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Streptomycin Resistance is Inherited as a Recessive Mendelian Trait in a *Nicotiana sylvestris* Line

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Summary. The SR180 cell line has been isolated in a callus culture derived from a haploid *Nicotiana sylvestris* (n = X = 12) plant by its ability to proliferate on a selective medium containing 2,000 μg/ml streptomycin sulphate. From the cell line diploid plants have been regenerated. The SR180 selfs are resistant to streptomycin. Streptomycin sensitivity in F1, and a 3:1 (sensitive to resistant) segregation in F2 indicate that resistance in the SR180 mutant is the result of a recessive Mendelian mutation.

Key words: Streptomycin resistance – Mendelian mutant – *Nicotiana sylvestris* – Callus selection

Introduction

Antibiotic resistant mutations in flowering plants would be helpful in elucidating the contribution of chloroplast and nuclear genes to chloroplast ribosomes, as indicated by studies of such mutants in the green alga *Chlamydomonas* (reviewed in Kirk and Tilney-Basset 1978). Resistance phenotypes could also be used as markers in somatic hybridisation (Schieder and Vasil 1980).

Cell lines resistant to various antibiotics have been reported in cultured plant cells (reviewed in Maliga et al. 1980; Maliga 1980). Sexual transmission, however, was confirmed only in case of streptomycin resistance which was inherited maternally in each of the three *N. tabacum* mutants studied so far (Maliga et al. 1973; 1980; Umiel 1979). In this paper a new type, a Mendelian streptomycin resistance mutation is described.

Materials and Methods

Primary callus from leaf sections of haploid (n = X = 12) *Nicotiana sylvestris* Spegaz et Comes plants was induced on RMNO medium. This medium is the Linsmaier and Skoog's (1965) RM medium modified to contain 0.04 mg kinetin, 0.1 mg 2,4 dichlorophenoxy acetic acid and 3 mg indole-3-acetic acid per liter (Márton and Maliga 1975). Resistant cell lines were selected on RMP medium (RM medium with 0.1 mg kinetin and 0.1 mg 2,4 dichlorophenoxy acetic acid per liter; Maliga et al. 1977). Plant regeneration, leaf resistance tests, and some of the seedling tests were carried out on RMO medium, which is the same RM medium but contains 0.5 mg benzylaminopurine and 2 mg indole-3-acetic acid per liter (Maliga et al. 1973). Seedlings were also tested for resistance on Linsmaier and Skoog's RM salts supplemented with 3% sucrose. Cultures were incubated at 28°C, and in light (1,500 lux, 16 h), except for callus induction from leaves and selection on streptomycin-containing RMP medium in which cases no illumination was provided. General tissue culture procedures have been described in previous publications (Maliga et al. 1973; Márton and Maliga 1975).

Results

Isolation of Resistant Cell Lines

Small pieces (50 mg) of calli, obtained from haploid leaves, were placed on RMP medium containing 2,000 μg ml^{-1} streptomycin sulphate. This selective medium prevents the growth of the inoculated calli, which turn brown. In six to eight weeks, however, a few white, proliferating sectors appeared. These white calli were isolated, regrown on the same selective RMP medium, then transferred to drug-free RMO medium for plant regeneration.

Regenerated plants could be divided into two groups. Leaf sections of those with a higher degree of resistance form green callus on RMO medium containing 2,000 μg ml^{-1} streptomycin sulphate, whereas plants with the lower resistance-level form calli which green only on a medium with up to 500 μg ml^{-1} streptomycin. This lower level is still selective since callus initiated from sensitive parental leaves is white. White shoots, or chimeras with white sectors were regularily found in a few percent of the cultures regenerating on non-selective medium. Pigment deficiency in these cases may be due to streptomycin-induced mutations (reviewed in Kirk and Tilney-Bassat 1980).

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Inheritance of Streptomycin Resistance

Inheritance of streptomycin resistance was studied in detail in diploid (2n = 24) SR180 regenerates with the lower resistance level. Regeneration of diploid plants from the initially haploid cultures is the result of spontaneous diploidisation of the cells, a phenomenon that frequently occurs in cultured cells (Bayliss 1980). The phenotype of the seedlings was scored by the ability to grown as a green plant (Fig. 1A, B) or form a callus (Fig. 1C) on RM and RMO media, respectively, containing 1,000 μg ml^{-1} streptomycin sulphate. Seedlings obtained from crosses (F1, RF1) between resistant regenerates and the sensitive diploid parental plants are sensitive (Fig. 1A). In F2 a 3:1 segregation was found indicating that in the SR180 line resistance is controlled by a recessive nuclear allele (Table 1). Upon selfing resistant regenerates no segregation was found.

In two other lines streptomycin resistance was not inherited, although regenerates were clearly resistant.

Discussion

Isolation of Mendelian streptomycin resistance mutants in a flowering plant species, *Nicotiana sylvestris*, was predictable since such mutants exist in unicellular algae (reviewed in Kirk and Tilney-Basset 1978). Mendelian inheritance is

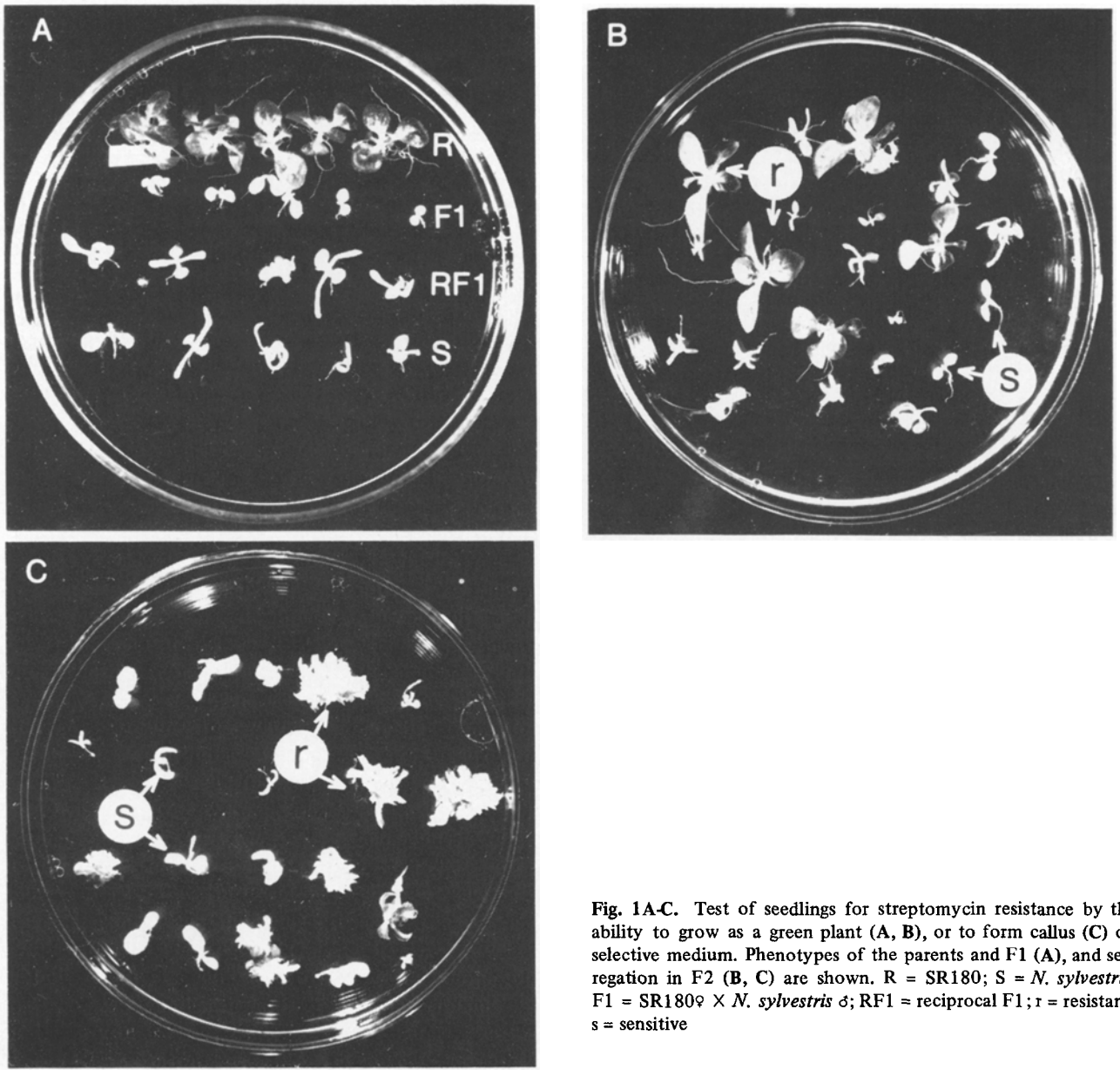

Fig. 1A-C. Test of seedlings for streptomycin resistance by the ability to grow as a green plant (**A, B**), or to form callus (**C**) on selective medium. Phenotypes of the parents and F1 (**A**), and segregation in F2 (**B, C**) are shown. R = SR180; S = *N. sylvestris*; F1 = SR180♀ × *N. sylvestris* ♂; RF1 = reciprocal F1; r = resistant; s = sensitive

Table 1. Inheritance of streptomycin resistance in selfs and F2

Way of testing		No. of seedlings tested	Sensitive		Resistant	
			Obs.	(Exp.)	Obs.	(Exp.)
Plant growth	*N. sylvestris* (selfed)	300	300			
	Ns SR180 (selfed)	300			300	
	N. sylvestris♀ × *Ns* SR180 ♂	300	221	(225)	79	(75)
	Ns SR180♀ × *N. sylvestris* ♂	300	227	(225)	73	(75)
Callus formation	*N. sylvestris* (selfed)	400	400			
	Ns SR180 (selfed)	300			300	
	N. sylvestris♀ × *Ns* SR180 ♂	400	297	(300)	103	(100)
	Ns SR180♀ × *N. sylvestris* ♂	380	268	(285)	112	(95)

Segregation ratios do not differ significantly from expected values ($P > 0.05$)

an accepted indication that the SR180 allele is located in the nucleus. Other streptomycin resistance mutations in *Nicotiana* (c.f. Introduction) are maternally inherited, in which case the resistance factor should be located in the cytoplasm. Studies on plastid segregation in somatic hybrids suggest that genetic determinants of the resistance are located in the chloroplasts in at least one of the cytoplasmic mutants (Menczel et al. 1981).

Mendelian streptomycin resistance in the SR180 line is recessive, as it is the case with similar mutants in the green alga *Chlamydomonas* (Lee et al. 1973). The use of true haploid cells therefore was a critical factor in obtaining this line. Further studies should elucidate, whether or not a mutation effecting chloroplast ribosomes is responsible for the resistant phenotype.

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