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Genetic Control of Embryo Production and Embryo Quality in Anther Culture of *Petunia*

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Summary. Anther cultures of *Petunia* lines and hybrids at different levels of heterozygosity were tested under similar conditions with the following results:

– the yield of androgenetic plants is conditionned in *Petunia* by two important distinct factors, i.e., yield of embryos and quality of these same embryos.

– yield of embryos is directly linked to the heterozygosity of the mother plant from which the anthers are harvested.

– quality of embryos is expressed as the capacity to give viable plants. This character is carried by certain lines and transmitted to their progeny. It does not appear as a monogenic factor.

– Non triploid plant production remains a rare, non-heritable event in the studied genotypes.

Key words: Androgenesis – Triploid plants – *Petunia* – Genetic control

Introduction

The theoretical and practical use of *Petunia* anther culture is strongly limited by two main factors. The first one is the low level of viable plants obtained through this technique. The second and probably the most important factor is the very high proportion of triploid plants arising from anther culture. All these triploid plants are selfed tri-haploids arising from haploid microspores (Raquin 1972, 1982; Wagner and Hess 1974). Despite a partial female fertility, their potential use is limited because of the lack of efficient pollen.

Many different approaches have been used to increase the yield of androgenesis of *Petunia:* a) determination of the optimal stage for anther culture (Engvild 1973); b) direct culture of microspores (Sangwann and Norreel 1975); c) pretreatment of the flowers at +6 °C (Malhotra and Maheswari 1977); d) selection of genotypes (Mitchell et al. 1980).

Using a defined pool of genes, our aim was double: a) to compare the influence on the androgenetic yield of two different states of the mother plant, i.e., heterozygous against androgenetic; b) to study the heritability of other ploidies than triploid plant production.

Materials and Methods

Plant Material

Plant material was developed as depicted in Figure 1. The slp_1 inbred line of *Petunia hybrida* was kindly provided by the Laboratoire de Mutagenèse de Dijon. This line is homozygous recessive for many genes, particularly for hf_1 (hypocotyl without anthocyanins). Using the technique described by Cornu and Singh (1976) a parthenogenic haploid plant was obtained. Through spontaneous chromosome doubling a strictly homozygous line slh_1, undistinguishable from slp_1, was obtained. Those lines contain *hybrida* cytoplasm.

Anther culture of a F_1 hybrid *Petunia axillaris* × *Petunia hybrida* produced a large number of triploid plants and one homozygous diploid. This was identified after genetical analysis as TL^+h_6 line. Anther culture of F_1 hybrid $TL^+h_6 \times slp_1$ resulted in another homozygous diploid plant, the PL^+d_1 line which is different from TL^+h_6 and from slp_1. Both PL^+d_1 and TL^+h_6 lines contain *axillaris* cytoplasm. For the following experiments, the hybrids used are constituted as indicated on Fig. 2.

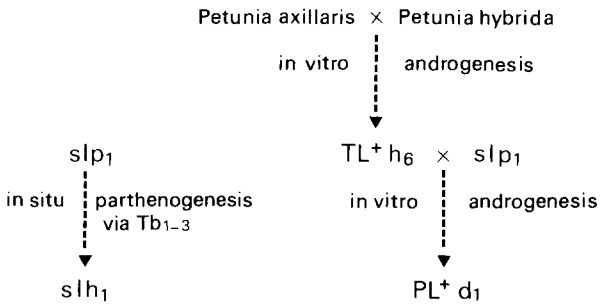

Fig. 1. Origin of the *Petunia* lines used in the experiment

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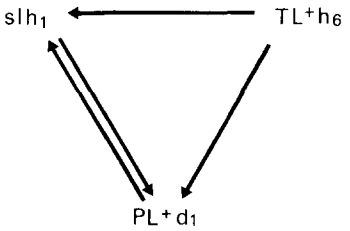

Fig. 2. Constitution of the hybrids of *Petunia* used in the experiment

The following hybrids were constituted:

$slh_1 \times TL^+h_6$
$PL^+d_1 \times TL^+h_6$
$slh_1 \times PL^+d_1$ on *hybrida* cytoplasm
$PL^+d_1 \times slh_1$ on *axillaris* cytoplasm.

PL^+d_1 contains only genes derived from slp_1 (identical to slh_1) and TL^+h_6. Therefore, the mean heterozygosity of $PL^+d_1 \times TL^+h_6$ and $slh_1 \times PL^+d_1$ or $PL^+d_1 \times slh_1$ is exactly the half of the heterozygosity of $slh_1 \times TL^+h_6$.

Culture Medium

The culture medium contained the macro nutriments of that of Murashige and Skoog (1962), only at half the original concentration, the micro nutriments from Heller's medium (1953) without $FeCl_3$, FeEDTA 10^{-4} M, the vitamins of Morel and Wetmore's medium (1951), 1 g/l meso-inositol, 0.1 mg/l α naphthalene acetic acid, 1 mg/l benzylaminopurine, 20 g/l glucose, 20 g/l sucrose, 8 g/l Bacto agar DIFCO. The pH was ajusted to 5.8 before autoclaving 20 min at 115 °C.

Anther Culture, Cytology

Flowers were harvested at the end of the first mitosis (length of corolla 8–12 mm). Sepals were eliminated and the flowers were surface-sterilized by emersion for 10 min in a solution of calcium hypochlorite (20 g/l). The sterilizing agent was then drained off but the flowers were not rinsed. This technique is very efficient for hairy plants. Anthers were plated in 60 mm Petri dishes and sealed with a PVC film "Scel-o-Frais". The culture room was regulated at 28 °C day and 24 °C night with a 16 h 1,500 lux photoperiod.

Embryos which developed directly to plantlets were transferred onto a basal medium without glucose (sucrose present), vitamins and growth substances. Young plants were transferred to the greenhouse. Chromosome numbers were counted on root tips by Feulgen staining with 5 N HCl hydrolysis, 35 minutes, at room temperature, according to the method of Itikawa and Ogura (1954).

About 5,000 anthers of each genotype were plated. Every day of culture the same number of anthers were cultivated for each genotype in order to minimize the effect of the very important but uncontrolled daily variations.

Results

Contaminations occurred more frequently in the homozygous lines than in the hybrids, particularly in slh_1 because of the easy opening of the flowers, and in TL^+h_6 which was difficult to sterilize because of its secretions.

Embryo Production

Only the successfully cultivated anthers are accounted for in this part. The results are reported in Table 1.

According to the number of embryogenic anthers, the genotypes may be classified as follows:

$slh_1 \times TL^+h_6 > PL^+d_1 \times TL^+h_6 > TL^+h_6 > PL^+d_1 \times slh_1$
$x^2 =$ 35 7,1 12,8
$\geqq slh_1, PL^+d_1, slh_1 \times PL^+d_1$
$x^2 =$ 4,9

According to the number of produced embryos, the genotypes may be be classified as follows:

$slh_1 \times TL^+h_6 > PL^+d_1 \times TL^+h_6 > TL^+h_6 > PL^+d_1 \times slh_1$
$x^2 =$ 49 22 6,7
$> PL^+d_1, slh_1 \times PL^+d_1, slh_1$
$x^2 =$ 7,7

These two classifications are practically the same. Embryo production of the most heterozygous hybrids (8.75%) is superior to the mean of embryo production of the other hybrids (3.48%), which is superior to the mean production of the starting lines (2.02%). Genotypes containing the TL^+h_6 parent are superior to the

Table 1. Genetic control of androgenetic yield in *Petunia:* a) embryo production

Genotypes	No. of anthers cultivated	Embryogenic anthers		Embryo production	
		No.	%	No.	%
slh_1	4,170	49	1.17	59	1.41
PL^+d_1	4,695	48	1.02	70	1.49
TL^+h_6	4,440	114	2.57	140	3.15
$slh_1 \times TL^+H_6$	4,845	297	6.13	424	8.75
$PL^+d_1 \times TL^+h_6$	4,710	167	3.54	241	5.12
$slh_1 \times PL^+d_1$	4,860	48	0.99	69	1.42
$PL^+d_1 \times slh_1$	4,605	70	1.52	105	2.28

Table 2. Genetic control of androgenetic yield in *Petunia:* b) embryo quality

Genotypes	No. of embryos	Viable plants obtained	
		No.	%
slh_1	59	4	6.8
PL^+d_1	70	2	2.9
TL^+h_6	140	30	21.4
$slh_1 \times TL^+h_6$	424	43	10.1
$PL^+d_1 \times TL^+h_6$	241	37	15.4
$slh_1 \times PL^+d_1$	69	4	5.8
$PL^+d_1 \times slh_1$	105	3	2.9

others, but amongst the TL^+h_6 genotypes the most important factor for embryo production is the heterozygosity of the hybrid.

$slh_1 \times TL^+h_6 > PL^+d_1 \times TL^+h_6 > TL^+h_6$.

Quality of Embryos

Embryos were harvested twice a week. All the embryos which did not turn brown or undergo proliferation into calluses were transferred to the basal medium. Results are reported on Table 2.

These results are quite different from those concerning embryo production. The genotypes may be classified as follows:

TL^+h_6, $PL^+d_1 \times TL^+h_6 > slh_1 \times TL^+h_6$, slh_1,
$x^2 =$ 3.94
$PL^+d_1 \times slh_1$, PL^+d_1, $slh_1 \times PL^+d_1$

When genotypes are grouped, it appears that: a) there is no significant difference for embryo quality between hybrids and lines $x^2 = 1.79$; b) there is a highly significant difference between the genotypes containing TL^+h_6 and the others $x^2 = 20.18$

Ploidy Level of Androgenetic Plants

The results are reported in Table 3. 87% of the plants are triploids. The production of plants at a different level of ploidy remains rare, even if the embryos come from such spontaneous diploid androgenetic plants as PL^+d_1, TL^+h_6, or from their hybrid.

Total Balance

If the total androgenetic yield is considered as

$$\frac{\text{no of viable plants}}{\text{no of cultivated anthers}},$$

the following classification may be proposed:

$slh_1 \times TL^+h_6$, $PL^+d_1 \times TL^+h_6$, $TL^+h_6 > slh_1$,
$x^2 =$ 17
$slh_1 \times PL^+d_1$, $PL^+d_1 \times slh_1$, PL^+d_1

Table 3. Genetic control of androgenetic yield in *Petunia:* c) ploidy level of viable plants

Genotypes	No. of viables plants	N	2N	3N	4N
slh_1	4		1		3
PL^+d_1	2			2	
TL^+h_6	30	2	3	24	1
$slh_1 \times TL^+h_6$	43	1	2	40	
$PL^+d_1 \times TL^+h$	37		2	35	
$slh_1 \times PL^+d_1$	4			4	
$PL^+d_1 \times slh_1$	3			3	

There is a significant difference between hybrids and lines $x^2 = 7.6$. There is a highly significant difference between genotypes containing TL^+h_6 and the others $x^2 = 108$. The two classifications are not independant.

Discussion

The main feature that arises from this study is evidence of two factors which have been shown to relate to the success of androgenesis with *Petunia*, i.e.

$$\frac{\text{no of viable plants obtained}}{\text{no of cultivated anthers}}:$$

1) embryo production $= \dfrac{\text{no of embryos produced}}{\text{no of cultivated anthers}}$

2) quality of embryos $= \dfrac{\text{no of viable plants obtained}}{\text{no of embryos produced}}$.

Embryo production seems to be essentially under sporophytic control. The most important fact resulting from our analysis of this defined gene pool is the heterozygosity of the anther donor plant. The presence of a better nurse effect of the anther wall in the hybrid genotypes may explain this observation. The importance of the conditioning brought about by the somatic tissue of Tobacco and *Petunia* in the androgenetic process has been established by Pelletier and Ilami (1972). In addition to the importance of heterozygosity, a favourable factor for embryo production is inherited by the TL^+h_6 line.

There is a slight but significant difference between reciprocal hybrids. This may be due to an inherent advantage of the *axillaris* cytoplasm. This result is similar to those reported by Mitchell et al. (1980).

As demonstrated by Wenzel and Uhrig (1981) on potato, the regeneration capacity of microspores depends upon the genotype and can be inherited. In our experiment, the quality of the embryos is inherited by the TL^+h_6 line.

If this character is monogenic we should find: a) $PL^+d_1 = TL^+h_6$ (actually they were found to be different) or b) $PL^+d_1 = slh_1$ (this is not coherent with $PL^+d_1 \times TL^+h_6 > slh_1 \times TL^+h_6$).

Therefore, this character is probably not monogenic.

Experiments have to be carried on in order to state if embryo quality is under sporophytic or gametophytic control. In contrary to one of the hypotheses of Sibi et al. (1979), it seems as if there is no contradiction between a high embryo production and a good quality of embryos. In this experiment both favourable factors are carried by the TL^+h_6 line.

Until now the production of nontriploid plants has remained a rare event in all genotypes of *Petunia* studied. Moreover, our results show clearly that androgenetic non-triploid plant production is not an heritable event. $PL^{+}d_1$ and $TL^{+}h_6$ derived from spontaneous diploid androgenetic plants as well as their hybrids give mostly triploids through androgenesis.

Inside a defined gene pool, the genetic control of embryo production and embryo quality has been estimated. The heterozygosity of the mother plant and its ability to give a good embryo quality must be taken in account if a high androgenetic yield is required. This is especially true for plant breeding. It remains to establish their relative importance compared with the other genetical and physiological conditions required for androgenesis and also to see if this condition applies to other species.

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