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Genetic analysis of in vitro shoot regeneration from cotyledonary petioles of *Brassica oleracea*

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Abstract Diallel analysis was used to investigate the genetic control of in vitro shoot regeneration in *Brassica oleracea*. Twelve doubled haploid (DH) lines, selected to include a range of genotypes with differing shoot regeneration potentials, were crossed reciprocally to produce 132 F_1 and 12 selfed, DH families. Cotyledonary petioles from 4-day-old seedlings, from all families, were excised and maintained on MS medium supplemented with 2 mg/l BAP. Explants were scored after 44 days for both the presence or absence of shoots and the number of regenerating shoots per explant. Diallel analysis showed both shoot regeneration and the production of multiple shoots to be controlled by additive and dominant gene effects, with additive effects being more important. Additive gene effects accounted for 71% and 77% of the genetic variation observed within the diallel for shoot regeneration and multiple shoot regeneration, respectively. By investigating the shoot regeneration potential of subsequent backcross and F_2 populations, the ability to introduce and increase shoot regeneration potential into otherwise recalcitrant lines was demonstrated.

Introduction

The ability to regenerate whole plants in vitro is an essential prerequisite for efficient and reliable plant transformation systems. Extensive screening of genotypes and tissue culture conditions has improved the frequency of shoot regeneration for most *Brassica* species. However, the effect of genotype still overrides most efforts to improve efficiencies, with some genotypes remaining recalcitrant to in vitro regeneration. By understanding the genetics behind regeneration, it may be possible to select for particular genes or introduce genes for regeneration ability into agronomically elite lines.

To better understand the genotype dependence of shoot regeneration, a number of groups have investigated shoot regeneration both between and within the *Brassica* species. Murata and Orton (1987) observed that *B. napus* (AACC) had a higher regeneration response than *B. rapa* (AA), thereby concluding that genes from the C genome may be influencing its greater regeneration response. Narasimhulu et al. (1988a, 1988b) looked at shoot regeneration in three diploid *Brassica* species and their synthetic amphidiploid hybrids. They found no significant difference between the B and C genomes in terms of regeneration potential, but concluded that the A genome was the most recalcitrant genome for regeneration. The synthetic hybrids *B. napus* (AACC) and *B. juncea* (AABB) both had lower regeneration responses than their better parent response, *B. oleracea* (CC) and *B. nigra* (BB), respectively, suggesting an inhibitory effect of the A genome. These studies suggest shoot regeneration to be a heritable trait. Hansen et al. (1999) reported on the genetic analysis of shoot regeneration from protoplasts of *B. oleracea* by crossing a high- and a low-regenerating line and looking at the regeneration response in the F_2. The frequency distributions observed suggested that at least three independent loci were responsible for regeneration. The finding that two or three genes control regeneration is consistent with other reports for crops such as rice (Peng and Hodes 1989; Taguchi-Shiobara et al. 1997) barley (Komatsuda et al. 1989) and tomato (Koorneef et al. 1987). Ono and Takahata (2000) looked at the genetic control of shoot regeneration in *B. napus* using a diallel cross; in a 5×5 diallel shoot regeneration from cotyledonary petioles was associated with additive and dominant gene effects, with additive gene effects accounting for the majority of the variation.

The present study looks at the genetic control of in vitro regeneration from cotyledonary petioles of *B. oleracea*. The ability to introduce shoot regeneration potential into recalcitrant lines is demonstrated. The

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possible conservation of 'regeneration' genes within the *Brassica* genus is discussed.

Materials and methods

Plant material

Based on the results of a previous study (Sparrow 2003), 12 doubled haploid (DH) *B. oleracea* lines, covering a range of shoot regeneration potentials from cotyledonary petioles, were selected for diallel analysis. The 12 DH lines were taken from a reference mapping population, derived from a cross between *B. oleracea* ssp. *alboglabra* (A12DHd) and *B. oleracea* ssp. *italica* (Green Duke GDDH33). The population was originally produced to create a RFLP map of *B. oleracea* by Bohuon et al. (1996), and public access to this material can be obtained via G. King of Horticultural Research International, Wellesbourne, UK. The 12 DH lines were crossed reciprocally to produce 132 F_1 crosses and selfs of the 12 DH lines. A subset of the F_1 were backcrossed reciprocally and selfed to produce backcross (BC) and F_2 populations.

Experimental procedure

Seeds were surface sterilised in 100% ethanol for 2 min, 15% sodium hypochlorite for 15 min and rinsed three times for 10 min in sterile, distilled water. Seeds were germinated on full-strength MS (Murashige and Skoog 1962) plant salt base, containing 3% sucrose, 0.8% phytagar at pH 5.6. After autoclaving, filter-sterilised vitamins were added to the medium: myo-inositol (100 mg/l), thiamine-HCl (10 mg/l), pyridoxine (1 mg/l) and nicotinic acid (1 mg/). Seeds were sown at a density of 15 seeds per 90-mm petridish, and transferred to a 10°C cold room overnight before being transferred to a 23°C culture room under 16-h day length of 70 μmol m^{-2} s^{-1}.

Cotyledonary petioles were excised from 4-day-old seedlings and maintained on regeneration medium (germination medium supplemented with 2 mg/l of 6-benzylaminopurine) in a 23°C culture room under 16-h day length of 70 μmol m^{-2} s^{-1}. Cotyledonary explants were excised with approximately 2–5 mm of petiole attached; petioles were embedded into the regeneration medium whilst ensuring the cotyledonary lamella were clear of the medium. Explants were sub-cultured onto fresh regeneration medium after 23 days in culture. One hundred cotyledonary petioles were established for each of the genotypes screened and approximately 200 cotyledons for each of the F_2 populations. Explants were scored individually after 44 days in culture for the presence or absence of shoots and the number of shoots produced per explant.

Statistical procedures

Two-way ANOVA using a random model was carried out to determine the amount of variation ascribed to both genetic and environmental effects (VSN International 1992). The diallel results were further analysed using methods described by Hayman (1954), and genetic component analysis was carried out using the methods described by Mather and Jinks (1987).

Results

The inheritance of shoot regeneration

Twelve DH lines and 132 F_1 hybrids from a 12×12 diallel were scored for the presence or absence of shoots after 44 days in culture. Shoot regeneration data (expressed as the number of explants forming shoots/the number of explants established) are presented in Table 1.

The heritability of shoot regeneration is clearly shown when a non- (or low-) regenerating line was crossed with a higher regenerating line; the regeneration response in the resulting F_1 hybrid was significantly higher than that of the lower regenerating parent (Table 1). This demonstrates the potential to introduce regeneration ability into recalcitrant lines by sexual hybridisation and suggests that high-regenerating genotypes are dominant over low-regenerating genotypes.

Two-way ANOVA revealed that just 15% of the variation observed within the diallel was a result of non-genetic or environmental effects, while 85% of the variation was due to genetic effects. Further analysis, following Hayman (1954), revealed the genetic control for shoot regeneration to be subject to both additive (a) and dominant (b) gene effects (Table 2), with additive gene effects being more important. The relatively high b_1 mean square (MS) indicates directional dominance and in comparing the mean of the F_1 (0.71) with the mean of the DH selfs (0.50), high shoot regeneration appears to be

Table 1 12×12 diallel table showing frequency of shoot regeneration from cotyledonary petioles, after 44 days in culture. The data are presented as mean frequencies (the number of explants forming shoots/the total number of explants) from five replicates. Parental values are shown in *bold*

Male / Female	5070	3070	5047	5117	4052	6024	4030	2072	5118	2069	1027	1002
5070	**1.00**	1.00	1.00	1.00	0.98	0.99	0.93	0.99	0.95	0.98	0.68	0.94
3070	1.00	**1.00**	0.98	1.00	1.00	1.00	0.98	1.00	1.00	1.00	0.89	0.86
5047	1.00	0.98	**0.99**	0.99	0.94	0.98	0.98	0.94	0.97	0.77	0.80	0.95
5117	1.00	1.00	0.99	**0.84**	0.90	0.71	0.92	0.71	0.55	0.77	0.53	0.49
4052	0.98	1.00	0.98	0.90	**0.90**	0.80	0.96	0.70	0.53	0.88	0.62	0.74
6024	0.99	1.00	1.00	0.88	0.80	**0.40**	0.67	0.66	0.61	0.73	0.09	0.57
4030	0.94	0.99	0.92	0.92	0.89	0.34	**0.30**	0.61	0.56	0.32	0.22	0.41
2072	0.99	1.00	0.94	0.89	0.56	0.53	0.62	**0.31**	0.20	0.46	0.04	0.15
5118	0.95	1.00	0.97	0.55	0.53	0.61	0.56	0.20	**0.18**	0.70	0.13	0.08
2069	0.97	1.00	0.61	0.71	0.81	0.73	0.38	0.42	0.68	**0.10**	0.08	0.12
1027	0.79	0.89	0.92	0.72	0.70	0.27	0.24	0.04	0.13	0.06	**0.01**	0.00
1002	0.94	0.86	0.95	0.49	0.86	0.58	0.30	0.15	0.08	0.12	0.00	**0.02**

Table 2 Analysis of variance of the 12×12 diallel table for shoot regeneration from cotyledonary petioles. *MS* Mean square, *df* degree of freedom

Item	MS	*df*	*F*-test
a	5.1299	11	288***
b_1	2.2933	1	129***
b_2	0.0532	11	2.99**
b_3	0.2547	54	13.8***
b	0.2520	66	14.16***
c	0.0225	11	1.26^{ns}
d	0.0128	55	0.72^{ns}
Block error	0.0178	572	–

Where (a) is additive, (b) is dominance and (c and d) are maternal effects, b_1 is a measure of directional dominance, b_2 of ambidirectional dominance and b_3 of the residual dominance
Significance probability levels: **1.0%, ***0.1%, ^{ns} not significant

dominant. Both maternal effects (c and d) were not significant, showing no significant differences were observed between reciprocal crosses.

A plot of the relationship between the variance of the F_1 offspring to the recurrent parental line (Vr) and their covariance with the non-recurrent parent (Wr) is shown in Fig. 1a. The Vr/Wr graph provides information on three points. First, it supplies a test for the adequacy of the model (the assumption being that the genetic control is due to an additive-dominance model, with additive environmental effects and independence of the genes in action and in distribution among the parents). If this model is adequate, then the linear regression of Wr on Vr has a slope of 1.0 (Jana 1975). Second, given that the model is adequate, a measure of the degree of dominance is provided by the departure from the origin where the regression line cuts the Wr axis. Finally, the relative order of the points along the regression line indicates the distribution of dominant and recessive genes among the parents.

The slope of the regression line was 0.95 (Fig. 1a) and, thus, was not significantly different to the line of unity (1.0), allowing the model to be further analysed. In Fig. 1a, DH lines 1002 and 1027 (both low-shoot regenerating lines) are associated with the upper end of the regression line indicating recessive alleles for shoot regeneration. DH lines 5070, 3070 and 5047 (high-shoot regenerating lines) were associated at the lower end of the regression line, indicating dominance for high shoot regeneration from cotyledonary petioles. The intercept of the regression line was above the origin and suggests incomplete dominance for shoot regeneration, thereby supporting the theory that additive gene effects play the significant role.

The plot of Wr + Vr against the mean parental value is shown in Fig. 1b. High shoot regeneration corresponded to a smaller Wr + Vr, again showing alleles for high shoot regeneration are dominant to those associated with low shoot regeneration. The plot of Wr + Vr against the mean parental value gave a positive correlation coefficient of r=0.97 (P<0.01), indicating dominant alleles act to increase expression of the character. All points also lie close to the line, indicating the dominance relationship holds true for all parents.

The relationship between Vr and Wr for shoot regeneration from cotyledonary petioles gave no reason to doubt the adequacy of the simple model. Therefore, the components of variation (D, H_1, H_2, F and E) were

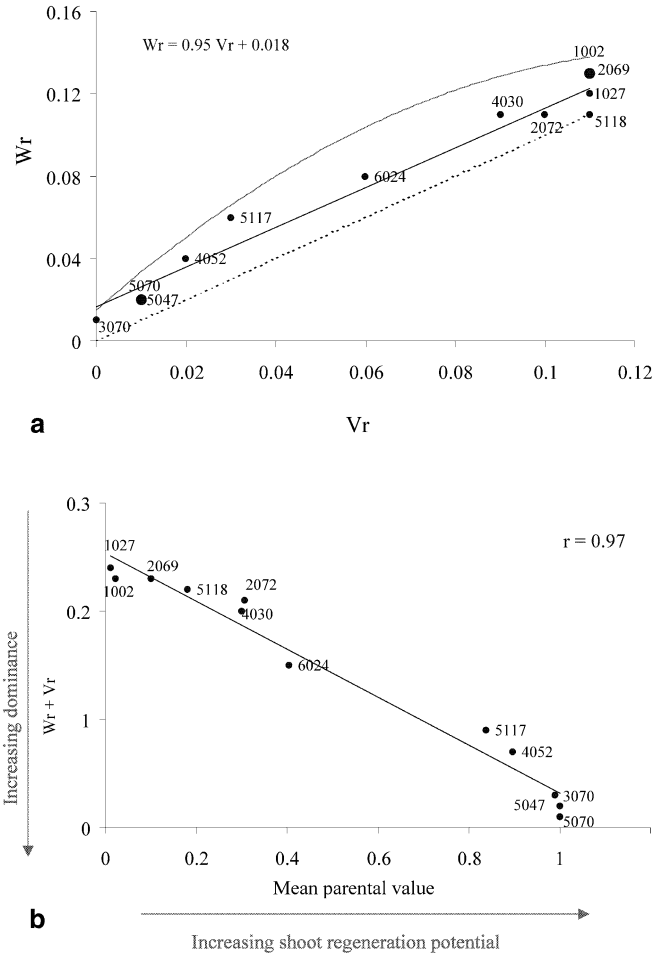

Fig. 1 **a** The relationship between the variance of the F_1, for each parental line (*Vr*) and their covariance with the non-recurrent parent (*Wr*) for shoot regeneration from cotyledonary petioles. **b** Wr + Vr from each array of the 12×12 diallel plotted against the mean of the common parent

Table 3 Genetic components analysis: shoot regeneration from cotyledonary petioles

Component	Values
D	0.148
H_1	0.056
H_2	0.066
F	−0.025
E	0.018
Mean degree of dominance $\sqrt{H_1/D}$	0.615
Proportion of dominance $H_2/4H_1$	0.295
Broad-sense heritability	0.849
Narrow-sense heritability	0.709

Table 4 12×12 diallel table showing frequency of multiple shoot regeneration from cotyledonary petioles after 44 days in culture. The data are presented as mean frequencies (the number of shoots formed per explant/the total number of explants shooting) from five replicates. A score of 8.0 is indicative of a multiple-shoot regenerating line. Parental values are shown in *bold*

Female \ Male	5070	3070	5047	5117	4052	6024	4030	2072	5118	2069	1027	1002
5070	**6.63**	8.00	8.00	6.31	6.61	6.73	5.10	6.43	5.32	4.99	4.46	5.45
3070	8.00	**7.89**	7.40	7.93	7.04	7.57	6.62	8.00	4.67	7.49	5.10	6.00
5047	8.00	7.40	**7.64**	6.20	6.89	6.36	7.59	6.71	5.31	5.03	4.54	6.07
5117	6.14	7.93	6.20	**4.49**	5.34	3.53	4.54	3.85	4.27	3.78	2.90	3.04
4052	6.69	7.04	6.41	5.34	**4.21**	4.84	6.17	4.43	3.08	4.14	3.99	4.79
6024	6.73	7.57	6.52	3.67	5.02	**3.80**	3.14	3.33	2.79	3.91	1.13	3.33
4030	4.90	6.62	5.68	4.54	5.20	2.85	**3.15**	3.71	3.07	2.65	2.43	3.42
2072	6.43	8.00	5.87	4.62	3.43	3.38	3.90	**3.53**	2.00	3.12	0.88	2.58
5118	5.32	4.67	5.31	4.27	3.08	2.79	3.07	2.00	**2.18**	2.81	1.98	2.30
2069	5.16	7.55	4.05	3.62	4.30	3.91	3.55	2.62	2.42	**1.89**	1.30	1.73
1027	4.46	5.10	6.47	2.99	4.53	2.05	4.00	0.88	1.98	1.60	**0.60**	0.00
1002	5.45	6.00	6.07	3.04	4.06	2.99	2.32	2.58	2.30	1.73	0.00	**0.20**

calculated to further investigate the genetic control of shooting from cotyledonary petioles (Table 3).

Genetic component analysis reveals, for the inheritance of shoot regeneration, additive genetic variation (D) is larger than the dominance genetic variances (H_1 and H_2). This is also noted in Table 2, with MS values far greater for additive effects (a) than dominant effects (b). D values greater than H_1 indicate incomplete dominance (which is expected if additive effects play a major role). The mean degree of dominance ($\sqrt{H_1/D}$) was 0.615 and, again, indicates incomplete dominance (a value of 1.0 indicates complete dominance and >1.0 would indicate over dominance), supporting the results of the graphical analysis (Fig. 1a). Broad- and narrow-sense heritability were 0.849 and 0.709, respectively, suggesting 85% of the phenotypic variation was heritable, with the remaining 15% being associated to environmental or non-heritable effects. Narrow-sense heritability provides a measure of the breeding value of a population and measures the proportion of the variation that is due to the additive gene effects of genes. The high, narrow-sense heritability value of 0.709 shows that around 71% of this trait is controlled by additive gene effects and, therefore, the potential to introduce this trait into breeding material is high.

Inheritance of multiple shoot regeneration potential

The number of shoots regenerating from each cotyledonary petiole was scored to determine whether regeneration of multiple shoots was dominant over the regeneration of just one or a few shoots per explant. Genotypes were scored for the total number of shoots formed/the number of explants that were shooting. A score of 8 shoots per explant was set as the maximum, as numbers greater than this were hard to score accurately. The average score for the five replicate screens are presented in Table 4.

The regeneration of multiple shoots appears to be associated with high shoot regeneration (when scored as just presence or absence of shoots) with a high correlation, r=0.8 (P<0.01), observed between the two diallel tables (Tables 1, 4). Two-way ANOVA revealed 22% of the variation observed within the diallel for multiple shoot regeneration was a result of non-genetic or environmental effects, and 78% of the variation was due to genetic effects. Further ANOVA, following Hayman (1954), revealed that both additive (a) and dominant (b) effects were significant for the genetic control of multiple shoot regeneration (Table 5), with additive effects being more important. The high MS of b_1 indicates directional dominance. Maternal effects (c) and (d) were non-significant.

Table 5 ANOVA of the 12×12 diallel for multiple shoot regeneration

Item	MS	*df*	*F*-test
a	226.15	11	195***
b_1	30.55	1	26.3***
b_2	2.18	11	1.88^{ns}
b_3	5.26	54	4.54***
b	5.13	66	4.42***
c	1.26	11	1.09^{ns}
d	0.61	55	0.53^{ns}
Block error	1.16	572	-

Where (a) is additive, (b) is dominance and (c and d) are maternal effects, b_1 is a measure of directional dominance, b_2 of ambidirectional dominance and b_3 of the residual dominance
Significance probability levels: ***0.1%, ^{ns} not significant

The relationship between the variance of the F_1 offspring to the recurrent parental line (Vr) and their covariance with the non-recurrent parent (Wr) for multiple shoot regeneration is shown in Fig. 2a. The slope of the regression line for the Wr/Vr graph was 0.91 and supports a simple model of additive-dominant genetic control. The smaller Wr and Vr values were associated with those DH lines that regenerated multiple shoots. The slope of the regression line intercepted the Wr axis at a level significantly above the origin, indicating incomplete dominance of the trait. As the regression line fell close to the line of the limiting parabola, this strongly suggests the majority of the genetic control is due to additive gene

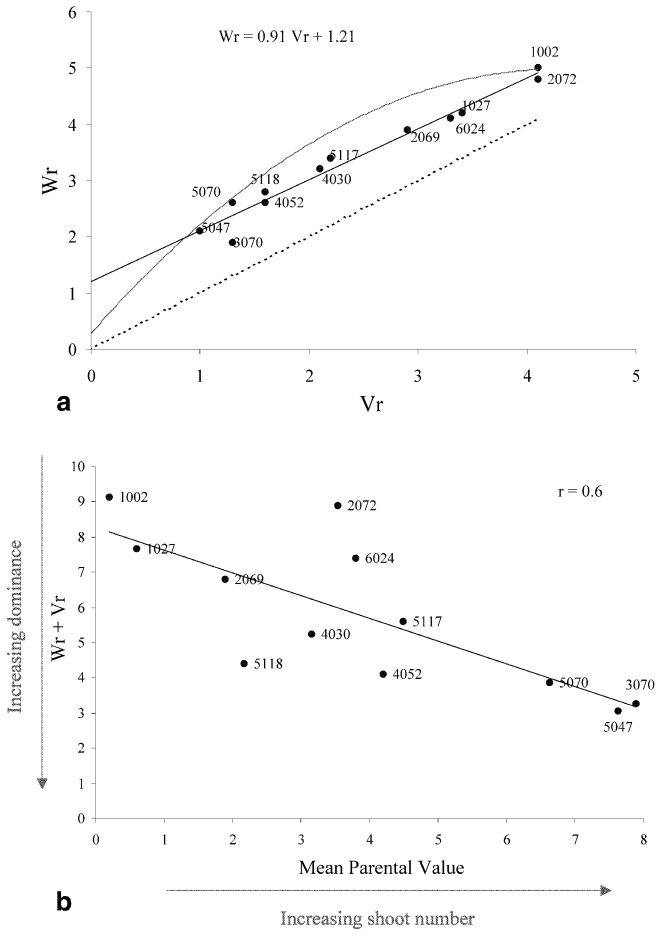

Fig. 2 **a** The relationship between the variance of the F_1, for each parental line (*Vr*) and their covariance with the non-recurrent parent (*Wr*) for multiple shoot regeneration from cotyledonary petioles. **b** Wr + Vr from each array of the 12×12 diallel plotted against the mean of the common parent, for multiple shoot regeneration from cotyledonary petioles

effects. A plot of Wr + Vr against the mean common parental value (Fig. 2b) gave a positive correlation coefficient of r=0.6 (P<0.01), indicating that dominant alleles act to increase expression of the character; in this case, dominant alleles are associated with multiple shoot regeneration. However, not all points lay close to the regression line, and this indicates that the dominance relationship does not hold true for all parents (in particular, DH 2072) and supports the interpretation of Fig. 2a: that additive gene effects play the major role.

The genetic components of variation D, H_1, H_2, F and E were calculated to further investigate the genetic control of multiple shooting from cotyledonary petioles and are presented in Table 6. The methods used to calculate the genetic components of variation support the theory that multiple shoot regeneration was controlled almost entirely by additive gene effects (D). Using this analysis, dominance effects became non-significant (H_1 and H_2). The high, narrow-sense heritability value accounts for all of the broad-sense heritability and

Table 6 Genetic components analysis: multiple shoot regeneration from cotyledonary petioles

Component	Values
D	5.22
H_1	0
H_2	0
F	−2.64
E	1.15
Mean degree of dominance $\sqrt{H_1/D}$	–
Proportion of dominance $H_2/4H_1$	–
Broad-sense heritability	0.77
Narrow-sense heritability	0.77

suggests that 77% of the variation observed within the diallel for multiple shoot regeneration was controlled by additive gene effects, with 23% of the variation accounted for by non-genetic or environmental effects. The high level of additive gene effects controlling this trait means the potential for introducing this trait into desirable material is high.

Inheritance of shoot regeneration potential: investigating BC and F_2 populations

The information gained from the DH and F_1 lines of the 12×12 diallel was used to make predictions on the inheritance of shoot regeneration in subsequent populations. The diallel screen suggested the ability to regenerate shoots from cotyledonary petioles was predominantly controlled by additive gene effects (71%), with dominance effects and environmental effects accounting for 14% and 15% of the total variation, respectively. Maternal effects were not significant and suggest the genetic control to be nuclear rather than cytoplasmic.

The DH parents of ten families were screened at the same time as the associated BC and F_2 populations; however, due to seed shortage, the F_1s were not re-screened alongside these populations; therefore, the first estimate made was that of the F_1 value. Assuming additive gene effects to be predominant, the F_1 was estimated as the mid-point value between the two DH parents [(parent A + parent B)/2]. As dominance effects also contribute to the inheritance of this trait, it could be assumed that regeneration rates slightly above the estimated value might be obtained. Likewise, the shoot regeneration response of the F_2 and BC populations was estimated as the mid-point of the relevant parents (using estimated F_1 values where appropriate).

The observed and the expected shoot regeneration responses for the ten families are presented in Table 7. Crossing two low-regenerating phenotypes together (families 2, 4, 5 and 10) resulted in a low shoot regeneration response in subsequent generations, as expected, and in all cases, the observed response was close to that of the estimated value.

Table 7 Inheritance of shoot regeneration potential in backcross (BC) and F_2 populations. Expected values were estimated based on the relevant mid-parent value

	Family 1 2072 × 4052 A × B		Family 2 1027 × 2069 A × B		Family 3 1027 × 4052 A × B		Family 4 2072 × 2069 A × B		Family 5 2072 × 1027 A × B	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
Parent A	0.17	–	0.00	–	0.00	–	0.17	–	0.17	–
Parent B	0.48	–	0.06	–	0.48	–	0.06	–	0.0	–
F_1	–	0.32	–	0.03	–	0.24	–	0.12	–	0.09
BC to parent A	0.12	0.25	0.06	0.015	0.04	0.12	0.16	0.14	0.09	0.13
BC to parent B	0.42	0.40	0.04	0.045	0.59	0.39	0.13	0.09	0.10	0.04
F_2	0.21	0.32	0.05	0.03	0.21	0.24	0.08	0.12	0.05	0.09
	Family 6 2069 × 5117 A × B		Family 7 1027 × 5117 A × B		Family 8 3070 × 4052 A × B		Family 9 2072 × 3070 A × B		Family 10 1002 × 1027 A × B	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
Parent A	0.06	–	0.00	–	1.00	–	0.17	–	0.00	–
Parent B	0.89	–	0.89	–	0.48	–	1.00	–	0.00	–
F_1	–	0.48	–	0.45	–	0.74	–	0.59	–	0.00
BC to parent A	0.27	0.27	0.05	0.23	0.91	0.87	0.11	0.38	0.00	0.00
BC to parent B	0.57	0.69	0.47	0.67	0.88	0.61	0.52	0.80	0.00	0.00
F_2	0.42	0.48	0.16	0.45	0.74	0.74	0.39	0.59	0.01	0.00

Inheritance of shoot regeneration potential was observed when a low shoot regenerating phenotype was crossed with an intermediate (families 1 and 3) with the resulting F_1 having a regeneration response higher than the lower regenerating parent. However, in these families, when the F_1 was backcrossed to the low regenerating parent, the shoot regeneration potential, although conserved, was lower than the estimated value. The significance of this is hard to estimate, due to the low regeneration response and environmental effects.

Families 6, 7 and 9 (Table 7) represent crosses between low- and high-shoot regenerating phenotypes. Shoot regeneration potentials in families 7 and 9 were lower than those of the expected values, while in family 6, they were close to those of the expected values. Crossing a high- and an intermediate-shoot regenerating line (as family 8, Table 7) gave observed regeneration rates approximately equal to, or slightly higher than, the expected values. The estimated values were made on the assumption of only additive effects being present. Had dominance effects also been a major component of the genetic variation, then values higher than the mid-parent value would have been observed. The fact that values in general behaved as expected, and sometimes slightly lower than expected, highlights that additive effects are more significant in the genetic control of shoot regeneration. The data from these families demonstrate how increased shoot regeneration potential could be passed on to subsequent generations (F_1 and F_2) and that by backcrossing the F_1 to the higher regenerating parent, regeneration rates could be increased significantly.

Discussion

The ability to introduce or increase the in vitro-shoot regeneration potential of a genotype by conventional breeding will help overcome restrictions to routine transformation programmes, where efficient shoot regeneration is a critical pre-requisite. Genotypes that regenerate multiple shoots (a response associated with a callus phase) are considerably more favourable to *Agrobacterium*-mediated transformation than genotypes that regenerate a small number of shoots directly from the petiole base (Sparrow 2003). Under the experimental conditions described, shoot regeneration from cotyledonary petioles appears to be under strong genetic control, with 85% of the variation accounted for by genetic variation, and the remainder a result of non-heritable or environmental influences. The majority of the genetic control was a result of additive gene effects, and high shoot regeneration was observed to be dominant over low shoot regeneration. The production of multiple shoots (in favour of just a few shoots) from regenerating cotyledonary petioles was also shown to be heritable, with additive gene effects accounting for the majority of the variation (77%) observed within the diallel. Such strong, additive genetic control will enable researchers to transfer shoot regeneration potential into current breeding lines for use in tissue culture and transformation programmes.

Narasimhulu et al. (1988 a, 1988b) demonstrated that crossing a low-regenerating *B. rapa* (AA) genotype with a high-regenerating *B. oleracea* (CC) resulted in an intermediate response in the AACC genome of *B. napus.* In this current paper, we demonstrate that crossing a low-regenerating line with a high-regenerating line results in an intermediate response in the F_1. It would appear that crossing genotypes of differing shoot-regeneration re-

sponse, whether between or within the *Brassica* species, will result in an intermediate response in the resulting F_1 hybrid. Previous reports indicate that there is no strong evidence for whether the BB genome is better at regenerating than the CC genome (Narasimhulu et al.1988 a, 1988b). These observations would suggest that both the BB and CC genomes have maintained genes associated with high shoot regeneration.

The results reported here support the hypothesis that genes associated with shoot regeneration may have been conserved across the genus. This idea is substantiated by the findings of Ono and Takahata (2000), who looked at the genetic control of shoot regeneration in *B. napus* and concluded that shoot regeneration from cotyledonary petioles was associated with additive and dominant gene effects. Dominant genes had a positive effect on shoot regeneration and, as with the findings presented here, dominance of this trait was incomplete and additive gene effects accounted for the majority of the variation (82% in the *B. napus* population screened). The similarity of the inheritance patterns observed for both *B. napus* and *B. oleracea* would suggest conservation of genes for shoot regeneration within the same genome (CC). Preliminary mapping of quantitative trait loci for shoot regeneration, using the DH mapping population described in this paper, indicates that genes associated with in vitro shoot regeneration may be located on linkage group O1 of *B. oleracea* (Sparrow 2003). Further work will enable the identification of markers associated with in vitro shoot regeneration and will facilitate comparative studies between the *Brassica* species to determine if these genes have been conserved within the *Brassica* genus.

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References

Bouhuon EJR, Keith DJ, Parkin IAP, Sharpe AG, Lydiate DJ (1996). Alignment of the conserved C genomes of *Brassica oleracea* and *Brassica napus*. Theor Appl Genet 93:833–839

Hansen LN, Ortiz R, Andersen SB (1999) Genetic analysis of protoplast regeneration ability in *Brassica oleracea*. Plant Cell Tissue Organ Cult 58:127–132

Hayman BI (1954) The analysis of variance of diallel crosses. Genetics 39:789–809

Jana S (1975) Genetic analysis by means of diallel graph. Heredity 35:1–19

Komatsuda T, Enomoto S, Nakajima K (1989) Genetics of callus proliferation and shoot differentiation in barley. J Hered 80:345–350

Koorneef M, Hanhart CJ, Martinelli L (1987) A genetic analysis of cell culture traits in tomato. Theor Appl Genet 74:633–641

Mather K, Jinks JL (1987) Biometrical genetics, 3rd edn. Chapman and Hall, London, pp 255–292

Murata M, Orton TJ (1987) Callus initiation and regeneration capacities in *Brassica* species. Plant Cell Tissue Organ Cult 11:111–123

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:437-497

Narasimhulu SB, Chopra VL (1988a). Species-specific shoot regeneration response of cotyledonary explants of brassicas. Plant Cell Rep 7:104–106

Narasimhulu SB, Prakash S, Chopra VL (1988b). Comparative shoot regeneration responses of diploid brassicas and their synthetic amphidiploid products. Plant Cell Rep 7:525–527

Ono Y, Takahata Y (2000) Genetic analysis of shoot regeneration from cotyledonary explants in *Brassica napus*. Theor Appl Genet 100:895–898

Peng J, Hodes TK (1989) Genetic analysis of plant regeneration in rice (*Oryza sativa*). In Vitro Cell Dev Biol 25:91–94

Sparrow PAC (2003) Plant morphogenesis and genetic transformation of horticultural brassicas. PhD thesis, Open University

Taguchi-Shiobara F, Komatsuda T, Oka S (1997) Comparison of two indices for evaluating regeneration ability in rice (*Oryza sativa*) through a diallel analysis. Theor Appl Genet 94:378–382

VSN International (1992) GenStat for Windows, release 6.1, 6th edn.