants. Similarly, the weight of 100 seeds remained unaltered at 127 mg for both transgenic and control plants. Two plants (Nos. 8, 18) that carried, but did not express *PcGA2ox*, had similar phenotypes to non-transformed plants.

Southern analyses of PCR and RT-PCR positive plants 1A, 3C, 15A and 19B (a clone of 19C) confirmed the presence of *npt*II and *PcGA2ox1* transgenes in these plants (data not shown). Northern analyses of transgenic plants 3B, 15A, 11C and 1A showed a differential expression pattern of *PcGA2ox1* that generally correlated with plant height (Fig. 4). Quantitative analysis of several GAs in shoots from PCR and RT-PCR positive plants 1A, 3B (a clone of 3C), 11C and 15A, confirmed reduced concentrations of the bioactive forms GA_1 and GA_4 compared with a non-transformed plant, except for GA_4 in plant 3B (Table 1). There were also reductions in the concentrations of GA_{19} and, particularly, GA_{20}, the precursors of GA_1, in the transgenic plants, while GA_{29} (2β-hydroxyGA_{20}) accumulated in these plants, consistent with greater 2-oxidase activity. There were, however, only very slight increases in the concentrations of GA_8 and GA_{34}, the 2β-hydroxylated analogs of GA_1 and GA_{34}, respectively.

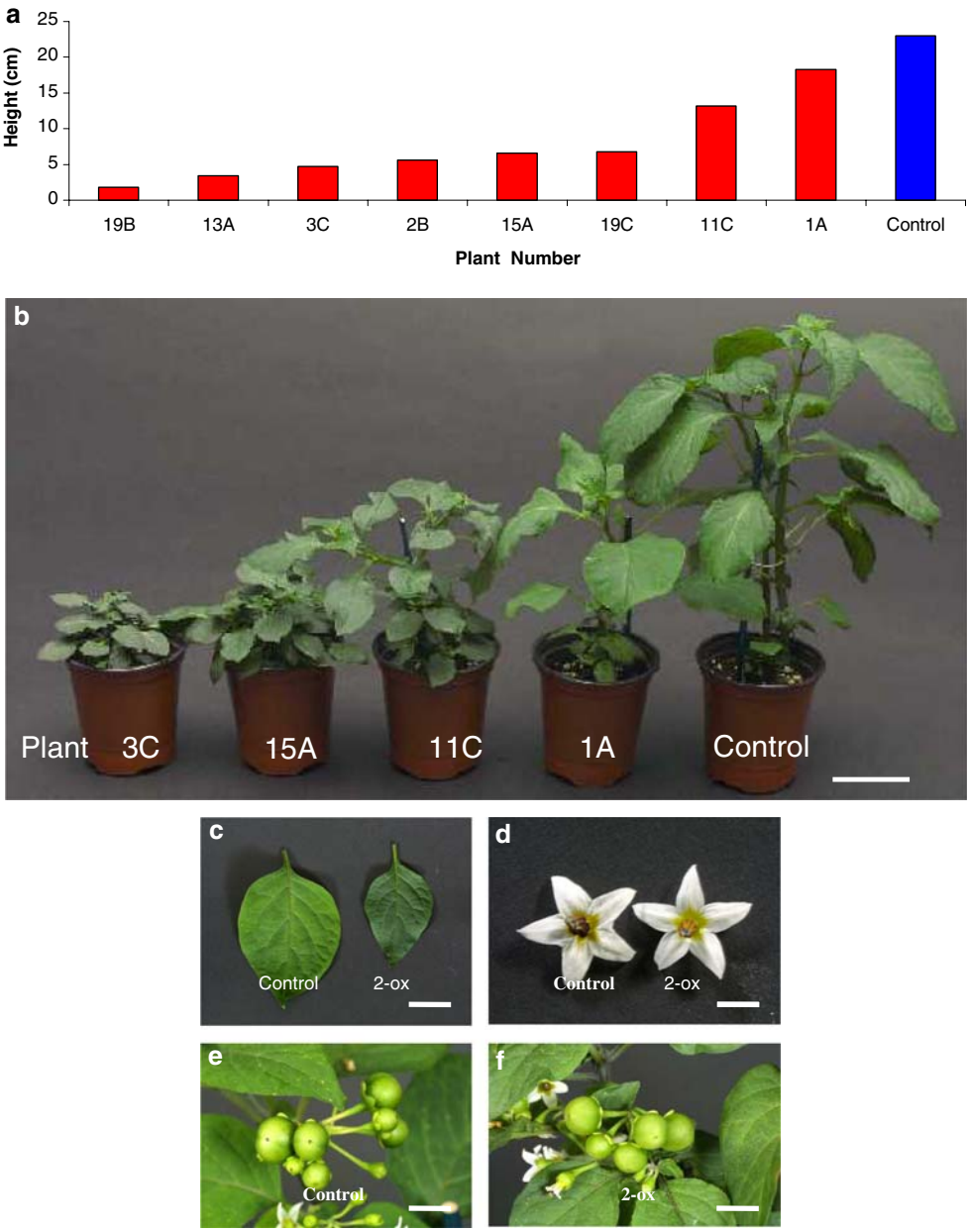

Fig. 2 **a**, **b** Comparison of the heights of *PcGA2ox1* transformed with non-transformed (*control*) plants at 21 days post-acclimation. **c** Leaf of a *PcGA2ox1* transformed plant (*right*) compared to a leaf from a non-transformed plant (*left*). (**d**–**f**) Similar morphology of flowers and fruits from transformed and non-transformed plants. *Bars* = 59, 31, 2, 5 and 5 mm for **b**–**f**, respectively

Solanum nigrum

The results for *S. nigrum* were similar to those for *S. melanocerasum*. Thirty-four kanamycin resistant plants regenerated from inoculated leaf explants, were PCR positive for *PcGA2ox1*. Ten plants regenerated from uninoculated explants cultured without kanamycin, were PCR negative. Of 16 PCR positive plants selected at random, 10 were RT-PCR positive for *PcGA2ox1*. RT-PCR positive plants exhibited a range of dwarfed phenotypes; RT-PCR negative plants were similar morphologically to non-transformed plants regenerated from leaf explants not inoculated with *Agrobacterium*. Student's *t*-tests of data for RT-PCR positive plants were highly significant ($P < 0.01$) for reductions in height, internode length, leaf length and leaf width ($P < 0.05$) compared to these parameters for non-transformed plants. As in the case of *S. melanocerasum*, transgenic plants had darker leaf pigmentation and similar changes in pigments compared to their non-transformed counterparts.

A limited GA analysis of transgenic plants (Nos 42, 68, 217), which were 28.6, 11.2 and 42.5 cm in height, respectively, showed GA_1 contents of 1.4, 1.3 and 1.1 ng g^{-1}, respectively, compared with a non-transformed plant, which was 62.6 cm in height and contained 1.7 ng g^{-1} GA_1. A better correlation between GA_1 content and height was obtained if the GA_1 content was expressed on a per shoot basis, giving 2.3, 0.8 and 2.3 ng $shoot^{-1}$,

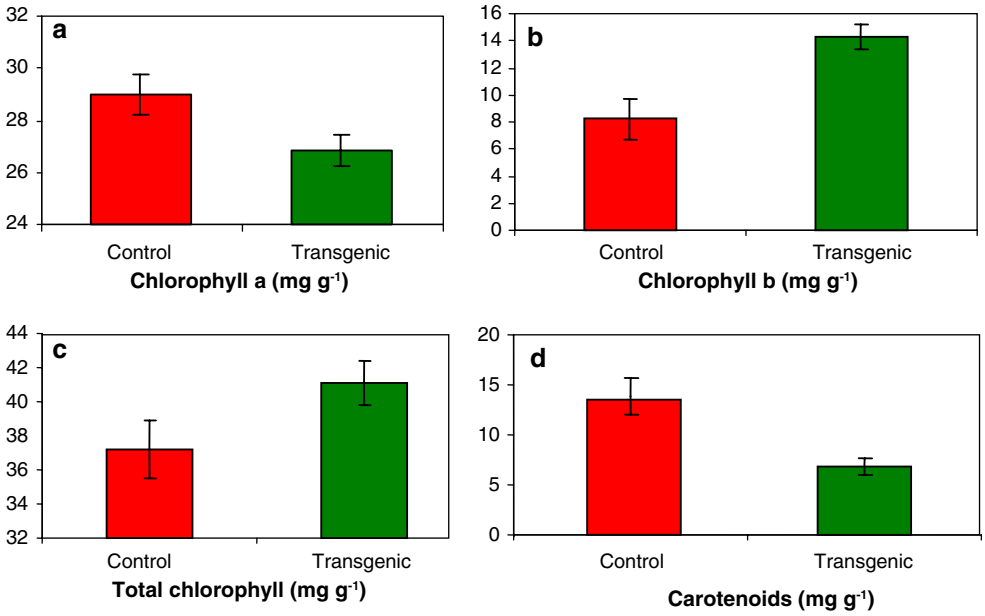

Fig. 3 **a** Chlorophyll *a*, **b** chlorophyll *b*, **c** total chlorophyll and **d** carotenoid contents in leaves of non-transformed and *PcGA2ox1* transformed plants of *S. melanocerasum*

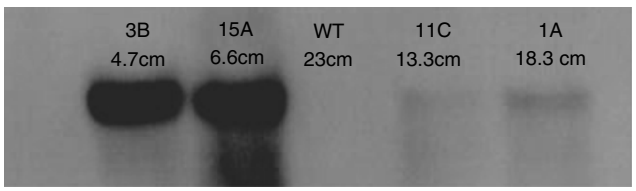

Fig. 4 Northern blot analysis showing differential gene expression of *PcGA2ox1* in transformed plants 3B, 15A, 11C and 1A; WT is a wild-type, non-transformed plant

Table 1 Concentrations of GAs in shoots of non-transformed and *PcGA2ox1*-transformed plants of *S. melanocerasum*

Plant	Stem height (cm)	GA concentration (ng g^{-1})						
		GA_{19}	GA_{20}	GA_{29}	**GA_1**	GA_8	**GA_4**	GA_{34}
Control	23.0	47.0	20.3	7.4	**22.6**	0.9	**2.2**	0.3
1A	18.3	8.6	1.3	17.0	**1.6**	0.3	**0.9**	5.1
3B	4.7	26.4	3.0	26.8	**12.5**	1.8	**3.7**	1.5
11C	13.3	20.9	1.0	22.9	**0.9**	ND	**0.8**	0.5
15A	6.6	44.0	1.3	45.3	**9.7**	1.1	**1.4**	0.3

Data for bioactive GAs are in bold

ND Not determined

respectively, for the transgenic lines compared with the control value of 8.1 ng $shoot^{-1}$. The GA_{20} content was also reduced to 1.4 ng g^{-1} (2.3 ng $shoot^{-1}$), 1.1 ng g^{-1} (0.7 ng $shoot^{-1}$) and 0.6 ng g^{-1} (1.3 ng $shoot^{-1}$) in plants Nos. 42, 68 and 217, respectively, compared with the control (2.4 ng g^{-1}; 10.9 ng $shoot^{-1}$), There was no significant difference in mean values for seed weight (112 mg 100 $seeds^{-1}$), and berry weight (450 mg $berry^{-1}$) for the ten plants expressing *PcGA2ox1* and ten non-transformed plants (112 mg 100 $seeds^{-1}$; 450 mg $berry^{-1}$), or from transgenic plants that carried, but did not express the transgene.

Discussion

Plants of both *Solanum* species transformed with *PcGA2ox1* exhibited phenotypic alterations characteristic of GA deficiency, namely reductions in height, internode lengths and leaf lengths and widths, that were associated with reduced concentrations of bioactive GAs. The major bioactive GA in *Solanum* species investigated was GA_1, which was reduced substantially in the transgenic lines. The concentration of its immediate precursor GA_{20} was reduced even more, with an associated increase in the concentration of its 2β-hydroxylated form GA_{29} in *S. melanocerasum*, consistent with high 2β-hydroxylation activity in the transgenic plants. However, the 2β-hydroxylated metabolites of GA_1 (GA_8) and GA_4 (GA_{34}) did not accumulate to the same extent, perhaps because the concentrations of their precursors were already low. It is also possible that GA_8 and GA_{34} were further converted to catabolites (Thomas et al. 1999), but it seems most likely that the transgene product acts primarily on GA_{20}. On the basis of concentration there was a poor correlation between plant height and bioactive GA content in the transgenic lines. The correlation improved when GA_1 content in *S. nigrum* was expressed on a per shoot basis and we propose that in the most severe dwarfs the larger numbers of shoots per unit weight elevates the apparent GA concentration, assuming that GAs are not distributed homogeneously within the tissues analysed. We do not have the data for comparing the GA content per shoot for the *S. melanocerasum* lines.

Sakamoto et al. (2001) obtained a range of heights and phenotypes characteristic of GA deficiency in rice plants over-expressing the rice *OsGA2ox1* gene. The shortest rice plant generated was 15 cm in height, compared to a mean height for non-transformed plants of 90 cm. The largest phenotypic change seen in the transgenic Solanaceae plants generated in the present investigation was in internode length, in agreement with the role of GA in controlling stem elongation (Hedden 1999). The mean internodal length in RT-PCR positive plants of *S. melanocerasum* was 0.45 cm, compared to 2.6 cm for non-transformed plants.

An increased chlorophyll concentration, as found in the present study, is commonly associated with reduced GA content (Biemelt et al. 2004) and may be a consequence of smaller cell size and greater cell density. Flowers, seeds and berries were unaltered following *PcGA2ox1* expression in the *Solanum* species, which contrasts with the report of Sakamoto et al. (2001) in which transgenic rice plants failed to flower. This difference may be due to the strength and tissue-specificity of the promoters used, namely CaMV35S in the current experiments, while an actin promoter was used to drive *OsGA2ox* expression in rice. It may also reflect differences in the dependence of these species on GA for flower induction. Expression of the *PcGA2ox1* transgene could not be detected by RT-PCR in some transgenic lines; these plants were significantly taller than plants that expressed the transgene at detectable levels, although they were shorter than non-transformed plants. This intermediate stature may be attributed to delayed gene silencing, with the extent of dwarfing being related to the period of time before transgene silencing. Such an effect seems probable in view of previous work in which chitinase transgene expression was detected early in development, but had ceased when plants were assessed at 6–7 weeks in tobacco (Kunz et al. 1996) and at 8 weeks in rice (Chareonpornwattana et al. 1999). Additionally, Lange and Lange (2006) suggested that GA internal homeostasis could alter the phenotype induced by 2-oxidase transgenes. Therefore, in order to counteract internal mechanisms, it may be necessary to use relatively strong promoters to produce sufficient changes in GA content to reduce stature. Radi et al. (2006) found that plants failed to set seed and produced abnormal pollen tubes when a 2-oxidase gene from *Cucurbita maxima* (*CmGA2ox1*) under the E12–35S-Ω promoter was expressed in *Arabidopsis*. Sakamoto et al. (2003) also reported that rice transformed with *OsGA2ox1* from rice, but driven by an actin promoter, failed to set seed. Tissue-specific promoters will be important in directing gene expression in vegetative parts, such as stems, and not floral organs, thereby ensuring uniform flowering and seed set. In order to facilitate selection of such promoters, *Arabidopsis* microarray databases can be screened to identify promoters with the optimum expression profile.

Many ornamental plants, such as chrysanthemum, are taller in stature than is desired by the consumer, and lack uniformity of growth. Consequently, the use of chemical growth retardants, such as daminozide, which act by disrupting the synthesis of GAs, is common in commercial production. However, such compounds are not only hazardous to workers and the environment, but expensive for the producer. Additionally, the effects of such compounds are only transient. Genetic manipulation may provide an alternative approach to the use of growth retardants, since plants generated in this study exhibited dwarf phenotypes similar to those following application of growth retardants (Onuegbu 1997; Kumar et al. 1998; Rademacher 2000). Future work should extend the present investigation to ornamentals such as chrysanthemum which, for retail production, are dwarfed by treatment with chemical growth retardants. Such investigations should also involve the use of constructs carrying the 2-oxidase gene, but lacking the *npt*II selectable marker gene, in order to facilitate acceptability of this genetic manipulation approach to control plant stature.

Acknowledgments AB was supported by a Dorothy Hodgkin Postgraduate Award sponsored by the Biotechnology and Biological Sciences Research Council (BBSRC) and British Petroleum, UK, AP by the Department of Environmental, Food and Rural Affairs (DEFRA) and SK by Fine Agrochemicals. Paul Hopkins is thanked for GA analysis. Rothamsted Research is sponsored by the BBSRC.

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