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Inheritance of Chloramphenicol Resistance, a Trait Selected in Cell Cultures of *Nicotiana sylvestris*. Speg. and Comes.

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ABSTRACT

Three cell lines with improved resistance to growth inhibition by chloramphenicol were selected from cell cultures of *Nicotiana sylvestris*. Resistance was retained in callus cultures of two out of three plants regenerated from one of the lines, but not in cultures of plants regenerated from the other two lines. Sexual progeny of the two resistant plants were either sensitive or showed slow segregation for chloramphenicol resistance. In callus from only two of the seedlings was inheritance of chloramphenicol resistance clearly demonstrated.

Key words: *Nicotiana sylvestris*, cell culture, choramphenicol resistance.

INTRODUCTION

Recognition of the value of *in vitro* selection procedures to obtain variant plant cell lines, both for crop improvement and for studies in plant cell genetics, has led to a rapid expansion of the efforts in this field. Progress in the selection of variant cell lines has been covered by several recent reviews (Widholm, 1977; Maliga, 1978, 1981; Dix, 1979). One area of special interest is the selection of lines resistant to antibiotics known to interfere with 70S ribosomal protein synthesis. Such lines could be valuable for investigations into the cytoplasmic genetics of higher plants.

Cell lines resistant to streptomycin (Binding, Binding and Straub, 1970; Maliga, Sz.-Breznovits and Márton, 1973; Umiel and Goldner, 1976; Dix, Joó and Maliga, 1977; and Maliga *et al.*, 1979) and kanamycin (Dix *et al.*, 1977) have been described. Maternal inheritance of the resistance has been demonstrated in plants regnerated from streptomycin-resistant lines (Maliga *et al.* 1973; Maliga, Sz.-Breznovits, Márton and Joó 1975; Umiel, 1978).

The present paper reports on the selection of lines resistant to chloramphenicol, another inhibitor of 70S ribosomal protein synthesis, and on the retention of resistance in regenerated plants and their sexual progeny.

MATERIALS AND METHODS

Plant material and culture conditions

Callus cultures were initiated from plants obtained by asexual propagation of a single haploid plant (SH13) of *Nicotiana sylvestris* Speg. and Comes. as described earlier (Dix *et al.*, 1977). The culture media used were (i) RMP (Maliga *et al.*, 1977) for routine callus and suspension culture, (ii) RMO (Maliga *et al.*, 1973) for greening and shoot regeneration, and (iii) Linsmaier and Skoog's revised medium (1965), lacking hormones and vitamins, for rooting of the regenerated shoots. Except when used for suspension culture these media were solidified with 7 g l^{-1} Difco Bactoagar. Further details of the culture techniques were described by Dix *et al.* (1977).

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RESULTS

Selection of variant cell lines

One line, designated CPR105, was selected from a callus culture maintained on RMP agar-medium containing 10 μg ml^{-1} chloramphenicol (Sigma) for 8 weeks (normal callus culture passage in the absence of chloramphenicol was 4 weeks). Two further lines, CPR102 and CPR104 were isolated from cell suspension cultures initiated from the haploid (SH13) callus. These arose as rapidly growing aggregates after prolonged (5 months) incubation in RMP medium containing 10 μg ml^{-1} chloramphenicol. (The normal cell suspension culture passage was 3 weeks). Individual regenerated plants, and callus cultures initiated from them, are designated by suffix letters, e.g. CPR102A, CPR102B etc.

Resistant lines all showed partial inhibition of growth in the presence of chloramphenicol at levels almost completely inhibitory (5 μg ml^{-1}) or completely inhibitory (10 μg ml^{-1}) for normal *Nicotiana sylvestris* callus (Table 1). Similar levels of growth were found when the resistant lines had been previously maintained on medium with no chloramphenicol (Table 1) or supplemented with 5 μg ml^{-1} chloramphenicol, but there was frequently a gradual decline in growth during a number of successive culture passages on medium containing 10 μg ml^{-1} chloramphenicol.

TABLE 1. *Growth of chloramphenicol resistant lines on RMP medium containing chloramphenicol compared with normal* N. sylvestris *callus (NS) of comparable age*

	Chloramphenicol (μg ml^{-1})	
	5	10
CPR102	0·503 ± 0·052	0·221 ± 0·029
CPR104	0·824 ± 0·121	0·327 ± 0·044
CPR105	0·335 ± 0·038	0·305 ± 0·050
NS	0·102 ± 0 018	0·037 ± 0·011

All lines had previously been grown for two culture passages on medium without chloramphenicol. Fresh weight (g) values are means of 20 replicates weighed 8 weeks after initiation. Mean fresh weight was 0·050 g. Final mean fresh weights in the absence of chloramphenicol were as follows: CPR102, 2·02 g; CPR104, 2 21 g; CPR105, 1·30 g; NS, 1·97 g.

Retention of resistance through plant regeneration

Three plants were regenerated from line CPR102, four from CPR104, and one from CPR105. Callus cultures were initiated from leaves of each of these plants, and also from two plants (NSA and NSB) regenerated from the parent cultures, and tested in the second culture passage for resistance to growth inhibition by chloramphenicol. All these calli grew well in the absence of chloramphenicol. They showed varying degrees of growth inhibition in the presence of the drug (Table 2). Two lines, CPR102A and CPR102C, showed clear retention of chloramphenicol resistance.

Inheritance of resistance

The two resistant regenerated plants CPR102A and CPR102C were selfed, and reciprocal crosses performed with wild-type *N. sylvestris*. Tests for chloramphenicol resistance were performed on seedlings (using RM medium) and on the initiation of callus

TABLE 2. *Growth of callus lines initiated from regenerated plants, on RMP medium containing 5 or 10 μg ml⁻¹ chloramphenicol*

	Chloramphenicol (μg ml⁻¹)	
	5	10
CPR102A	0·455±0·040	0·247±0·023
CPR102B	0·156±0·018	0·099±0·013
CPR102C	0·381±0·032	0·219±0·022
CPR104A	0·161±0·021	0·060±0·009
CPR104B	0·125±0·015	0·035±0·007
CPR104C	0·130±0·016	0·048±0·007
CPR104D	0·182±0·020	0 046±0·006
CPR105A	0·166±0 017	0·039±0·007
NSA	0·178±0·022	0·045±0·006
NSB	0·125±0·013	0·053±0·008

Fresh weight (g) values are means of 20 replicates weighed 8 weeks after initiation. Mean initial fresh weight was 0·050 g. Final mean fresh weights in the absence of chloramphenicol varied between 1·45 and 2·22 g.

from seedlings (using RMP medium). Resistance to chloramphenicol was not shown in either case (data not shown). When callus was initiated in the absence of chloramphenicol, however, subsequent tests for chloramphenicol resistance did reveal differences between the seedlings. A total of 125 seedlings from selfs or crosses involving CPR102A or CPR102C were tested in this way, alongside 35 seedlings of selfed *N. sylvestris*.

The results, summarized in Table 3, showed that the majority of seedling calli were either completely sensitive or exhibited only limited sectorial growth in the presence of chloramphenicol. The calli from only two seedlings (CPR102C, selfed, seedling 19; and CPR102♀ × NS ♂ seedling 21) had a level of resistance comparable with that of the parent cell line. The resistance was retained over three successive culture passages in the presence of 10 μg ml⁻¹ chloramphenicol (Table 4).

TABLE 3. *Growth of callus lines derived from the sexual progeny of plants CPR102A and CPR102C*

Progeny	Number of seedling calli tested	Number of seedling calli showing growth in the presence of chloramphenicol		
		In 1 or more replicate	In all 5 replicates	In 3rd passage on chloramphenicol
CPR102A (selfing)	15	12	1	0
CPR102A♀ × NS♂	15	11	1	0
NS♀CPR102A♂	20	8	1	0
CPR102C (selfing)	25	16	2	1
CPR102C♀ × NS♂	25	19	1	1
NS♀ × CPR102C♂	25	11	0	0
NS (selfing)	35	6	0	0

Pieces (0·30 g) from each callus were placed on RMP medium containing 0 or 10 μg ml⁻¹ chloramphenicol, 5 replicates of each treatment. Growth was assessed visually after 6 weeks, pale healthy callus contrasting with brown or black dying callus, and confirmed after 8 weeks by fresh weight increase. All calli grew in the absence of chloramphenicol.

TABLE 4. *Growth of resistant seedling calli in a third successive (6 week) culture passage on medium containing 10 μg ml⁻¹ chloramphenicol*

Origin of callus	Fresh weight (g)
CPR102C, selfed, 19	0·204 ± 0·020
CPR102C♀ × NS♂, 21	0·182 ± 0·021
NS (previously on chloramphenicol free medium)	0·040 ± 0·008

Other experimental conditions are as described in footnote to Table 1.

DISCUSSION

Several cell lines of *Nicotiana sylvestris* have been obtained with improved resistance to growth inhibition by chloramphenicol. Compared to that of cell lines selected for resistance to other antibiotics, such as streptomycin (Maliga *et al.*, 1973) or kanamycin (Dix *et al.*, 1977), the increase in resistance in the present case is small. 3 μg ml⁻¹ is a permissible level of chloramphenicol for growth of normal callus, but resistance is best demonstrated at 5–10 μg ml⁻¹ chloramphenicol, at which levels the resistant lines themselves grow at very much reduced rates.

Chloramphenicol resistance appears to be stable in callus cultures of all three resistant lines, but is completely lost on plant regeneration from two of them. This suggests that resistance in these lines is due either to an epigenetic change, stable in culture but reverting on plant regeneration, or to a change in the nuclear or cytoplasmic genome resulting in lines which are continually segregating for chloramphenicol resistance. In the latter case sensitive plants would have been regnerated from spontaneously-arising sensitive regions of the callus.

Segregation is further manifested in the loss of resistance in one of the three CRP102 plants, and in the bulk of the sexual progeny of the resistant plants. These findings suggest that it is difficult to completely eliminate the sensitive genome, and emphasize the probable value of a single cell cloning step in the establishment of variant cell lines.

The occurrence of seedlings in which chloramphenicol resistance has been retained suggests a genetic basis for the resistant phenotype although no conclusions can be drawn as to whether it is a nuclear or cytoplasmic trait. This will be investigated in clonal regenerants from the two seedling callus lines exhibiting resistance.

LITERATURE CITED

BINDING, H., BINDING, K. and STRAUB, J., 1970. Selection in Gewebekulturen mit Haploiden. *Naturwissenschaften* **57**, 138–39.

DIX, P. J., 1979. Cell structure manipulations as a potential breeding tool. In *Low Temperature Stress in Crop Plants*, eds. J. M. Lyons, D. Graham and J. K. Raison, pp. 463–72, Academic Press, New York.

——, JOÓ, F, and MALIGA, P., 1977. A cell line of Nicotiana sylvestris with resistance to kanamycin and streptomycin. *Molec. gen. Genet.* **157**, 285–90.

LINSMAIER, E. M. and SKOOG, F, 1965. Organic growth factor requirements of tobacco tissue culture. *Physiologia Pl.* **18**, 100–27.

MALIGA, P., 1978. Resistance mutants and their use in genetic manipulation. In *Frontiers of Plant Tissue Culture*, ed. T. A. Thorpe, pp. 381–92, IAPTC, Calgary.

——, 1981. Isolation, characterisation and utilisation of mutant cell lines in higher plants. In *Recent Advances in Plant Tissue Culture*, Suppl. Int. Rev. Cytol., eds. I. K. Vasil and D. G. Murphy, Academic Press, New York.

——, LÁZÁR, G., JOÓ, F., H.-NAGY, Á. and MENCZEL, L., 1977. Restoration of morphogenic potential in *Nicotiana* by somatic hybridisation. *Molec. gen. Genet.* **157**, 291–6.

——, R.-Kiss, Zs., Dix, P. J. and Lázár, G., 1979. A streptomycin-resistant line of *Nicotiana sylvestris* unable to flower. Ibid. **172**, 13–5.

——, Sz.-Breznovits, Á. and Márton, L., 1973. Streptomycin-resistant plants from callus culture of haploid tobacco. *Nature* (*New Biol.*) **24**, 29–30.

—— —— —— and Joó, F., 1975. Non-Mendelian streptomycin-resistant tobacco mutant with altered chloroplasts and mitochondria, *Nature; Lond.* **255**, 401–2.

Umiel, N., 1978. The isolation of mutants in plant tissue cultures and their use in genetic studies and plant breeding. In *Production of Natural Compound by Cell Culture Methods*, eds. A. W. Alfermann and E. Reinhard., pp. 346–52, München: Gesellschaft für Strahlen und Umweltforschung MBH.

—— and Goldner, R., 1976. Effects of streptomycin on diploid tobacco callus cultures and the isolation of resistant mutants. *Protoplasma* **89**, 83–9.

Widholm, J. M., 1977. Selection and characterization of biochemical mutants. In *Plant Tissue Culture and its Bio-technological Applications*, eds. W. Barz, E. Reinhard, and M. H. Zenk, pp. 112–22, Springer-Verlag, Berlin, Heidelberg and New York.