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Cross-resistance in Cell Lines of *Nicotiana sylvestris* Selected for Resistance to Individual Antibiotics

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ABSTRACT

Cell lines initially selected for resistance to the antibiotics kanamycin, streptomycin and chloramphenicol, were each tested for resistance to several different antibiotics. Only the kanamycin resistant lines showed any cross-resistance to other antibiotics. The three lines tested were resistant to streptomycin and neomycin, while one of them, KR103, was also resistant to chloramphenicol early in its history, although this resistance was subsequently lost. None of the lines showed any resistance to cycloheximide.

Key words: *Nicotiana sylvestris*, cell culture, cross-resistance, antibiotics, chloramphenicol, kanamycin, streptomycin, neomycin, cycloheximide, cytoplasmic mutants, callus.

INTRODUCTION

From the demonstration (Dix, Joó and Maliga, 1977) that cell lines of *N. sylvestris* selected as resistant to kanamycin were also resistant to streptomycin rose the question of specificity of resistance to related drugs in other variants.

In addition to the kanamycin resistant lines other cell lines were available, which had been selected as resistant to either streptomycin (Maliga, R.-Kiss, Dix, and Lázár, 1979), or chloramphenicol (Dix, 1981). The resistance of all of these lines to each of the following antibiotics was investigated: kanamycin, streptomycin, neomycin, chloramphenicol and cycloheximide. The patterns of cross-resistance are discussed in relation to the structural similarities, and sites of action, of the different antibiotics.

MATERIALS AND METHODS

Plant material and culture conditions were as described in the preceding paper (Dix, 1981). All antibiotics except kanamycin were filter-sterilized and added to autoclaved medium. As far as possible growth tests were performed on the media supporting both unorganized growth (RMP) and organized growth and greening (RMO), discussed in that paper.

Variant cell lines

The selection of the three kanamycin resistant lines, KR103, KR115 and KR116, was described earlier (Dix *et al.*, 1977). They were all defective in morphogenesis but retained their resistance to kanamycin through an extended period of growth in the absence of the drug.

The streptomycin resistant line, SR155, has also been described previously (Maliga *et al.*, 1979). Callus used in the present tests was initiated from a regenerated SR155 plant, and retained its streptomycin resistance on organizing (RMO) medium.

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The chloramphenicol resistant line CPR102 was that described in the preceding paper (Dix, 1981). Two sub-lines were used, CPR102A and CPR102C, each initiated from a different regenerated plant and showing clear retention of the resistance phenotype.

RESULTS

Kanamycin resistant lines

Callus cultures of lines KR103, KR115 and KR116 have already been shown to be resistant to streptomycin (Dix *et al.*, 1977). On a number of separate occasions they have now been repeatedly tested for resistance to streptomycin, neomycin, chloramphenicol and cycloheximide, on RMP medium. KR103 has also been tested on RMO medium (lines KR115 and KR116 grow very poorly on RMO medium). The results of one such test are given in Table 1. Tests were performed at a range of antibiotic concentrations but data are presented for only a single critical level of each antibiotic. All three lines showed an enhanced level of resistance to streptomycin and neomycin, and KR103 was also clearly resistant to chloramphenicol. The same tests performed 15 and 18 months later gave similar results except that the chloramphenicol resistance of line KR103 had been completely lost (data not shown).

None of the lines was resistant to cycloheximide, an inhibitor of 80S ribosomal protein synthesis (Gillham, 1978).

Streptomycin resistant line

Line SR155 was tested for resistance to the same range of antibiotics on both RMP and RMO medium and key results are shown in Table 2. The line was clearly resistant to streptomycin on RMO medium, where there was substantial proliferation of green SR155 callus at a level of streptomycin sulphate (500 μg ml^{-1}) which did not allow growth or greening of normal *N. sylvestris* callus. On RMP medium, however, resistance to streptomycin could not be demonstrated. SR155 was sensitive to all the other antibiotics, irrespective of the medium used.

Chloramphenicol resistant lines

Data pertaining to sub-lines CPR102A and CPR102C are shown in Table 3. Resistance was found only to chloramphenicol, on both RMP and RMO media.

DISCUSSION

Three lines selected as resistant to kanamycin under completely heterotrophic conditions are also resistant to streptomycin and neomycin, other aminoglycoside antibiotics thought to interact with the 30S subunits of organelle ribosomes (Gillham, 1978). Resistance of one of the lines, KR103, to chloramphenicol which acts on the 50S sub-unit (Gillham, 1978), was transient, being lost after a prolonged culture period, and was probably not associated with the resistance to the other antibiotics. None of the lines was resistant to cycloheximide, an inhibitor of cytoplasmic (80S) ribosomal protein synthesis.

The other lines appeared to be resistant solely to those antibiotics used for selection (streptomycin or chloramphenicol) although resistance to other antibiotics, not used in the present work cannot be excluded.

We therefore have two phenotypically-distinct types of streptomycin-resistant line.

TABLE 1. *Growth of kanamycin resistant lines on media containing five different antibiotics*

	No antibiotics	Kanamycin sulphate 50 mg l^{-1}	Streptomycin sulphate 500 mg l^{-1}	Neomycin 300 mg l^{-1}	Chloramphenicol 7 mg l^{-1}	Cycloheximide 5 mg l^{-1}
KR103	1·598	1·432	0·719	1·124	0·613	0·064
KR103‡	1·160	0·846	0·458	0·696	0·495	0·081
KR115	1·273	0 972	0 572	0·850	0·059	0·132
KR116	0·943	0·642	0·349	0 767	0·121*	0·129*
NS	1·730	0·185	0·129	0·094	0·171*	0·115

‡ Tested on RMO medium.

Except where indicated RMP medium was used. Normal N. sylvestris callus (NS) was used as control. Each fresh weight (g) value is the mean of 20 replicates weighed 6 weeks after initiation. Initial mean fresh weight was 0·050 g. S.e.m. was always less than 10 per cent of the mean, except * where it was in the range 10–20 per cent.

TABLE 2. *Growth of streptomycin resistant line, SR155, on media containing five different antibiotics*

	No antibiotics	Kanamycin sulphate 50 (RMP) or 10 (RMO) mg l^{-1}	Streptomycin sulphate 500 mg l^{-1}	Neomycin 300 mg l^{-1}	Chloramphenicol 7 mg l^{-1}	Cycloheximide 2 (RMO) or 5 (RMP) mg l^{-1}
RMP						
SR155	1·382	0 114	0·157	0·138	0·061	0·085
NS	1·462	0·159	0·216*	0·121	0·138	0·118
RMO						
SR155	0·779	0·145	0·257	0·210	0·116	0·154
NS	1·190	0·257	0·056	0·237	0·159	0 220

Details are as described in footnote to Table 1.

TABLE 3. *Growth of chloramphenicol resistant lines on media containing five different antibiotics*

	No antibiotics	Kanamycin sulphate 50 (RMP) or 30 (RMO) mg l^{-1}	Streptomycin sulphate 500 mg l^{-1}	Neomycin 300 mg l^{-1}	Chloramphenicol 7 mg l^{-1}	Cycloheximide 5 mg l^{-1}
RMP						
CPR102A	1·492	0·188	0·269	0·089	0·460	0·060
CPR102C	1·414	0·094	0·197	0·114	0·362	0·076*
NS	1·480	0·160	0·219	0·121	0·194	0 073
RMO						
CPR102A	1·460	0·146	0·140	0·178	0·511	0·037
CPR102C	1·732	0·169	0·169	0·312*	0·386	0·057
NS	1·425	0·215	0·102	0·194	0·097	0·079

Details are as described in legend to Table 1.

One, SR155, is resistant solely to streptomycin and the resistance, only observed on RMO medium, is characterized by greening, as well as improved growth. The other, typified by KR103, is also resistant to kanamycin and neomycin and is equally, or better, expressed on medium which promotes growth of non-pigmented, undifferentiated tissue. SR155 resembles the SR1 line of *N. tabacum* in which streptomycin resistance is associated with a change in a chloroplast ribosomal protein (Yurina, Odintosova and Maliga, 1978).

The effect of the 'mutation' producing KR103 was to prevent several related antibiotics from interfering with ribosomal function. Mutation resulting in structural changes in ribosomes and conferring multiple resistance to antibiotics would not be surprising in view of the clustering of ribosomal genes in *Escherichia coli* (Brown and Apririon, 1974) and more recent evidence for close linkage between the antibiotic resistance loci in the mitochondrial DNA of fungi (Lazarus and Turner, 1977; Morimoto, Merten, Lewin, Martin and Rabinowitz, 1978). Alternatively, a change in an organelle membrane protein could influence uptake of several related antibiotics into the organelle. It is clearly desirable to discover which sub-cellular component has been modified in KR103.

Considerable progress has been made in mapping the mitochondrial genome in lower eukaryotes, particularly yeast (Bandlow, Schweyen, Wolf and Kaudevitz, 1977). The use of rho^- petite mutants containing various antibiotic resistance markers has been central to this work (Schweyen, Weiss-Brummer, Backhaus and Kaudevitz, 1977).

While we are a long way from the level of experimental sophistication required to obtain similar information from cultured plant cells, the isolation and characterization of a range of cytoplasmic mutants is an important initial step. All the variant cell lines described here are resistant to known inhibitors of 70S ribosomal protein synthesis, but it has still to be shown that their resistance results from a mitochondrial or plastid mutation.

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