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GENETIC TRANSFORMATION AND HYBRIDIZATION

Comparative expression of β-glucuronidase with five different promoters in transgenic carrot (*Daucus carota* L.) root and leaf tissues

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Abstract Tissue-specific patterns and levels of protein expression were characterized in transgenic carrot plants transformed with the β-glucuronidase (GUS) gene driven by one of five promoters: *Cauliflower mosaic virus* 35S (35S) and double 35S (D35S), *Arabidopsis* ubiquitin (*UBQ3*), mannopine synthase (*mas2*) from *Agrobacterium tumefaciens* or the rooting loci promoter (*rolD*) from *A. rhizogenes*. Five independently transformed carrot lines of each promoter construct were assessed for GUS activity. In leaves, activity was highest in plants with the D35S, 35S and *UBQ3* promoters, while staining was weak in plants with the *mas2* promoter, and only slight visual staining was present in the leaf veins of plants containing *rolD* promoter . Strong staining was seen in the lateral roots, including root tips, hairs and the vascular tissues of plants expressing the 35S, D35S and *UBQ3*. Lateral roots of plants containing the *rolD* construct also showed staining in these tissues while the *mas2* promoter exhibited heightened staining in the root tips. Relatively strong GUS staining was seen throughout the tap root with all the promoters tested.. When GUS expression was quantified, the *UBQ3* promoter provided the highest activity in roots of mature plants, while plants with the D35S and 35S promoter constructs had higher activity in the leaves. Although plants containing the *mas2* promoter had higher levels of activity compared to the *rolD* plants, these two promoters were significantly weaker than D35S, 35S and UBQ3. The potential for utilization of specific promoters to target expression of desired transgenes in carrot tissues is demonstrated.

Keywords Carrot · β-Glucuronidase · Promoter · Organ specific expression

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Introduction

Carrot (*Daucus carota* L. subsp. *sativa*), a member of the family Apiaceae, is grown for its edible taproot, which contains high levels of β-carotene (provitamin A), vitamins B_1 and C, and provides a good source of dietary fiber (Ammirato 1986). Commercial cultivars of carrot have been developed using traditional breeding methods for improved root growth, root shape and colour, β-carotene levels and smooth skin (Ammirato 1986). Carrot is a model system for tissue culture studies and previous research has demonstrated the utility of somatic embryogenesis, bioreactor scale-up of suspension cultures and protoplast culture and fusion for carrot improvement (Ammirato 1986; Zimmerman 1993; Komamine et al. 2005). In addition, transgenic technology has been used to enhance fungal disease resistance in carrot (Gilbert et al. 1996; Melchers and Stuiver 2000; Takaichi and Oeda 2000; Chen and Punja 2002; Jayaraj and Punja 2007), to create herbicide-resistant plants (Chen and Punja 2002), or for metabolic engineering of designer medical products (Bouche et al. 2003; Marquet-Blouin et al. 2003; Kumar et al. 2004) and novel antioxidant compounds (Jayaraj et al. 2007). In the majority of these studies, the *Cauliflower mosaic virus* 35S (CaMV 35S) (Odell et al. 1985) was used, while the maize ubiquitin promoter (Christensen et al. 1992) was also

utilized to drive the constitutive expression of transgenes in carrot (Chen and Punja 2002). The regulation of transgene expression is crucial for successful commercial genetic engineering to ensure expression levels are high and in the desired tissues. A comparative assessment of promoter tissue specificity and strength in different tissues has not been previously conducted in carrot as they have in other plants (Schledzewski and Mendel 1994; Horloft et al. 1995; Ni et al. 1995; Gandhi et al. 1999; Kamo and Blowers 1999; Samac et al. 2004). In particular, the utility of promoters to provide expression in carrot tap roots, has not been previously assessed.

The objective of this study was to characterize β-glucuronidase (*uidA*) expression in transgenic carrot tissues under control of three constitutive promoters: the *Arabidopsis* Ubiquitin promoter 3 (*UBQ3*) (Norris et al. 1993), the CaMV 35S promoter (Odell et al. 1985) and domain B duplicated CaMV 35S (D35S) (Kay et al. 1987) The promoters *mannopine synthase* (*mas2*) (Feltkamp et al. 1995) from *Agrobacterium tumefaciens* and the rooting loci gene promoter *rolD* (Leach and Aoyogi 1991) from *A. rhizogenes* were also evaluated, since previous reports indicated that these promoters had enhanced root activity. Relative strengths of these promoters were measured in the leaves, lateral roots and tap roots of mature greenhouse-grown carrot plants as well as in vitro grown calli, leaves and roots of five independently-derived transgenic lines for each promoter.

Materials and methods

Plasmid DNA and plant transformation

The p*CAMBIA* 1391 Z plant transformation vector containing the hygromycin resistance gene under control of the CaMV 35S promoter and the two exons of *uid*A with the catalase intron in front of the puc9 multi-cloning site (MCS) with a *nopaline synthase* terminator (CAMBIA, Australia) was used. Plasmid DNA was isolated from 2 ml of overnight grown cultures of *E. coli* using the Qiagen Qiaquick spin column isolation kit (Qiagen, Maryland, USA). The CAMV 35S, D35S (Dr. Shawn Mansfield, University of British Columbia), *UBQ3* (Syngeta, Canada), *mas2* (Dr. Stephane Garberk, INRA, France) and *rolD* (Francesca Leach, INRA, France) promoters were cloned into the *Hin*dIII and *Bam*HI sites of the MCS (Fig. 1). The ligated plasmids were transformed by electroporation into electromax LBA4404 competent *A. tumefaciens* cells (Invitrogen, Carlsbad, CA, USA) using established methods (Wally et al. 2006). Sterile 'Nantes Coreless' carrot petiole explants were transformed and regenerated as described by Wally et al. (2006). The transgenic callus was maintained on full-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 100 mg/l hygromycin and 0.5 mg/l 2,4-D. Regenerated plantlets were maintained on hormone-free half-strength MS with 100 mg/l hygromycin, and transferred to soil and grown in the greenhouse (Wally et al.

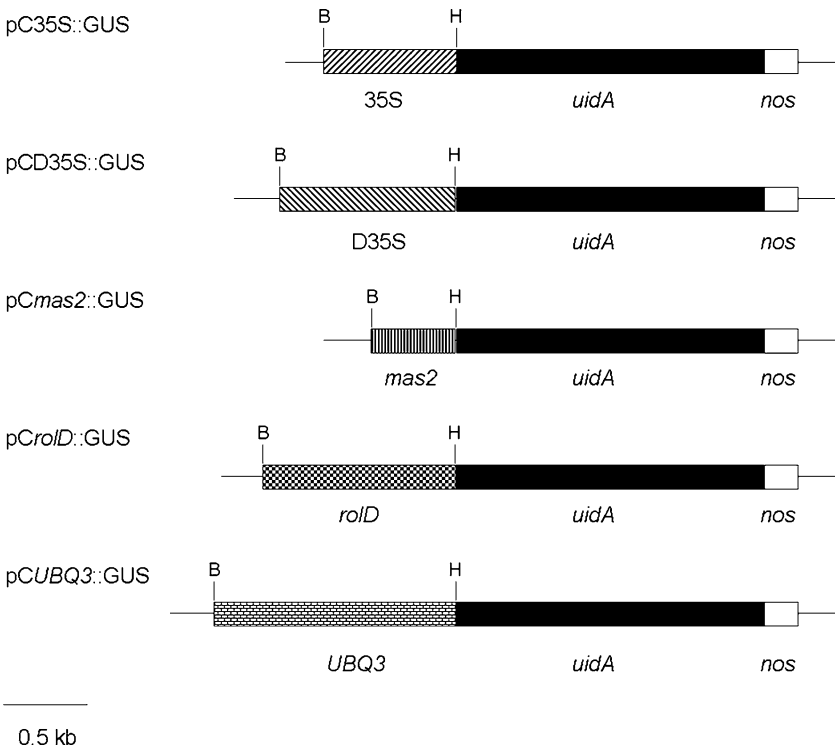

Fig. 1 Plasmid DNA constructs used for *Agrobacterium* transformation of carrot. *From top to bottom* the *uidA* fusion constructs in pCambia1391Z plasmid, under the control of CaMV 35S promoter (pC35S::GUS), enhanced 35S promoter (pCD35S::GUS), the *A. tumefaciens mannopine synthase* (*mas2*) promoter (pCmas2::GUS), the A. rhizogenes rooting loci gene (*rolD*) promoter (pCrolD::GUS) and the *Arabidopsis* ubiquitin (*UBQ3*) promoter (pCUBQ3::GUS). Restriction sites are indicated on figure *Bam*HI (*B*) and *Hin*dIII (*H*)

2006). Root and leaf tissues from 4–5 month old greenhouse-grown plants were harvested and used for analysis of GUS expression and molecular analysis. In addition, lateral root and leaf tissues from 4–8 week old tissue-culture derived plantlets were also included in the analysis.

Confirmation of gene integration

The presence of the *hygromycin phosphotransferase* (*hph*) gene using PCR, was used as the first step for confirmation of transformation. Total genomic DNA was isolated from 200 mg (fresh weight) of leaf tissue that was lyophilized prior to being extracted using established protocols (Wally et al. 2006). The primers used to amplify a 1025 bp fragment of the *hph* gene were hph-F2 (5′-CTA TTT CTT TGC CCT CGG AC-3′) and hph-R2 (5′-AAG CCT GAA CTC ACC GCG AC-3′). Each reaction (25 µl) contained 50 ng carrot DNA, 50 p*M* primers, 0.5 units *Taq* polymerase (Invitrogen) and 1.5 mM $MgCl_2$. The PCR conditions included a 55°C annealing temperature and proceeded for 35 cycles using a PTC-200 thermocylcer (MJ Research, Waltham, MA, USA). Subsequently, Southern analysis was used to demonstrate integration of the *uidA* gene. Total genomic DNA (12 µg) was digested for 16 h at 37°C with 80 units of either *Bam*HI or *Hin*dIII (Promega, Madison, WI, USA), to cut at sites that are both unique in the transgene construct . The digested DNA was run on a 1.2% agarose TBE gel for 20 h and transferred to a nylon membrane under alkaline conditions using established protocols (Sambrook et al. 1989). The blots were probed with a ^{32}P labelled 740 bp fragment of the *uidA* gene generated by PCR using the primers GUSA-F1 (5′- TGA AGA TGC GGA CTT ACG TG -3′) and GUSA-R1 (5′- CCA GCC ATG CAC ACT GAT AC-3′) under the above PCR conditions with a 58°C annealing temperature. Random primers were used to label the probe using [α-^{32}P] dCTP and Prime-A-gene kit (Promega) following manufacturers protocols. The blots were hybridized with the probes for 16 h at 65°C following a 2 h pre-hybridization at 55°C using Ekono hybridization buffer (Research Products International Corp, Mt. Prospect, IL, USA). Following hybridization, the blots were washed 3 times at room temperature with 2× SSC, 0.1% (w/v) SDS for 5 min each, followed by 2 washes with 1× SSC, 0.1% (w/v) SDS at 60 and 65°C, respectively, for 20 min (Sambrook et al. 1989). The blots were exposed to X-ray film at –80°C for 3–7 days with an intensifying screen.

GUS expression

Histochemical staining of petioles, leaves, lateral and tap roots was performed according to Jefferson et al. (1987). The plant tissues were placed in fixative (10 mM MES, pH 5.6, 0.3 M mannitol and 0.3% formaldehyde) on ice for 30 min, followed by washing in 50 mM NaH_2PO_4, pH 7.0. The samples were vacuum infiltrated (20 mBar) for 15 min and stained overnight at 37°C in 50 mM NaH_2PO_4 , 0.5% (v/v) Triton X-100 and 1 mM 5-bromo-3-chloro-3-indoyl-β-D-glucuronide cyclohexamide sodium salt (X-gluc, Inalco Pharmaceuticals, Italy) dissolved in dimethylformamide. Petioles, leaves and orange roots were destained by repeated washings with 70% ethanol for 24–48 h, or until all the coloured pigments were removed. Sections were visualized using 40× magnification under a light microscope with white light (Zeiss, Axioskop, Germany).

Specific expression of GUS for each promoter was determined using five independent lines derived from different transformation events and confirmed to be transformed by Southern analysis. Tissue samples were taken a minimum of three times at 1-week intervals from mature greenhouse-grown shoots and roots, callus and tissue-cultured shoots and roots. GUS activity was determined according to Jefferson et al. (1987) by measuring the accumulation of the fluorescent substrate 4-methylumbelliferone (4-MU) cleaved from 4-methylumbelliferone-glucuronide (4-MUG). Samples of 250–750 mg fresh weight of the various carrot tissues were macerated using an ice-cold mortar and pestle with a pinch of sea sand with a 2:1 (v/w) buffer:tissue ratio. The extraction buffer contained 50 mM NaH_2PO_4, pH 7.0, 10 mM EDTA, 0.1% (v/v) Triton X-100, 0.1% (w/v) sodium lauryl sarcosine, 4% (w/v) polyvinylpolypropadine (PVPP) and 5 mM dithioretitol (DTT) (Jefferson et al. 1987). Macerated samples were centrifuged twice at 4°C for 15 min at 14,000*g*, and the supernatant was transferred to a new 1.5 ml microcentrifuge tube. Clarified protein extracts were quantified using the Bradford reagent (Sigma, USA) using a Bio-Tek 1200 microplate reader (Fisher, USA). Five µg of protein extract was assayed in a total volume of 50 µl in a 1 mM MUG solution (50 mM NaH_2PO_4, 10 mM EDTA, 0.1% (v/v) Triton X-100 and 5 mM DTT) on a 96 well micro-titre plate. The reactions were stopped at 1.5, 3 and 4.5 h by adding 250 µl of 0.2 M sodium carbonate. Fluoresence was measured using a 96 well fluorometer (Isoplate-96 Perkin-Elmer Corporation, Norwalk, CT, USA) set at 365 nm for excitation and 455 nm for emission. Non-transformed 'Nantes Coreless' carrot roots and leaves served as negative controls/blanks for both histochemical and fluorometric assays.

Results

Molecular analysis

Of the 76 total lines confirmed to be positive for the presence of the *hph* gene by PCR (not shown), 70

contained one to three copies of the *uid*A gene, as determined by *Hin*dIII and *Bam*HI digestion and Southern blot hybridization (Fig. 2). Up to ten independent lines, with 1–3 *uidA* copies, from each of the five promoter constructs were then analyzed for GUS activity by histochemical staining (Fig. 3). The majority (65 of 70) had detectable levels of X-gluc staining, and the five visually strongest expressing lines from each promoter were selected for further analysis. The percentage of regenerated plantlets that grew on MS medium with 100 mg/l hygromycin and showed *uid*A expression was 100% for *UBQ3* and *mas2*, 80% for *rolD*, 75% for 35S and 50% for D35S. The number of *uidA* copies had no correlation to the intensity of GUS activity (data not shown).

Visualization of GUS expression

There were significant differences in the intensity of staining of carrot tissues with X-gluc in plantlets containing the different constitutive promoter constructs. Strong GUS activity was found in the leaves of plants expressing *uidA* under control of the D35S, *UBQ3* and 35S promoters. All leaf tissues stained very darkly, including the trichomes, mesophyll and vascular tissues (Fig. 3). There were only slight visually observable differences between the promoters, with the D35S lines appearing stronger overall. When the lateral roots were stained, these three promoters provided expression throughout the length of the root, including the root tip, root hairs and the vascular tissues (Fig. 3). Cross-sections of the tap roots revealed similar patterns of staining; however, the 35S promoter provided slightly less intense and non-uniform staining throughout the length of the root compared to *UBQ3* and D35S (Fig. 3). There was intense GUS staining in the root parenchyma cells, phloem rays, xylem and cambium in plants containing the 35S, D35S and UBQ3 promoters (Fig. 3).

Plantlets expressing *uidA* under control of the *mas2* and *rolD* promoters showed significant differences in staining intensities in different tissue types of greenhouse-grown plants compared to the smaller tissue culture-grown plants. Only slight staining was visible in the veins of leaves of *rolD* plants (Fig. 3), while the *mas2* promoter showed weak staining throughout the leaves, with more staining near the tip of the leaf. (Fig. 3). In *mas2* lines, the cotyledons and hypocotyl tissues exhibited GUS activity, while no activity was seen in these tissues in the *rolD* lines (not shown). Lateral roots of *mas2* and *rolD* lines stained darkly, with enhanced staining observed at root tips, vascular bundles and root hairs. The *rolD* roots stained slightly darker than roots of the *mas2* lines; however, expression was still significantly lower when compared to the constitutive promoters. The taproots of plants with *mas2* and *rolD* promoters were also stained throughout the different tissue types, with the weakest staining observed in the periderm and more intense staining in the phloem and cambium (Fig. 3).

Quantification of promoter strength

GUS enzyme activity assays were performed on the five lines selected for each of the different promoter constructs. For the CaMV 35S promoter, GUS activity was lowest in callus tissue and highest in leaves of tissue-cultured plants (Fig. 4). High GUS activity was observed in the roots and leaves of mature greenhouse-grown plants, which was similar to the levels measured in the roots of tissue-cultured plants. There was more variation among individual 35S promoter plant lines in leaf expression, compared to root or callus tissue.

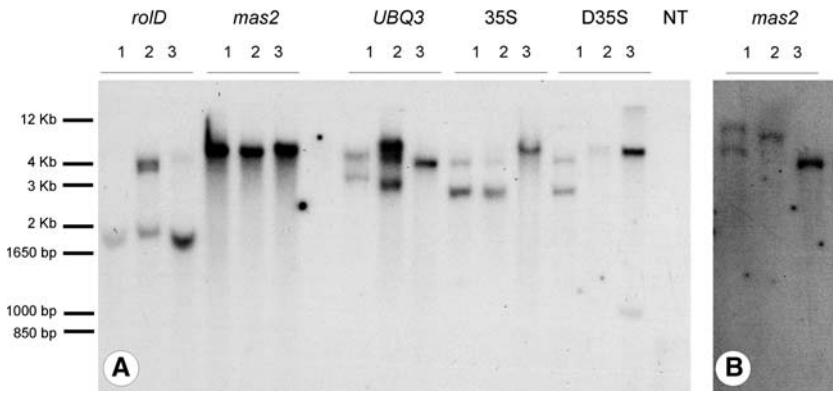

Fig. 2 Southern hybridization analysis of transgenic carrot lines containing the *uidA* fusion gene under control of either the *rolD*, *mas2*, *UBQ3*, CaMV 35S or D35S promoters. Genomic DNA was digested with *Bam*HI (**a**) or *Hin*dIII (**b**), the DNA blot was hybridized with the 740 bp *uidA* gene fragment. Three different lines were shown for each promoter. Size markers from the 1 Kb+ (Invitrogen) ladder are shown on the *left*. *NT* non transgenic control plant

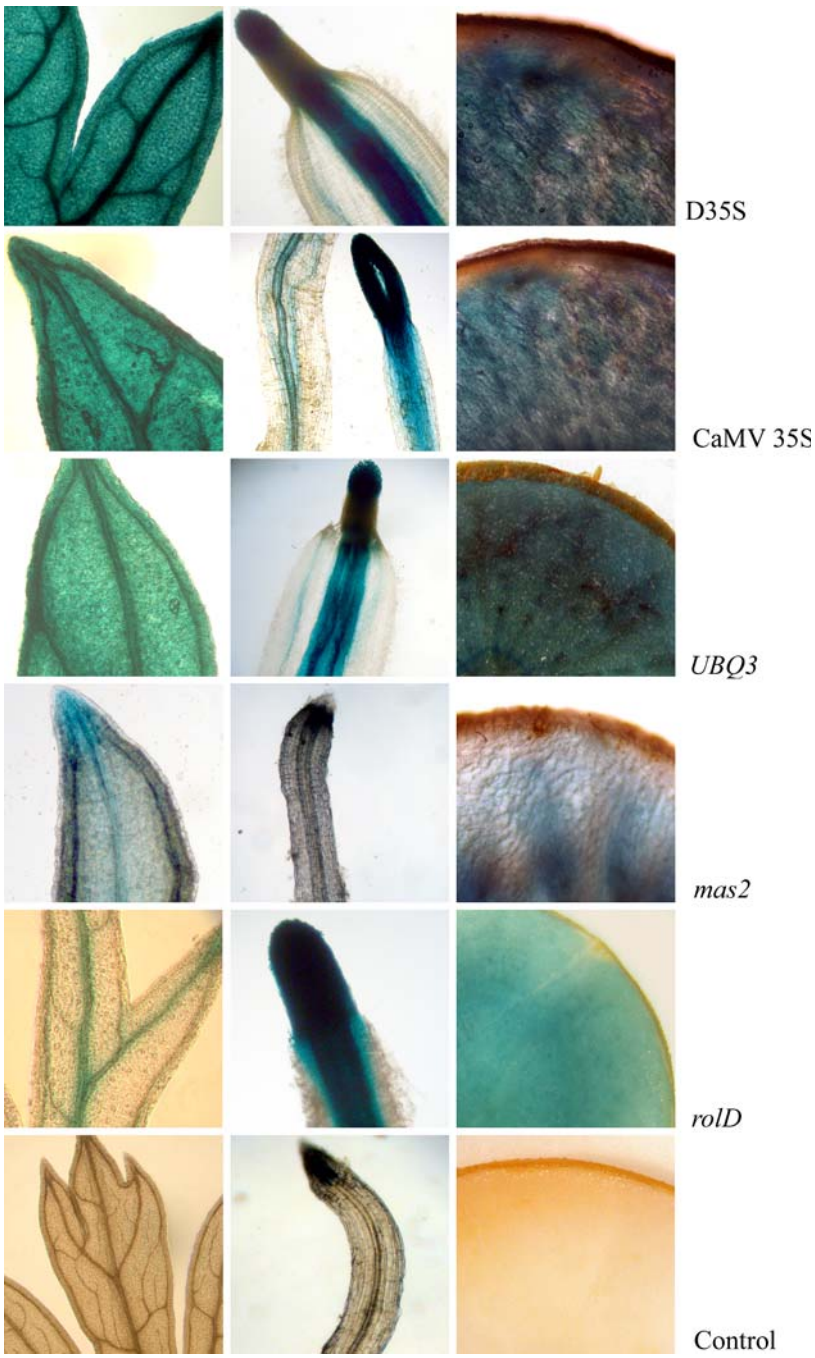

Fig. 3 Histochemical assay for GUS activity in leaves, seedling roots and tap roots of carrot shown from *left* to *right*. The various promoters used in this study are indicated

With the D35S promoter, there was very high GUS activity in all tissue types examined, with highest activities in mature greenhouse-grown leaves and in leaves and roots of tissue culture-grown plants (Fig. 4). The lowest activity was found in callus tissue, which had approximately 50% of the GUS activity compared to the leaves. The D35S promoter had higher GUS activity than the 35S promoter in all tissues examined, ranging from an average of 1.5-fold higher activity in tissue-culture grown leaves to an average of over threefold higher activity in callus tissues (Table 1).

With the *UBQ3* promoter, the highest GUS activity was observed in the roots of mature greenhouse-grown plants, with an average of 2.5-fold higher activity than the 35S promoter. The GUS activity was also significantly higher compared to the 35S promoter in the callus tissue, with nearly an average increase of twofold. The *UBQ3* promoter GUS activity was significantly lower than 35S promoter in the leaves of tissue-cultured plants, and was similar to that in roots of tissue culture-grown plants. However, in greenhouse-grown plants, the roots had an average of nearly threefold higher GUS activity compared to the leaves.

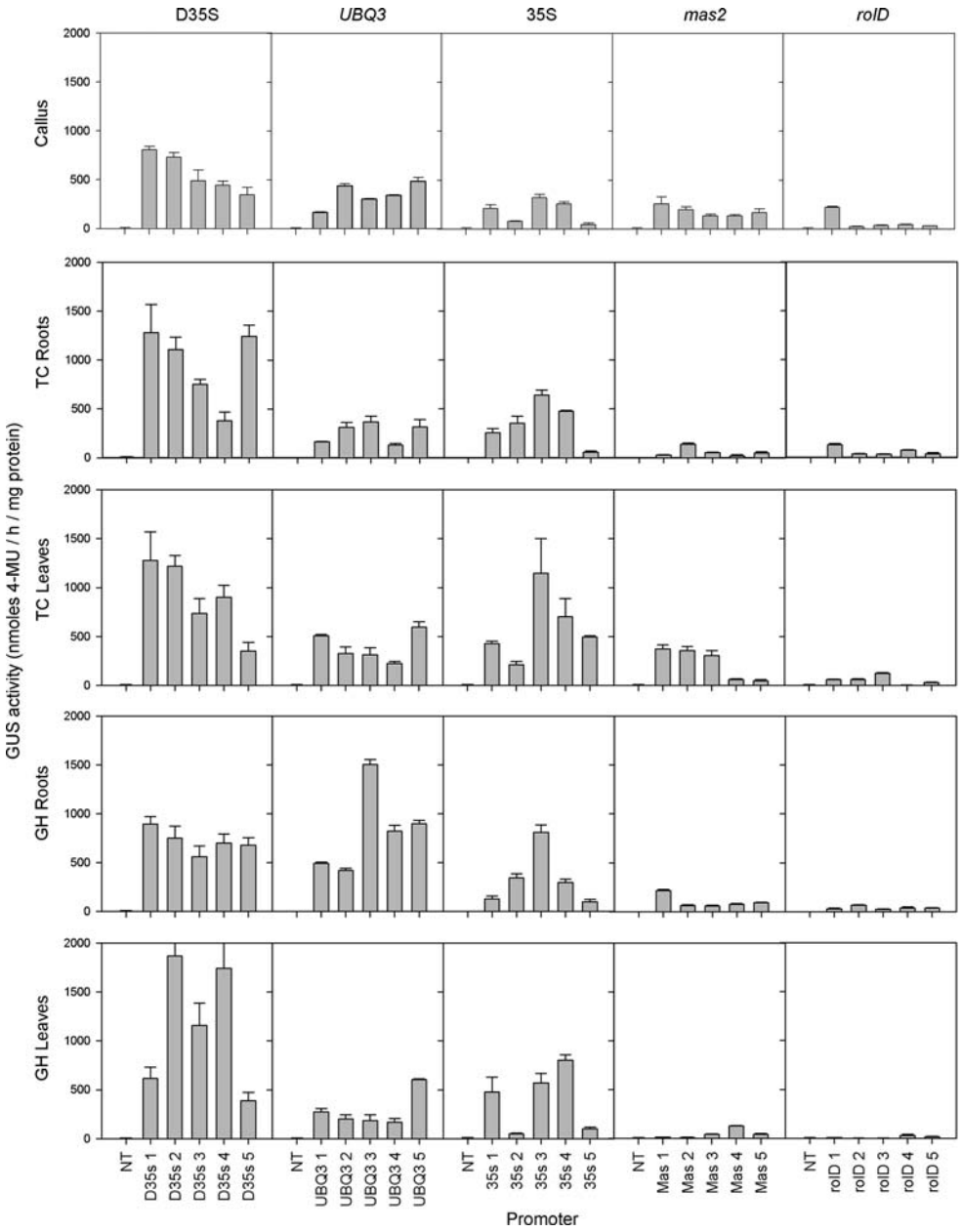

Fig. 4 Specific activity of GUS in callus, tissue-cultured (TC) roots, tissue-cultured leaves, greenhouse-grown (GH) roots and leaves of transgenic carrot plants expressing the *uidA* gene under control of five promoters (D35S, *UBQ3*, 35S, *mas2* or *rolD*). Values represent the mean specific activity in five individual lines for each promoter, with a minimum of three replicates ± standard error for each transgenic line

With the *mas2* promoter, the average GUS activity was highest in leaves of tissue culture-grown plants (Fig. 4). There was also fairly high GUS activity in the callus tissue with levels similar to that of the 35S promoter (Table 1). There was an average of fourfold higher activity in tissue-culture grown leaves as compared to roots of the *mas2* lines examined. However, in mature greenhouse-grown plants, there was 50% less GUS activity in leaves compared to roots. There was an average of twofold higher activity in mature roots compared to young tissue-culture grown roots. There was approximately fourfold higher activity in young tissue-culture grown leaves compared to mature greenhouse-grown leaves.

With the *rolD* promoter, GUS activity was highest in callus tissues (Fig. 4). The activity was relatively low in all of the other tissues tested, with GUS levels at only 3–12% of that in the roots and leaves of 35S promoter plants (Table 1). There were no significant differences in GUS activity between tissue-culture grown roots and leaves. However, in mature greenhouse-grown roots, there was nearly a fourfold increase in GUS activity compared to the leaves. GUS activity was similar in greenhouse-grown roots and tissue-cultured roots.

Table 1 Average levels of GUS expression in callus, leaves, and roots of carrot plants expressing the *uidA* gene under control of CaMV 35S, D35S, *UBQ3*, *mas2* or *rolD* promoters

Promoter	Callus	Tissue culture-grown plants		Greenhouse-grown plants	
		Leaves	Roots	Leaves	Roots
D35S Mean[a]	561.7 ± 48.9 (3.08)	896.8 ± 94.7 (1.51)	950.7 ± 95.6 (2.70)	1154.3 ± 164.4 (2.89)	715.6 ± 30.2 (2.13)
Range[b]	345–804	350–1280	377–1278	390–1870	560–891
UBQ3 Mean	350.5 ± 30.9(1.92)	391.9 ± 38.5 (0.66)	350.6 ± 26.1 (0.98)	286.3 ± 45.5 (0.72)	827.1 ± 107.5 (2.47)
Range	171–487	223–600	129–365	166–603	420–1500
35S Mean	182 ± 29.1 (1.00)[c]	595 ± 88.3 (1.00)	355.9 ± 55.24 (1.00)	399.2 ± 80.2 (1.00)	335.3 ± 16.2 (1.00)
Range	45–320	210–1145	55–640	99–801	100–800
mas2 Mean	178.1 ± 12.8 (0.98)	227.0 ± 40.3 (0.38)	57.3 ± 11.6 (0.16)	48.2 ± 12.1 (0.12)	101.1 ± 16.2 (0.30)
Range	135–257	47–372	20–136	10–129	56–214
rolD Mean	71.4 ± 21.1 (0.39)	54.1 ± 11.0 (0.09)	61.2 ± 10.2 (0.17)	11.1 ± 2.5 (0.03)	39.2 ± 4.1 (0.12)
Range	32–221	3–120	31–127	3–27	25–66

[a] Values are the means of specific activity (nmoles 4-MU/h/mg protein) ± standard error of five independently transformed lines for each promoter. The assays were repeated 3–6 times

[b] Range of GUS activity (nmoles 4-MU/h/mg protein), with highest and lowest values

[c] Value in parenthesis indicates the relative GUS expression level compared to the CaMV 35S level for the particular tissues

Discussion

The CaMV 35S promoter is the most widely used promoter for transgene expression in plants, and provides very high constitutive levels of expression in dicotyledon species and slightly weaker expression in monocotyledon species (Gandhi et al. 1999). The enhanced D35S promoter has a duplication of the –343 to –90 domain B which has been shown to enhance transgene expression by up to tenfold when compared to the 35S promoter (Kay et al. 1987). As with previous reports from other dicotyledon plants (Comai et al. 1990; Holtorf et al. 1995), the CaMV 35S and enhanced D35S promoters were found to be the strongest promoters overall in carrot tissues. The duplication of the domain B increased the overall expression levels in carrot by an average of 3.1-fold in callus, 2.7 and 2.1-fold in roots, and 1.5 and 2.9-fold in leaves under different growing conditions. These increases are within the ranges reported for other transgenic plants (Potenza 2004). Both 35S and D35S promoters provided strong GUS expression in all carrot tissues examined, including leaves, petioles, cotyledons, lateral roots and tap roots. Both promoters had highest activity in leaves, followed by roots and callus tissue. These findings are consistent with those from *Arabidopsis* and tobacco, where petiole and leaf expression levels were significantly higher than in roots (Holtorf et al. 1995; Malik 2002) but differs from the expression levels reported in gladiolus and alfalfa (Kamo 2003; Samac et al. 2004), in which the 35S promoter had the highest activity in roots. Overall, the D35S promoter provided the highest level of gene expression in all carrot tissue types tested, except for mature roots.

Ubiquitin is highly conserved across plant species, is highly abundant in the cytoplasm and is involved in many crucial cellular processes. Many of the Ubiquitin genes are constitutively expressed, including the maize (*ubi-1*) and *Arabidopsis* (*UBQ3*) genes (Christensen et al. 1992; Norris et al. 1993). Typically, monocotyledon plants have highest constitutive levels of transient gene expression with monocot-derived actin or ubiquitin promoters, while dicots typically have highest expression with viral promoters or dicot-derived ubiquitin promoters (Horloft et al. 1995; Gandhi et al. 1999; Kamo 2003). In this study, the *Arabidopsis UBQ3* promoter provided significantly higher expression levels compared to the CaMV 35S promoter in callus (2.0-fold higher) and mature greenhouse-grown roots (2.5-fold higher). The *UBQ3* driven GUS expression levels were very similar to the 35S promoter in tissue-cultured young roots, and slightly lower in both tissue-cultured (0.65 of the 35S) and mature greenhouse leaves (0.7 of the 35S). These findings are similar to those made in transiently-expressing *Arabidopsis*, where comparable expression was observed in the leaves with genes driven by the 35S and *UBQ3* promoters (Norris et al. 1993). The *UBQ3* driven GUS expression was very prominent in carrot root tissues, with enhanced activity when compared to the 35S and D35S promoters. When quantified, the GUS activity provided by the *UBQ3* promoter in mature roots was significantly higher than that of the D35S promoter. The heightened overall root activity indicates that *UBQ3* is ideal for expressing proteins in tap roots, and for post-harvest roles, including suppression of post-harvest diseases by over-expression of pathogenesis-related proteins.

The *mannopine synthase* gene is a bi-directional gene from *Agrobacterium tumefaciens*, which requires the activity of a *mannopine conjugase* (*mas2*) and *reductase* (*mas1*). The *mas2* promoter has been analyzed in detail, and contains two enhancer sequences and is reported to provide significantly higher levels of gene expression than the *mas1* promoter, which contains only a single enhancer sequence (Guevera-Garcia et al. 1999). In this study, the *mas2* promoter drove high levels of GUS expression in callus tissue cultured in vitro, with GUS expression levels approaching that of the 35S promoter. The *mas* promoters are sensitive to auxin and activity increases when the auxin:cytokinin ratio is increased (Langridge et al. 1989). The carrot calli were maintained on medium containing 2,4-D (0.5 mg/l); therefore, enhanced expression in the callus tissue was expected. Similar to transgenic potato and tobacco transformed with *mas2*::*uidA* fusion constructs, there was higher GUS activity in mature greenhouse-grown carrot roots compared to the shoot or leaf tissues (Feltkamp et al. 1995). However, these levels were substantially less than in transgenic tobacco, where root activity levels exceeded those of the 35S promoter (Comai et al. 1990). Our results also differed from the *mas2* activity reported in transgenic rapeseed varieties, which exhibited high *mas2* driven GUS activities in the leaves, with reduced activity in roots, substantially lower than for the 35S promoter (Pauk et al. 1995). In carrot, the *mas2* promoter is potentially useful for use in suspension culture bio-reactors, reflecting the high GUS expression levels observed in callus tissues, and for expression of transgenic proteins in mature roots.

The rooting loci gene (*rolD*) isolated from the root-inducing plasmid of *Agrobacterium rhizogenes* has been reported to drive high levels of expression in both the leaves and roots of young transgenic tobacco seedlings (Leach and Aoyagi 1991) and transgenic pea (Fei et al. 2003). The transgenic carrots examined had very low levels of GUS expression in all of the tissues tested. Strongest expression was seen in the callus, roots and leaves of tissue-cultured plants. In mature plants, there was fourfold higher activity in the roots compared to the leaves. Conversely, mature transgenic tobacco containing the *rolD*::*uidA* construct had 30-fold higher GUS expression in roots compared to the shoots (Elmayan and Tepfer 1995). In carrot, the overall strength of *rolD* in mature root tissues was substantially lower than that reported from other plants. Transgenic *Gladious* plants exhibited strong GUS root expression with *rolD*, and expression levels were comparable to that of the 35S promoter (Kamo 2003). In transgenic *N. plumbaginifolia*, a 3–5 fold higher root expression was seen with *rolD* compared to that of the 35S promoter (Fraisier et al. 2000). Despite lower GUS activity, histochemical staining of GUS was still evident with the *rolD* promoter in carrot taproots. However, these findings indicate that the *rolD* promoter will likely not be very useful for expressing transgenic proteins in carrots.

In conclusion, the D35S promoter provided highest levels of GUS activity in carrot leaves followed by the 35S promoter, while the *UBQ3* promoter from *Arabidopsis* provided high levels of GUS activity in all tissues, especially in the tap roots. The previously reported root enhanced promoters *mas2* and *rolD* provided proportionally lower levels of GUS activity in mature carrot roots. Understanding the GUS expression profiles of the different promoters will allow for more precise control of expression levels and organ targeting in both in vitro and field grown transgenic carrots.

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