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EMS-induced streptomycin resistance in *Solanum melongena*

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Abstract. Streptomycin-resistant mutations were induced in *Solanum melongena* by exposing seeds to ethyl methane sulphonate (EMS). Seed mutagenesis resulted in a high frequency of chlorophyll-deficient mutations and a low frequency of resistant shoots, both of which retained their resistance on subsequent testing. Reciprocal crosses between streptomycin-resistant and -sensitive plants showed a non-Mendelian transmission of the resistance trait. Streptomycin resistance is the first selectable and maternally inherited organelle marker described in brinjal.

Key words: *Solanum melongena* – Mutagenesis – Ethyl methane sulphonate – Streptomycin resistance

Introduction

In most higher plants, organelles are inherited uniparentally. Thus, the combining of cytoplasmic organelles with different genetic traits is not possible by sexual hybridization. One advantage in analyzing organelle transfer and interaction in cybrids is the presence of selectable and easily screened genetic markers on the organelles. The value of plastome-encoded antibiotic resistance markers for organelle inheritance and interactions between nuclear and cytoplasmic genomes in higher plants have been alluded to by a number of workers (Cseplo and Maliga 1984; Fluhr et al. 1985; Jansen et al. 1990). Other plastid mutants which have generated interest include those resistant to herbicides inhibiting photosynthesis (Cseplo et al. 1985; Menczel 1986) and chlorophyll deficiency (Hosticka and Hanson 1984; Svab and Maliga 1986). Streptomycin resistance is the most extensively used marker in higher plants and has already been induced in *Onobrychis vicifolia* (Hamill et al. 1986), *Lycopersicon peruvianum* (Jansen et al. 1990; McCabe et al. 1989) and *Nicotiana* species (To et al. 1989; Fluhr et al. 1985; Cseplo and Maliga 1984). Streptomycin binds to the 30S ribosomal subunit, inhibits polypeptide synthesis and causes misreading of the genetic code (Davis et al. 1974; Edwards 1980). Single base-pair changes leading to streptomycin resistance have been mapped to 16S rRNA genes of the chloroplast genome (Fromm et al. 1987, 1989; Etzold et al. 1987). If by analogy to other species a C-T transition is required to obtain streptomycin resistance in brinjal, NMU (nitroso methyl urear) and EMS (ethyl methane sulphonate), are potential inducers of this mutation. NMU and EMS induces mutations in cpDNA (chloroplast DNA) (Chia et al. 1986; Miller et al. 1984; Hagemann 1982). The plastid DNA mutations reported include changes that reduce plastid pigmentation and produce variegated plants. The present article reports for the first time the efficiency of EMS in making plastids antibiotic resistant and describes the induction of streptomycin resistance in eggplant.

Materials and methods

Seeds of *Solanum melongena* cv 'Purple round' (after imbibition for 24 h in sterile distilled water at 25 °C) were incubated for 18 h in culture medium in which 0.1% EMS had been dissolved and then washed 3 times with fresh medium. Mutagenized seeds were surface sterilized with 0.1% $HgCl_2$ for 3–5 min followed by three rinses with sterile, distilled water, then germinated on the surface of MS basal medium.

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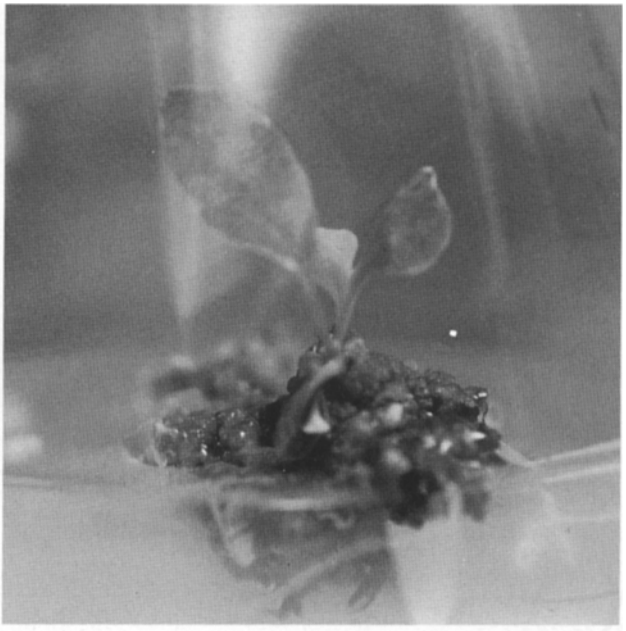

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Figs. 1 and 2. Green shoot developing from *S. melongena* cotyledon explant placed on medium supplemented with 500 mg l^{-1} streptomycin sulphate after mutagenesis with EMS. **2** The occurrence of green nodular callus with differentiating shoots as leaf explant on medium containing 1000 mg l^{-1} streptomycin sulphate

Cotyledons from 3-week-old seedlings were implanted on shoot regeneration medium containing MS salts, 20 gm sucrose, 100 mg l^{-1} meso-inositol, 0.5 mg l^{-1} IAA, 3 mg l^{-1} BAP, pH adjusted to 5.7 and solidified with 8 g l^{-1} Difco-Bacto agar supplemented with 500 mg l^{-1} streptomycin sulphate. A single explant was placed in each culture tube and incubated at 25° ± 2 °C under a 16-h photoperiod for 6 weeks.

Results

Both mutagenized and control seeds were germinated in the absence of antibiotic. After 3 weeks cotyledonary explants were placed in shoot regeneration medium containing 500 mg l^{-1}. Plant regeneration was suppressed and was associated with efficient bleaching in non-mutagenized explants. Cotyledons from mutagenized seeds produced green nodules and shoots as resistant mutants (Fig. 1). EMS mutagenesis of brinjal seeds resulted in a very high frequency of chlorophyll deficient mutants (20%) and a low frequency of streptomycin-resistant shoots. The data on the appearance of resistance are given in Table 1, where both the percentage of explants with resistant shoots and the mean number of shoots per explants are recorded. However, resistant mutants could also be isolated as green adventitious shoots on cotyledon explants (4.16%). Shoots arising on explants were rooted by transfer to MS medium supplemented with 0.1 mg l^{-1} IAA, without streptomycin; this experiment was repeated to confirm the results obtained. The stability of streptomycin resistance was studied by a leaf assay. The plants regenerated from mutagenized cotyledon explants retained streptomycin resistance as indicated by the appearance of the shoots on leaf sections of the regenerates grown on selective medium containing concentrations of up to 1 mg/ml streptomycin (Fig. 2). Ploidy estimations were carried out on adventitious shoots arising from cotyledon explants, all of the material was found to be diploid.

Table 1. Production of chlorophyll deficiency mutants and streptomycin-resistant shoots in *S. melongena*

	Explant bleached (%)	Chlorophyll deficiency mutant (%)	Streptomycin-resistant shoots (%)	Mean number of shoots per explants
1. Control	100	–	–	–
2. EMS	75.84	20	4.16	3.2 ± 0.21

Table 2. The inheritance of streptomycin resistance in *S. melongena*

Cross	% Germination	Number of seedlings tested	
		Resistant	Sensitive
♀ SR × SS ♂	75	580	0
♀ SS × SR ♂	82	0	450

The transmission of streptomycin resistance to the progeny was studied as evidence of the mutational origin of streptomycin resistance. Reciprocal crosses were made between one flowering streptomycin-resistant plant and the original streptomycin-sensitive plants. When the streptomycin-resistant plant was the female parent, all of the offspring were streptomycin resistant (Table 2). Conversely, all of the progeny were streptomycin sensitive when the pollen of the resistant plant was used. These results confirm the genetic nature of the streptomycin resistance trait and demonstrate that the resistance is controlled by a maternally inherited mutation.

Discussion

This investigation was initiated to test the efficiency of different mutagenic agents for in vitro mutagenesis and selection of antibiotic resistance encoded on the cpDNA. The successful isolation of mutants was dependent upon establishing conditions where bleaching was not accompanied by severe growth limitation. This certainly relates to the complex kiloploid nature of the plant chloroplast genome (Medgyesy 1990). Streptomycin resistance has also been shown to be the result of recessive mutations in the nucleus (Maliga et al. 1981). One possible explanation for our failure in obtaining nuclear mutations is that our sample sizes may not have been large enough to screen for nuclear mutations. Because of the polyploid nature of the chloroplast and mitochondrial genomes, it is expected that mutations would occur more frequently in the organelles than in the nucleus. Moreover, nuclear-encoded streptomycin resistance is always inherited as a recessive trait and has only been isolated using haploid material. Thus, it is unlikely to expect a dominant, nuclear streptomycin-resistant mutant and even less likely to expect a homozygous recessive, nuclear mutation in diploid plant material.

The inhibition of greening at lower antibiotic concentrations might be explained by a destabilization of the plastid protein complex by mis-sense proteins; the inhibition of cell multiplication at high drug concentrations might be the result of a complete inhibition of plastid protein synthesis. Antibiotic-resistant plastids in a cell and cells with resistant plastids, therefore, should have a selection advantage during multiplication in the presence of antibiotics that inhibit protein synthesis (Cseplo et al. 1992).

Mutants resistant to several different antibiotics have been successfully isolated in members of the solanaceae by the plastome-targeted mutagen NMU. This has been the mutagen of choice in more recent investigations on the production of chloroplast mutants (McCabe et al. 1989, 1990; Dix et al. 1990), although interesting exceptions are the investigations of To et al. (1989) and Sadanandam and Farooqui (1991), in which efficient plastome mutagenesis was achieved with *N*-methyl-*N*-nitro-*N*-nitroso guanidine and r-irradation in *Nicotiana* and *Solanum melongena*, respectively.

No cross resistance of the streptomycin-resistant clones to other antibiotics could be determined; these clones were not resistant to lincomycin. This is in accordance with the fact that aminoglycoside-type antibiotics interact with the small subunit of the prokaryotic ribosome, whereas lincomycin acts on the large ribosomal subunit (Neirhaus and Wittmann 1980). We speculate that the streptomycin-resistant mutants obtained in this study may result from a change in the chloroplast 16S-rRNA gene. This speculation is consistent with the findings that in a number of eukaryotes streptomycin resistance is caused by a single point mutation in the chloroplast 16S-rRNA gene (Harris et al. 1987; Lemieux and Lee 1987; Gauthier et al. 1988; Fromm et al. 1989).

In conclusion, cotyledon explants from EMS-mutagenized seedlings give a higher yield of streptomycin-resistant plants, and this adds another effective mutagenic procedure for routinely obtaining antibiotic-resistant plants in *Solanum melongena*. With a judicious selection of mutagenic treatment, there are good prospects for using this approach to obtain mutants of crop plants with improved agronomic traits.

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