Theor Appl Genet (1985) 71:231–237

Sporophytic-gametophytic herbicide tolerance in sugarbeet

G. A. Smith and H. S. Moser *
USDA – ARS Crops Research Laboratory, Colorado State University, Fort Collins, CO 80523, USA

Accepted April 18, 1985
Communicated by A. L. Kahler

Summary. In vitro selection procedures for herbicide tolerance were initially developed in the sporophytic generation of sugarbeet (*Beta vulgaris* L.), and then tested in the gametophytic generation. The primary objective of our study was to develop and evaluate in vitro techniques for identifying genotypes within heterogeneous seedling populations tolerant to specific herbicides, and to use meristematic cloning procedures to synthesize clones genetically tolerant to the herbicide. Seed from cloned selections tolerant to the herbicide ethofumesate were obtained and compared to plants from seed of the original population (using germination, central bud development, and root dry weight). Verification for in vitro selection accuracy was accomplished by pollen germination studies in the gametophyte. The results indicate that in vitro selection of germinated seedlings in the presence of the proper concentration of challenging agent can be effective in identifying genotypes tolerant to ethofumesate. Such identification was accomplished in fully differentiated tissue, but without the necessity of mature plants. Gametophytic studies, via pollen germination, indicated an association between genes operating in the sprophyte and those in the gametophyte. Cloning the seedlings identified as tolerant genotypes, and subsequent intercrossing of these clones provided a convenient method of synthesizing populations with gene frequencies shifted in the direction desired.

Key words: Cloning – *Beta vulgaris* L. – Herbicide tolerance – In-vitro-selection – Resistance-breeding

* Joint contribution of the Agricultural Research Service, USDA, and the Colorado State University Experiment Station. Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Series Paper No. 2952

Introduction

The rationale for developing crop cultivars that can tolerate herbicide has been discussed at length (Faulkner 1982; Gressel 1980; Gressel et al. 1982; Hughes 1983; Meridith and Carlson 1982). Perhaps, the most convincing argument for such development is that, with traditional methods, the cost of developing a new crop cultivar is only 1–5% of the developmental cost of a new herbicide (Faulkner 1982). However, selection for herbicide resistance or tolerance in the field by traditional breeding technique is very time (years) and labor-intensive and, at best, very inexact. Low frequency of resistant individuals, low heritability of tolerance, escapes – all associated with high environmental variability – account for severely limited progress.

Cell culture systems appear to offer a good alternative approach. Cell culture systems facilitate the use of large numbers of cells (potentially 'totipotent' plants) in small volumes with relatively short generation times. The uniformity of cell cultures allows greater selection pressures, which may lead to rapid selection for resistance (Gressel et al. 1978). Although many variant cell lines have been isolated, in only a few cases have plants been regenerated from cell cultures. For many species, regenerating plants is extremely difficult with normal cell culture, let alone with variants. Often, plants that are regenerated lose the tolerance expressed in cell culture (Meridith and Carlson 1982). Tobacco (*Nicotiana tobacum* L.) is the only species in which herbicide tolerance, selected in vitro, has been expressed in regenerated plants and transmitted to the progeny of these plants (Chaleff and Parsons 1978; Chaleff and Ray 1984; Hughes et al. 1984). Even with this success, Chaleff and Parsons (1978) stated, "The value of cell culture as a means for selecting and introducing agronomically desirable new alleles has remained unproved because in too few cases has the expression of a characteristic selected at the cellular level been demonstrated in the mature plant." Of course, cultured cells and whole plants represent different developmental states which should

be characterized by specific gene expression (Meridith and Carlson 1982). Undoubtedly, many genes may be expressed in both cell culture and in plants. However, many others are restricted to one state or the other, and it is obvious that cell cultures lack many functions associated with differentiated cells.

In our search for techniques that might be used to exploit the genetic variance for herbicide tolerance in sugarbeet (*Beta vulgaris* L.), we considered several other factors in addition to those discussed above. Totipotency (the ability to regenerate a whole plant from a single cell) has not been demonstrated in sugarbeet, although regeneration from callus is possible (Saunders and Daub 1984). Further, the majority of reports of differential herbicide sensitivity among clones, ecotypes, or crop cultivars show a more or less continuous pattern of variation, suggesting that their responses to herbicides are most often controlled quantitatively (Faulkner 1982; Gressel et al. 1982; Smith et al. 1982). Recently, we reported differential cultivar response to several important preplant and postemergence herbicides used for weed control in sugarbeet (Smith and Schweizer 1983). These studies established the existence of quantitative genetic variation for herbicide tolerance in *Beta vulgaris.*

Since single cell totipotency has not been demonstrated in sugarbeet and herbicide tolerance appears to be quantitative, we examined other in vitro methods for capitalizing on the genetic variation for herbicide tolerance. It has been suggested that a substantial number of genes expressed in the gametophyte (pollen) also are expressed in the sporophyte (Mulcahy 1971; Ottaviano et al. 1980). Recent evidence in maize (*Zea mays* L.) (Mulcahy 1971, 1979; Mulcahy and Mulcahy 1975; Ottaviano et al. 1980) and in tomato (*Lycopersicon esculentum*) (Tanksley et al. 1981) have supported the hypothesis that a portion of the genes (60% in tomato) expressed in the sporophyte also are expressed in the gametophyte.

The primary objective of our present study was to develop and evaluate in vitro techniques for identifying genotypes within heterogeneous seedling populations that are tolerant to specific herbicides, and to use meristematic cloning procedures to synthesize clones genetically tolerant to the herbicide. Our in vitro selection procedures were developed initially with the sporophytic generation, and then tested in the gametophytic generation in accordance with the proposal that a correlated effect between generations might result from selection of genes expressed in both stages (Mulcahy 1979).

Materials and methods

In vitro screening for ethofumesate tolerance

Several experiments were conducted, hereafter referred to as initial, experiment 1, experiment 2 and experiment 3. The source population for all selections in this study was FC 901. FC 901 is a moderately heterogeneous multigerm breeding line developed at Ft. Collins, CO for resistance to the fungal disease *Cercospora beticola.* Our initial experiment was conducted to determine the response of germinated seedlings grown on a medium containing a common sugarbeet herbicide, ethofumesate [(±)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate]. Germination of surface-disinfested seed from the source population was initiated to establish the selection criteria and expected phenotypic response to ethofumesate-enriched medium. Murashige-Skoog (MS) minimal organic media (Huang and Murashige 1976) supplemented with 22.0 mg/L $FeSO_4$:7 H_2O, 30.0 mg/L Na_2 EDTA, 10 g/L sucrose, and 9.0 g/L Bacto-agar, was used as a basal medium. The pH of the medium was adjusted to 6.0 with 1N NaOH or 1N HCL. Ethofumesate, as an emulsifiable concentrate (0.18 kg ai/L, 1.5 lb ai/gal) was added at concentrations of 0, 3, 6, 9 and 12 mg/L active ingredient to the base medium before autoclaving. The medium was dispensed into 300-ml beakers (40 ml per beaker), and the beakers were covered with aluminum foil and autoclaved at 117 kPa and 121 °C for 20 min. Powdered MS medium was obtained from Carolina Biological Supply, Burlington, NC, and Difco-Bacto agar from Difco Laboratories, Detroit, MI[1].

Seed was surface disinfected in 70% ethanol for three to 4 s, soaked in 1.05% sodium hypochlorite (pH adjusted to 5.0 with 6 N HCL) for 20 min, and rinsed three times with sterile distilled water (10 min per rinse). Five beakers of each ethofumesate concentration were plated with 25 seeds by placing the seeds directly on the medium. After plating, beakers were covered with foil and placed in a growth chamber under a mixture of fluorescent and incandescent light (190 µmol m^{-2} s^{-1}) at 22 °C. The experimental design was a randomized, complete block with five replications.

Total germination and the number of seedlings with normal true leaf development were determined after 17 days. Data for both parameters were subjected to variance and regression analysis using least squares and orthogonal coefficients.

Subsequent screenings, based on observations from our initial experiment, were conducted with seedlings cultured on medium containing 12 mg/L ethofumesate. Generally, 30 beakers of selective medium, each containing 30 seed, were tested. An additional five beakers with nonselective medium were plated to serve as checks. Central bud development has been used in the field as an indicator of ethofumesate injury in sugarbeet (Schweizer 1980). Seedlings were selected after 21 days if they displayed normal morphological development, including minimal stunting and central bud development as evidenced by normal true leaf growth with leaves at least 5 mm long.

Immediately following selection, roots and cotyledons were removed from selected seedlings, and the remaining hypocotyl and true leaves were plated (Hussey and Hepher 1978). MS basal medium supplemented with 0.2 mg/L 6-Benzylaminopurine was used for clonal multiplication. When the desired number of ramets were obtained (after three to six subcultures), they were transferred to rooting medium consisting of MS basal medium supplemented with 3.0 mg/L naphthalene acetic acid. After rooting in vitro, ramets were

1 Trade names and company names are included for the benefit of the reader and imply no indorsement or preferential treatment by the USDA or Colorado State University of the product listed

transplanted to Jiffy 7 peat pellets (pH 6.0–6.3) and placed in growth chambers at 20 °C and 95% relative humidity. After furter rooting and plant development, (2–3 weeks) plants were transplanted to 10-cm round pots containing soil. Plants were maintained under normal greenhouse culture for 4–6 weeks after which time they were placed in photo-thermal induction for 90 days to induce flowering (Smith 1980). Following photo-thermal induction, plants that had been screened on 12 mg/L ethofumesate were placed in isolation to allow flowering and pollen production. Mature seed were harvested in bulk.

Progeny evaluation of in vitro selections

In experiment 1, seed was harvested from nine interpollinated plants which had been selected for ethofumesate tolerance and tested in the greenhouse against the seed from the original FC 901 population. The experiment was duplicated using steam-sterilized soil and non-sterilized soil directly from the field. Because results were similar and because filed soil may be more representative, only field soil results are presented. Ethofumesate was incorporated into soil (a Nunn clay loam, Aridic argiustoll, fine montmorillonitic measic with 1.2% organic matter and a pH of 7.7) at concentrations of 0, 3, 6, 12 and 18 mg/kg soil. Four 10-cm round plastic pots with 10 seeds each were established for each of the five concentrations for both the screened and unscreened populations. Pots were placed in the greenhouse under sodium vaporlamps set for a 16 h photoperiod. The experimental design was a randomized complete block with four replications.

Total emergence, and the presence or absence of normally developed central buds and true leaves were determined at 14 days. The numbers of seedlings with true leaves were converted to percent emergence for each pot. These percentages gave an estimate of the percent of the population with normal central bud development and growth of true leaves. Seedlings then were thinned at random to five plants per pot. These remaining five plants were harvested at 21 days. Root and top dry weights were determined following 48 h oven-drying at 70 °C. Root, top, and total plant dry weights were converted to percent of untreated check and subjected to an analysis of variance.

Gametophytic (pollen) and sporophytic association studies

In vitro experiments were conducted to determine the effect of ethofumesate on pollen germination and growth and to study the association of response between the sporophyte and gametophyte. In experiment 2, pollen germination from three FC 901 clones (N15–19, N3–3 and N2–4) developed from screened selections on 12 mg/L ethofumesate, and a clone (also from FC 901) that had not been screened were tested. In experiment 3, pollen from six different FC 901 clones (N3-3, N3-6, N15-2, N15-8, N15-10 and N15-18) also developed from screened selections on ethofumesate and another unscreened clone from FC 901 were tested. A liquid formulation of Brewbaker and Kwack (BK) medium (Brewbaker and Kwack 1963) supplemented with 320 g/L sucrose and adjusted to pH 5.0 as suggested by Hecker (1984), was used in all tests. Pollen was sampled from plants of each clone, divided into three equal samples, and then added to 4.0 ml of medium containing 0, 10, or 20 mg/L ethofumesate and plated on 60 mm – diameter petri plates. Two or more plants within each clone were sampled on succeeding days for a total of four replications in experiment 2 and three replications in experiment 3. Plates then were placed under flourescent lights (6.4 μmol m^{-2} s^{-1}) at 20 °C. After 24 h, one drop of solution (well mixed) from the petri plates was examined on a glass slide. Between 400 and 500 pollen grains were observed under the microscope. A pollen grain was considered germinated when the pollen tube was twice the diameter of the pollen grain. Average percent pollen germination was converted to percent of control with the untreated pollen from each clone serving as its own control. For both experiments a split-plot design was used with clones being the main-plots and herbicide concentration the subplots. Main plots were treated as completely random. Data for pollen germination percent were subjected to analyses of variance.

Results

In vitro screening for ethofumesate tolerance

The effect of varied concentrations of ethofumesate (0–12 mg/L) on germination and central bud development of the original unscreened FC 901 seed was determined 17 days after plating, which was the earliest that reliable readings were possible. Three mg/L is approximately equal to the normal rate of ethofumesate recommended for weed control in the field. Similarly, 12 mg/L represents approximately four times the normal field rate. Consistent linear reductions in both germination and central bud development occurred as ethofumesate concentrations increased. Seed germination ranged from 84% of control at 3 mg/L to only 37% of control at 12 mg/L. Central bud development ranged from 32% at 3 mg/L to 9% at 12 mg/L. Analysis of variance for central bud development indicated significant ($P=0.01$) linear response with a mean square about 30 times the mean square for deviations from linearity. In addition to reductions in germination and central bud development, reductions in vigor and an increased number of abnormal seedlings were noted with increasing concentrations. Based on these results, screening for identification of tolerant genotypes was conducted at 12 mg/L. Actual selections were made at 21 days to insure adequate true-leaf expansion, resulting in selections with normally developed true leaves at least 5 mm long. Seedlings selected for tolerance were cloned, and, following photo-thermal induction to induce flowering, were intercrossed to produce seed for progeny testing.

Progeny evaluation of in vitro selections

Results from experiment 1 progeny seed of the nine interpollinated selections (selected at 12 mg/kg) are presented in Figs. 1 and 2. Compared with seedlings from the original population, screened seedlings had

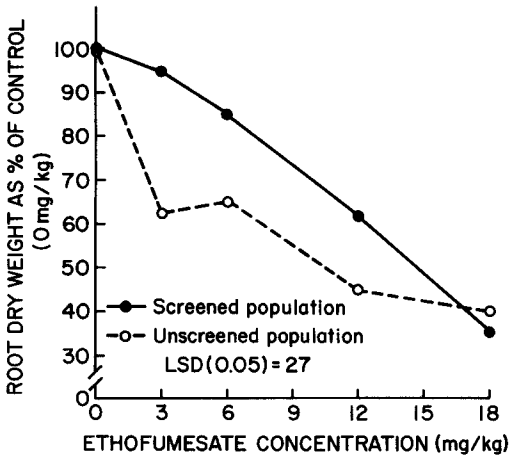

Fig. 1. Root dry weight (21 days) of seedling progeny from screened and unscreened (original population) populations under various concentrations of ethofumesate

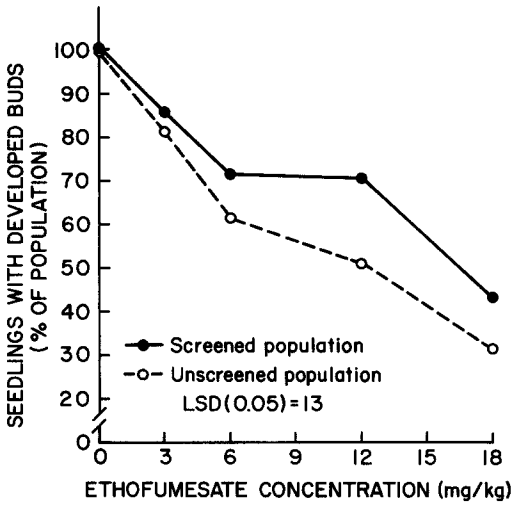

Fig. 2. Central bud development (14 days) of seedling progeny from screened and unscreened (original population) populations under various concentrations of ethofumesate

higher root dry weights at all ethofumesate concentrations through 12 mg/kg (Fig. 1). The largest difference for 21-day root dry weight was at 3 mg/kg. The difference was significant at 3 mg/kg and approached significance at the $P=0.05$ for 6 and 12 mg/kg (exact probability = 0.06 to 0.07). Root dry weights ranged from 18% greater at 12 mg/kg to 31% greater at 3 mg/kg. For central bud development, screened progeny exhibited less inhibition of true leaf development at all concentrations of ethofumesate (Fig. 2). The largest significant difference occurred at 12 mg/kg. At both 6 and 18 mg/kg, differences between screened and original population progeny approached significance at the 0.05 level (exact probability = 0.06). Screened progeny also exhibited greater top dry weights and total plant dry weights, although differences were not significant at the $P=0.05$ (data not presented).

Gametophytic (pollen) and sporophytic association studies

Results from our preliminary experiments designed to determine the effects of ethofumesate on in vitro pollen germination and development established that pollen germination was reduced at both 10 and 20 mg/L of ethofumesate. In no case, however, did ethofumesate completely inhibit pollen germination. In addition to germination inhibition, pollen tube morphology was affected by ethofumesate. Many of the germinated pollen grains produced pollen tubes that were distinctly shorter than those in the untreated check. These inhibitions of growth most often ranged from 10–50% of the length of normal tubes. Also, the surface of these reduced tubes was noticeably undulated.

Two pollen germination and development studies (experiments 2 and 3) were conducted to determine the potential association between the sporophyte and gametophyte in terms of tolerance to ethofumesate. Results from experiment 2 were similar to those of experiment 3 except that in the latter, pollen germination was greater and more consistent for all three herbicide levels. This led to overall differences in germination as a percent of control (0 mg/L), but the clonal response to the herbicide relative to the unscreened clone was the same in both experiments. In experiment 2, pollen of two of three clones (N15-19 and N3-3), which were developed from seedlings screened and selected for ethofumesate tolerance as sporophytes, germinated significantly better at 10 mg/L of ethofumesate than did pollen from a clone developed from the same FC 901 source population but without challenge by the herbicide. The better of these two clones, N15-19, germinated 70% greater at 10 mg/L (143% of control vs 73% of control) and 63% greater at 20 mg/L of ethofumesate (89% of control vs 26% of control) than did the unscreened clone. One clone (N2-4), which had been screened for tolerance, did not produce pollen which germinated any better than pollen from the unscreened clone at either herbicide concentration. Results from experiment 3 are presented in Fig. 3. Five of six clones developed from seedlings screened and selected as sporophytes, produced pollen that germinated significantly better at 10 mg/L than did pollen from the UNC clone which was developed from an unscreened selection. These five clones (N3-3, N3-6, N15-8, N15-10 and N15-18) averaged 65.3% of their respective controls compared to 33.8% for the UNC clone. Pollen from the best clonal

Table 1. Mean squares from the analyses of variance (fixed effects model) for percent germination of pollen from ten[a] clones under three herbicide concentrations

Source of variation	Experiment 2		Experiment 3	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Clones	3	184*	6	323.75**
Error (a)	12	42.25	14	70.41
Herbicide concentration	2	828.10**	2	4,212.67**
Clone × herbicide concentration	6	49.99**	12	53.24**
Error (b)	24	12.06	28	17.05

*,** Significant at the 0.05 and 0.01 probability level, respectively
[a] One clone (N3-3) was common to both experiments

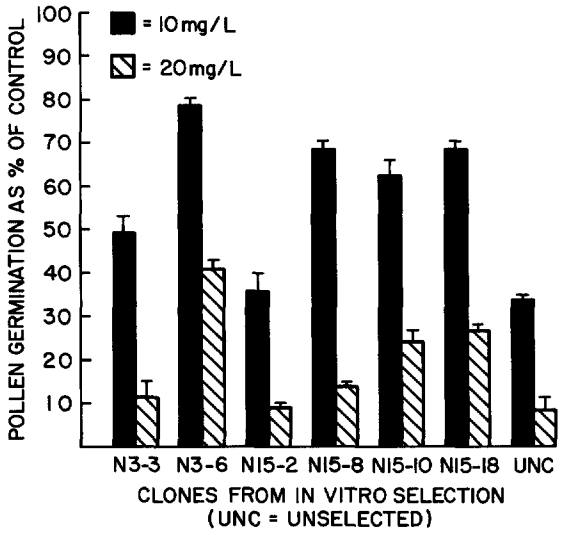

Fig. 3. Germination of pollen from each of seven clones cultured in media containing 10 and 20 mg/L ethofumesate. Values for each clone are given as percent of that clones' control. One SE shown at top of each bar. The $LSD_{0.05} = 10.2$ for comparison of entries within the same level of herbicide

selection, N3-6, germinated 44% better than pollen from the unscreened UNC clone (78% of control vs 34% of control). Pollen from clones N3-6, N15-10 and N15-18 also germinated significantly better than the UNC clone at 20 mg/L (average = 31% of control vs 9% of control). As in experiment 2, one clone (N15-2) produced pollen with germination about equal to the UNC clone at either herbicide concentration (Fig. 3). An analysis of variance of pollen germination for both experiments 2 and 3 (Table 1) indicated significant main effect mean squares for clones and for herbicide concentration. This indicated innate differences among one or more of the clones and demonstrated unequal effects of ethofumesate concentrations. A significant first-order interaction was found in both experiments for the clone X herbicide concentration component of variance, suggesting differential pollen germination among the clones for one or more ethofumesate concentrations.

Discussion

Our initial research to find an effective in vitro ethofumesate screening procedure utilizing seedlings was successful in establishing a satisfactory medium, concentration of challenging agent, proper seed preparation procedures, and the correct selection criteria (i.e. central bud development and relative vigor). Once the seed from cloned selections was obtained, selected clonal progeny were compared with plants from seed of the original population via root dry weight, and central bud development. In addition to greenhouse progeny evaluation, verification of in vitro selection accuracy was accomplished by gametophyte (pollen germination) studies. Our preliminary gametophytic-sporophytic association studies determined the effet of ethofumesate on pollen germination and development. Once satisfactory procedures for pollen collection, incubation and ethofumesate concentration were established, it was possible to compare pollen from selected and unselected clonal families. The results indicate that in vitro selection of germinated seedlings in the presence of the proper concentration of challenging agent can be effective in identifying genotypes tolerant to ethofumesate. Such identification was accomplished in fully differentiated tissue, but without the necessity of mature plants.

Since ethofumesate tolerance has been identified as a quantitative trait, it was imperative that we screen sufficient numbers of individuals to identify those that had the correct gene combination. The minimum

number of diploid individuals that must be screened (assuming perfect selection accuracy) is a function of the number of gene pairs controlling the trait, and is represented by 4^n, where n is the number of gene pairs. Because the exact number of genes controlling tolerance to ethofumesate is not known, we hypothesized the number to be at least four. Accordingly, the minimum number of individuals that we screened in each of our selection cycles was 600–1000.

Perhaps of most significance was the finding that gametophytic studies, via pollen germination, indicated an association between genes operating in the sporophyte and those in the gametophyte. The commonality of gene expression in the sporophyte and gametophyte indicates that further research is needed to determine if selection might be made in the gametophyte via pollen germination. Although results from this research indicate that such an approach is plausible, further refinement of techniques should promote more consistency. Some of our evidence indicated that not all sporophytes selected for ethofumesate tolerance demonstrate this tolerance in the gametophyte. For example, clone N2-4, in experiment 2 and clone N15-2 in experiment 3, which had been screened at 12 mg/L, did not produce pollen germination results better or worse than pollen from unscreened clones from the same original population when tested at 10 and 20 mg/L of ethofumesate. However, we suspect that errant selections were made in our sporphytic screening procedure. These results underline the importance of timely and accurate selection of sporophytes. On the other hand, because perfect selection in the sporophyte cannot be guaranteed (most likely with any selection scheme), verification in the gametophyte should certainly increase selection accuracy.

Cloning the seedlings identified as tolerant genotypes and subsequent intercrossing of these clones presents a convenient method of synthesizing populations with gene frequencies shifted in the direction desired. In those cases where self incompatibility (Smith 1980) precludes good seed production from clonal intercrossing, two or more less closely related clones, selected for the same trait, can be intercrossed for adequate seed production.

The screening technique described in this research obviously is not limited to ethofumesate tolerance screening. However, certain considerations are necessary before other in vitro challenging agents are used. Among the more important considerations are the following: the challenging agent must be amenable to in vitro manipulation. For example, the chemical must be water soluble, autoclavable (heat stable) or of such viscosity to allow sterilization by filtration. In addition, the chemical must be absorbed through the root or hypocotyl and transported to the site of activity. Many contact herbicides are not taken up through the roots and, hence, would not likely be amenable to this technique. The chemical also must be persistent enough in vitro to be active at the correct developmental phase of the seedling. Absolute surface disinfestation of seed germinated in vitro is a prerequisite for avoiding fungal or bacterial contamination. Sugarbeet seed is especially difficult to surface disinfest due to a thick, corky, porous, cortical seed coat. With further refinement, we believe that the techniques described herein can identify desired genotypes with an increased degree of accuracy.

References

Brewbaker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot 50:859–865

Chaleff RS, Parsons MF (1978) Direct selection in vitro for herbicide-resistant mutants of *Nicotiana tobacum*. Proc Natl Acad Sci USA 75:5104–5107

Chaleff RS, Ray TB (1984) Herbicide-resistant mutants from tobacco cell cultures. Science 223:1140–1143

Faulkner JS (1982) Breeding herbicide-tolerant crop cultivars by conventional methods. In: LeBaron HM, Gressel J (eds) Herbicide resistance in plants. Wiley and Sons, New York, pp 235–256

Gressel J (1980) Uses and drawbacks of cell cultures in pesticide research. In: Sala F, Parisi B, Cella R, Ciferri O (eds) Plant cell cultures: results and perspectives. Elsevier/North Holland Bio Med Press, pp 379–387

Gressel J, Ezra G, Jain SM (1982) Genetic and chemical manipulations of crops to confer tolerance to chemicals. In: McLaren JS (ed) Chemical manipulation for crop growth and development. Butterworth Scientific, London, pp 79–92

Gressel J, Zilkah S, Ezra G (1978) Herbicide action, resistance and screening in cultures vs plants. In: Proc 4th Int Congr Plant Tissue and Cell Culture, p 113

Hecker RJ (1984) Personnal communication. USDA-ARS Crops Research Laboratory, Colorado State University, Ft Collins CO (USA)

Huang LC, Murashige T (1976) Plant tissue culture media: major constituents, their preparation and some applications. TCA Manual 3:539–548

Hughes K (1983) Selection for herbicide resistance. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture. Macmillin, New York, pp 442–460

Hughes KW, Negrotto D, Daub ME, Meeusen RL (1984) Free-radical stress response in paraquat-sensitive and resistant tobacco plants. Environ Exp Bot 24:151–157

Hussey G, Hepher A (1978) Clonal propagation of sugarbeet plants and the formation of polyploids by tissue culture. Ann Bot 42:477–479

Meridith CP, Carlson PS (1982) Herbicide resistance in plant cell cultures. In: LeBaron HM, Gressel J (eds) Herbicide resistance in plants. Wiley and Sons, New York, pp 275–291

Mulcahy DL (1971) Correlation between gametophytes and sporophytic characteristics in *Zea mays* L. Science 171: 1155–1156

Mulcahy DL (1979) The rise of the angiosperms: a genecological factor. Science 206:20–23

Mulcahy DL, Mulcahy GB (1975) The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. Theor Appl Genet 46:277–280

Ottaviano E, Sarri-Gorla M, Mulcahy DL (1980) Pollen tube growth rates in *Zea mays:* Implications for genetic improvement of crops. Science 42:437–438

Saunders JW, Daub ME (1984) Shoot regeneration from hormone-autonomous callus from shoot cultures of several sugarbeet (*Beta vulgaris* L.) genotypes. Plant Sci Lett 34:219–223

Schweizer, EE (1980) ersonal communication. USDA-ARS Crops Research Laboratory, Colorado State University, Ft Collins CO (USA)

Smith, GA (1980) Sugarbeet. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. Am Soc Agron, Madison, pp 601–616

Smith GA, Schweizer EE (1983) Cultivar × herbicide interaction in sugarbeet. Crop Sci 23:325–328

Smith GA, Schweizer EE, Martin SS (1982) Differential response of sugarbeet populations to herbicides. Crop Sci 22:81–84

Tanksley SD, Zamir D, Rick CM (1981) Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon esculentum*. Science 213:452–455