IN VITRO ISOLATION OF BIOCHEMICAL MUTANTS IN HAPLOID CELL CULTURES OF NICOTIANA TABACUM

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INTRODUCTION

Biochemical mutants in higher plants have several attractions. 1. They provide genetic markers for the selection of heterokaryons in studies with somatic hybridization and transformants in studies with DNA uptake (2). 2. They provide useful material for cell biological studies. 3. Some of the mutants like the pathotoxin resistant, amino-acid-analogue resistant (3) and herbicide resis-tant (4) could be of direct applied value.

The higher plant cells present some difficulties in the selection of mutants particularly the auxotrophs (5, 6) and thus what seems to be most desirable to develop suitable techniques for isolation of mutants in a workable system. The present studies were undertaken to make an attempt to isolate auxotrophs and mutants resistant to PFP in haploid cell cultures of Nicotiana tabacum.

MATERIALS AND METHODS

Micro-colonies obtained by culturing protoplasts of androgenically obtained haploid plants of Nicotiana tabacum on liquid Ohyama and Nitsch's (7) medium were used as the source of the cells for mutant selection. Mutagen treatment to the shake cultures was offered in the doses of 0.025%, 0.05% and 0.1% with EMS and 1.0 kR, 1.5 kR and 2.5 kR with x-rays. The cells were resuspended in liquid Ohyama and Nitsch's medium after washing them in the medium. The suspension was distributed in a number of petri dishes containing liquid Ohyama and Nitsch's medium.
After six weeks colonies from those petri dishes which showed approximately 50% lethality were separated. The mutagen doses which showed about 50% lethality were 0.05% of EMS and 1.5 kR of x-rays. The 0.5% EMS treated colonies were used for isolation of auxotrophs for vitamin 'B' components, i.e., thiamine Hcl, pyridoxine Hcl, biotin, folic acid and nicotinic acid, while those treated with 1.5 kR x-rays used for isolation of mutants resistant to the amino acid analogue parafluorophenyl alanine. The procedure of selection of the two types of variants was as follows.

Selection of Auxotrophs

The EMS (0.05% treated colonies, growing on the complete Ohyama and Nitsch's liquid medium, (hereafter referred to complete medium or the CM) were individually transferred to test-tubes containing Ohyama and Nitsch's agar medium without vitamins, (hereafter referred to as the minimal medium or MM). After six weeks, the colonies which had ceased to grow were separated and individually transferred to tubes containing solid CM. When these colonies grew into callus masses each callus mass was sub-divided into a number of small parts and separately cultured on the following media:

(I) CM-thiamine Hcl referred to as thia- (II) CM-biotin, bio- (III) CM-pyridoxine Hcl, pyr- (IV) CM-nicotinic acid, NA- and (V) CM-follic acid, FA.

After 8 weeks of incubation colonies which did not show growth on these media were picked up and further tested for the requirement by plating callus pieces from them on MM and MM + the suspected requirement and comparing their growth.

The process of screening was repeated for 10 sub-cultures and only those variants which consistently exhibited growth inhibition on the deficient media while growing normally on the supplemented media were selected. The calli differentiated into plantlets, during the course of sub-cultures. Cytological examination of these plantlets was carried out by the leaf squash technique of Burns (8).

Selection of Variants Resistant To Parafluorophenyl Alanine PFP

Colonies treated with 1.5 kR x-rays were plated on Ohyama and Nitsch's medium with agar containing increasing concentrations of PFP ranging from 0 to 15 μg/ml. The colonies which showed continued growth on these doses were further carried to media containing 20 μg/ml to 50 μg/ml of PFP. Controls were also grown on media without PFP. A number of sub-cultures of those calli which showed consistent resistance were made on these media. Plantlets got differentiated during the course of these sub-cultures. Chromosome numbers of the variants showing resistant to these con-