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ANTIBIOTIC RESISTANCE IN NICOTIANA

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INTRODUCTION

The mode of action, and mechanism of resistance of many antibiotics are known since antibiotic resistance markers are commonly used in microbial genetics. Some of them, such as streptomycin, kanamycin and chloramphenicol selectively inhibit protein synthesis on the "bacterial type" ribosomes of the chloroplasts and mitochondria. Resistance to these antibiotics is, in some cases, coded by the organellar DNA, so these mutations are convenient markers in studies on organelle segregation, recombination and function in fungi and algae[1].

The need for marker mutations in plant cell genetics, and our interest in cytoplasmic organelles, suggested to us the selection of antibiotic resistant cell lines in cell cultures of two species belonging to the genus *Nicotiana, N. tabacum and N. sylvestris.* Streptomycin, kanamycin and chloramphenicol resistant lines described in flowering plants (*N. tabacum, N. sylvestris, Petunia hybrida*) and the moss, *Physcomitrella patens,* have been reviewed[2, 3]. In the next sections some recent results on streptomycin, chloramphenicol and kanamycin resistance from our laboratory will follow.

CHLORAMPHENICOL RESISTANCE

Chloramphenicol resistant lines have not been reported in flowering plants. Three cell lines of *N. sylvestris* able to grow in the presence of inhibitory levels of chloramphenicol (5 and 10 $\mu g\ ml^{-1}$) were isolated. Two, designated CPR102 and CPR104 were

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selected after 5 months incubation of an initially haploid cell suspension culture (SH13) in liquid RMP medium[4] supplemented with 10 µg ml^{-1} chloramphenicol. The third, CPR105 was selected from an SH13 callus culture on RMP medium containing the same level of chloramphenicol. All three lines were partially inhibited by levels of chloramphenicol completely inhibitory for sensitive callus, and resistance was stable through at least two subcultures in the absence of chloramphenicol.

Plants were regenerated from all three lines, 3 from CPR102, 4 from CPR104, and 1 from CPR105. Callus initiated from the regenerated plants was tested for chloramphenicol resistance. Two of the CPR102 plants gave resistant callus, while callus from all the remaining plants was sensitive. The growth data at 10 µg ml^{-1} chloramphenicol are presented on Table 1., for one regenerant of each line only, compared to that for the three lines prior to plant regeneration.

TABLE 1
CHLORAMPHENICOL RESISTANCE TEST OF THE CELL LINES, AND CALLUS INITIATED FROM REGENERATED PLANTS.

	Callus line	Callus from regenerant
CPR102	221 ± 29	247 ± 23
CPR104	327 ± 44	60 ± 9
CPR105	305 ± 50	39 ± 7
NS	37 ± 11	45 ± 8

Resistance was tested by growth on selective (10 µg ml^{-1}) chloramphenicol medium. Values are mean fresh weight (mg ± s.e.) of 20 replicates weighted 8 weeks after initiation. NS Ts normal *N. sylvestris*. Initial mean fresh weight was 50 mg. Final values in the absence of chloramphenicol varied between 1300 (CPR105) and 2.220 mg. All tests were performed using RMP medium[4].

When the two resistant CPR102 plants flowered, selfs and reciprocal crosses with *N.sylvestris* were performed. Callus from 135 seedlings has so far been tested but only two seedling calli were clearly resistant to chloramphenicol.

STREPTOMYCIN RESISTANCE

We report here on two new results in the streptomycin resistance work: recovery of a recessive nuclear mutation which was possible because haploids of a diploid species, *N. sylvestris* were used, and application of protoplast-derived colonies, instead of callus, for screening.

Streptomycin resistant cell lines of *N. sylvestris* have been isolated in callus cultures by their ability to proliferate on RMP medium containing 2000 µg ml^{-1} streptomycin sulphate. Streptomycin resistance of the plants regenerated from the resistant cell lines was tested by innoculating leaf selections on to RM0 medium[4] containing 500, 1000 and 2000 µg ml^{-1} streptomycin. These antibiotic concentrations severely inhibit callus growth, and the callus formed on the leaf sections is white unlike callus on drug-free medium, which is green. So far two degrees of resistance have been found. The low-resistance group forms green callus only on medium containing 500 µg ml^{-1} streptomycin sulphate whereas the high-resistance group gives green callus up to 2000 µg ml^{-1}. By further cloning callus of a "low resistance" line, SR180, plants with a high degree of resistance could be obtained.

Crosses have so far been carried out only with the SR180 regenerates which belong to the low-resistance group. Resistance in that line seems to be controlled by a recessive mendelian mutation since only selfed progeny, and not the F1, is resistant to streptomycin (Fig. 1b). The mode of inheritance in the high-resistance group remains to be seen.

A spontaneous streptomycin resistant mutant, SR201, was found in a culture derived from "diploid" (2n=4x=48) *N. tabacum* c.v. Xanthi nc. protoplasts. Resistant callus was recovered by P. Medgyesy as a green colony after plating the aggregates into RMB medium[4] containing 500 µg ml^{-1} streptomycin in which sensitive cells form white callus. Resistance in this line is inherited uniparentally (Fig. 1a).

A previously isolated kanamycin resistant line of *N. sylvestris*, KR103, was found to be cross-resistant to streptomycin[5]. Preliminary tests indicate that some of the new *N. sylvestris* isolates

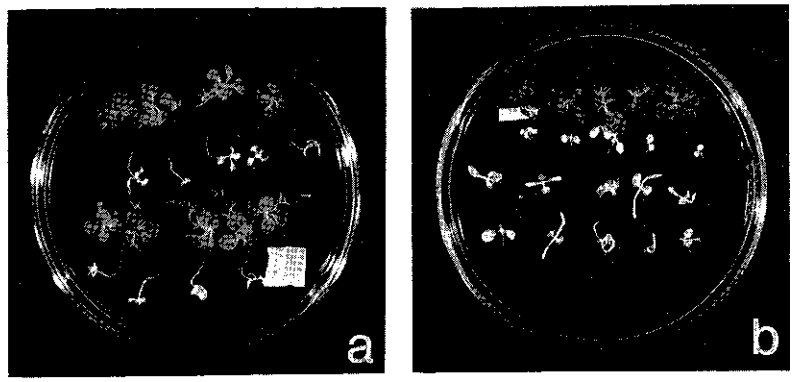

Fig. 1. Maternal (a) and mendelian (b) inheritance of streptomycin resistance in a *N. sylvestris* line, SR180 (a) and a *N.tabacum* line, SR201 (b). From the top: resistant parent, F1 (sensitive parent used as female), RF1 (resistant parent used as female) sensitive parent. Seedlings have been grown for six weeks on RM medium[6] (hormone-free) containing 1000 µg ml^{-1} streptomycin sulphate.

(e.g. SR157) are, while others (SR133, SR180) are not cross-resistant to kanamycin, and other aminoglycoside antibiotics. It is hoped that the differences in the spectrum of cross-resistances can be explored for selection in intraspecific fusion experiments.

KANAMYCIN RESISTANCE

Kanamycin resistant lines have been described[5], but this is the first report on plant regeneration from such a line.

For screening resistant lines mesophyll protoplasts were isolated from *diploid* (2n=4x=48) *N.tabacum* cv. Xanthi plants, cultured in K3 medium[7] for two weeks, and the resulting cell aggregates plated on to solid medium containing selective (50 µg ml^{-1}) concentrations of kanamycin sulphate. From two independent experiments, 39 resistant calli were isolated, all of which could be regenerated into plants on RMO medium[4]. These plants were subsequently tested for resistance.

Of the 32 lines from which regenerates have been obtained 11 were found to be sensitive (e.g. KR 227) and 19 lines to be of

intermediate resistance (e.g. KR 220) able to growth on 50 µg but not 100 µg ml^{-1} kanamycin sulphate. Lines KR 214 and KR 228 are termed "strongly" resistant since, although slow growing in general, they can be maintained at both levels of kanamycin.

Of 24 flowering regenerants, from distinct cell lines, 7 exhibit an abnormal type of flowering. This "single bud" phenotype is illustrated on Fig. 2b. A high frequency of plants with the

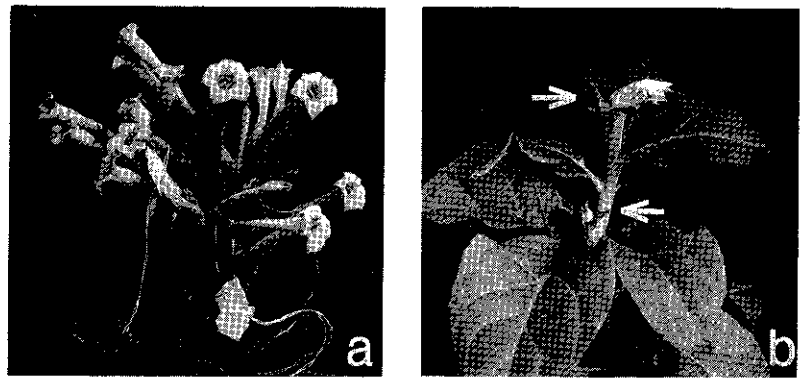

Fig. 2. Normal flowering (a) and the "single bud" phenotype (b) in *Nicotiana tabacum*.

"single bud" phenotype seems to result from the kanamycin treatment, but cannot be correlated with kanamycin resistance, since single bud plants have been regenerated from both resistant and sensitive lines.

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