Theor Appl Genet (2005) 111: 479–488
DOI 10.1007/s00122-005-2037-1

ORIGINAL PAPER

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QTLs controlling the production of transgenic and adventitious roots in *Brassica oleracea* following treatment with *Agrobacterium rhizogenes*

Received: 12 November 2004 / Accepted: 11 April 2005 / Published online: 8 June 2005

Abstract *Brassica oleracea* can be genetically engineered using *Agrobacterium rhizogenes*. The initial stage of this process is the production of transgenic ('hairy') roots; shoots are subsequently regenerated from these roots. Previous work using *gus* and *gfp* reporter genes has shown that genotypes of *B. oleracea* vary in their performance for transgenic root production. Quantitative trait loci (QTLs) controlling this trait have been located in one mapping population. The current study provides evidence that performance for transgenic root production is associated with performance for adventitious (non-transgenic) root production in *B. oleracea* across a second mapping population. This is shown by regression analyses between performance for the two traits and the demonstration that QTLs controlling the two traits map to the same positions within the genome. Since the rate of adventitious root production does not differ significantly in the presence and absence of *A. rhizogenes*, there is no evidence that the expression of *Agrobacterium* genes induces adventitious root production. It is apparent that genotypes exhibiting high adventitious root production in the absence of *A. rhizogenes* will also tend to show high transgenic root production, thereby allowing the selection of lines that are more efficiently transformed.

Introduction

Agrobacterium-mediated transformation has become the most commonly used method for plant genetic engineering (Puddephat 2003). The interactions between *Agrobacterium* and plant cells are complex, but considerable progress has been made determining the functions of *virulence* (*vir*) genes in the bacterium (Gelvin 2000). In recent years, a number of plant genes, proteins and metabolites that act to control components of the plant-*Agrobacterium* interaction have also been discovered (Tzfira and Citovsky 2002; Gelvin 2003). In the early stages of the interaction, a vitronectin-like protein (Wagner and Matthysse 1992; Swart et al. 1994) and an arabinogalactan protein (Nam et al. 1997, 1999) have been shown to play a role in *Agrobacterium* attachment to plant cells, while a component of a plant exudate has been demonstrated to specifically inhibit *vir* gene expression (Zhang et al. 2000). The bacterial proteins VirD2 and VirE2 play a role in delivering T-DNA into the plant nucleus, and there is evidence that several plant proteins interact with these and influence the efficiency of this process. For example, VirD2 binds to three members of a cyclophilin-chaperone family (Deng et al. 1998), to a protein phosphatase (Gelvin 2000) and to a karyopherin α-protein (Ballas and Citovsky 1997), while VirE2 binds to proteins denoted as VIP1 (Tzfira et al. 2001) and VIP2 (Tzifira and Citovsky 2000). The final stage of the interaction is the integration of T-DNA into the plant genome by illegitimate recombination. Histone H2A has been shown to be involved in this process (Mysore et al. 2000), and it is probable that plant DNA repair enzymes also play key roles (Ziemienowicz et al. 2000). While it seems likely that only a proportion of the plant genes that influence the efficiency of T-DNA transfer have been identified to date, variation at such loci must be responsible for some of the differences in the efficiency of *Agrobacterium*-mediated transformation commonly encountered among the genotypes (breeding lines or varieties) of individual plant species (see, for example, Sangwan et al. 1991; Barg et al. 1997; Cogan et al. 2001).

Previous work on *Brassica oleracea* has resulted in the development of transformation protocols employing both *Agrobacterium tumefaciens* and *A. rhizogenes*

Communicated by G. Wenzel

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(Poulson 1996; Puddephat et al. 1996). Anther culture can be used to produce doubled haploid (DH) plants of *B. oleracea*, and a DH mapping population, known as the AG population (Sebastian et al. 2000), has been used for the genetic analysis of transformation-associated traits using both *Agrobacterium* species. When *A. tumefaciens* is used in genetic engineering protocols, the wild-type T-DNA has been deleted from the Ti plasmid and an engineered T-DNA, without the tumorigenic genes but normally containing a selectable marker and/or reporter gene, is delivered to the plant—often from a binary plasmid. This strategy is necessary because it is extremely difficult to regenerate whole plants from tumour cells (Guerche et al. 1987), and even where this is possible, the regenerants are likely to be chimeric. The *A. tumefaciens*-based protocol for *B. oleracea* transformation has employed just such a binary plasmid system to deliver transgenes to plant cells; shoot regeneration from these transformed cells follows. Diallel analyses carried out using the AG population have indicated that the variation observed in susceptibility to *A. tumefaciens* was attributed to both additive and dominant gene effects and that a highly significant quantitative trait locus (QTL) is located on linkage group (LG) 9 (Sparrow et al. 2004a). A subsequent diallel analysis demonstrated that both shoot regeneration and the production of multiple shoots are controlled by additive and dominant gene effects (Sparrow et al. 2004b). The demonstration that both shoot regeneration and susceptibility to *A. tumefaciens* are good phenotypic markers for performance in transformation has allowed their use in the selection of genotypes from within the AG population that can be transformed with high efficiency (Sparrow et al. 2004c).

Parallel genetic analyses carried out using an *A. rhizogenes*-based transformation protocol with the AG DH population of *B. oleracea* have revealed that the transformation process is different from that with *A. tumefaciens*-induced tumours. When *A. rhizogenes* is used, it is frequently possible to regenerate plants from the transgenic roots—named "hairy roots" by Tepfer (1990)—which are the visible symptom of wild-type T-DNA transfer by this species of *Agrobacterium*. Furthermore, since each root is produced from a single cell, all cells of a regenerated plant will have the same (transgenic) genotype. The regeneration of whole plants from hairy roots has been demonstrated for *B. oleracea* (Puddephat et al. 1996). These hairy roots have been co-transformed with T-DNAs from both the wild-type Ri (root-inducing) plasmid and from a binary plasmid bearing a reporter gene. Using the *green fluorescent protein* (*gfp*) transgene, Cogan et al. (2001) showed that there are significant differences in the efficiency of *A. rhizogenes*-induced production of transgenic roots across a set of *B. oleracea* genotypes. QTLs controlling the production of transgenic roots have been identified on linkage groups 1, 3 and 7 (Cogan et al. 2002). Further analysis of these QTLs has been carried out using a set of substitution lines in which small segments of the GDD33 genome have been introgressed into the A12DHd background (Ramsey et al. 1996; Rae et al. 1999), thereby confirming and redefining the genetic locations of the QTLs (Cogan et al. 2004). The use of a *β-glucuronidase* (*gus*) reporter gene in time-course experiments and an "orthogonal set" of genotypes (containing all eight possible combinations of "positive" and "negative" alleles at the three QTLs) also allowed the determination of the stage of transformation at which these genes have their effects. During these analyses, QTLs for both transgenic and non-transgenic adventitious root production have been identified in *B. oleracea*. Two of the QTL regions identified were common to both transgenic and non-transgenic root production (Cogan et al. 2004).

In the investigation reported here, we have used an independent *B. oleracea* mapping population (the "NG" rather than the "AG" population) to identify QTLs controlling transgenic root production following the incubation of *B. oleracea* explants with *A. rhizogenes*. The previous genetic analyses have suggested that genotypes with superior performance for adventitious root production are easier to transform using *A. rhizogenes*. A major objective of this work was to test this hypothesis by determining whether the performances of DH progeny lines for transgenic root production and for non-transgenic (adventitious) root production are associated and by determining whether QTLs for the two traits map to the same genomic positions. An additional objective was to test the possibility that the production of phenotypically normal, non-transgenic roots is enhanced by treatment with *A. rhizogenes* as has previously been reported in some other species (Van Wordragen et al. 1992; Yibrah et al. 1996; Falasca et al. 2000). To investigate this, adventitious root production was monitored in *B. oleracea* lines in both the presence and absence of *A. rhizogenes*.

Materials and methods

Plant material and culture conditions

Plant material included seeds of the DH cauliflower line Nedcha (N) (*Brassica oleracea* var. *botrytis*) and the DH Brussels sprout line Gower (G) (*B. oleracea* var. *gemmifera*) as well as of 48 genotypes of the DH NG mapping population that were produced by microspore culture using F_1 progeny following the crossing of the Nedcha and Gower lines (Sebastian et al. 2000). The seeds were first surface-sterilised by submersion in a 1.7% (w/v) solution of sodium dichlorciscyanurate for 6 min, rinsed twice in sterile purified water and air-dried under aseptic conditions for at least 4 h. The dried seeds were then stored at 5°C until required. For germination, the seeds were placed in 25-compartment, 100-mm^2 repli-dishes (Bibby Sterilin; Barloworld Scientific, Stone, Staffordshire, UK). Each compartment contained 1 ml of water agar (6 g/l) and three seeds of each genotype.

The seeds were initially incubated for 3 days at 15 ± 2°C under a 12/12-h (light/dark) photoperiod with light supplied at an intensity of 250 μmol/m^2/s^2); this was followed by a further 3 days at 22°C under a 16/8-h (light/dark) photoperiod (at 80 μmol/m^2/s^2).

Transformation procedure

Agrobacterium rhizogenes strain LBA9402 (Spano et al. 1982) harbouring the binary plasmid pBIN-*m*-*gfp*-5ER (Haseloff et al. 1997) containing the *gfp* reporter gene driven by a cauliflower mosaic virus (CaMV) 35S promoter was used for inoculation purposes. Cultures were maintained on semi-solid YMB medium (Gelvin and Liu 1994) supplemented with 50 mg/l kanamycin for maintenance of the binary plasmid. For plant transformation, *Agrobacterium* cells were suspended in MGL broth (Walkerpeach and Velten 1994) and incubated overnight at 25°C on a shaking platform (175 rpm). The bacterial cells were then pelleted by centrifugation at 11,600 *g* for 5 min and resuspended in liquid Murashige and Skoog (1962) medium containing 30 g/l sucrose (MS30) and 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) to produce an optical density ($A_{600\ nm}$) of 1.0 ± 0.1.

The method of transformation was as described in Puddephat et al. (2001). Explants for the transformation experiments were excised from 6-day-old aseptic *B. oleracea* seedlings by cutting the hypocotyl approximately 5 mm below the cotyledons. The explants were then inverted, with the cut end of the hypocotyls facing upwards, and placed onto MS30 medium supplemented with 200 mg/l cefotaxime in 5-cm-deep petri dishes with three explants per dish. The explants were inoculated with either a 2-μl drop of resuspended *Agrobacterium*, which was placed onto the cut surface of the hypocotyls, or with 2 μl of an inoculum consisting only of culture medium and 2,4-D and no *A. rhizogenes* and incubated at 22°C under a 16/8-h (light/dark) photoperiod (as described for germination). Thirty-five days following inoculation, all explants were scored for the number of adventitious roots produced and, in the case of those inoculated with *A. rhizogenes*, for the number of transgenic roots. Transformation events were detected by illuminating samples with long-wave UV light, using a hand-held lamp (UVP, B-100 AP) and a UV face-shield (UVC-803). Transgenic roots showed lime-green fluorescence due to the presence of GFP. Roots that did not show any visible fluorescence were scored as adventitious (non-transgenic).

Experimental design and analysis

A total of 15 replications were performed on separate occasions, each consisting of three explants from each of the 48 genotypes and both parents (a total of 2,250 individual explants). Cut hypocotyls were inoculated, one with only culture medium and two with medium containing *A. rhizogenes*. Predicted mean data sets for the number of GFP-fluorescing and adventitious roots produced per explant for each DH line were calculated using a generalised linear model (McCullaugh and Nelder 1987). The predicted means obtained were analysed with Poisson error and log link functions to assess the validity of the values and normality of the distribution.

QTL analysis

Prior to QTL analysis, an analysis of variance was performed on the means for numbers of transgenic and adventitious roots produced per explant for the parents and the NG lines. This confirmed that the lines differed significantly for each of these quantitative traits and produced a value for the additive genetical variance, V_A. Square root transformation of the data was carried out before QTL analysis in order to improve the normality and fit of the data to the QTL models in comparison to normally distributed but untransformed data.

A subset of marker loci was selected from the genetic linkage map for the NG population developed by Sebastian et al. (2000). This provided an even coverage of the genome, with the markers spaced approximately 10 cM apart using the consensus map distances provided by Sebastian et al. (2000) and marker scores for individual NG genotypes at each locus. The predicted mean data sets for GFP-fluorescent root production and adventitious root production for all genotypes were analysed by ANOVA for each linkage group in order to detect correlations between the sum of the mean scores and marker alleles (marker means). Analysis of the marker means considered all individual associations between marker scores and trait performance. These tests are significant to the 5% level and highlight areas of significance for each trait along each linkage group, thereby providing an initial estimate of QTL presence and location. To investigate these regions in more detail, QTL analysis was performed by marker regression and interval mapping using the QTL CAFÉ software (http://www.biosciences.bham.ac.uk/labs/kearsey).

The marker regression method proposed by Kearsey and Hyne (1994) was used to provide estimates of additive effects and identify QTLs using ANOVA. A single predicted QTL was only accepted when the residual mean square figure was not significant at the 5% level, and the regression mean square was significant at the 1% level. Where a single predicted QTL was significant using multiple marker regression, the predicted location, along with centiMorgan confidence intervals and additive effects, were all calculated using 1,000 simulations. If the residual test statistic was significant, genetic models with additional QTL were examined.

The interval mapping approach followed the method described by Haley and Knott (1992), which uses flanking markers for "interval mapping" based on individual observations rather than marker means (Kearsey and Pooni 1998). Interval mapping (Lander

and Botstein 1989; Haley and Knott 1992; Jansen and Stam 1994) was performed on the data set using the same software to confirm the presence of the QTL when the residual F test statistic was 0.01.

Results

Hypocotyl stumps of two parental lines of *B. oleracea* were incubated in the presence or absence of *A. rhizogenes* and subsequent root production was scored. The mean total numbers of roots produced by Nedcha and Gower across all treatments were 15% and 42%, respectively. When Nedcha and Gower were incubated in the presence of *A. rhizogenes*, the proportions of these roots that were transgenic (GFP-positive) were 10.9% and 5.7%, respectively. The same analyses were carried out with DH mapping lines of the NG population (produced following a cross of Nedcha and Gower). In the absence of the bacterium, the mean numbers of adventitious (non-transgenic) roots produced by explants of genotypes of the DH mapping population varied from 2.27 (±0.51) to 20.21 (±5.46) per explant (Fig. 1a) with an overall mean of 9.00. In the presence of *A. rhizogenes*, the mean numbers of adventitious roots produced ranged from 3.29 (±0.395) to 13.00 (±0.785) per explant (Fig. 1b), with an overall mean of 8.84. Table 1 shows the generation means for adventitious root production both in the presence and absence of *A. rhizogenes* as well as estimated additive effects. In both the presence and absence of *Agrobacterium* there was significant variation in adventitious root production across the NG population ($P < 0.001$). Following incubation with *A. rhizogenes*, the numbers of transgenic roots were assessed by scoring the fluorescence of GFP produced as a result of transgene delivery from a binary plasmid. The values across the NG population ranged from 0.43 (±0.13) to 5.04 (±0.50) per explant (Fig. 2), with an overall mean of 2.46 and significant variation between genotypes ($P < 0.001$). Table 1 also shows the generation means for transgenic root production as well as estimated additive effects.

Using the predicted mean data sets, analyses were carried out to test for significant correlations between the three traits: transgenic root production and adventitious root production in the presence and absence of *Agrobacterium*. All of these traits showed significant correlation ($P < 0.001$; Table 2).

Adventitious root production in the absence of *A. rhizogenes*

Preliminary marker means analysis identified regions of significance for adventitious root production in the absence of *A. rhizogenes* on LGs 1, 8 and 9, with no significant genetic variation associated with any markers on any other linkage group (Table 3). Interval mapping identified a single QTL on the same linkage groups

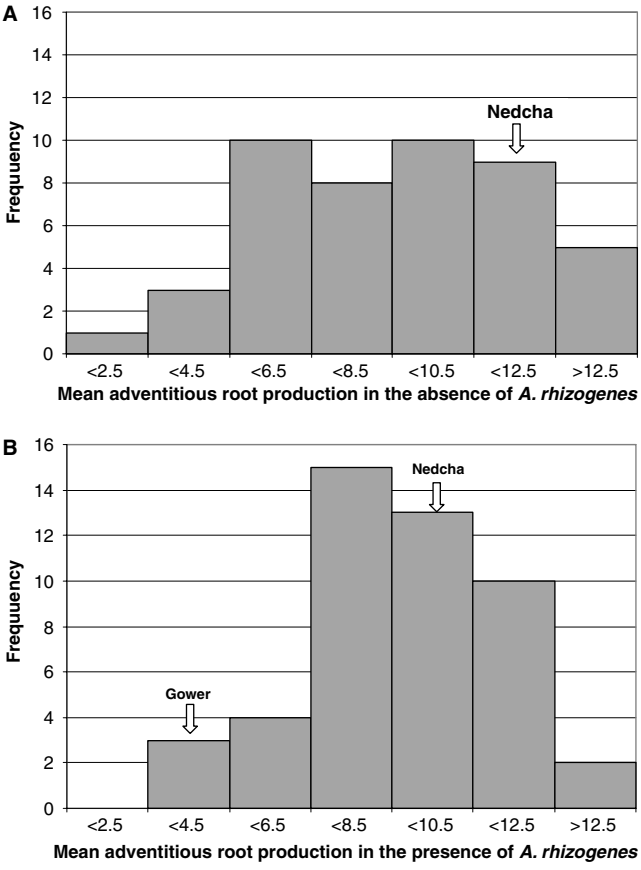

Fig. 1 **a** Frequency histogram showing mean scores of adventitious root production in the absence of *Agrobacterium rhizogenes* from seedling explants of the NG DH mapping population. Explants were scored 35 days after inoculation. The mean adventitious root production in the presence of *A. rhizogenes* was 10.69 for Nedcha. Insufficient data were obtained for Gower due to germination and contamination problems. **b** Frequency histogram showing the mean scores of adventitious root production in the presence of *A. rhizogenes* from seedling explants of the NG DH mapping population. Explants were scored 35 days after inoculation. The mean adventitious root production in the presence of *A. rhizogenes* was 10.38 and 3.07 for Nedcha and Gower, respectively

(Table 4), while marker regression analysis identified highly significant regression scores on LGs 1, 8 and 9 (Table 4). The residual regression was not significant ($P > 0.05$) on LGs 1 and 8, however it was significant on LG 9 ($P = 0.03$). LG 9 was subjected to analysis with a two-QTL model. This reduced the F statistic from 188.97 to 21.58, although this was still highly significant ($P \leq 0.001$) and the residual variation was non-significant ($P > 0.095$). Subsequent interval mapping for a two-QTL model on LG 9 reduced the F value only slightly, from 10.23 to 3.02, and was not significant ($P = 0.059$). Although marker regression analysis identifies a large proportion of unexplained variation with the single-QTL model and strongly indicates the presence of a second QTL on LG 9, it is not possible to conclude with any confidence that there are two QTLs present because the estimated positions of the QTLs differ too greatly between the analyses (Table 5). Therefore, the results

Table 1 Generation means of adventitious root production both in the presence and absence of *Agrobacterium rhizogenes* and GFP-fluorescent root production for the DH parental genotypes, Nedcha and Gower, and DH lines from the NG mapping population. Explants were scored by illumination under a UV lamp, which allowed the identification of transgenic, GFP-fluorescing roots

Genotype	Additive mode[a]	Adventitious root production in the absence of *A. rhizogenes*		Adventitious root production in the presence of *A. Rhizogenes*		Transgenic root production	
		× Mean	Estimate of *a*	× Mean	Estimate of *a*	× Mean	Estimate of *a*
Nedcha	$m+a$	10.69	–	10.28	3.605	1.82	0.380
Gower	$m-a$	–[b]		3.07		2.58	
NG mapping population	M	9.00		8.84		2.46	

[a]Estimates of additive genetic effects are derived from the generation means, where *m* is the mean and *a* is the estimate of additive genetic effects
[b]–, Missing values

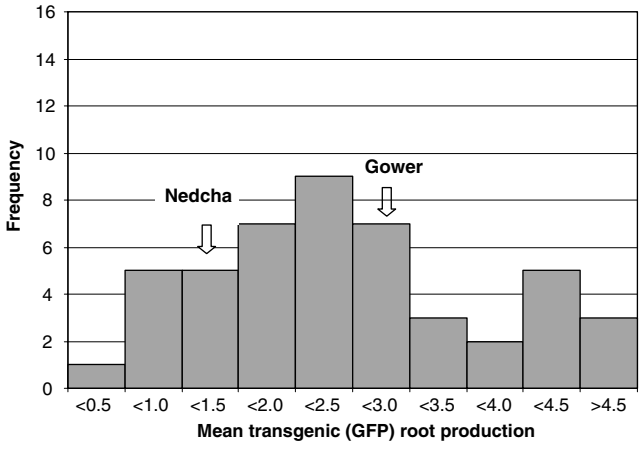

Fig. 2 Frequency histogram showing mean scores of GFP root production from seedling explants of the NG DH mapping population. Explants were scored 35 days after inoculation. *Arrows* indicate the group into which each parent belongs, with mean a GFP root production of 1.82 and 2.58, for Nedcha and Gower, respectively

indicate that there is a single QTL for adventitious root production in the absence of *Agrobacterium* on LGs 1 and 8 but that there is insufficient evidence to distinguish between the one-QTL and two-QTL models with respect to LG 9.

Adventitious root production in the presence of *A. rhizogenes*

Marker means analysis of adventitious root production in the presence of *A. rhizogenes* identified one region on each of LGs 1 and 8 and two regions, one at either end, of LG 9. No other linkage group showed evidence for the presence of QTL (Table 3). Additive regression scores from interval mapping were highly significant for this trait on both LG 1 and LG 8 (Table 4). With a single-QTL model, LG 9 was highly significant, but when investigated with a two-QTL model it was not significant at the 5% level. Marker regression analysis showed that the additive regression score for LG 1 closely approached significance ($P=0.02$) and was highly significant for LG 8, both with non-significant residual scores. For the LG 9 single-QTL model, marker regression analysis did not identify a significant additive regression score, but the residual variation was highly significant, suggesting that not all the variation has been explained. The additive regression for a two-QTL model was highly significant, with a non-significant for the residual.

These data and estimated positions (Table 5) suggest that there are single QTLs present on LGs 1 and 8 at roughly 34 cM and 8 cM, respectively. The data for LG 9 are more difficult to interpret; all three analyses identified similar estimated positions of the two QTL (Tables 3, 5), although the two-QTL model was not significant with interval mapping analysis (Table 4). The low significance of a two-QTL model with interval mapping may be due to the limited population size.

Transgenic root production

For the production of GFP-fluorescent, transgenic roots, marker means analysis identified significant regions on LGs 1, 8 and 9 (Table 3). Further analysis by interval mapping for a single-QTL model confirmed the regions on LGs 1 and 8 to be highly significant although the region on LG 9 was not significant (Table 4). Marker regression analysis of the data was consistent with this. Additive regression was significant for QTLs on LG 1, close to significance on LG 8 and non-significant on LG 9. The residual value was not significant for LGs 1 or LG 8 but was significant for LG 9 (Table 4). The data were then reanalysed to determine whether a two-QTL model for LG 9 would provide a better fit. Interval mapping did not provide any evidence for two QTLs. Marker regression found the additive regression to be highly significant with non-significant residual variation (Table 4). It was concluded that there is a single QTL on each of LG 1 and LG 8 as all three

Table 2 Summary of the correlation analysis between the traits

	Adventitious root production in the presence of *Agrobacterium*	Adventitious root production in the absence of *Agrobacterium*
Transgenic root production	$r = 0.491$***	$r = 0.537$***
Adventitious root production in presence of *Agrobacterium*		$r = 0.658$***

*** $P < 0.001$

Table 3 Marker means analysis scores for each trait (square root transformed) on linkage groups displaying regions of significance

Linkage group	QTL region	Marker means		QTL position (cM)
		F	*P*	
Adventitious root production in the absence of *A. rhizogenes*				
1	1	6.63	0.014	38
8	1	18.01	< 0.001	11.2
9	1	9.58	0.004	91.2
Adventitious root production in the presence of *A. rhizogenes*				
1	1	10.21	0.003	38
8	1	24.58	< 0.001	11.2
9	1	5.54	0.024	4.8
	2	5.78	0.021	91.2
Transgenic root production				
1	1	11.35	0.002	38
8	1	16.09	< 0.001	11.2
9	1	4.93	0.032	104

Table 4 Marker regression and interval mapping ANOVA statistics for significant linkage groups of the NG mapping population screened for the identification of QTLs for adventitious root production in the presence and absence of *A. rhizogenes* and for transgenic (GFP-fluorescent) root production (software QTL CAFÉ (http://web.bham.ac.uk/g.g.seaton/)

Linkage group	QTL model	Marker regression				Interval mapping	
		Additive regression		Residual			
		F	*P*	*F*	*P*	*F*	*P*
Adventitious root production in the absence of *A. rhizogenes*							
1	1	53.16	0.01	0.41	0.47	6.89	0.012
8	1	53.77	0.01	2.13	0.11	18.11	< 0.001
9	1	188.97	0.01	15.19	0.03	10.23	0.003
	2	21.57	< 0.001	1.88	0.10	3.02	0.059
Adventitious root production in the presence of *A. rhizogenes*							
1	1	86.91	0.02	2.85	0.06	10.82	0.002
8	1	76.32	0.01	1.55	0.13	27.08	< 0.001
9	1	26.77	0.13	4.53	0.03	5.85	< 0.001
	2	19.77	< 0.001	0.53	0.82	2.88	0.067
Transgenic roots production							
1	1	76.46	< 0.001	0.80	0.27	12.61	< 0.001
8	1	45.25	0.03	2.23	0.14	17.54	< 0.001
9	1	55.00	0.07	5.34	0.04	3.44	0.07
	2	23.03	< 0.01	0.61	0.76	1.749	0.186

analyses support this and provide close estimates of the positions (Table 5).

The regions on LG 9 were more difficult to interpret. Interval mapping and marker means analyses suggest the presence of a QTL around 104 cM (Tables 3, 5). Marker regression analysis is highly significant for a two-QTL model, with suggested locations of 4 cM and 58 cM. The first of these would overlap with the region identified in a two-QTL model for adventitious root production in the presence of *Agrobacterium*. For transgenic root production with respect to LG 9, a single-QTL model does not fully explain all the variation, but there is not enough evidence to confirm the presence of two QTLs.

The locations and additive effects of each QTL identified from the analyses are summarised in Table 5. The predicted locations of the QTLs are consistent between the two methods of analysis (interval mapping and marker regression), and any differences fall within the confidence intervals. The contribution of alleles from the parents in the NG population at each QTL can be determined from the additive effect (Table 5). A positive additive effect indicates that the Nedcha allele enhances that trait, and a negative additive effect indicates that the

Nedcha allele depresses the response. Adventitious root production both in the presence and absence of *A. rhizogenes* and transgenic root production were favoured by the presence of alleles from parent one, Nedcha. Gower only had a contributing effect on LG 9 (Table 5) where there is a suggestion of QTLs for adventitious root production in both the presence and absence of *A. rhizogenes*. Both the estimate of the additive genetic effect at each QTL (V_A) compared to the total genetic effect observed in the population and the broad-sense heritability are shown in Table 5. As there was not sufficient evidence to confirm the presence of QTLs on LG 9 for any of the traits, the "total" calculated only takes account of the % V_A from LGs 1 and 8.

Table 5 Summary of the QTL positions and effects on adventitious root production—in both the presence and absence of *A. rhizogenes*—transgenic root production and the proportion of transgenic roots produced, from the NG DH mapping population

Linkage group		Position in centiMorgans[a]	+/−[b]	Additive effect[a]	+/−	% V_A[c]	h^2 b[d]
Marker regression							
Adventitious root production in the absence of *A. Rhizogenes*							
1		38	15.998	0.241	0.110	24.54	0.025
8		2	14.615	0.366	0.125	56.57	0.059
Total						81.11	
9	1 QTL	100	28.154	−0.298	0.157	37.50	0.038
9	2 QTL	0		0.176			
9	2 QTL	88		−0.361			
Adventitious root production in the presence of *A. Rhizogenes*							
1		34	16.744	0.153	0.07	21.11	0.021
8		8	10.802	0.254	0.07	40.15	0.039
Total						61.26	
9	1 QTL	104	35.148	−0.129	0.093	15.17	0.015
9	2 QTL	0		0.163			
9	2 QTL	86		−0.200			
Transgenic root production							
1		38	14.003	0.1647	0.075	24.46	0.019
8		4	10.558	0.211	0.071	40.16	0.032
Total						64.62	
9	1 QTL	64	28.39	−0.135	0.076	16.44	0.013
9	2 QTL	4		0.181			
9L	2 QTL	58		−0.249			

Linkage group		Position in centiMorgans	Effect	% V_A	h^2 b
Interval mapping					
Adventitious root production in the absence of *Agrobacterium*					
1		40	0.2607	28.67	0.032
8		12	0.3659	56.57	0.062
Total				85.24	
9	1 QTL	87	−0.3176	42.59	0.050
9	2 QTL	2	0.5401		
9	2 QTL	21	−0.5716		
Adventitious root production in the presence of *A. Rhizogenes*					
1		37	0.1914	33.02	0.033
8		3	0.2694	65.50	0.062
Total				98.52	
9	1 QTL	5	0.143	18.04	0.018
9	2 QTL	5	0.1359		
9	2 QTL	91	−0.1335		
Transgenic root production					
1		36	0.2159	42.06	0.033
8		8	0.2385	51.35	0.041
Total				93.41	
9	1 QTL	104	−0.117	3.86	0.002
9	2 QTL	4	0.2101		
9	2 QTL	24	−0.2248		

[a]The analyses were performed using QTL CAFÉ, with confidence intervals calculated by marker regression for the position (centiMorgan) intervals and additive effects using 1,000 simulations. Data for both the one- and two-QTL models for LG 9 are shown, but these have not been included under "Totals"; neither have calculations of V_A or h^2 b been included, since these QTLs have not been confirmed

[b] +/−, Confidence intervals; +, Nedcha (P1); −, Gower (P2)

[c] V_A, Additive variance

[d] h^2 b, Broad-sense heritability

Discussion

The analysis of the *B. oleracea* NG mapping population has revealed significant relationships ($P < 0.001$) between the performances of individual lines for three classes of root production by hypocotyls stumps in culture. These classes are adventitious (non-transgenic) root production in the absence of *A. rhizogenes*, adventitious root production in the presence of *A. rhizogenes* and transgenic (GFP-fluorescent) root production in the presence of *A. rhizogenes*. The genetic basis for the association between different specific root production traits has been investigated using QTL analysis, and the data have been analysed using marker means, marker regression and interval analysis. The results of all three analytical methods are consistent with the occurrence of a single QTL on LG 1, with a most probable position of 34–38 cM, that is responsible for a proportion of the difference in performance for all three classes of root production between the two parents. Hence, the same QTL (in so far as the resolution of such experimentation allows) has been shown to play a role in controlling both adventitious and transgenic root production. Similarly, the results of all three analysis methods are consistent with the occurrence of a single QTL on LG 8, with a most probable position of 2–8 cM, that is again responsible for a proportion of the difference in performance for all three classes of root production between the two parents. The only other indication of a QTL is for LG 9, and here the results of the analyses are more complex. Taken overall, it appears that there is/are a locus/loci controlling all three classes of root production on LG 9 and that the same loci control all three root production traits. However, it is not clear whether there is one locus or two loci. These are the only (putative) QTLs where the additive effect is from parent 2 (Gower). Gower is the responsible parent on the one-QTL model. With the two-QTL model, the P2 effect is always on the second QTL although estimates of the position vary. This is probably because the population size used was not large enough to allow resolution of the QTLs implicated on LG 9.

Previous studies on the production of adventitious and transgenic roots in *B. oleracea* have been carried out using the AG mapping population (Cogan et al. 2002). In this population, QTLs on LGs 3 and 7 were shown to influence both adventitious and transgenic root production. A QTL influencing adventitious root production alone was detected on LG 7, and another influencing transgenic root production alone was detected on LG 1; however, this latter QTL mapped at around 100–105 cM rather than at the 34–38 cM position of the QTL identified in the current study. The current results are an independent confirmation that individual QTLs can control the production of both adventitious and transgenic roots, but it is not surprising that different QTLs have been detected in the *B. oleracea* AG and NG mapping populations. Only loci that happen to bear differing alleles in the two parents can be detected in such mapping populations, and one would not expect the two sets of parents to differ at the same loci.

Our studies show that genotypes that are predisposed to high performance for adventitious root production tend also to perform well in experiments designed to produce transgenic roots induced by *A. rhizogenes*. Work on other plant species has suggested that treatment with *A. rhizogenes* can induce the production of adventitious roots as well as lead to the production of transgenic roots. For example, Van Wordragen et al. (1992) reported adventitious root production by chrysanthemum explants and suggested that transient expression of T-DNA may be sufficient to induce the proliferation of roots, the cells of which do not contain integrated copies of T-DNA. Transient expression of TR-DNA genes would include those encoding enzymes involved in indoleacetic acid synthesis, and it has been proposed that a short-lived increase in the level of this hormone could lead to increased adventitious rooting. However, the role of plant growth regulators in the *Agrobacterium*-plant interaction is complex; it has also been shown that the competence of explants for transformation can be increased by a phytohormone pretreatment (e.g. Chateau et al. 2000). As well as auxin, the concentration of cytokinins may be influenced by the presence of *Agrobacterium*. While transient expression of the T-DNA-borne cytokinin-related genes (Costacurta and Vanderleyden 1995) may change the local concentration of this class of regulators, the latter may also be affected by the chromosomal *miaA* locus that encodes a DMAPP:tRNA transferase (Morris et al. 1993). However, in the present study, *B. oleracea* hypocotyls were incubated in the presence and absence of *A. rhizogenes,* and the results show that the presence of the bacterium had no significant effect on adventitious root production. Consequently, there is no evidence that transient expression of T-DNA genes influences root production in *B. oleracea*. It has been possible to identify superior lines—ones with performances higher than that of either parent—from the NG population. Hence, with respect to future work on other *B. oleracea* germplasm, our results suggest that screening genotypes for adventitious root production in the absence of *A. rhizogenes* would be a useful method for selecting lines that will undergo higher rates of transgenic root production in the presence of the bacterium. However, it must be borne in mind that *A. rhizogenes*-based transformation protocols also involve the regeneration of shoots from transgenic roots; it would also be beneficial to assess performance for this independent trait in order to identify genotypes exhibiting superior characteristics for the whole transformation process.

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