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Plant regeneration and variants from calli derived from immature embryos of diploid barley (*Hordeum vulgare* L.) and *H. vulgare* L. × *H. bulbosum* L. crosses

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Summary. Plant regeneration from calli was carried out at two locations using several parental genotypes and environments. Selfed immature diploid (VV) barley (*Hordeum vulgare*) embryos and immature haploid (V) or hybrid (VB) embryos from crosses between *H. vulgare* and *H. bulbosum* were used as explants. 'Golden Promise', 'Emir' and 'CB 7432' were the best cultivars for plant regeneration, and 15 °C tended to be more suitable for plant development than higher temperatures. A total of 844 regenerants were obtained, and over 200 fertile progenies were screened agronomically. Apart from the occurrence of polyploidy and albinism, three variants were identified. One showed maternal inheritance for yellow leaf striping while the other two were controlled by single recessive genes. One of these possessed increased resistance to scald (*Rhynchosporium secalis*) compared with the donor parent cv 'Triumph', and one resembled a *chlorina* mutant.

Key words: Callus tissue – Embryo culture – Wide hybrids – *Hordeum* spp. – Somaclonal variation

Introduction

Somaclonal variation may be one way of fulfilling the need for increasing the diversity within crop plants which the breeder can usefully exploit. The technique which has been reviewed by Evans and Sharp (1986) involves culturing various explants, such as embryos, ovaries and spikes on a callus-inducing medium prior to plant regeneration. Some of the regenerants have shown differences in both qualitative and quantitative characters compared with the parental cultivar, and the cause of this variation has usually been attributed to cytological and mutational events in vitro (Evans and Sharp 1986).

In the Triticeae, variants have been observed in wheat (Larkin et al. 1984) and triticale (Lapitan et al. 1984), but only infrequently in barley (Karp et al. 1987). Although there have been no dramatic improvements in the characteristics of these cultivated cereals, useful variants possessing disease resistance have been selected in, e.g., maize (Gengenbach et al. 1977).

The present investigation was aimed at improving the agronomic performance of spring barley. Plants were regenerated from calli derived from immature selfed embryos (VV) of *Hordeum vulgare* L., and haploid (V) and hybrid (VB) embryos from a cross between *H. vulgare* L. $2n=2x=14$ and *H. bulbosum* L. $2n=2x=14$ (Kasha and Kao 1970). Haploid embryos were cultured both to assess whether recessive mutants could be rapidly identified and to compare regeneration rates with VV embryos as explants. The reason for using VB embryos, which contain both parental genomes, was to identify barley-like regenerants possessing some useful characters transferred from *H. bulbosum*. A few variants were obtained and will be described, but only one exhibited any potentially useful characteristics.

Materials and methods

The project was carried out at the Welsh Plant Breeding Station, Aberystwyth, UK and DSIR, Crop Research Division, Christchurch, New Zealand. In general, the procedures for growing and hybridizing plants of *H. vulgare* and *H. bulbosum* were as described previously (Pickering and Morgan 1985). Diploid (VV) embryos of *H. vulgare* were obtained after selfing, whereas haploid embryos were excised from developing seeds 9–18 days after pollinating *H. vulgare* with *H. bulbosum*. After fertilization of the *H. vulgare* egg by the *H. bulbosum* sperm, the chromosomes of the latter species are usually eliminated in subsequent mitotic divisions leading to haploid (V) embryo formation (Kasha and Kao 1970). However, when certain *H. vulgare* geno-

types are pollinated at temperatures less than 17.5 °C, a proportion of the developing embryos retain both parental genomes, resulting in hybrid (VB) embryos (Pickering 1985). V and VB embryos can be identified morphologically at the time of culture and either or both of these types were used. In all cases, immature embryos (0.75–1.5 mm in length) were cultured with their scutella facing upwards initially on B5 medium with 2 mg/l 2,4-D in darkness at 22° ± 1 °C, to induce callus formation. Any germinating embryos were removed and discarded. Nodular compact yellowish calli were subcultured usually after 3–4 weeks onto B5 without 2,4-D, for plant regeneration, and transferred to an artificially illuminated chamber maintained at 22° ± 1 °C; 16 h daylength supplied by cool white fluorescent tubes provided an irradiance of 20–25 μE/m²/sec. Developing plantlets were separated, when possible, and repotted either into a John Innes No. 3 compost (UK) or bark/sand mixture containing added fertilizer (New Zealand), prior to growing in the glasshouse.

Cytological analyses and chromosome doubling using colchicine solution were carried out according to the methods of Thomas and Pickering (1983). Electrophoretic techniques for protein pattern comparisons on seeds were similar to those described by MacGibbon and Cross (1982). Arcsine transformed data of plant regeneration rates from the original explants were analysed using a conventional analysis of variance. In the text, R_0 refers to the original regenerant and R_1, R_2, etc. to the subsequent selfed generations. Callus derived from haploid, hybrid and diploid embryos is designated V, VB and VV callus, respectively.

Three experiments were carried out, one in Wales, one in New Zealand and one at both sites. They differed in the parental genotypes and environments used, and varied with respect to colchicine treatment.

Experiment 1. Plant regeneration from callus derived from embryos of eleven cultivars of H. vulgare ♀ × H. bulbosum

H. vulgare cv 'Akka', 'Allegro', 'CB7432', 'Firlbeck III', 'Maris Mink', 'Melody', 'Proctor', 'Sultan', 'VDH 479-72', 'Universe 2/6/3/4/1' and 'Universe 2/6/3/5/1' (two selections from 'Universe' showing incompatibility and compatibility with *H. bulbosum*, respectively). *H. bulbosum* genotype: Cb 2929/1, a selection from a population (GBC 281) obtained from Prof. K.J. Kasha, Guelph University, Canada.

All plants were grown in a glasshouse at the Welsh Plant Breeding Station, maintained at 16° ± 2 °C day/9° ± 2 °C night with a 16 h day length. Emasculated *H. vulgare* spikes were pollinated with *H. bulbosum* after the plants had been transferred to a controlled environment room at a temperature of 15° ± 0.25 °C with a 16 h day length, supplied by cool white fluorescent tubes providing an irradiance of 250–300 μE/m²/sec at spike height. All embryos were cultured without making a distinction between V and VB types. In order to determine the rate of spontaneous chromosome doubling, the regenerated plants were not treated with colchicine. Seed from the fertile R_0 plants was sown in 2-m field rows at Crop Research Division for agronomic assessment. Somatic chromosome counts were performed on root tips of each regenerant and electrophoretic analysis of the seed was carried out.

Experiment 2. Plant regeneration from V and VV callus of eight H. vulgare cultivars either selfed or crossed with H. bulbosum

H. vulgare cv 'Emir', 'Golden Promise', 'Goldmarker', 'Goldspear' (V callus only), 'Kym', 'Mata', 'Triumph' and 'Zephyr'. *H. bulbosum* genotype: Cb 2920/4, a selection from a population (I–5) obtained from Prof. C.J. Jensen, Risø National Laboratory, Denmark.

All plants were grown throughout in a glasshouse at Crop Research Division, maintained at 20° ± 2 °C day/12° ± 1 °C night, with a 16 h day length. Spikes were hybridized or selfed to obtain V and VV embryos for culture. All VB embryos were discarded on the basis of their morphology at the time of excision. Regenerants from V callus were colchicine-treated to restore their fertility, and R_1 plants were assessed agronomically in 2-m field rows. Any variants (except albinos) were investigated further, both cytologically and electrophoretically.

Experiment 3. Plant regeneration from callus derived from embryos of cv 'Golden Promise' and 'Emir' crossed with various H. bulbosum genotypes in several environments

H. bulbosum genotypes: Cb 2920/4; Cb 2929/1; Cb 3811/7 selected from a diploid population obtained from Estacao Agronomica Nacional, Oeiras, Portugal; CPI 18968 and HB2082 obtained from Dr. J.W. Snape, Institute of Plant Science Research, Cambridge, UK.

H. vulgare plants were grown in the glasshouse and either retained therein or transferred to controlled environment rooms for crossing at temperatures of 15° ± 0.25 °C or 25° ± 0.5 °C. One series of crosses was also carried out in the glasshouse at a temperature of 20°–25 °C day/12°–15 °C night. Only those regenerants from V callus produced in New Zealand were colchicine-treated to restore fertility. All R_1 plants were then assessed as in experiment 2.

Results

Experiment 1. Plant regeneration rates from calli derived from embryos which developed at 15 °C are presented in Table 1. Regeneration of plants was higher from 'CB 7432' compared with all the remaining cultivars except 'Akka' and 'Firlbeck III' ($P < 0.05$). As expected, a temperature of 15 °C during embryo development resulted in the regeneration of varying proportions of VB hybrids which contain both sets of parental chromosomes (Pickering 1985). The rates of spontaneous doubling ranged from 0%–33.3% and all (except for one amphidiploid from 'CB 7432') possessed 14 chromosomes. There was no phenotypic variation amongst the plant progenies, and their seed protein patterns all appeared similar to the barley cv from which they originated.

Experiment 2. The rates of plant regeneration from V and VV callus are presented in Table 2. Significantly greater regeneration ($P < 0.05$) was obtained from V compared with VV calli for all cultivars except 'Golden Promise' and 'Mata'. 'Golden Promise' was the most successful cultivar from which to obtain diploid and haploid regenerants ($P < 0.05$), although 'Emir' and 'Triumph' did not differ significantly from 'Golden Promise' in their regeneration rates from V calli. Several variants were recorded which will be described in the next section.

Experiment 3. Data from experiment 2 for 'Golden Promise' and 'Emir' crossed with *H. bulbosum* were analysed together with the data obtained for these cultivars crossed with *H. bulbosum* in the environments outlined in

Table 1. Mean plant regeneration (arcsine transformed in radians) and standard error of the mean (SEM) from embryo-derived calli of 11 cv of *H. vulgare* crossed with *H. bulbosum* (Cb 2929/1) at 15 °C; expt. 1

Cultivar	No. of embryos cultured	No. of plants regenerated				Mean plant regeneration	SEM
		V	VV[a]	VB	VVBB[a]		
'Akka'	62	14	3	4	0	0.649	±0.054
'Allegro'	47	3	0	2	0	0.252	±0.105
'CB7432'	40	13	7	3	1	1.026	±0.183
'Firlbeck III'	47	12	6	1	0	0.624	±0.129
'Maris Mink'	75	9	0	5	0	0.397	±0.091
'Melody'	55	11	0	2	0	0.431	±0.138
'Proctor'	73	8	2	3	0	0.436	±0.062
'Sultan'	62	16	2	0	0	0.427	±0.121
'VDH479-72'	37	3	0	4	0	0.392	±0.212
'2/6/3/4/1'	66	10	0	0	0	0.299	±0.097
'2/6/3/5/1'	65	5	0	2	0	0.252	±0.093

[a] Spontaneously chromosome doubled

Table 2. Mean plant regeneration (arcsine transformed in radians) from embryo-derived calli of 8 cv of *H. vulgare* either selfed, or crossed with *H. bulbosum* (Cb 2920/4) in the glasshouse; expt. 2. The number of VV embryos cultured excludes those which were discarded because of germination

Cultivar	Embryo[a] explant	No. of embryos cultured	No. of plants regenerated	Mean plant regeneration	SEM
'Emir'	V	112	26	0.420	±0.099
	VV	122	8	0.099	±0.054
'Golden Promise'	V	92	35	0.653	±0.053
	VV	123	31	0.446	±0.124
'Goldmarker'	V	82	9	0.286	±0.072
	VV	120	1	0.034	±0.034
'Goldspear'	V	69	10	0.295	±0.095
'Kym'	V	74	11	0.304	±0.086
	VV	46	0	0.00	±0.00
'Mata'	V	37	3	0.102	±0.102
	VV	82	1	0.033	±0.034
'Triumph'	V	62	17	0.496	±0.093
	VV	95	1	0.037	±0.037
'Zephyr'	V	80	10	0.270	±0.073
	VV	51	1	0.052	±0.052

[a] VV – diploid embryo explant; V – haploid embryo explant

'Materials and methods'. Results are presented in Table 3. Plant regeneration from V and VB calli of 'Golden Promise' was higher than that from 'Emir' at 15 °C and 25 °C ($P<0.05$) during embryo development. However, there were no significant differences between the cv when grown throughout in the glasshouse. The glasshouse maintained at the lower of the two temperatures was a more suitable environment from which to obtain responsive embryos of both cv ($P=0.01$). There was also a similar, but non-significant trend for the lower (15 °C) of the two growth room temperatures to be more favourable in this respect. At this temperature, the regeneration rates from VB callus were not significantly different from V callus. In crosses with 'Emir', greater regeneration rates from VB callus were obtained from Cb 2929/1 as pollinator compared with HB 2082 ($P=0.05$).

A total of 13 spontaneously doubled haploids were obtained, all but one from 'Golden Promise' crossed at 25 °C. Only three spontaneously doubled fertile amphidiploids (VVBB) were recorded, one from 'Golden Promise' and two from 'Emir'.

Regeneration rates and R_0 variants

A total of 543 and 301 R_0 plants were regenerated from V and VB callus derived from the interspecific cross.

Table 3. Mean plant regeneration (arcsine transformed in radians) from embryo-derived calli of 'Golden Promise' (GP) and 'Emir' (E) crossed with five different *H. bulbosum* genotypes in several environments; expt. 3

♀ Parent	♂ Parent	Environment	Embryo explant	No. of embryos cultured	No. of plants regenerated		Mean plant regeneration	SEM
					V	VB		
E	Cb2929/1	15 °C	VB	363	1	182	0.811	±0.040
E	HB2082	15 °C	VB	59	1	16	0.563	±0.105
GP	HB2082	15 °C	VB	138	0	76	0.894	±0.088
E	HB2082+Cb3811/7	25 °C	V	96	17	0	0.379	±0.067
GP	HB2082+Cb3811/7	25 °C	V	172	87	0	0.756	±0.077
E	Cb2929/1+CPI 18968	15 °C	V	38	11	0	0.484	±0.099
GP	Cb2929/1+CPI 18968	15 °C	V	60	37	0	0.927	±0.104
E	Cb2920/4	20/12 °C	V	112	26	0	0.420	±0.099
GP	Cb2920/4	20/12 °C	V	92	35	0	0.653	±0.053
E	Cb2920/4	20–25/12–15 °C	V	767	65	0	0.219	±0.031
GP	Cb2920/4	20–25/12–15 °C	V	761	80	0	0.281	±0.033

Forty-three R_0 regenerants were obtained from VV callus. Nineteen R_0 plants from V callus (3.5% of those regenerated) were observed which were either albino or possessed leaves with longitudinal white stripes. Three albino R_0 plants from VV callus (7.0% of regenerants) were recorded, but none from VB callus.

Two of the 301 regenerants from VB callus involving crosses between 'Emir' × HB2082 and 'Golden Promise' × HB 2082 had awnless spikes which could not be maintained stably by vegetative propagation. A meiotic analysis of pollen mother cells (PMCs) at metaphase 1 (MI) revealed no anomalous features. PMCs with a trivalent and 15 chromosomes were sometimes observed in both VBs, but pairing was otherwise well within the limits expected in VB hybrids from similar crosses (Thomas and Pickering 1985). One of these VBs was colchicine-treated and its fertility restored. Chromosome pairing at MI was as expected for a VVBB hybrid, with a proportion of multivalents in addition to uni- and bivalents being observed (cf. Kasha and Sadasivaiah 1971). Selfed progeny comprised plants resembling 'Emir' (after elimination of the *H. bulbosum* chromosomes), but the majority remained hybrid-like, presumably still retaining chromosomes from both parental genomes.

Eleven regenerants from VB callus possessed haploid and hybrid sectors, one of which (828H6), from 'Triumph' × Cb 2920/4, had a haploid sector with white longitudinal leaf stripes (Fig. 1 a), but the hybrid sector was similar to a normal VB hybrid in morphology. After colchicine treatment, fertility was restored and R_1 and R_2 plants resembled normal barley, but were homozygous for several morphological characters which distinguished them from cv 'Triumph', their female parent. These were (a) denser spike with fewer grains (Fig. 1 b); (b) marked necrotic blotching, especially on later developing leaves (Fig. 1 c); (c) increased resistance to scald *(Rhynchosporium secalis)* in the field, compared with 'Triumph'. There were no cytological abnormalities at meiosis and seven ring bivalents were regularly formed at MI in 50 PMCs examined. Protein patterns obtained by electrophoresis on seeds of 828H6 were similar to the stock of 'Triumph' from which it was derived.

Reciprocal crosses were made between 828H6 and both 'Triumph' and 'Fleet', and there was no evidence of cytoplasmic inheritance, as all F_1 plants were normal in appearance. F_2 populations from 'Triumph' × 828H6 and 'Fleet' × 828H6 were grown, and 193 normal versus 51 828H6 types were recorded. These data did not differ from a 3:1 ratio expected for a single recessive gene ($\chi^2 = 1.97$ at 1 *df*).

Thirty-four (12.3%) spontaneously chromosome-doubled plants were regenerated from 276 non-colchicine-treated R_0 plants derived from V callus, compared with only four (1.3%) out of 301 regenerants from VB callus. One triploid R_1 plant was obtained after colchicine treatment of a regenerant from V callus of 'Golden Promise'. There were no spontaneous tetraploid R_0 plants obtained from VV callus.

R_1 variants

Overall, 219 R_1 progeny rows were screened in the field. These comprised 186 from V, 28 from VV and five from VB callus which included the variant 828H6 described previously. The other four VB R_1s, which had spontaneously doubled their chromosome number, behaved as expected, with a proportion of plants resembling the *H. vulgare* parent and the remainder appearing hybrid-like. Of the 214 R_1s from V and VV callus, 208 resembled their respective *H. vulgare* parents agronomically, but six progeny rows possessed one of the following chlorophyll anomalies: (1) segregating green: albino plants (ex V cal-

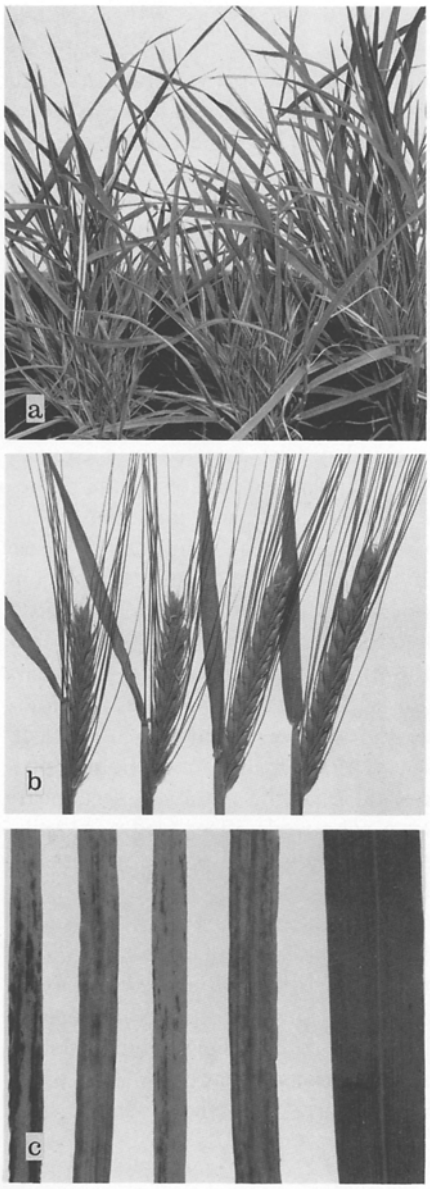

Fig. 1 a–c. Variant 828H6 derived from 'Triumph' × Cb 2920/4; **a** R_0 generation, illustrating chlorophyll deficient leaf striping; **b, c** R_1 generation: **b** two dense spikes of 828H6 (left) compared with two spikes of 'Triumph', **c** necrotic leaf blotching on four leaves of 828H6 (left) compared with 'Triumph' (right). (Note also differences in leaf width)

lus); (2) white longitudinal leaf striping (ex V callus); (3) pale purple leaves (ex V callus); (4) yellow-purple leaves (ex V callus); (5) chlorophyll deficient (ex VV callus; code E1/1); (6) segregating albinos and plants with yellow longitudinal stripes on the leaves (ex V callus; code 826Y12/6). Apart from E1/1 and 826Y12/6 (and progeny from the row with green and albino plants, which was not examined further), these characteristics were not transmitted sexually to the R_2 generation.

Variant E1/1 was derived from VV callus of 'Emir' in experiment 2. In the R_1 generation, three out of seven progeny rows in the field segregated normal versus chlorophyll deficient plants, two of which survived. Selfed progeny from the latter were all yellow as seedlings but became greener during later development and matured several weeks after the normal plants. Seven ring bivalents were observed at MI in 43 out of 50 PMCs, the remainder possessing six rings and one rod bivalent. Electrophoretic analyses performed on seeds of E1/1 and 'Emir' revealed no differences in protein banding patterns. Reciprocal crosses were made with 'Emir' and all F_1 plants were green. Pooled data from two F_2 populations derived from these crosses segregated 46 green : 13 yellow, not significantly different from a 3:1 ratio for a single recessive gene ($\chi^2 = 0.141$ at 1 *df*). These yellow plants were similar to *chlorina* mutants (gene symbol *f*) which are also under the control of single recessive genes (Tsuchiya et al. 1973).

Variant 826Y12/6 was a colchicine-treated regenerant from V callus of 'Golden Promise' in experiment 2. One progeny row in the R_1 generation comprised eight albino and three plants with yellow longitudinal stripes on the leaves. Selfed seed was harvested and 554 seedlings were classified as 172 normal green, 265 striped and 117 with more severe chlorosis and stunting. At tillering, all 345 surviving plants had developed yellow striping on the leaves, which could still be observed until leaf senescence. Fertility of some of the poorer plants was reduced and some of the seeds (<15%) were much smaller than normal at maturity. Only three out of 81 of these germinated on moistened filter paper and produced typical chlorotic plants. Twenty-six PMCs of an R_4 stunted chlorotic plant were examined cytologically, but no abnormalities were observed and seven ring bivalents were regularly formed at MI. There were no clear differences in protein banding patterns obtained after carrying out electrophoresis on seeds of 826Y12/6 and 'Golden Promise'.

Reciprocal crosses were made between 'Golden Promise' and several R_5 plants with different degrees of stunting. Eighty-one F_1 plants from 826Y12/6 (♀) × 'Golden Promise' and eleven F_2 populations all showed yellow leaf striping. In contrast, 205 F_1 plants from the reciprocal cross and 20 F_2 populations all appeared normal. From these data it appears that the inheritance of these abnormalities is completely maternal.

Discussion

Plant regeneration was markedly influenced by environmental conditions, parental genotype and genomic constitution of the explant.

Environmental influence. In experiment 3, the higher of the two temperatures in the glasshouse was not conducive for obtaining responsive immature embryos. Significantly better results were attained at a constant 15 °C, which is close to that recommended for the growth of donor plants for anther culture (Lyne et al. 1986).

Genotype effects. The influence of genotype has been shown to be an important factor in the success of barley anther culture programmes (Foroughi-Wehr et al. 1976) and for obtaining satisfactory results from callus cultures derived from embryos (Lührs and Lörz 1987; Bayliss and Dunn 1979). In the present paper, 'Golden Promise' and to some extent 'Emir' appeared to be outstanding for plant regeneration from V and VV callus, which confirms previous reports for diploid embryos (Lührs and Lörz 1987; Karp et al. 1987). The *H. bulbosum* genotype also influenced plant regeneration from VB callus, and Cb 2929/1 was superior to HB 2082 in crosses with 'Emir'.

Genome constitution. Only a small amount of published data exist on plant regeneration from callus of *H. bulbosum* or *H. vulgare* × *H. bulbosum* hybrids. Breiman (1985) compared one population of *H. bulbosum* and 14 of *H. spontaneum,* and found that plant regeneration from subcultured 'differentiated' callus was similar in both species. Using explants of immature spikes, Jørgensen et al. (1986) did not succeed in regenerating plants from VB hybrids of *H. vulgare* × *H. bulbosum* ($2n=2x=14$) or VBB hybrids from *H. vulgare* × *H. bulbosum* ($2n=4x=28$).

In experiments 1 and 3, regenerants were obtained from VB callus without much difficulty. Moreover, in experiment 3, plant regeneration rates from VB callus derived from 'Emir' × Cb 2929/1 were not significantly different from those obtained using V callus from the same cross. Bearing in mind the influence that parental genotype has on plant regeneration, a larger-scale investigation with several *H. bulbosum* genotypes is needed to assess the influence of the *H. bulbosum* genome itself on regeneration rates.

The use of haploid embryos for callus induction and plant regeneration has rarely been reported, which is surprising in view of the relative ease of obtaining the explant and the potential value of haploid embryos for the rapid identification of recessive mutants. However, Séguin-Swartz et al. (1984) and Jensen (1981) regenerated plants from haploid explants and, thus, there are no insurmountable barriers to obtaining plants from V callus. Indeed, in experiment 2 calli from haploid embryos were more responsive than those from diploid embryos, and significantly more regenerants were obtained. It is possible that the greater ability of V callus to regenerate plants may be a function of ploidy level. This could be tested by comparing the response of haploid, diploid and tetraploid explants.

Variants

Morphological. Although phenotypic variation in regenerants has been described in other species in the Triticeae (Larkin et al. 1984), there have been few reports of any morphological variants arising from callus cultures derived from diploid embryos of *Hordeum* species (Breiman 1985; Karp et al. 1987). The vast majority of the regenerants obtained in these experiments were also phenotypically identical to their barley parents. Three distinct variants were obtained but only one of these (828H6) appeared to have any agronomic value, namely an increased resistance to *R. secalis* compared with cv 'Triumph' from which it was derived.

This resistance could not be detected in a conventional glasshouse test using single isolates of the pathogen present in New Zealand, but was identified in the field as reduced percentage leaf area infected. Disease nursery tests during 1988 in Wales and New Zealand confirmed that levels of infection were around 35%–50% less than 'Triumph' (R.B. Clothier and C.A. Munro, personal communication). Attempts to transfer the resistance to *H. vulgare* by screening 65 doubled haploids (DHs) from F_1s between 828H6 × 'Triumph' and 'Fleet' failed, and only DHs with the mutant morphology possessed increased resistance to the pathogen. It appears that the gene exerts a pleiotropic effect, and poor spike conformity and leaf necrosis will be associated with the disease resistance.

828H6 and the *chlorina* variant E1/1 are both governed by single recessive genes and are likely to be mutations which occurred during tissue culture. A cytoplasmically inherited mutant was also identified in 'Golden Promise'. To the author's knowledge, this is the first report of such a mutant arising in barley through tissue culture, although they have occurred spontaneously (Ahokas 1976).

Polyploidy. Despite the presence of polyploid cells in callus from diploid explants of barley (Lupotto 1984), spontaneous doubling of chromosome number has rarely been observed in the regenerants themselves (Karp et al. 1987; Lupotto 1984) compared with those from haploid explants (Jensen 1981; Saalbach and Koblitz 1977). Regarding interspecific and intergeneric hybrids, chromosome doubling has been observed after plant regeneration from callus in some hybrid combinations in the Triticeae (Fedak 1984; Jørgensen and Andersen 1989). Polyploidy in callus cultures of *H. vulgare* × *H. jubatum* has been reported by Orton (1980), although chromosome counts from ovary wall cells of regenerants were relatively normal.

In these experiments, regenerants from V and, more rarely, VB callus were obtained with spontaneously doubled chromosome numbers as well as a triploid

($2n=3x=21$) derived from V callus, but there was no spontaneous doubling in plants derived from VV callus. These data confirm the relatively frequent occurrence of chromosome doubling in plants obtained from V callus compared with those from VB and VV embryos as explants, and is probably a reflection of the high frequency and regenerative capacity of diploid cells in callus derived from haploid explants (Séguin-Swartz et al. 1984).

Other chromosomal changes. Despite reports on the occurrence of chromosomal rearrangements, aneuploidy and pairing anomalies at meiosis in regenerants from various explants in species and hybrids within the Triticeae (Fedak 1984; Lapitan et al. 1984), there have been few records in diploid *Hordeum* spp. Novak (1980) observed structural rearrangements and chromosome fragments in callus tissue from haploid embryos, but was unable to regenerate any plants. Saalbach and Koblitz (1977) also noted aneuploidy and polyploidy in root tips of ten regenerants from callus derived from apical meristems of haploid barley. Karp et al. (1987) identified 1 plant out of 42 regenerants with partial sterility resulting from chromosome breakage and irregular pairing at MI. Regarding these disturbances in wide hybrids, Orton (1980) observed the probable presence of chromosomal rearrangements in callus tissue derived from *H. vulgare* × *H. jubatum,* and increased pairing at MI in PMCs of regenerants. However, in a recent extensive programme involving many hybrids between seven wild *Hordeum* species and *H. vulgare,* Jørgensen and Andersen (1989) found little suggestion of any translocations after plant regeneration from callus, although several karyotypic variants were observed.

In the cytological analyses performed herein, there was no evidence of cytological disturbances in plants regenerated from V or VV callus, apart from the polyploidy already mentioned. This may be due to the inability of a diploid species to tolerate as many structural and numerical changes in the chromosome complement compared with polyploids. Meiotic analyses carried out on the VB regenerants also revealed no cytological anomalies other than those to be expected in unstable VB hybrids. Thus, the possibility of introgressing genes into barley from *H. bulbosum* by means of callus culture appear somewhat limited.

In conclusion, although several hundred regenerated plants were screened, only a few variants were obtained which were mainly chlorophyll mutants, some of which were not transmitted to subsequent generations. The frequency with which these occurred compares favourably with spontaneous mutation rates in barley (Nilan 1964), but the yield of recessive mutants is still likely to be far greater by means of artificial mutagenesis (Nilan 1981). Unfortunately, no useful agronomic types were selected apart from one variant with increased resistance to *R. secalis*. Despite this lack of success, much data were obtained which will help in the choice of suitable genotypes and environments for future research.

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