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Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis

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Summary. Plants were regenerated from leaf, cotyledon, and hypocotyl explants of tomato cv Moneymaker. Various phenotypic alterations were observed among regenerated plants (R_1), but were not transmitted to the progenies, except for ploidy variation. Variation in ploidy level, mainly tetraploidy, occurred in R_1 plants and their R_2 progenies, and the frequency of polyploid plants depended on the explant source. More than 50% of the regenerants derived from hypocotyl explants were found to be polyploid. A correlation was observed between the percentage of polyploid cells present in the explant material in vivo and the frequency of polyploid plants. Several monogenic mutations were recovered in the R_2, four of which were shown to be allelic to known, recessive, single-gene mutants. No significant effect of explant source or duration of tissue culture period on mutant frequency or spectrum was found. For several mutant types that could be scored unambiguously, somaclonal variation was compared to variation induced by treatment of seeds with ethyl methane sulphonate (EMS). The results showed that the mutant frequencies were higher after EMS treatment than those generated through tissue culture. With respect to the mutant spectrum, no clear differences were observed between the spectra obtained after EMS treatment and those after tissue culture. However, tissue culture gave rise to polyploid plants, whereas no ploidy variants occurred after EMS treatment.

Key words: *Lycopersicon esculentum* – Somaclonal variation – Chemical mutagenesis – Mutants – Polyploidy

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Introduction

Genetic variation among plants regenerated from cells or tissues has been observed in many plant species. This variation, designated somaclonal variation (Larkin and Scowcroft 1981), may be caused by gross alterations in chromosome number and structure, point mutations, mitotic recombination, amplification, deletion, transposition, or methylation of DNA sequences in nuclear, mitochondrial, or chloroplast genomes (Karp and Bright 1985; Lörz and Brown 1986; Lee and Phillips 1988). These karyological and molecular changes can result in genetically stable mutants that sexually transmit the mutant characteristics to their progeny, as has been described in several extensive reviews (e.g., Larkin and Scowcroft 1981; Scowcroft et al. 1983; Evans et al. 1984; Karp 1989; Wersuhn 1989).

To evaluate the effectiveness of somaclonal variation, the frequency and spectrum of mutants can be compared with those obtained from classical mutagenic treatments. For tomato, such a comparison has been made by Gavazzi et al. (1987), who concluded that somaclonal variation, obtained after plant regeneration from cotyledon tissue, gives a higher mutation frequency and a different spectrum of variation than chemical mutagenesis of seeds or pollen with ethyl methane sulphonate. However, they did not report data on chromosomal variation.

Several examples of recessive, and some dominant, monogenic mutations, obtained through plant regeneration from cells or tissues, have been published in various cultivars of tomato. These mutations affected, e.g., fruit colour (*tv* and *r-2*), jointless pedicel (*j*) and mottling (*m*) (Evans and Sharp 1983; Evans 1987), alcohol dehydrogenase (*Adh-1*, Medina-Filho and Tanksley 1983), and phytochrome content (*au*, Lipucci di Paola et al. 1988). Moreover, mutants interesting for plant breeding were

also obtained, e.g., mutants with resistance to *Fusarium* wilt (Miller et al. 1985; Shahin and Spivey 1986) and to tomato mosaic virus (TMV) (Barden et al. 1986; Smith and Murakishi 1987). Apparently, the potential of somaclonal variation for crop improvement looks promising and, consequently, should be explored.

The present investigation is directed towards the isolation of desirable mutants for breeding research, as well as towards selecting mutants with novel resistances to various diseases. For this purpose, cv Moneymaker, a commercial variety of importance for tomato breeding in The Netherlands, was chosen as source material. This report describes the recovery of several genetically stable variants, obtained through plant regeneration from tissue explants, and the comparison with mutants obtained after treatment of seeds with the chemical mutagen ethyl methane sulphonate. In addition, the effect of different explant sources and of different duration of the tissue culture period on the ploidy level of regenerants and the mutant frequency and spectrum was investigated.

Materials and methods

Plant regeneration from different explant sources

Seeds of the homozygous tomato cv 'Moneymaker', from the CPO seed collection, were surface-sterilized for 7 min in a 2% solution of sodium hypochlorite with 0.01% (v/v) Tween-20 and rinsed in sterile, demineralized water. The seeds were placed on MS medium (Murashige and Skoog 1962) containing 2.0% sucrose and 0.8% agar (Oxoid), pH 5.8, and were allowed to germinate at 26 °C in the dark. After emergence, seedlings were placed under a 16-h photoperiod (15 W/m^2). For cotyledon and hypocotyl explants, 11-day-old seedlings were used. For leaf explants, 10-day-old seedlings were excised below the cotyledons, transferred to fresh MS medium, and grown for another 3 weeks.

Leaf explants were prepared by sectioning leaflets of the first unfolded leaves into two halves, avoiding the midvein and removing the leaf margins. The same procedure was followed for cotyledons. Hypocotyls were cut into pieces approximately 10 mm in length. All explants were plated onto MS medium supplemented with 2.0% sucrose, 4.5 μ*M* zeatin, 0.5 μ*M* IAA, and 0.7% agar (pH 5.8). The explants were incubated in the dark (25 °C) for 1 week and subsequently under cool white light (15–18 W/m^2), to induce shoot regeneration. The first shoots (present after 4–5 weeks, designated regeneration cycle 1) were transferred to rooting medium when their length was about 10 mm. All remaining shoots and shoot primordia were removed, whereafter the explants plus callus were placed on fresh regeneration medium to induce shoot regeneration for the second time (regeneration cycle 2, shoots were excised after another 5–6 weeks). Shoots were rooted in glass jars with MS agar medium (0.6% agar) supplemented with 2.0% sucrose and 0.25 μ*M* IAA (16-h photoperiod, 15 W/m^2, 24 °C). Rooted plantlets were transferred to Jiffy pots, acclimatized in a growth chamber (day/night temperature: 24/20 °C; light intensity: 20 W/m^2) to normal growth conditions by gradually lowering the relative humidity, and planted into the soil in a heated greenhouse. R_1 plants and their progeny (R_2, obtained after selfing) were screened for phenotypic variation.

Chemical mutagenesis

Seeds of tomato cv 'Moneymaker' were submerged in a 60-m*M* aqueous solution of ethyl methane sulphonate (EMS) for 24 h in darkness at 24 °C. The seeds were washed with tap water and sown. The resulting M_1 plants were grown in a greenhouse. The plants were selfed and the progeny (M_2) was evaluated for mutant phenotypes.

Screening for phenotypic variation

The regenerated R_1 plants were evaluated in the greenhouse for the following traits: ploidy level, chimerism (leaf morphology, leaf colour, hairless spots), rogue plants (characterized by greater sideshoot growth, narrower leaf segments, and shorter internodes), growth (strong or weak, the latter category including dwarfs), reduced fertility (weakly fertile or sterile), and 'determinate' types. In the latter case, growth of the main stem is restricted, because the main stem ends with a terminal inflorescence.

The ploidy level was scored on the basis of leaf morphology, i.e., plants with darker green, less serrated and thicker leaves were considered to be polyploid. A number of these plants was checked for ploidy level by flow cytometric analysis of the nuclear DNA content in leaves. For this analysis, leaf tissue was chopped in an ice-cold, commercial Partec buffer (Partec AG, Arlesheim, Switzerland), supplemented with the fluorescent dye DAPI (4,6-diamino-2-phenyl indole) at 2 mg/l, to isolate and stain interphase nuclei. The sample was filtered through a 30-μm nylon filter and analyzed in a flow cytometer (ICP 22, Ortho Diagnostic Systems, Beerse, Belgium). The DNA content of nuclei was determined in arbitrary units by fluorescence measurements, and expressed as relative *C*-values. The DNA content of nuclei isolated from tomato plants with a known diploid chromosome number ($2n=2x=24$) was used as a standard. Control plants for the screening, raised in vitro from seeds, were transplanted to the greenhouse and evaluated according to the same procedures.

The R_2 progeny of the regenerants and the M_2 progeny of plants from EMS-treated seeds were evaluated for variants at the seedling stage. Samples of 20 seeds for each R_2 line and 40 seeds per M_2 seed lot, obtained by pooling seeds from ten M_1 plants, were sown. The seedlings were scored for the presence of the following traits: tetraploidy, lethality, reduced chlorophyll content, altered leaf morphology, anthocyanin deficiency, hairlessness, ABA deficiency, variegation, and dwarfs. Seedlings were rated as tetraploid when their cotyledons were broader than usual. The ploidy level was checked for a number of such plants by determination of the average number of chloroplasts per guard cell pair, counting guard cells of ten stomata in epidermis strips from fully expanded leaves. In tetraploid plants, the number of chloroplasts per guard cell pair was shown to be consistently higher than in diploid plants (Koornneef et al. 1989).

Mutant frequency was expressed as the fraction of mutants among the total number of M_2/R_2 plants screened. Genotypes homozygous recessive for well-defined mutant alleles used in allelism tests were obtained from C.M. Rick, Tomato Genetics Stock Center, Davis CA, USA.

Results

Analysis of R_1 plants

Regenerated plants (R_1) showed considerable variation in morphology when examined in the greenhouse. Variant phenotypes that were observed frequently are pre-

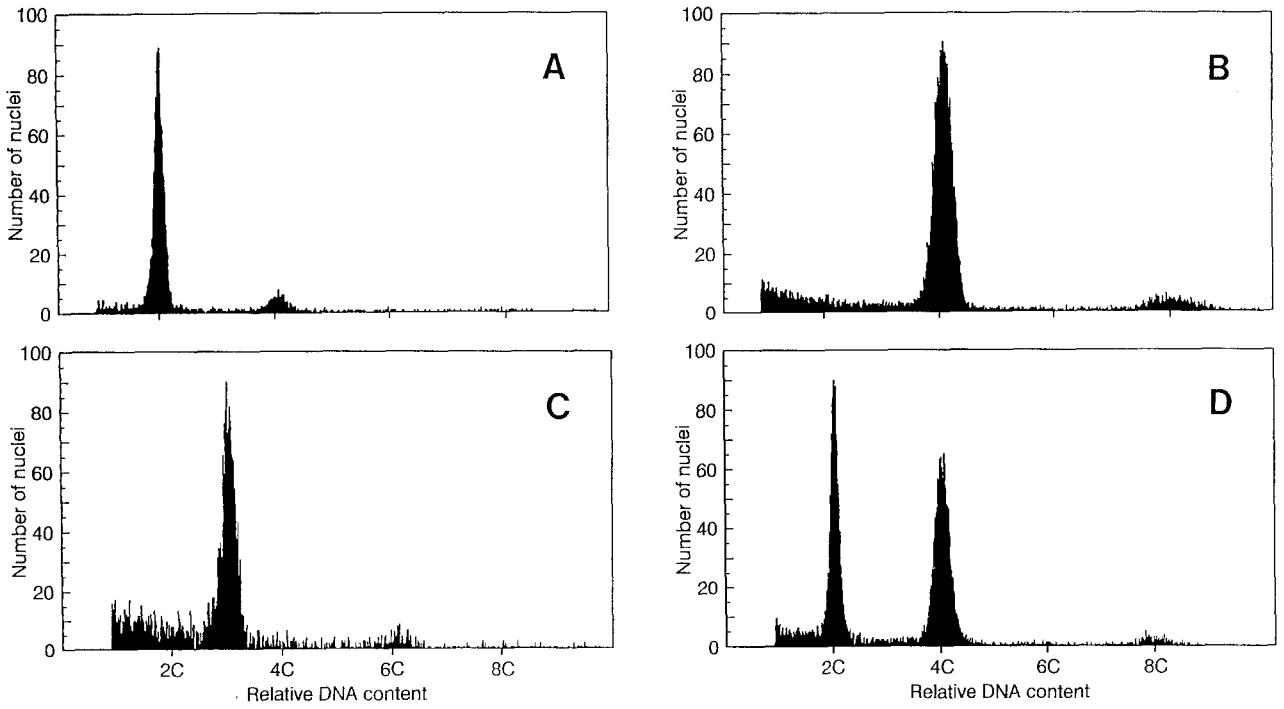

Fig. 1 A–D. Frequency distribution of relative nuclear DNA contents, determined by flow cytometry, in plants differing in ploidy level. **A** a diploid regenerant; **B** a tetraploid regenerant; **C** a triploid regenerant; **D** a mixoploid regenerant

Table 1. Frequency (%) of different phenotypic variants observed in the greenhouse among plants (R_1) regenerated from various explant types

Phenotypic trait	Explant source[a]		
	Leaf	Cotyledon	Hypocotyl
Polyploidy	1.5[b]	11.9	58.0
Chimerism	1.7	1.2	2.0
Rogue	1.1	1.2	0
Growth[c] – strong	3.0	2.4	1.0
– weak	4.4	6.7	4.5
Reduced fertility[d]	4.6	3.6	5.5
'Determinate' type	1.7	2.4	8.5
Total no. of plants scored	710	252	200

[a] The results for plants from the first and the second regeneration cycle are pooled
[b] Figures represent percentages of total number of plants scored
[c] Compared to control plants
[d] Percentage of diploid plants with reduced fertility. Polyploid plants, which showed mostly reduced fertility, are not included in this category

Table 2. Flow cytometric determination of relative nuclear DNA contents in various explant sources

Explant source[a]	% Nuclei showing various DNA *C*-values			
	2C	*4C*	*8C*	*16C*
Leaf	93±1	7±1	–	–
Cotyledon	60±7	36±5	4±2	–
Hypocotyl	22±1	44±2	29±2	5±1

[a] Analyses for each explant source were performed in triplicate with material of three different plants. The average percentage±standard error is given

sented in Table 1. Since the overall percentages of variants for the first and second regeneration cycle were practically similar, the results were combined. Most pronounced was the category of plants visually rated as polyploid. Flow cytometric analysis of 36 of these plants showed only 1 plant to be misrated, as it turned out to be a diploid. The other plants checked were tetraploid (25 plants), triploid (2 plants), or mixoploid, consisting of diploid and tetraploid cells (8 plants). As an example, the frequency distributions of relative DNA contents of nuclei isolated from leaf material of plants differing in ploidy level are shown in Fig. 1. Cytological analysis of pollen mother cells in young anthers of the triploid ($2n=3x=36$) plants confirmed the presence of 36 chromosomes (nine to ten trivalents could be observed at metaphase I, indicating autotriploidy).

The influence of the source of the explants on the percentages of plants with a different ploidy level is quite obvious from the data given in Table 1, showing a maximum of 58% polyploids for hypocotyl regenerants. The explant sources were also analyzed for variation in ploidy levels, by flow cytometric measurement of nuclear DNA

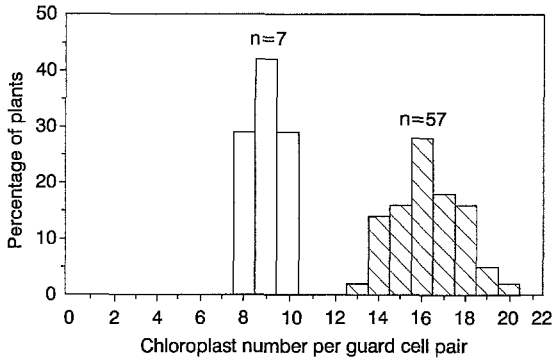

Fig. 2. Frequency distribution of chloroplast numbers counted per guard cell pair of diploid R_2 plants (*white bars*) and R_2 plants visually classified as tetraploid (*hatched bars*); *n*: no. of plants analyzed

Table 3. Number of tested R_2 lines and the percentage of tetraploid lines

	Origin[a]		
	Leaf	Cotyledon	Hypocotyl
No. of tested R_2 lines[b]	681	229	142
% Tetraploid lines	0.7	5.2	42.3

[a] Results from the first and the second regeneration cycle are pooled

[b] Not all tetraploid R_1 plants produced (enough) seed for testing

content in material of the same age as that used for regeneration (Table 2). The results indicated that the explants of hypocotyls contained a mixture of diploid and polyploid cells (polysomatic condition) at higher frequency, whereas the other explant types contained none or less polyploid cells.

For the other variant traits (Table 1), no distinct differences were observed between plants originating from different explant sources. Part of the variation observed was not stable, as cuttings taken from regenerants did not always show the variant trait, e.g., for growth (weak or strong), 'determinate type', and rogues.

Analysis of R_2 plants and a comparison with M_2 plants

The variation observed among the R_1 plants might be partly due to epigenetic effects. To establish heritable variation, the R_2 progenies were analyzed for phenotypic variation at the seedling stage. To compare mutant frequency and spectrum, results of a screening for mutants in M_2 progenies obtained after EMS treatment of seeds were included. This screening was performed under the same conditions and by the same observer (MK).

Ploidy level. Polyploid plants were identified in the R_2 through their characteristically different phenotype of the cotyledons and leaves. The ploidy level was verified for 57 plants, rated polyploid by their phenotype, by counting the number of chloroplasts per guard cell pair in epidermal strips. No overlap was present between the frequency distributions of average number of chloroplasts, for diploid R_2 plants and polyploid R_2 plants (Fig. 2), showing that these plants were correctly classified as polyploids. Considering the average number of chloroplasts, all polyploid plants are tetraploids. Comparable with the results in the R_1, R_2 lines originating from hypocotyl explants showed the highest percentage of tetraploids (Table 3). All R_1 plants scored as tetraploid produced R_2 lines that were also tetraploid, with the exception of 2 out of 80 R_2 lines. Not all tetraploid R_1 plants produced (enough) seed to be tested in the second generation, explaining the lower percentages in Table 3. No differences in percentage were observed between R_2 lines originating from the first and the second regeneration cycle (results not shown). The tetraploid R_2 lines were omitted in further mutant screening, as the probability of finding tetraploid recessive mutants is too small owing to the used seed sample size of 20 seeds, both in the case of duplex (AAaa) or triplex (AAAa) tetraploids. No tetraploids were observed in the progenies of a total of 1,650 M_1 plants derived from EMS treatment.

Mutant frequencies. A comparison between the different treatments can only be made for those phenotypic traits that can be scored unambugiously. These traits were in our case: lethal seedlings, a pale-green/yellow colour of the leaves (reduced chlorophyll content) or of leaf sections (e.g., as in Fig. 3A), abnormal leaf morphology, wilting symptoms similar to ABA-deficient mutants, hairless hypocotyls, variegated and anthocyanin-free. Dwarfs are less useful for the comparison of mutant frequencies, because their classification is relatively difficult and also the genetic basis for the various 'dwarf mutants' seems different (see later). The EMS experiments were designed to search specifically for certain mutant types, such as hormone and photoreceptor mutants, and therefore not all phenotypic variants observed were evaluated. The mutant types mentioned above were used in the EMS experiments as reference classes for the overall mutation rates. Since the estimation of mutant frequency is independent of M_2/R_2 progeny size and the degree of M_1/R_1 chimerism (Frydenberg 1963), the comparison of frequencies obtained from bulked M_1 plants and single-plant R_1 progenies is allowed.

For nearly all mutant classes, the mutant frequencies are higher after EMS treatment compared with somaclonal variation (Table 4). Two mutant phenotypes (hairless and ABA-deficient) were not found among the somaclones. However, since the frequencies per mutant class are low, it cannot be concluded that these mutants are specific for the EMS treatment. One mutant group

Table 4. Frequency ($\times 10^{-3}$) of various mutant phenotypes observed in the R_2 and M_2 generation at seedling stage

Mutant phenotype	Origin[a]				
	Leaf	Cotyledon	Hypocotyl	Weighted average	EMS
Lethal seedlings	2.0	3.0	2.0	2.2	5.5
Reduced chlorophyll (viable)	3.6	4.1	0	3.4	14.3
Altered leaf morphology	1.7	2.0	0	1.6	4.9
Anthocyanin-free	0.5	0.8	0	0.5	0
Hairless	0	0	0	0	0.7
ABA deficient	0	0	0	0	0.3
Variegated	2.2	0.3	0.7	1.7	1.7
Subtotal	10.0	10.2	2.7	9.4	27.4
Dwarfs[b] – '57 type'	2.1	11.7	5.4	4.4	0
– total	3.2	14.2	5.4	5.7	10.1
Various types[c]	0.6	1.3	0	0.7	–
Total	13.8	25.7	8.1	15.8	37.5
Total number of R_2 and M_2 plants scored[d]	12,667	3,939	1,487	18,093	2,883

[a] Results from the first and the second regeneration cycle are pooled
[b] The category 'various types' contains: mutants with 'scurfy' cotyledons, mutants with an elongated hypocotyl, and mutants with necrotic spots. The progenies obtained after EMS treatment were not screened for mutants of this category
[c] A special type of dwarf, designated '57 type', could be distinguished from the usual phenotype of dwarfs; see text for details
[d] Tetraploid plants have been omitted

Table 5. Number of R_2 lines, obtained from R_1 plants originating from various explant sources, that show segregation for specific mutant phenotypes and overall segregation ratio for these mutant phenotypes in the segregating R_2 lines

Mutant phenotype	No. of segregating R_2 lines per explant source[a]			Overall ratio of mutants for segregating R_2 lines	$\chi^2_{3:1}$[b]
	Leaf	Cotyledon	Hyocotyl		
Lethal seedlings	6 (0.89)	3 (1.38)	1 (1.22)	0.216	0.95
Reduced chlorophyll	10 (1.48)	4 (1.84)	0 (0)	0.238	0.13
Altered leaf morphology	6 (0.89)	3 (1.38)	0 (0)	0.180	3.83
Anthocyanin-free	1 (0.15)	1 (0.46)	0 (0)	0.275	0.01
Variegated	7 (1.04)	1 (0.46)	1 (1.22)	0.169	5.70*
Dwarfs[c] – '57 type'	14 (2.07)	18 (8.29)	4 (4.88)	0.117	63.08**
– other	7 (1.04)	2 (0.92)	0 (0)	0.151	7.80**
Various types[d]	2 (0.30)	1 (0.46)	0 (0)	0.250	0.02
Total no. of R_2 lines scored	676	217	82		

[a] Excluding tetraploid lines. Percentages of the total number of R_2 lines scored per explant source are given between parentheses
[b] Values of χ^2 were calculated with Yates correction factor; * = significant deviation from 3:1 ratio ($0.01 \leq P \leq 0.05$); ** = significant deviation from 3:1 ratio ($P \leq 0.01$)
[c,d] See details in Table 4

(anthocyanin-free) was not observed among the plants derived from the EMS material. In the same EMS experiment, however, another genotype (GT, a breeding line similar in fruit and growth characteristics to cv Moneymaker) was also evaluated (results not shown). Among M_2 plants of this genotype, anthocyanin-free mutants were found at a frequency of 2.2×10^{-3}.

For the easily recognizable mutant types of anthocyanin-free, hairless hypocotyls and ABA deficiency, a relatively large collection of mutants is available for reference. From the genetic characterization of these mutants, an estimate of minimum number of loci involved can be made. This is ten for anthocyanin-free (von Wettstein-Knowles 1968; Mutschler et al. 1987), three for hairless hypocotyl (Dempsey and Sherif 1987), and three for ABA deficiency (Tal and Nevo 1973). Taking these mutant types together, an (over-)estimate of the mutation frequency per locus per diploid cell was calculated, which was 0.69×10^{-3} for material obtained from EMS treatment and 0.12×10^{-3} for the tissue-culture-derived material.

Small differences between explant sources were observed, especially with regard to hypocotyl explants. This is mainly due to the absence of mutants in the categories

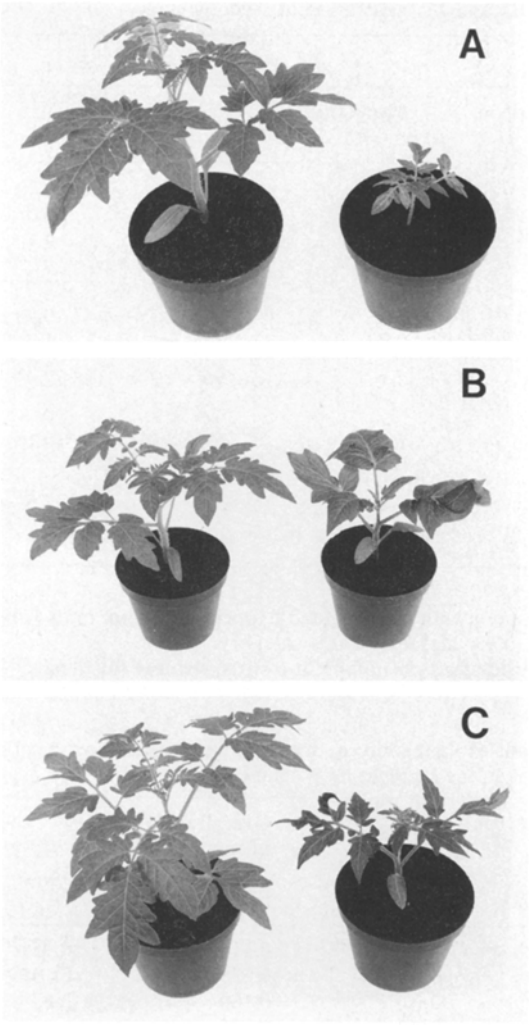

Fig. 3A–C. Phenotypic variants recovered in the R_2 progeny of tomato cv Moneymaker regenerants, viz., a chlorophyll mutant showing chlorosis around the veins (**A**), the leaf morphology mutant 'Entire' (**B**), and the '57-type' dwarf (**C**). A normal 'Moneymaker' plant is included in each photograph on the left

of reduced chlorophyll, altered leaf morphology, and anthocyanin-free mutants. Again, due to the low mutant frequencies per mutant class, no conclusions can be drawn about the differences in mutant spectrum and mutant frequencies between explant sources. As regards the frequency of polyploids, there was no distinct increase in overall mutant frequency among the R_2 progenies due to a longer tissue culture period.

Genetic analysis of somaclonal variants. The number of R_2 lines per explant source segregating for specific mutant phenotypes and the overall segregation ratio for these mutant phenotypes per 'mutated' R_2 line are given in Table 5. Most of the mutant groups fit a segregation ratio of 3:1, and therefore are likely to be monogenic-recessive mutants ($P \leq 0.05$). Ten mutants from different R_2 mutant categories (e.g., anthocyanin-free, altered leaf morphology, reduced chlorophyll, 'various types') were selfed and showed the expected transmission of the recessive mutant phenotype to all plants of their R_3 progeny. Four of these recessive mutants were tested for allelism to known homozygous recessive mutants. All plants derived from crosses with specific representatives of the same phenotypic class showed the mutant phenotypes, demonstrating that complementation did not occur. Hence, the four selected somaclonal mutants were similar to known single-gene mutants, i.e., an anthocyanin-free mutant (*ae*; Clayberg et al. 1960), an anthocyanin-gaining mutant (*ag*; Rick and Butler 1956), a mutant with 'scurfy' cotyledons (*scf*; Clayberg et al. 1966), and a mutant with fewer leaf segments and a distorted midvein ('Entire', Fig. 3B, *e*; Rick and Butler 1956).

Among the dwarf mutants of the somaclones a special phenotype, constituting the majority of this category, was observed (Fig. 3C). This phenotype was designated '57-type' dwarf, because of the first observation of its appearance in the progeny of somaclone no. 57. It is characterized by a reduced height, dark-green cotyledons, leaf distortions, and a chlorotic appearance at a later plant stage. This type of dwarfism was not observed in the progeny derived from EMS-treated seeds.

The segregation ratios for dwarfism and variegation show that these traits are not monogenic recessively inherited. Further analysis of '57-type' dwarfs in succeeding generations showed that R_3 and R_4 progenies continued segregating for this phenotype. Out of 52 R_3 lines tested, 8 lines did not show dwarfism, 5 lines showed dwarfism in more than 50% of the plants, and 39 lines showed it in less than 50% of the plants. Among the R_4 progenies of '57-type' dwarfs selected in the R_3, 19 lines did and 5 lines did not show dwarfism. Only one dwarf showed 100% transmission to the R_3 and R_4 and thus behaved as a normal recessive mutant. Dwarfs of the '57 type' were not observed among the progenies of other R_2 plants.

Discussion

The results obtained confirm the possibility of recovering mutations in tomato after plant regeneration from explants, as described before (Evans and Sharp 1983; Buiatti et al. 1985; Gavazzi et al. 1987). Variant traits were observed in the population of R_1 plants, but were not transmitted to the R_2 progeny, except for chromosomal variation. Moreover, cuttings taken from these plants often did not show the variant trait. Hence, this variation should be considered epigenetic (Meins 1983). For 'rogue' plants it is suggested that their appearance depends strongly on environmental factors, especially light and temperature. Rogues may even be found among plants derived from the same seed batch if sown at differ-

ent times or under different conditions. They did not occur, however, in the controls. Their offspring are usually normal (Grimbly 1986), as was also the case in our study.

A multicellular origin of adventitious shoots of *L. esculentum*, obtained after in vitro regeneration, has been shown by Monacelli et al. (1988) through histological analysis and by others in the same or different plant species (Sree Ramulu et al. 1976, 1986). As a consequence, some regenerants may be chimeras. Indeed, in the present study chimeras were found among R_1 plants. Thus, mutations must have occurred in one of the cells involved in the initiation of shoot regeneration prior to or after the onset of organogenesis. Chimerism was not observed at a higher frequency in plants obtained after a longer tissue culture period. Also, most mutant phenotypes fitted a 3:1 segregation ratio in the R_2 progeny, confirming that in the corresponding R_1 plants not much chimerism had occurred, at least not within the trusses that were harvested. Therefore it is likely that most mutations observed in this study were induced early, prior to shoot regeneration, or were already present in the explant.

Chromosomal variation, mainly tetraploidy, was observed frequently among the regenerants. This type of variation was stable, since R_2 lines obtained from polyploid R_1 plants were also polyploid. The selection of polyploid plants can be done on the basis of phenotypic characteristics, because verification by flow cytometric analysis or determination of the number of chloroplasts in guard cells showed visual classification to be reliable. However, aneuploidy cannot be detected by these methods.

The percentage of polyploid plants was obviously influenced by the type of explant source. This was apparently due to the occurrence of polyploid cells at higher frequency in the source material, due to endoreduplication, as has been reported in potato (Sree Ramulu and Dijkhuis 1986; Pijnacker et al. 1989). In other crops also, e.g. tobacco, it was demonstrated that callus cultures derived from plant tissues with a higher percentage of polyploid cells contained more chromosome variation (Murashige and Nakano 1967). Tetraploidy was also observed by Evans and Sharp (1983) in tomato plants regenerated from leaf explants and by Buiatti et al. (1985) in plants derived from cotyledons. However, Gavazzi et al. (1987) did not report any data on ploidy variation in the progeny of plants regenerated from cotyledons. The occurrence of triploid plants after explant regeneration was, to our knowledge, not reported before. The basis of this phenomenon is unknown.

Among the progeny of regenerants, monogenic-recessive mutations were observed. Four of the recessive mutations were allelic to known single-gene mutations and were not reported before to be obtained by regeneration from cells or tissue. Mutations, homozygous already in the originally regenerated plants as found by Evans and Sharp (1983) and Gavazzi et al. (1987), were not found in this study. Two distinct mutant phenotypes observed in the R_2, dwarfs and variegated plants, were not inherited monogenically. The segregation data of '57-type' dwarfs in R_3 and R_4 suggest that these types of genetic changes are instable. One can only speculate about the basis for this instability, e.g., changes in the state of methylation of DNA or the activity of transposable elements. One dwarf mutant fitted a 3:1 segregation ratio in the R_2 and bred true in the R_3 and R_4, indicating that a single, stable gene controls this trait. Buiatti et al. (1985) also found segregation data for several dwarf mutants that fitted a 3:1 ratio.

No apparent differences between the mutant frequencies and spectra for the three different explant sources were observed. The longer duration of the tissue culture period had no effect on either the occurrence of mutants or on the frequency of polyploids. In general, accumulation of variation is correlated with duration of culture. This has been demonstrated in tomato for chromosomal instability of shoots developed immediately on leaf explants versus shoots formed after a prolonged callus culture of 3 months (Koornneef et al. 1989), although an effect of different medium composition for the latter regeneration procedure, especially with regard to hormones used, cannot be excluded. An increased number of polyploid plants was also found for plants regenerated from protoplasts as compared to leaf explant regeneration (O'Connell et al. 1986; Koornneef et al. 1989). In our tissue culture system, a much longer culture period of the callus used for the second regeneration cycle might have resulted in a higher degree of somaclonal variation.

In order to evaluate its effectiveness, somaclonal variation was compared to a classical mutagenic (EMS) treatment. For the mutant types that could be scored unambiguously, it appeared that the mutant frequencies were higher after the EMS treatment. With respect to the mutant spectrum, no conclusive evidence was found, demonstrating that the spectrum of somaclonal variants is different from that obtained with chemical mutagenesis. The possible recovery of somaclonal, single-gene mutants of tomato not previously reported using conventional mutagenesis was mentioned by Evans et al. (1984). A direct comparison of somaclonal variation with mutagen (EMS) application has, so far, only been made by Gavazzi et al. (1987). They concluded that somaclonal variation gave a higher mutation frequency and a different spectrum. However, the use of different genotypes, different procedures to induce mutants by mutagen application, and different tissue culture procedures complicates the comparison of their study with ours. Different procedures of mutagenic treatments can especially result in large differences in mutation frequency. Moreover, their findings that potato leaf mutants were unique

for somaclonal variation are contradicted by the results of Robinson (1988), who found 2 potato leaf mutants, allelic to *c*, among 400 tomato mutants obtained in the M_2 after treatment of seed with 1% EMS for 24 h. This indicates once more that definite conclusions cannot be drawn from such comparative studies.

From the present data, it can be concluded that mutants obtained after regeneration of cells or tissue can be recovered with reasonable frequencies compared to mutagenic treatments, and it appears that the mutant spectrum for somaclonal variation is at least as broad as the spectrum found after chemical mutagenesis. Therefore, somaclonal variation can be useful for plant breeding research to recover new or known traits in valued genotypes. Somaclonal variation may result in more unwanted polyploids which, however, can be avoided to a large extent by using leaf material as a source of explant.

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