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Short communication

# Genotypes and their response to in vitro production of haploids in $F_1$ lines of *Nicotiana tabacum*

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**Summary.** The present article reports on pollen embryogenesis resulting in haploid plants in 14 different $F_1$ lines of *Nicotiana tabacum*. The response for pollen embryogenesis was high in cultures where the $F_1$ lines were derived from mutants. Present observations are in agreement with previous reports on *Secale cereale* where one of the parents is from a mutant $M_{II}$. These observations indicate that plant genotype is the main factor for differences in the induction of pollen embryogenesis.

**Key words:** Anther culture – *Nicotiana* – Haploids – Genotype – Mutants

## Introduction

The demonstration by Guha and Maheshwari (1964) that immature anthers of *Datura innoxia* when cultured in artificial nutritive media can give rise to plantlets with haploid numbers of chromosomes opened up a new approach to the induction of haploidy in plants.

This technique was later effectively used in different crops *Nicotiana* (Bourgin and Nitsch 1967; Nitsch 1969; Melchers and Labib 1970), *Oryza* (Niizekii and Oono 1968; Guha et al. 1970) and *Brassica* (Kameya and Hinata 1970). In *Oryza sativa*, Guha (1973) observed that differences in response were dependent on the variety used.

The objectives of the present research were to obtain haploids from selected $F_1$ lines of *N. tabacum* varieties and to isolate the fertile dihaploids with desirable characters for better yield and quality. During these investigations observations were recorded for response of $F_1$ lines of different genetic makeup to pollen embryogenesis.

## Materials and methods

The $F_1$ lines from 14 hybrid combinations were used. All the above lines were grown in the field. Selected plants from each line were taken for flower bud collection. The approximate size of the flower bud where the first pollen mitosis takes place as per Nitsch (1969) was confirmed by the proprianocormine smear method. Fifty flower buds were collected from each line between the 10th and 25th day after the first flower opening. The flower buds were surface sterilized following the procedure of Yeoman and MaCleod (1977).

*Medium*

For all cultures the medium used was that of Nitsch (1969), with the pH adjusted to 5.8. The cultures were first kept at $25 \pm 2$ °C in darkness for 7 days and then subsequently transfered to light conditions (photoperiod, 10 h). The number of anthers which showed plantlet formation was recorded on the 4th, 5th and 6th week after inoculation.

## Observations

Development of the embryo was observed on the 5th day; enlargement of anthers was noticed 10 days after inoculation. During the initial stages of development, pollen embryoids attach themselves to the wall of the anthers by suspensor-like outgrowths. The anther wall is apparently acting as a placentum. Later the embryoids get detached and grow independently. There was variation in the 14 $F_1$ lines studied with regard to their potential for producing plantlets (Table 1). The $F_1$ lines from mutant selections Cy 37, Cy 55, Cy 85, Cy 86, Cy 89, Cy 25 and Cy 34 gave good response.

## Discussion

One important prerequisite for the haploid method of breeding is to get a large number of plants as many are lost at different stages of maintenance. The genome

**Table 1.** Response of pollen embryogenesis in the $F_1$ lines of *Nicotiana tabacum* varieties

| No. | Line | No. of anthers inoculated | No. of anthers responding |
|---|---|---|---|
| 1. | Cy 18 (MDS × LP 54) | 250 | 40 |
| 2. | HD 19 (Sp G 28 × Mammoth) | 250 | 15 |
| 3. | Cy 25 (GSH 3 × Line 149) | 250 | 95 |
| 4. | Cy 54 (ALSF × Spl FCV) | 250 | 65 |
| 5. | Cy 34 (Spl FCV × MDS 13) | 250 | 75 |
| 6. | Cy 35 (Spl FCV × MDS 2) | 250 | 35 |
| 7. | Cy 37 (GSH 3 × ALSF) | 250 | 135 |
| 8. | Cy 40 (CP 25 × 16/103) | 250 | 70 |
| 9. | Cy 41 (CP 25 × Virginia bright) | 250 | 70 |
| 10. | Cy 55 (GSH 3 × Virginia bright) | 250 | 115 |
| 11. | Cy 86 (M 4 × Cy 77) | 250 | 135 |
| 12. | Cy 85 (M 4 × Cy 82) | 250 | 120 |
| 13. | Cy 89 (M 4 × M 3) | 250 | 145 |
| 14. | HD 16 (Sp G 28 × H 65–35) | 250 | 15 |

of the parent used for production of a $F_1$ hybrid appears to be very important in determining the subsequent success rate of anther culture. In the present study, an increase in microspore embryogenesis could be observed in hybrids where both parents were derived from mutants. Similar observations have been reported for *Secale cereale* hybrids by Wenzel et al. (1977) who increased the number of plantlets obtained by using the mutant II selection as one of the parents. In rice, Guha-Mukherjee (1973) assessed 21 rice varieties, where there was wide differences in response between the varieties for pollen embryogenesis. It was concluded that there was variation in genotypic response of varieties in the plantlet formation.

The present observations also support that genotype influences the response for pollen embryogenesis.

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