Theor Appl Genet (1985) 71:159–165

Large scale selection of aluminum-resistant mutants from plant cell culture: expression and inheritance in seedlings

A. J. Conner and C. P. Meredith
Department of Viticulture and Enology, University of California, Davis, CA 95616, USA

Received April 10, 1985; Accepted August 5, 1985
Communicated by P. Maliga

Summary. A large number of aluminum-resistant variants, selected from non-mutagenized homozygous diploid cell cultures of *Nicotiana plumbaginifolia* Viv., are characterized. Of 115 variants cloned and reselected from single cells, 67 retained Al resistance in callus cultures after 6–9 months of growth in the absence of Al. There was no association between Al resistance and callus growth in the absence of Al, suggesting that the Al-resistant phenotype is not detrimental in the absence of Al challenge and that Al resistance is not the result of increased vigor. Plants regenerated from initially resistant callus lines that subsequently lost their resistance failed, with one exception, to transmit resistance to their seedling progeny. Fertile plants were regenerated from 40 of the 67 variants that retained stable Al resistance in callus culture. All 40 transmitted Al resistance to their seedling progeny (selfed and backcrossed) in segregation ratios expected for a single dominant mutation. The selfed progeny of many variants also segregated for recessive lethal mutations which were attributed to independent mutations that occurred during cell culture.

Key words: Cell culture – Mutant selection – Variant stability – Aluminum resistance – *Nicotiana plumbaginifolia*

Introduction

It is well established that mutants can be selected from plant cell culture (Chaleff 1981; Maliga 1984a). Although many variants for agriculturally important traits have been selected from cell culture (Flick 1983), examples demonstrating expression of the selected trait in regenerated plants and transmission to sexual progeny are scarce.

Unequivocal evidence for the selection of agriculturally important mutants from cell culture is limited to a few instances of herbicide resistance (Chaleff 1980; Chaleff and Parsons 1978a; Chaleff and Ray 1984), pathotoxin resistance (Carlson 1973; Gengenbach et al. 1977; Brettell et al. 1980) and lysine overproduction selected via resistance to amino acids and their analogues (Hibberd and Green 1982; Negrutiu et al. 1984). A major limitation to the application of cell selection for crop improvement is the poor understanding of the molecular and cellular basis of many agricultural traits (Chaleff 1983a, b; Meredith 1984). Improved resistance to mineral stress has been identified as one class of phenotypes with sufficient expression at the cellular level such that selection in cell culture may be possible (Meredith 1984; Meredith and Conner 1985).

Aluminum toxicity is one of the most important environmental stresses limiting world food production (National Academy of Sciences, USA 1977). It is a major growth-limiting factor for most crop plants in acid soils throughout the world, and is especially severe in developing countries of tropical regions (Foy 1983; Sanchez et al. 1982). If aluminum resistant genotypes selected in cell culture are to be useful in a field setting, it is important that the selection strategies used closely simulate the conditions existing in the soil environment (Meredith 1984; Meredith and Conner 1985). Since the mineral milieu of Al-toxic acid soils is quite different from that of commonly used plant cell culture media, several modifications to the inorganic composition of the medium are necessary to maximize the expression of Al toxicity in *Nicotiana plumbaginifolia* Viv. cell cultures (Conner and Meredith 1985a).

Using this modified medium, supplemented with a concentration of Al that completely inhibits the growth of wild-type cells, we have isolated 246 Al-resistant variants of *N. plumbaginifolia* (Conner and Meredith 1985b). In this paper we describe the stability of Al resistance in callus cultures of these variants and the expression and inheritance of Al resistance in the seedling progeny of regenerated plants.

Materials and methods

Variant selection and maintenance

The initial selection of the Al-resistant variants from non-mutagenized homozygous diploid *N. plumbaginifolia* cell cultures has been described (Conner and Meredith 1985b). The variants were selected for survival in the presence of 600 μM Al, supplied as $Al_2(SO_4)_3 \cdot 18\ H_2O$, added to culture medium (designated MSMT-1) in which the phosphate concentration, the calcium concentration, and the pH were reduced to 10 μM, 0.1 mM, and 4.0, respectively, and the iron was supplied in unchelated form (Conner and Meredith 1985a). Twenty-nine variants were obtained by direct selection, in which cells were plated directly onto Al-toxic medium and variants recovered as growing colonies after 123 days. The other 217 variants were obtained by rescue selection, in which cell suspensions were cultured for 10 days in Al-toxic medium and then plated onto RMNO medium (Marton and Maliga 1975) for recovery of survivors after 21 days (Table 1). After 6–12 weeks of growth in the absence of Al, each variant was cloned and reselected from single cells. For each of the 119 variants that were successfully reselected, a single small callus colony was chosen for further characterization and transferred to RMNO medium. As soon as it reached sufficient size (approximately 5 mm diameter after about 4 weeks), each colony was divided into 2 parts. One part was maintained as callus on RMNO medium, and the other was transferred to RMOP medium (Maliga 1984b) for plant regeneration. All media were autoclaved for 20 min at 103 kPa. Cultures were incubated at 25–26 °C under cool-white fluorescent light (50–70 μmol m^{-2} s^{-1}, 16 h light : 8 h dark daily).

Stability of Al resistance in callus

Each variant was maintained as callus on RMNO medium in the absence of Al. Every 3–4 weeks callus of each variant was selected on the basis of friability for subculture. Callus of all the variants was uniformly friable after 5–6 months, such uniformity being an important prerequisite for accurately comparing cell lines for Al resistance (Conner and Meredith 1985b). After 6–9 months of growth in the absence of Al, each variant was rechallenged with Al. Two days after subculture (see Conner and Meredith 1985a), 0.1 g of callus was smeared onto 4.5 cm diameter Whatman No. 2 filter paper supported by polyurethane foam (Conner and Meredith 1984) saturated in MSMT-1 medium with and without 600 μM Al (5 replicates per treatment). At 10 days, relative growth (RG) was calculated as:

$$RG = \frac{100\,(Wf - Wi)}{Wi}$$

where Wi and Wf are the initial and final fresh callus weights, respectively. A resistance index (RI) was used to compare the Al resistance of individual variants:

$$RI = \frac{RG_{600}}{RG_0}$$

where RG_0 and RG_{600} represent the mean relative growth of each variant after 10 days at 0 and 600 μm Al.

Plant regeneration

Callus was transferred to RMOP medium for plant regeneration. Every 3–4 weeks the most morphogenic callus regions of

Table 1. Summary of the selection and characterization of Al-resistant variants from cell cultures of *Nicotiana plumbaginifolia*

	Experiment 1	Experiment 2	Experiment 3		Total
Selection method[a]	direct	rescue	rescue	rescue	
Feeder cells	no	no	no	yes	
Age of cell culture at initiation of selection	6 weeks	35 weeks	6 weeks	6 weeks	
No. of selection plates (10^5 colony units/plate)	100	15	50	50	215
Total selected	29	32	98	87	246
Cloning and reselection unsuccessful	20	17	46	44	127
Cloned and reselected from single cells	9	15	52	43	119
Lost through contamination or senescence	0	0	2	2	4
Primary callus Al-sensitive	5	4	21	18	48
Fertile plants not regenerated	3	3	10	7	23
Fertile plants regenerated	2	1	11	11	25
Seedling progeny Al-sensitive	2	1	10	11	24
Seedling progeny segregate for resistance	0	0	1	0	1
Primary callus Al-resistant	4	11	29	23	67
Fertile plants not regenerated	3	9	7	8	27
Fertile plants regenerated	1	2	22	15	40
Seedling progeny Al-sensitive	0	0	0	0	0
Seedling progeny segregate for resistance	1	2	22	15	40
Variant frequency ($\times 10^{-6}$)[b]	2.9	21.3	19.6	17.4	11.4
Mutant frequency ($\times 10^{-6}$)[b]	0.4	7.3	5.8	4.6	3.1

[a] See text for explanation of direct and rescue selection

[b] Frequencies based on total number isolated divided by total number of colony units plated; all variants with stable Al-resistant primary callus were considered mutants (see text)

each variant were selected for subculture. If plants failed to regenerate after 3 months, cultures were transferred to RMB medium (Maliga 1984b). As soon as small shoots appeared they were dissected out and transferred to half-strength MS salts (Murashige and Skoog 1962) for rooting and establishment of photoautotrophic growth. Once rooted, plants were transplanted into sterilized peat-sand (60 : 40) mix and acclimatized to greenhouse conditions.

Testing Al resistance in seedling progeny

When the regenerated variant plants flowered, controlled pollinations were made, and the resulting progeny screened for Al resistance. Seeds were soaked overnight in 1 mM gibberellic acid, rinsed in distilled water twice, and sown onto Whatman No. 2 filter paper supported by polyurethane foam (Conner and Meredith 1984) saturated in MSMT-3 salt solution (Conner and Meredith 1985b) with and without 600 μM Al (up to 50 seeds per petri dish). Since Al has no effect on the germination (radicle protrusion) of *N. plumbaginifolia* seeds (Conner and Meredith 1985b), all seeds that failed to germinate were considered inviable and disregarded during examination of segregating progenies. Seedlings surviving after 3 weeks were considered Al-resistant. The observed segregation numbers were statistically compared with those expected under the hypothesis that the variants were heterozygous for a single dominant mutation (i.e. 3 : 1 and 1 : 1 segregation rations of Al-resistant and Al-sensitive seedlings in selfed and backcrossed progeny respectively). The expected number of alive (A) and dead (D) seedlings was calculated as:

$A = 0.75\,(n - np) \text{ and } D = 0.25\,(n - np) + np$ for selfed progeny, and
$A = 0.50\,(n - np) \text{ and } D = 0.50\,(n - np) + np$ for backcrossed progeny,

where n = the total number of seedlings assayed on 600 μM Al, and p = the proportion of seedlings dying on control medium (no Al).

Results

Stability of Al resistance in callus

From the mean relative growth of callus at 0 and 600 μM Al, the resistance index was calculated for each of the 115 (4 lost) variants that were reselected from single cells (data not shown). Since relative growth on control medium (no Al) varied markedly among the variants (e.g. from 181–479 after 10 days), the resistance index was used in comparing the Al resistance of individual variants. On this basis, variants fell into 2 distinct classes (Fig. 1). One class of 48 variants showed an Al response similar to that of wild-type callus, and these variants were therefore no longer considered Al-resistant. The other class of 67 variants clearly expressed greater Al resistance than wild-type callus after growing for 6–9 months in the absence of Al. There was no association between callus growth on control medium (no Al) and resistance index (Fig. 2), suggesting that the Al-resistant phenotype is not detrimental in the absence of Al challenge and that Al resistance is not the result of increased vigor.

Expression and inheritance of Al resistance in seedling progeny

Although all variants showed at least partial regeneration, complete and fertile plants could only be obtained from 65 variants (Table 1), despite repeated transfers every 3–4 weeks to fresh regeneration medium for 9 months. The seedling progeny from these 65 variants were screened regardless of (and in some cases prior to the determination of) the stability of resistance in callus. Of the 65, 40 expressed stable resistance as callus and 25 were sensitive. The data for all 65 variants is not shown. Instead, only 5 examples are presented, each representing a different pattern of expression of Al resistance in seedling progeny (Table 2).

Seedlings from selfed wild-type plants were all sensitive to Al. In many of the Al-resistant variants (e.g. Alr 086 and Alr 148 and 29 other variants) a large proportion of the selfed progeny died on the control medium (no Al). We attribute this to the segregation of recessive lethal mutations, independent of Al resistance,

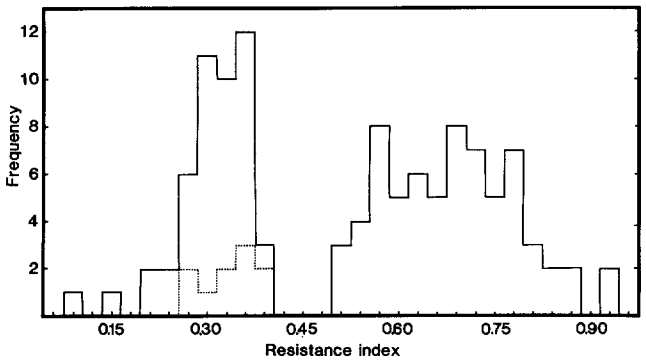

Fig. 1. Frequency distribution of resistance index in callus culture of 115 initially Al-resistant variants (*solid outline*) after 6–9 months of growth in the absence of Al, compared with wild-type (*dotted outline*)

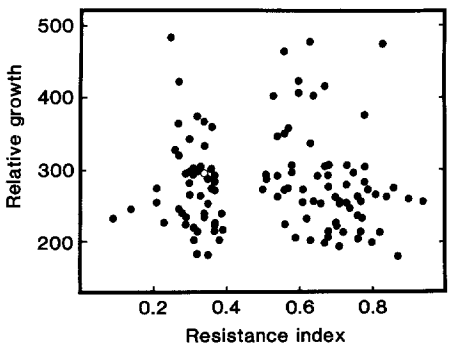

Fig. 2. Scatter diagram showing the lack of association between relative growth in the absence of Al and resistance index in callus culture of 115 initially Al-resistant variants (●) and wild-type (○). Spearman's rank correlation coefficient = −0.15 ($t_s = 1.61$; $P = 0.1$–0.2)

Table 2. Segregation of Al resistance in seedling progeny of plants regenerated from variants selected for Al resistance in cell cultures of *Nicotiana plumbaginifolia*. Results are presented for 2 separate seed collections from each cross

Cross (×)	Observed no. of seedlings				Expected no.[a]		Chi-square	*P*
	0 μM Al		600 μM Al		600 μM Al			
	Alive	Dead	Alive	Dead	Alive	Dead		
Wild-type (selfed)	210	6	0	154	–	–	–	–
	141	8	0	138	–	–	–	–
Alr 062 (selfed)	53	4	42	20	43	19	0.08	0.7–0.8
	47	6	57	23	53	27	0.89	0.3–0.5
Wild-type × Alr 062	24	1	17	19	17.5	18.5	0.03	0.8–0.9
	33	0	21	17	19	19	0.42	0.5–0.7
Alr 076 (selfed)	no viable seeds produced				–	–	–	–
Wild-type × Alr 076	no viable seeds produced				–	–	–	–
Alr 076 × Wild-type	36	0	27	19	23	23	1.39	0.2–0.3
	21	4	39	44	35	48	0.73	0.3–0.5
Alr 086 (selfed)	74	8	38	28	45	21	3.42	0.05–0.1
	34	17	28	31	30	29	0.27	0.5–0.7
Wild-type × Alr 086	40	3	26	28	25	29	0.07	0.7–0.8
	21	2	16	12	13	15	1.29	0.2–0.3
Alr 148 (selfed)	137	79	60	119	85	94	14.0	<0.01
	101	50	47	121	84	84	32.6	<0.01
Wild-type × Alr 148	27	3	12	19	14	17	0.52	0.3–0.5
	18	4	15	14	12	17	1.28	0.2–0.3
Alr 163 (selfed)	83	17	0	100	–	–	–	–
	118	14	0	126	–	–	–	–
Wild-type × Alr 163	93	17	0	108	–	–	–	–
	59	13	1	74	–	–	–	–

[a] Expected numbers of seedlings were adjusted as described in "Materials and methods"

that arose during cell culture. As expected under this hypothesis, the high incidence of lethality disappeared on backcrossing to wild-type plants. There were only a few variants (e.g. Alr 062 and 4 other variants) in which seedling death on control medium was equivalent to the negligible level observed in wild-type plants. In the analysis of segregation ratios, therefore, expected numbers were adjusted to account for those seedlings expected to die in the presence of Al for reasons other than Al sensitivity.

In most instances (e.g. Alr 086 and 23 other variants) the correction of the expected numbers permitted the observed results to be interpreted as the segregation of a single dominant mutation. In a few instances (e.g. Alr 148), the frequency of observed Al-resistant seedlings in the selfed progeny, although considerably higher than wild-type seedlings, was much lower than expected for a single dominant allele. However, backcrosses fitted the expected 1 : 1 segregation ratio for a single dominant allele. Since there was also a high proportion of lethality on control medium in each instance, the selfed progeny segregation may represent linkage between a single dominant Al-resistant gene and independent recessive lethals. However, various cytogenetic abnormalities could also be responsible for the aberrant ratios.

Many of the plants regenerated from Al-resistant variants showed greatly reduced fertility. In some instances this was overcome by regenerating additional plants from the variant line in question. In other instances only small numbers of viable seeds were obtained. In extreme situations (e.g. Alr 076 and 3 other variants) viable seeds could only be obtained after backcrossing as a female parent to the wild-type. Whether chromosomal aberrations contributed to the reduced fertility is unknown since the chromosomes of regenerated plants were not examined.

Fertile plants were regenerated from 25 variants with transient resistance (i.e. callus became Al-sensitive after 6–9 months of growth in the absence of Al). The seedling progeny of these plants (e.g. Alr 163, Table 2) were all sensitive to Al with one exception (Alr 092) (Table 1). Repeated tests on the primary callus cultures of Alr 092 confirmed sensitivity to Al. Since the primary callus culture and the regenerated plant were cloned from the same single cell, the segregation for Al resistance in the seedling progeny of Alr 092 may be the result of a mutation that occurred in cell culture subse-

quent to cloning and reselection. We are screening selfed progeny from a large number of plants regenerated from unselected cells to determine whether Al resistance can arise without selection.

Discussion

This investigation has clearly demonstrated the feasibility of selecting mutants from cell culture with increased resistance to Al toxicity, one of the most important environmental stresses limiting world food production. There is only one other example in which variants selected for resistance to a mineral stress in cell culture (NaCl-resistant cell lines of *N. tabacum*) have expressed the trait in regenerated plants and their sexual progeny (Nabors et al. 1980). However, segregation ratios were irregular and various non-genetic hypotheses were not eliminated (Nabors et al. 1980).

The variant and mutant frequencies shown in Table 1 were based on the total number of variants or mutants isolated divided by the total number of colony units initially plated. All 246 lines initially selected were considered to be variants. The 67 variants that retained their resistance after 6–9 months in the absence of Al were considered mutants, even those from which fertile plants were not regenerated (since every one for which fertile plants were regenerated transmitted Al resistance to progeny as a single dominant mutation, Table 1). The frequencies reported for rescue selection are probably overestimates due to the nature of this selection strategy. Any Al-resistant cells present at the initiation of selection may have undergone up to 2–3 mitotic divisions during the 10 day suspension culture in Al-toxic medium (Conner and Meredith 1985 b). If some of the resulting cells dissociated prior to plating, they could have given rise to separate cell colonies and have been identified as distinct variants. Even if this increased the mutant frequency 4–5 times, the isolation efficiency of rescue selection is still higher than that of direct selection.

Of the original 246 variants, 119 could be reselected from single cells (Table 1) and thus had retained their Al resistance after 6–12 weeks of growth in the absence of Al (Conner and Meredith 1985 b). However after a further 6–9 months of growth in the absence of Al, 48 of these lost their resistance (Fig. 1). This illustrates that short term maintenance in the absence of selection pressure is insufficient to establish the stability of variant phenotypes. The instability of these 48 variants is unlikely to have resulted from escape of the selection pressure due to cell density/aggregate effects (see Conner and Meredith 1985 b), since they were reselected by plating single cells at very low density (Conner and Meredith 1985 b). Instead they probably represent a transient physiological adaptation to Al stress that was lost after extended culture in the absence of selection pressure, similar to that reported for unstable cycloheximide resistance (Maliga et al. 1976).

Abnormal segregation patterns within the progeny of plants regenerated from variants selected in cell culture have been frequently observed (Marton and Maliga 1975; Chaleff and Parsons 1978 b; Nabors et al. 1980; Owens 1981; Thomas and Pratt 1982; Singer and McDaniel 1984). In these instances variants were not cloned from single cells after selection, and the abnormal segregation ratios may have resulted from chimeral plants (as well as from gross chromosomal abnormalities). All the Al-resistant variants characterized in this paper were cloned from single cells (Conner and Meredith 1985 b). While the possibility of regenerated plants being chimeral for Al resistance was virtually eliminated, many may have still been chimeral for other unselected mutations that occurred subsequent to cloning. The segregation of recessive lethals on control medium (no Al) in the selfed progeny of many variants only rarely fitted any conventional inheritance patterns (Table 2). Furthermore, in many variants these segregation patterns differed markedly between separate seed lots collected from the same plant (e.g. Alr 086, Table 2). Both these observations suggest that variants were chimeral for recessive lethal mutations.

One of the most significant features of this study is the large scale on which the selection and characterization of variants was undertaken. From the information summarized in Table 1 a number of important conclusions can be made which may be of general importance to plant cell genetics.

Of the 246 Al-resistant variants initially selected, only 27% retained their Al resistance in callus culture after growing for 6–9 months in the absence of Al. In other large scale selection experiments, low percentages of stable variants have also been reported: only 8.4% of 250 initially selected for resistance to p-fluorophenylalanine (Flick et al. 1981), 62% of 140 for paraquat resistance (Miller and Hughes 1980), and 17% of 178 for methylglyoxal-bis(guanylhydrazone) resistance (Malmberg and McIndoo 1984) in *N. tabacum*, and 14% of 110 for ethionine resistance in *Medicago sativa* (Reisch et al. 1981). The high proportion of unstable variants among those initially selected emphasizes the importance of isolating large numbers of independent variants during selection experiments.

N. plumbaginifolia is one of the most highly regenerable plant species from cell culture (Maliga 1984 b). Despite this, complete plants could only be regenerated from 57% of the cloned and reselected variants (Table 1). The problem was especially severe when the direct selection method was used (Experiment 1), or when selection was applied to older cell cultures (Ex-

periment 2). When plants could be regenerated in each of these cases, they showed greatly reduced fertility and their seedling progeny segregated with a high proportion of lethality. These observations are consistent with the emerging concept that stress can cause genetic changes in plants (Cullis 1983; Freeling 1984). In the case of direct selection, cells were exposed to long term Al-stress (102 days) compared with short term exposure (10 days) in rescue selection (Conner and Meredith 1985 b). The problems associated with poor regeneration, infertility and segregation of recessive lethals in selected variants emphasizes the importance of isolating large numbers of variants in short term selection experiments (such as the rescue selection strategy), from recently initiated cultures.

All 40 variants in which callus remained resistant and regeneration of plants was possible proved to be simply inherited Mendelian mutations. The 27 stable Al-resistant variants in which fertile plants could not be regenerated therefore probably also represent mutants. No novel phenotypes with an epigenetic basis (stable events persisting through mitotic divisions, but reversed by processes of differentiation and meiosis (Chaleff 1981, 1983 a)), such as those associated with habituation (Binns and Meins 1973) were observed. Al resistance was expressed in intact plants of all 40 Al-resistant variants confirmed as mutants. There were no instances involving the expression of Al resistance at the cellular level, but not at the whole plant level as has been observed in *N. tabacum* for 5-bromodeoxyuridine resistance (Marton and Maliga 1975), glycerol utilization (Chaleff and Parsons 1978 b), paraquat resistance (Miller and Hughes 1980), hydroxyurea resistance (Keil and Chaleff 1983) and methotrexate resistance (Chaleff 1983 a), and in *Lycopersicon esculentum* for paraquat resistance (Thomas and Pratt 1982). This reflects the fundamental cellular basis of Al toxicity (Haug 1984), and suggests that Al resistance mechanisms involve the expression of "housekeeping" genes associated with basic cell metabolism.

Acknowledgements. This study was supported by a Jastro-Shields Research Scholarship awarded to AJC and a grant from the American Vineyard Foundation to CPM. AJC, currently on study leave from Crop Research Division, DSIR, Lincoln, New Zealand, thanks the National Research Advisory Council for financial assistance in the form of a Post-graduate Research Fellowship. We also thank Drs. D. Pratt, A. E. Lauchli, and R. W. Michelmore for their comments on the manuscript.

References

Binns A, Meins F (1973) Habituation of tobacco pith cells for factors promoting cell division is heritable and potentially reversible. Proc Natl Acad Sci USA 70:2660–2662

Brettell RIS, Thomas E, Ingram DS (1980) Reversion of Texas male-sterile cytoplasm maize in culture to give fertile T-toxin resistant plants. Theor Appl Genet 58:55–58

Carlson PS (1973) Methionine sulfoximine-resistant mutants of tobacco. Science 180:1366–1368

Chaleff RS (1980) Further characterization of picloram-tolerant mutants of *Nicotiana tabacum*. Theor Appl Genet 58:91–95

Chaleff RS (1981) Genetics of higher plants: applications of cell culture. Cambridge University Press, Cambridge, 184 pp

Chaleff RS (1983 a) Considerations of developmental biology for the plant cell geneticist. In: Kosuge T, Meredith CP, Hollander A (eds) Genetic engineering of plants: an agricultural perspective. Plenum Press, New York, pp 257–270

Chaleff RS (1983 b) The isolation of agronomically useful mutants from plant cell cultures. Science 219:676–682

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

Chaleff RS, Parsons MF (1978 b) Isolation of a glycerol-utilizing mutant of *Nicotiana tabacum*. Genetics 89: 723–728

Chaleff RS, Ray TB (1984) Herbicide-resistant mutants from tobacco cell cultures. Science 223:1148–1151

Conner AJ, Meredith CP (1984) An improved polyurethane support system for monitoring growth in plant cell cultures. Plant Cell Tissue Organ Cult 3:59–68

Conner AJ, Meredith CP (1985 a) Simulating the mineral environment of aluminum-toxic soils in plant cell culture. J Exp Bot 36:870–880

Conner AJ, Meredith CP (1985 b) Strategies for the selection and characterization of aluminum-resistant variants from cell cultures of *Nicotiana plumbaginifolia*. Planta 166 (in press)

Cullis CA (1983) Environmentally induced DNA changes in plants. CRC Crit Rev Plant Sci 1:117–131

Flick CE (1983) Isolation of mutants from cell culture. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture, vol 1. Techniques for propagation and breeding. Macmillan, New York, pp 393–441

Flick CE, Jensen RA, Evans DA (1981) Isolation, protoplast culture and plant regeneration of PFP-resistant variants of *Nicotiana tabacum*. Z Pflanzenphysiol 103:239–245

Foy CD (1983) Plant adaptation to mineral stress in problem soils. Iowa State J Res 57:339–354

Freeling M (1984) Plant transposable elements and insertion sequences. Annu Rev Plant Physiol 35:277–298

Gengenbach BG, Green CE, Donovan CM (1977) Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc Natl Acad Sci USA 74: 5113–5117

Haug A (1984) Molecular aspects of aluminum toxicity. CRC Crit Rev Plant Sci 1:345–373

Hibberd KA, Green CE (1982) Inheritance and expression of lysine plus threonine resistance selected in maize tissue culture. Proc Natl Acad Sci USA 79:559–563

Keil RL, Chaleff RS (1983) Genetic characterization of hydroxyurea resistant mutants obtained from cell cultures of *Nicotiana tabacum*. Mol Gen Genet 192:218–224

Maliga P (1984 a) Isolation and characterization of mutants in plant cell culture. Annu Rev Plant Physiol 35:519–542

Maliga P (1984 b) Cell culture procedures for mutant selection and characterization in *Nicotiana plumbaginifolia*. In: Vasil IK (ed) Cell culture and somatic cell genetics of plants, vol 1. Laboratory procedures and their applications. Academic Press, Orlando, pp 552–562

Maliga P, Lazar G, Svab Z, Nagy F (1976) Transient cycloheximide resistance in a tobacco cell line. Mol Gen Genet 149:267–271

Malmberg RL, McIndoo J (1984) Ultraviolet mutagenesis and genetic analysis of resistance to methylglyoxal-bis(guanylhydrazone) in tobacco. Mol Gen Genet 196:28–34

Marton L, Maliga P (1975) Control of resistance in tobacco cells to 5-bromodeoxyuridine by a simple Mendelian factor. Plant Sci Lett 5:77–81

Meredith CP (1984) Selecting better crops from cultured cells. In: Gustafson JP (ed) Gene manipulation in plant improvement. Plenum Press, New York, pp 503–528

Meredith CP, Conner AJ (1985) In vitro selection for agriculturally significant traits: resistance to mineral stress. In: Shao Q (ed) Proc Int Symp Genet Manipulation in Crops. Science Press, Beijing (in press)

Miller OK, Hughes KW (1980) Selection of paraquat-resistant variants of tobacco from cell cultures. In Vitro 16:1085–1091

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nabors MW, Gibb SE, Bernstein S, Meis ME (1980) NaCl-tolerant tobacco plants from cultured cells. Z Pflanzenphysiol 97:13–17

National Academy of Sciences USA (1977) World food and nutrition study: the potential contributions of research. National Academy of Sciences, Washington

Negrutiu I, Cattoir-Reynearts A, Verbruggen I, Jacobs M (1984) Lysine overproducer mutants with an altered dihydrodipicolinate synthase from protoplast culture of *Nicotiana sylvestris* (Spegazzini and Comes). Theor Appl Genet 68:11–20

Owens LD (1981) Characterization of kanamycin-resistant cell lines of *Nicotiana tabacum*. Plant Physiol 67:1166–1168

Reisch B, Duke SH, Bingham ET (1981) Selection and characterization of ethionine-resistant alfalfa (*Medicago sativa* L.) cell line. Theor Appl Genet 59:89–94

Sanchez PA, Bandy DE, Villachica JH, Nicholaides JJ (1982) Amazon basin soils: management for continuous crop production. Science 216:821–827

Singer SR, McDaniel CN (1984) Selection of amitrole-tolerant tobacco calli and the expression of this tolerance in regenerated plants and progeny. Theor Appl Genet 67:427–432

Thomas BR, Pratt D (1982) Isolation of paraquat-tolerant mutants from tomato cell cultures. Theor Appl Genet 63: 169–176