Plant Cell Rep (2001) 20:755–762
DOI 10.1007/s00299-001-0395-y

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The effects of anther culture and plant genetic background on *Agrobacterium rhizogenes*-mediated transformation of commercial cultivars and derived doubled-haploid *Brassica oleracea*

Received: 21 May 2001 / Revision received: 27 July 2001 / Accepted: 27 August 2001 / Published online: 18 October 2001

Abstract The production of transgenic roots was scored for eight *Brassica oleracea* cultivars from broccoli, cabbage, cauliflower and kale following inoculation with an *Agrobacterium rhizogenes* cell line carrying a binary plasmid bearing the green fluorescence protein (*gfp*) gene in the T-DNA. Significant differences in the numbers of explants producing transgenic roots were observed between cultivars, ranging from 1.4% for Marathon F1 to 57.8% for the Green Duke F1. Three F1 cultivars were subjected to anther culture, and doubled-haploid (DH) lines were used for transformation. The DH lines produced showed considerable variation for transgenic root production with some lines showing increased efficiency compared to the parental F1 cultivar. Grouping of the DH lines into response classes with respect to transgenic root production allowed the development of potential genetic models to explain the variation in performance released from each F1 cultivar. No apparent segregation distortion for transgenic root production was observed in the DH lines following anther culture.

Keywords *Agrobacterium rhizogenes* · *Brassica oleracea* · Transformation · Double haploid · Genetic analysis

Abbreviations *BA*: 6-Benzylaminopurine · *2,4-D*: 2,4-Dichlorophenoxyacetic acid · *DH*: Doubled haploid · *GFP*: Green fluorescence protein · *2iP*: 6-(γ,γ-Dimethylallylamino)purine · *NAA*: α-Naphthaleneacetic acid

Communicated by G. Phillips

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Introduction

The improvement of crop plants can be achieved by conventional breeding or genetic transformation. The methods of choice for transformation of *Brassica* species have been based upon the natural genetic engineering capabilities of *Agrobacterium tumefaciens* and *A. rhizogenes* (Poulsen 1996; Puddephat et al. 1996). Similar transformation mechanisms operate in both *Agrobacterium* species, mediated by genes conserved in the *vir* (virulence) region of the large extra-chromosomal plasmids found in virulent bacteria of both species (Tepfer 1990). Numerous studies have shown that transformation efficiency is restricted by the choice of target genotype, suggesting that plant genetic factors determine responses to *Agrobacterium*-mediated transformation (reviewed in Gelvin 2000). The first step in developing an *Agrobacterium*-mediated protocol is usually to identify plant genotypes that are competent for transformation; however, such genotype screens are time-consuming. There are at least four steps in *Agrobacterium*-mediated transformation where plant genes may regulate the process. These are: bacterial attachment to the plant cell surface, transfer of the T-DNA from the bacterium to plant cell, transport of the T-DNA to the plant nucleus and stable integration of the T-DNA into the host genome (Sheng and Citovsky 1996; Zupan and Zambryski 1995).

Whilst the *Agrobacterium* genes controlling the T-DNA transfer have been subject to detailed investigation (Sheng and Citovsky 1996; Zupan and Zambryski 1995, 1997), relatively little work has been done on identifying plant genes that regulate the process, despite the strong evidence for a genetic basis to transformation competence. Genotypic variation for susceptibility to *Agrobacterium*-mediated transformation has been widely reported (reviewed in Klee et al. 1987; van Wordragen and Dons 1992). Heritable variation for tumour induction by *A. tumefaciens* has been found in *Pisum sativum* (Robbs et al. 1991) and in *Arabidopsis thaliana* with respect to bacterial attachment to plant cells and reduced T-DNA integration (Nam et al. 1997). Both ease of transforma-

tion and competence for shoot regeneration have been identified in tomato lines following crosses of *Lycopericon peruvianum* with *L. esculentum* (Barg et al. 1997; Koornneef et al. 1986). In *Solanum tuberosum*, molecular markers linked to transformation efficiency have been identified on chromosome 5 (El-Kharbotly et al. 1995).

Brassica oleracea is a convenient crop species in which to study the genetic control of plant transformation. *B. oleracea* is closely related to the model species *A. thaliana*, for which detailed molecular genetic resources are available. Furthermore, genetic analysis in the diploid C genome of *B. oleracea* provides data readily transferable to the amphidiploid AC genome of its crop relative, *B. napus* (U 1935). Genetic analyses in *B. oleracea* are facilitated by the availability of a number of DH populations with associated genetic maps (Bohuon et al. 1996; Sebastian et al. 2000) and overlapping substitution lines (Rae et al. 1999; Ramsay et al. 1996). Although common procedures for the transformation of *B. oleracea* have yet to emerge (Puddephat et al. 1996), techniques based on the use of *A. rhizogenes* are well-developed (Berthomieu and Jouanin 1992; Christey and Sinclair 1992; Puddephat et al. 2001).

DH lines are powerful tools for plant breeding and genetic analyses. For the purposes of genetic analyses, DH lines ideally should represent a random selection of the genetic variability in the parents. However, the utility of DH lines can be limited by segregation distortion (Niemirowicz-Szczytt 1997), which can occur when DH lines are produced, following either the anther culture (AC) or microspore culture (MC) process (Cloutier and Landry 1994), from parents with different capacities for AC or MC (Murigneux et al. 1993). Distortion results from the preferential transmission of alleles from the responsive parent that control the AC or MC capacity (Cloutier et al. 1995). Any other loci linked to regions conferring AC or MC capacity would also be preferentially selected.

In this paper we report the results of our investigation on the efficiency of production of transgenic roots across eight genotypes from four crop types of *B. oleracea* following *A. rhizogenes*-mediated transformation. We applied anther culture to three F1 cultivars and assessed the efficiency of transformation among the DH lines produced. We also tested whether any of the DH lines exhibited greater transformation efficiency than the parental F1 cultivar and developed some simple genetic models to help explain the variation in performances exhibited by the DH lines. In addition, we tested whether the application of anther culture leads to any segregation distortion for transgenic root production in the derived DH populations.

Materials and methods

Plant material and culture conditions

A range of commercial cultivars of vegetable brassicas and AC-derived DH breeding lines was used in transformation experiments; these were broccoli cvs. Marathon F1, Trixie F1, Corvet F1, Green Duke F1, along with cabbage cv. Hawke F1, cauliflower cv. White Rock and kale cv. Kaliaan. The DH lines were produced by AC from the F1 cvs. Marathon, Trixie, Corvet and Hawke. Seeds of each cultivar or DH line were surface-sterilized by immersion in 1.7% (w/v) sodium dichloroisocyanurate for 6 min, followed by two rinses in sterile purified water. Seeds were then germinated for 3 days on moist filter paper in 9-cm Petri dishes at 15°C and under a 12/12-h (light/dark) photoperiod with light supplied by an even mix of white and warm-white fluorescent tubes at an irradiance of 250 µmol m^{-2} s^{-1}. Germinating seedlings were then incubated for a further 3 days at 22°C under a 16/8-h (light/dark) photoperiod with light provided by a mix of 70-W white and 65/80-W Grolux fluorescent tubes at an irradiance of 80 µmol m^{-2} s^{-1} at the culture level.

Plant transformation

A. rhizogenes strain LBA 9402 pRi1855 (Spano et al. 1982) was used, which harbours the binary vector pBIN *m-gfp*5-ER (Haseloff et al. 1997), with the *gfp* gene driven by a CaMV 35S promoter. Prior to the plant transformation experiments, LBA 9402 was subcultured on semi-solid YMB medium (10 g/l mannitol, 0.4 g/l yeast extract, 0.1 g/l NaCl, 0.2 g/l $MgSO_4.7H_2O$, 0.5 g/l K_2HPO_4, with the pH adjusted to 7.0–7.2) supplemented with 50 mg/l kanamycin for selection of the binary plasmid, and incubated at 25°C overnight. Three to four 10-µl loops of *Agrobacterium* were taken from this overnight culture and used to inoculate 10 ml of MGL broth (2.5 g/l yeast extract, 5 g/l tryptone, 5 g/l mannitol, 5 g/l NaCl, 1.16 g/l Na-glutamate, 0.25 g/l KH_2PO_4, 0.1 g/l $MgSO_4$, 1.0 mg/l biotin, with the pH adjusted to 7.0). Broths were incubated for 16 h at 25°C on a shaking platform (150–200 rpm). The *Agrobacterium* cells were then pelleted by centrifugation at 13K rpm for 5 min and the cells subsequently resuspended in liquid Murashige and Skoog (MS; 1962) medium with 30 g/l sucrose and supplemented with 1 mg/l 2,4-D to produce an optical density ($A_{600\ nm}$) of 1.0 (±0.1).

Explants for use in the transformation experiments were excised from 6-day-old seedlings grown under aseptic conditions by cutting the hypocotyl approximately 5 mm below the cotyledonary petioles. The explants were inverted and placed on MS medium containing 30 g/l sucrose and supplemented with 200 mg/l cefotaxime (to limit over-growth of *Agrobacterium*) in 5-cm deep Petri dishes, with three explants per dish. A 3-µl drop of resuspended *Agrobacterium* was placed on the cut surface of the hypocotyl. Inoculated explants were incubated as previously described for germinating seedlings.

Identification of co-transformed hairy roots

Transgenic root production was determined 35 days after inoculation. Explants were illuminated under long-wave UV radiation (UVP BLAK-RAY lamp, model B100 AP) to detect GFP fluorescence in roots.

Anther culture

DH lines of cabbage F1 cv. Hawke were supplied by D.J. Ockendon (HRI, Wellesbourne), and broccoli DH lines were produced by anther culture from broccoli F1 cvs. Marathon, Trixie and Corvet following the method similar to that described by Ockendon (1984). Donor plants of the F1 hybrids were raised in an air-conditioned glasshouse at a constant temperature of 15°C. Supplementary lighting for day length extension to 14 h was used in the period October to March. The ratio of petal to anther length was used as an external staging method for assessing bud age. Typically, 4- to 5-mm long buds were selected for anther culture with a petal to anther ratio between 1:2 and 1:1. Buds were removed from the plants when the first two to five flowers were open and surface-sterilized in a 1.7% (w/v) solution of sodium dichloroisocyanurate for 6 min, followed by three washes in sterile distilled water. Anthers were excised from

the buds under a binocular microscope, and care was taken to remove the filament to avoid subsequent callus formation. Thirty anthers were placed filament side down onto culture medium in 9-cm Petri dishes. For culture initiation, we used a medium based on a modification of Gamborg's B5 medium (Keller et al. 1975), with 10% (w/v) sucrose and supplemented with 0.1 mg/l 2,4-D, 0.1 mg/l NAA, 0.08% glutamine, 0.06% (w/v) calcium chloride, 3.0 mg/l silver nitrate and 0.4% (w/v) agarose (N.L. Biddington, personal communication). Cultures were then incubated at 35°C for 16 h in the dark, after which the temperature was reduced to 25°C.

Embryos were removed from anthers upon emergence and transferred to growth regulator-free modified B5 medium with 2% (w/v) sucrose (Keller et al. 1975). Cultures were placed in an illuminated growth room at 22°C under a 16/8-h (light/dark) photoperiod with light provided by a mixture of warm-white and Grolux fluorescent tubes at an intensity of 80 µmol m^{-2} s^{-1} at the culture level. After 3–4 weeks, individual embryos were transferred to modified B5 medium supplemented with 2% (w/v) sucrose and 1.0 mg/l BA to promote multiple shoot formation. After a further 2–3 weeks, individual shoots were transferred to B5 medium supplemented with 2% (w/v) sucrose and 0.3 mg/l 2iP in order to encourage shoot elongation. As the shoots became large enough (longer than 10 mm), they were transferred to growth regulator-free modified B5 medium supplemented with 2% (w/v) sucrose in 100-ml screw-cap specimen jars to form roots. Where possible, two shoots per embryo were regenerated to produce genetically identical offspring; such clones were designated as 'a' and 'b'. When plantlets had produced well-developed root systems, they were transferred to 4×4-cm modules containing compost (Levington M2) and placed in propagators for acclimatization. Established plants were transferred to 12-cm pots and grown further in the glasshouse.

Ploidy levels of the plants were determined by measuring guard-cell lengths following the methods reported by Ockendon (1988) and/or by flow cytometry. Extraction of nuclei and flow cytometry analysis were performed by Plant Cytometry Services (Schijnel, The Netherlands). Diploid plants were grown to flower, and the buds self-pollinated to produce seed. Lines that produced more than 36 seeds were used directly in transformation experiments, but some lines were selfed further in order to obtain sufficient seed for experimentation.

Experimental design and data analysis

All experiments were repeated at least three times. In each experiment, 48 explants of Green Duke F1 (broccoli), White Rock (cauliflower), Shogun F1 (broccoli) and Kailaan (kale) were inoculated with *Agrobacterium*. For broccoli cvs. Marathon F1, Corvet F1 and Trixie F1, cabbage cv. Hawke F1 and all of the DH lines derived from these cultivars, a total of 12 explants were inoculated with *Agrobacterium* in each experiment. The number of available explants was restricted in these experiments by seed availability, germination rate and contamination. The number of explants of each genotype with one or more GFP-fluorescent roots was scored and the number of GFP-fluorescing roots per explant determined. The proportions of explants producing GFP-fluorescing roots were analysed using generalized linear models (McCullaugh and Nelder 1989) with logit link and binomial error in the software package GENSTAT 5 for Windows release 4.2.

Results and discussion

Production of GFP-positive roots in commercial cultivars

The inoculation of explants from all eight cultivars of *B. oleracea* with *A. rhizogenes* LBA 9402 produced roots with GFP fluorescence (putative transgenic roots) after 35 days (Fig. 1). As in our earlier work on the optimization of *A. rhizogenes*-mediated transformation of *B. oleracea* (Puddephat et al. 2001), we found that inoculated explants also produced adventitious roots that did not fluoresce under long-wave UV. Significant differences [$\chi^2(df)=122.8(7)$, $P<0.001$] in the proportion of inoculated explants producing roots with GFP fluorescence (referred to here as transformed explants) were found between all of the cultivars tested, ranging from 1.4% in explants of Marathon F1 to 57.8% in explants of Green Duke F1. Differences in the proportion of transformed explants between the five broccoli cultivars were also significant [$\chi^2(df)=70.1(4)$, $P<0.001$], and F1 cultivars Marathon, Trixie and Corvet were identified as having poor, intermediate and good transformation responses, respectively. Genotypic variation in response to *Agrobacterium*-mediated transformation is common (Gelvin 2000).

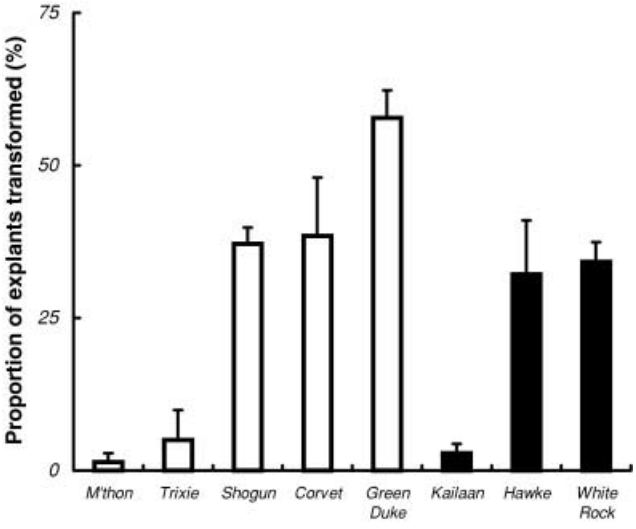

Fig. 1 Transgenic root production from *Agrobacterium rhizogenes*-inoculated explants of *Brassica oleracea* cultivars. Transgenic roots were identified by GFP fluorescence 35 days after inoculation of explants. Data have been ranked for broccoli cultivars (*white columns*) and other cultivars (*black columns*). *Bars*: Standard error of each mean

In each of the cultivars tested, transformed explants produced one or more GFP-fluorescing roots, with the mean numbers ranging from 1.0 to 4.6 (Table 1). There was no apparent relationship between the proportion of transformed explants and the mean number of transgenic roots produced per transformed explant. Transformation efficiencies are potentially increased when inoculated explants produce more than one GFP-fluorescing root, provided these originate from independently transformed cells.

Transformation efficiencies have been improved by manipulating the virulence of the bacterium through the use of feeder layers or phenolic compounds that induce the expression of genes in the *vir* region, as has been reported recently for *A. rhizogenes*-mediated transformation of broccoli (Henzi et al. 2000). The improved trans-

Table 1 Transgenic root production from seedling explants of commercial *Brassica oleracea* cultivars. Explants were inoculated with *Agrobacterium rhizogenes* strain LBA 9402 carrying the vector pBIN m-*gfp*5-ER. Explants with transgenic roots were identified by GFP-fluorescence in root tissues under long-wave UV illumination 35 days after inoculation

Crop type and Cultivar	Number of inoculated explants	Explants producing roots with GFP fluorescence		Number of GFP roots per expressing explant (mean ±SE)
		Total no.	Percentage	
Broccoli				
Marathon F1	72	1	1.4	1.0
Trixie F1	20	1	5.0	1.0
Shogun F1	332	127	38.0	3.5±0.23
Corvet F1	26	10	39.0	2.3±0.4
Green Duke F1	116	67	57.8	3.0±0.23
Kale				
Kailaan	108	3	2.8	3.0±1.53
Cabbage				
Hawke F1	28	9	32.1	4.6±0.78
Cauliflower				
White Rock	222	76	34.4	3.8±0.3

Table 2 Production of spontaneous double-haploid plants from anther cultures of F1 hybrid cultivars of broccoli

Cultivar	Number of anthers cultured	Number of anthers to respond	Embryo yield per 100 anthers	Number of plants successfully regenerated	Ploidy analysis (no. of plants)		
					Haploid	Diploid	Polyploids[a]
Corvet	870	10	5.86	31	5	23	3
Trixie	810	46	8.64	51	16	27	8
Marathon	480	24	15.21	75	7	47	21

[a] Polyploids include plants that were either triploid, tetraploid or mixoploid

formation obtained by these researchers resulted from manipulating the virulence of *A. rhizogenes* strain A4T, which is not the strain used in this study. In our laboratory, *A. rhizogenes* strain LBA 9402 has been used successfully to transform a wide range of *B. oleracea* genotypes (unpublished data). It has been suggested that the limits of extending host range by manipulating the virulence of the bacterium may have been reached and that further improvements will result from manipulation of the plant (Gelvin 2000). Genotypic variation for rates of explant transformation between broccoli F1 cultivars led us to ask whether the transformation response would segregate in DH lines produced by AC from these F1 cultivars and whether DH lines with improved rates of transformation could be detected.

Production of anther-cultured DH lines of broccoli

Embryos were obtained from cultured anthers of each hybrid cultivar (Table 2). Embryo induction rates from cultured anthers of Trixie and Marathon were similar (5.6% and 5%, respectively) and higher than those from Corvet (1.1%). Marathon produced the greatest number of embryos per cultured 100 anthers followed by Trixie and Corvet, and the number of plants regenerated through culture reflected these responses (Table 2). A high proportion of regenerated plants were found to be spontaneous DHs; this ranged from 49% of AC-derived lines of Trixie to 72% of AC-derived lines of Corvet. DH lines were bud-self-pollinated to produce seed for the genetic analysis of the transformation traits. Nine DH lines of Corvet and 11 DH lines each of Trixie and Marathon produced sufficient seed for the transformation experiments.

Genetic analysis of transformation efficiency

A wide variation in the proportion of transformed explants of AC-derived DH lines from each of the three F1 cultivars of broccoli was found. In contrast to the results with the F1 cultivars, DH lines were found that produced no transgenic roots from inoculated explants. More importantly, DH lines in each population were also identified that had significantly increased numbers of transformed explants compared to the parental F1 cultivar. Five DH lines derived from cv. Marathon produced no GFP-fluorescing roots. Explants of the internal control duplicate and DH lines 6a and 6b produced comparable rates of transformation that were similar to Marathon (1.4%) (Fig. 2a). The remaining four Marathon DH lines

all produced rates of transformation ranging from 11 to 29 times higher than that of Marathon.

In the DH lines derived from the intermediate F1 cultivar Trixie, inoculated explants of three DH lines produced no GFP-fluorescing roots. Explants of five DH lines produced rates of transformation comparable to that of the F1 parent (5%). Superior rates of transformation, 5–5.5 times higher than that of Trixie, were observed in three DH lines (Fig. 2b).

GFP-fluorescing roots were observed from inoculated explants of all DH lines derived from Corvet. For six DH lines, the rates of transformation were reduced compared to explants of Corvet. In the remaining three DH lines, the rates of transformation were either comparable or greater than that of the F1 parent (Fig. 2c). Explants of the genetically identical DH lines, 1a and 1b and 7a and 7b, which form an internal control, produced comparable rates of transformation to each other.

Segregation for the proportion of explants with GFP-fluorescing roots was evident in each of the three DH populations of the broccoli F1cultivars and indicated that individual genotypes could be grouped into distinct response classes. For the F1 cultivars Marathon and Trixie, the parental genotype and their respective DH lines were categorized into three response classes that were similar to, lower than or higher than the F1 response (see Fig. 2a, b). For F1 cultivar Corvet and associated DH lines, only two classes were used – either equal to or lower than the F1 response (see Fig. 2c).

Analysis of deviance was used to test whether the classification of genotypes into response classes explained the variation for GFP-fluorescent root production. Grouping DH lines into two or three response classes was sufficient to explain the genotypic variation observed in rates of explant transformation; in each case, differences between the response classes were significant and no significant residual genotype effects were detected (Table 3). The relatively low number of response classes used to explain the transformation responses suggests that relatively few genetic factors are segregating in the DH lines derived from the three F1 cultivars of broccoli used. Unfortunately, the numbers of DH lines available in each population limited our genetic analyses of explant transformation. However, based on the categories used, segregation for explant transformation is consistent with Mendelian models for the action of either one genetic factor (1:1 segregation) in the DH population

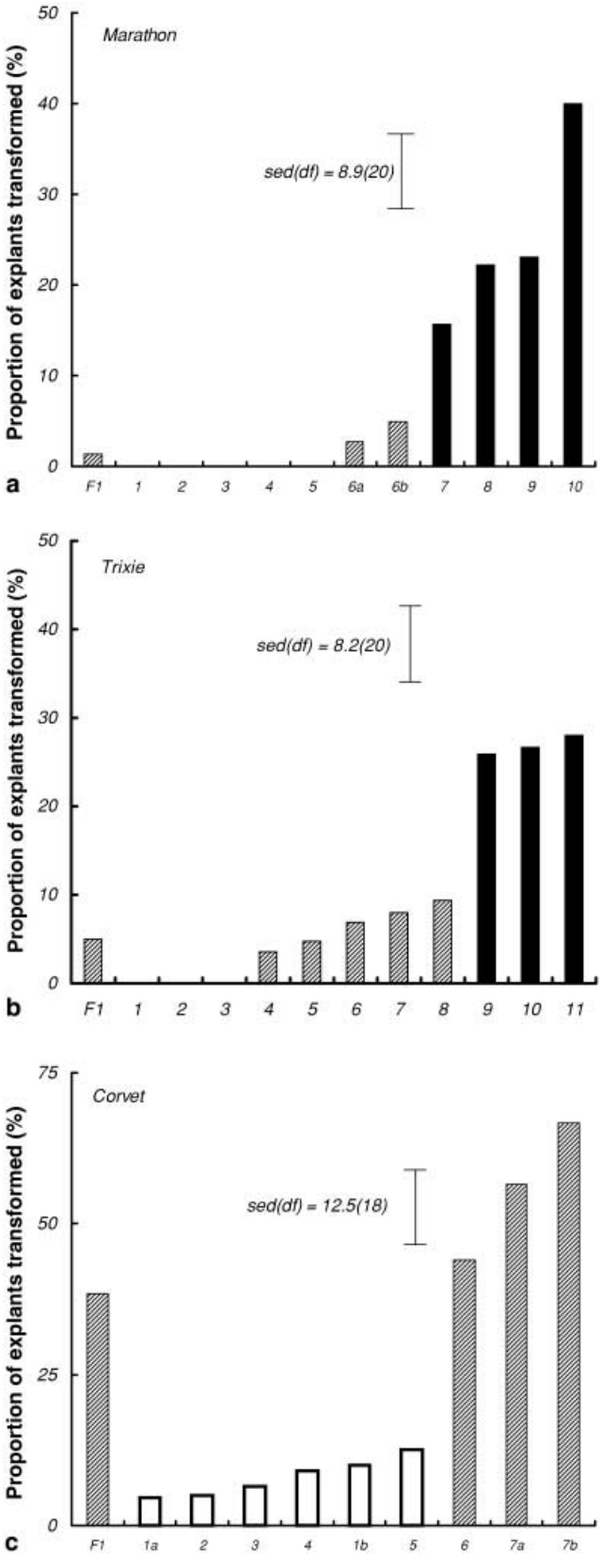

Fig. 2a–c Transgenic root production from *A. rhizogenes*-inoculated explants of F1 cultivars of broccoli and derived DH lines. **a** Marathon and 11 DH lines, **b** Trixie and 11 DH lines, **c** Corvet and nine DH lines. DH lines are *numbered sequentially*; *numbers* followed by either *a* or *b* identify genetically identical offspring. Transgenic roots were identified by GFP fluorescence 35 days after inoculation of explants. Genotypes with similar rates of transgenic root production are grouped into distinct response classes that are equal to (*hatched columns*), lower than (*white columns*) or higher than (*black columns*) the F1 response. The original F1 cultivar response is provided for comparative purposes; *bar* represents the sed value determined by ANOVA for comparison of means

Table 3 Summary of analysis of deviance for the comparison of response classes used to group genotypes on the basis of transgenic root production. Responses classes were analysed to validate the genotype groups identified and to establish segregation for transgenic root production in explants of F1 cultivars and anther-cultured DH-derived lines

F1 cultivar and DH lines	Number of response classes	Differences between classes [$\chi^2(df)$]	Residual genotype differences [$\chi^2(df)$]
Marathon	3	66.8(2)***	4.76(9), n.s.
Trixie	3	26.6(2)***	1.49(9), n.s.
Corvet	2	59.6(1)***	6.41(8), n.s.

***P=0.001; *n.s.* not significant

of Corvet or two genetic factors (1:2:1 segregation) in the DH populations of Trixie and Marathon. It should be noted that some DH lines in each broccoli population could have been placed into alternative response categories without affecting either the result of the analysis or the conclusions drawn.

As with the broccoli populations, AC-derived DH lines from cabbage cv. Hawke segregated for explant transformation following *A. rhizogenes* inoculation. The proportion of inoculated explants with GFP-fluorescing roots varied between lines from 5.6% to 85.2% (Fig. 3). Comparable rates of transformation were observed in inoculated explants from DH lines 17a and 17b, which were genetically identical. In contrast, the genetically identical DH lines 20a and 20b produced significantly different rates of explant transformation, suggesting that either an 'environmental effect' was operating or that there were maternal differences between the seeds of each line, even though no differences had been observed initially during seed germination.

In contrast to the broccoli screens, the 21 DH lines of Hawke produced a near-continuous distribution of explants with transformation events, although variation due to 'environmental factors' may have masked the grouping of DH lines into discrete response categories. Differences between genotypes were analysed based on three grouping structures, with DH lines 1–3 representing the initial class, being lines producing fewer inoculated explants with GFP-fluorescing roots than the F1 cultivar. DH lines 4–16 formed the second group, having produced a similar rate of transformation to that of the parent F1. DH lines 17a–21 represented the final response class, comprising genotypes with higher rates of transformation than that of the F1 cultivar (see Fig. 3) The duplicates of lines 17 and 20 used as internal controls reduced the number of unique genotypes in this response class from seven to five. This grouping structure was assessed by analysis of deviance and explained a highly significant proportion [$\chi^2(df)$=94.1(2), P<0.001] of the differences between genotypes, although there were significant residual differences between genotypes in the response classes [$\chi^2(df)$=43.5(21), P=0.003]. Two random subsets of the Hawke DH lines were also analysed; these were of similar sizes to those used for the analysis of the broccoli DH lines. Even in these smaller populations it proved possible to describe a significant proportion of the observed variation with three response classes, but

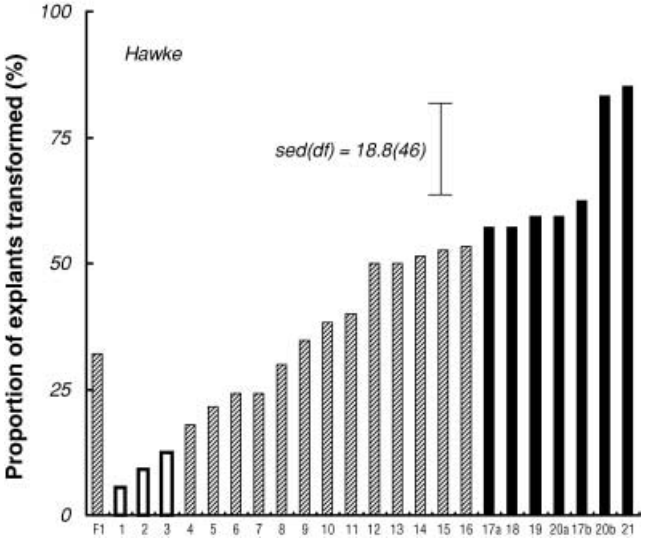

Fig. 3 Transgenic root production from *A. rhizogenes*-inoculated explants of AC-derived DH lines of cabbage F1 cv. Hawke. DH lines are *numbered* sequentially; *numbers* followed by either *a* or *b* identify genetically identical offspring. Transgenic roots were identified by GFP fluorescence 35 days after inoculation of explants. Genotypes with similar rates of transgenic root production are grouped into classes that approximate a response equal to (*hatched columns*), lower than (*white columns*) or higher than (*black columns*) the F1 response. The original F1 cultivar response is provided for comparative purposes; *bar* represents the sed value determined by ANOVA for comparison of means

residual differences were also significant (data not shown). Based on the three response categories used for all DH lines of Hawke, the observed segregation of 3:13:5 fits a Mendelian model [$\chi^2(df)$=1.57(2), P=0.46] for the action of two genetic factors (expected segregation ratio 1:2:1). However, several DH lines could have been classified into an alternative response category, and the significant residual genotypic effects indicate that the categories used and the Mendelian model do not fully explain the genetic variation observed.

Mendelian genetic studies of tissue culture traits have suggested that only a few genes control in vitro responses, whereas more sophisticated genetic analyses implicate the action of several genetic factors, suggesting that they are polygenic traits (reviewed in Henry et al. 1994). Since Mendelian genetic studies only reveal allelic differences between two genotypes or given, as in this case,

that in the DH lines derived from a single genotype only allelic differences at heterozygous loci are revealed, the action of other genes can not be excluded. The near-continuous nature of the distribution of explant transformation responses in cv. Hawke is also indicative of the action of several genetic factors, with a smaller additive effect typical of a quantitative trait under complex genetic control. Further resolution of the genetic control of explant transformation mediated by *A. rhizogenes* will require screening a larger population of DH lines with an associated genetic map, in order to assign quantitative trait loci (QTL).

DH lines have been used for the genetic analysis of regeneration responses in plant tissue cultures (Henry et al. 1994). Indeed, efficient regeneration of plant cells into whole plants is a prerequisite for most transformation systems. DH lines are only of value in genetic analyses if they represent a random array of pre-existing genetic variability of the parental genotype responses. A major question that has arisen with the development of techniques to produce DH plants was whether in vitro culture induces selection pressures leading to significant distortions (Cloutier and Landry 1994). Selection for regeneration capacity can result following AC, such that genes tightly linked to the trait are also preferentially selected for (Murigneux et al. 1993). Separate genetic locations have been established for genes implicated in different in vitro regeneration processes in a number of species (reviewed in Henry et al. 1994). Whether these regions are also linked to genetic factors determining transformation competence in *Brassica* is not known. We found DH lines with rates of explant transformation that were either lower and/or higher than that of the parental line in each population screened, suggesting that segregation for the trait occurs independently of the AC process used to produce DH lines.

Conclusions

Producing DH lines from F1 cultivars by AC is an effective means of identifying genotypes with both improved and reduced transformation capacities, irrespective of the responsiveness of the initial F1 cultivar. The ability to identify DH lines with improved explant transformation efficiencies indicates that AC is an effective means of manipulating the plant genotype for this purpose. Our results also confirm the assertion that manipulation of the plant genotype can be used to improve transformation efficiency (Gelvin 2000) as DH lines of cvs. Corvet and Hawke were identified with higher rates of explant transformation than any of the F1 cultivars screened. Our data show that rates of explant transformation segregate in AC-derived DH lines of cv. Hawke in a quantitative manner that lends itself to QTL analysis. Consequently, the genetic basis to transformation efficiency in *B. oleracea* may be further resolved using DH mapping populations and a QTL approach. The genetic resources available in *B. oleracea* and exploitation of the synteny with that of *A. thaliana* mean that QTL analyses of transformation efficiency offer the eventual prospect of identifying and characterizing the genes involved.

Acknowledgements Financial support from the UK Biotechnology and Biological Research Council is gratefully acknowledged. *Brassica* transformation work at HRI is carried out under MAFF Plant Health Licence No. PHL 5G/3749(2/2001).

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