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In vitro multiplication of *Saintpaulia ionantha* Wendl. by homogenization of tissue cultures

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ABSTRACT

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A homogenization technique was developed for the multiplication of *Saintpaulia ionantha*. Petals from unopened flower buds were used as initial explants, which were homogenized with a blender in distilled water after the formation of adventitious shoots. The homogenate was plated on solidified Murashige and Skoog (MS) medium where it formed clumps with multiple shoots. The effect of benzylaminopurine and α-naphthalene acetic acid, in the initial and plating media, on shoot production was studied. Five commercial cultivars tested were easily multiplied, transferred to soil and grown to flowering plants. The frequencies of 'off-type' plants observed were between 0 and 4%.

Keywords: homogenization; in vitro; micropropagation; *Saintpaulia ionantha*.

Abbreviations: BA = 6-benzylaminopurine; MS = Murashige and Skoog (1962); NAA = α-naphthalene acetic acid.

INTRODUCTION

Methods of micropropagation have become economically important for the rapid multiplication of selected clones and disease-free material of many horticultural species. Most economically acceptable methods for the micropropagation of higher plants are based on the capacity of initial explants to form clumps of multiple shoots, which are subsequently divided manually several times to obtain enough shoots before re-establishment in soil. These methods have been employed successfully for the production of *Saintpaulia ionantha* (Grunewaldt, 1976; Start and Cumming, 1976; Cooke, 1977; Vasquez et al., 1977; Bilkey et al., 1978; Reist and Lê, 1987; Schulze, 1988). The methods are generally labour intensive and, therefore, relatively expensive. The intro-

duction of methods suitable for mechanization, in order to reduce the cost of labour in micropropagation would, therefore, be desirable.

For several species of ferns, methods have been established for low-cost multiplication in vitro through the homogenization of in vitro established tissues, followed by regeneration of multiple plants from the homogenate plated on solid media (Cooke, 1979; Janssens and Sepelie, 1989; Fernandez et al., 1990). Encouraged by these achievements, we have investigated the possibility of employing homogenization in vitro for the cloning of an angiosperm, the African violet.

MATERIALS AND METHODS

The study includes five commercial African violet (*Saintpaulia ionantha* Wendl.) cultivars (Holtkamp Nos. 19, 49, 69, 282 and 283). In an initial experiment, another species, *Saintpaulia intermedia*, was used for establishing the technique. Petals were used as the initial explant because of their low contamination with bacteria and fungi, as compared to other plant parts.

For all experiments, autoclaved MS medium (Murashige and Skoog, 1962) solidified with 0.3% gelrite (Kelco), and containing 3% sucrose and different concentrations of benzylaminopurine (BA) and α-naphthalene acetic acid (NAA), was used.

The cultures were incubated at 23 ± 1 °C, under a 16 h photoperiod and irradiation of 15 W m^{-2} provided by Osram Cool-white.

Unopened flower buds (5 mm long) were surface sterilized in 70% ethanol containing 0.01% Tween-20™ for 30 s, followed by immersion in 3% Korsolin™ (aldehyde product, Ferrosan, Copenhagen) for 15 min. After rinsing three times in sterile water, the petals were excised and cultured in 10 cm Petri dishes. After 8 weeks of culture, the petals were covered with adventitious shoots. For homogenization, 2.0 g of this plant material were transferred to a 25 mm test tube containing 20 ml of sterilized distilled water. An Ultra-Turrax™ homogenizer (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) equipped with a S 25-18 G shaft was used. The standard rotor with two teeth was compared to a special rotor in which the teeth had been substituted by replaceable scalpel blades. The latter rotor was constructed at this laboratory (Fig. 1). Homogenization was carried out at 24 000 rev min^{-1} for 5 s and timed automatically by the use of an exposure timer. A pipette was used for transferring 1 ml of the homogenate to each 10 cm Petri dish. The homogenate was spread over the surface of the medium using a Drigalski spatula.

Numbers of clumps and shoots in each Petri dish were recorded 10 weeks after plating the homogenate and $\log_e(x+1)$ transformed before analysis of variance. Residuals were checked by graphical means.

Shoots were transferred to soil 3 months after homogenization and accli-

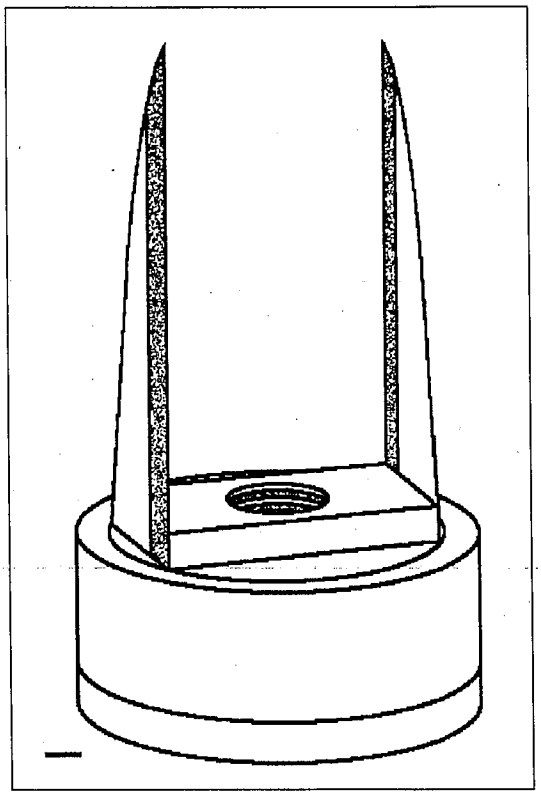

Fig. 1. Homogenization rotor in which the standard teeth have been substituted by replaceable scalpel blades (bar = 1 mm).

matized to greenhouse conditions under plastic cover. The flowering plants were examined for 'off-type' plants after another 6 months by comparing flower, leaf and whole-plant morphology.

RESULTS

The initial experiment with *S. intermedia*, comparing homogenization with a standard rotor and scalpel blade rotor, showed homogenates prepared with the latter rotor to be superior for both initial clump formation and final shoot formation (Table 1). While the homogenates prepared using the standard rotor produced an average of 26.5 initial clumps and 11.7 shoots per dish, the homogenates prepared with the scalpel rotor yielded 91.8 initial clumps and 63.7 final shoots per dish. The differences in clump and shoot formation were shown to be highly significant following *F*-test from analysis of variance. Therefore, the scalpel blade rotor was used in all subsequent experiments.

TABLE 1

Effect of the type of rotor used for homogenization of *S. intermedia* cultures on the numbers of clumps and shoots formed per Petri dish using MS medium with 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA

	Standard rotor		Scalpel rotor	
	Clumps	Shoots	Clumps	Shoots
	24	15	82	59
	20	7	113	68
	44	25	88	56
	33	14	82	58
	23	4	72	49
	22	6	100	82
	17	9	99	78
	25	6	82	54
	27	16	108	69
Mean	26.5	11.7	92.2***	65.4***

***$P<0.001$.

TABLE 2

Analysis of variance for number of clumps and shoots per Petri dish after homogenization of *S. ionantha*

Factor	Degrees of freedom	Mean squares	
		Clumps	Shoots
Genotype (G)	4	1.5***	6.5***
Initial medium (I)	2	18.6***	33.9***
Plating medium (P)	3	24.8***	21.2***
I×P	6	0.6*	1.6*
G×I	8	0.4	6.3***
G×P	12	0.6**	1.9**
Error	112	0.2	0.7

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

The effect of NAA and BA in the medium on which the plant material is grown prior to homogenization (initial medium), and the effect of the same hormones in the medium on which the homogenates are plated (plating medium), were studied using three different initial media, four different plating media and five different genotypes of *S. ionantha* in a 3×4×5 factorial experiment, replicated three times. The analysis of variance (Table 2) showed highly significant effects of both genotype and initial and plating media on both initial clump formation and final shoot formation. For the formation of shoots, there was a highly significant interaction between genotype and initial media, whereas for the formation of clumps this interaction was not signifi-

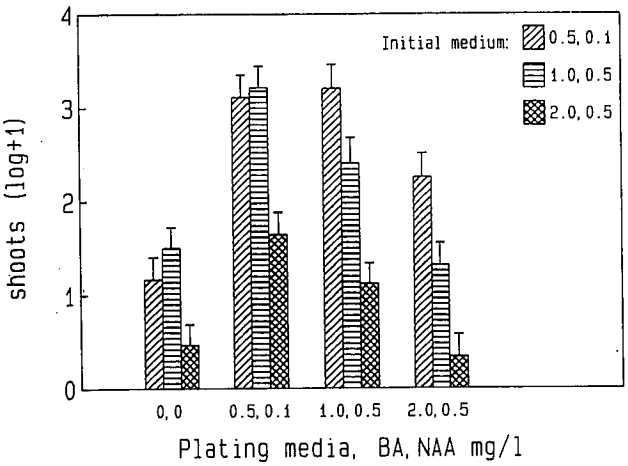

Fig. 2. Effect of growth hormones in the initial and plating media for shoot formation from homogenates of *S. ionantha*.

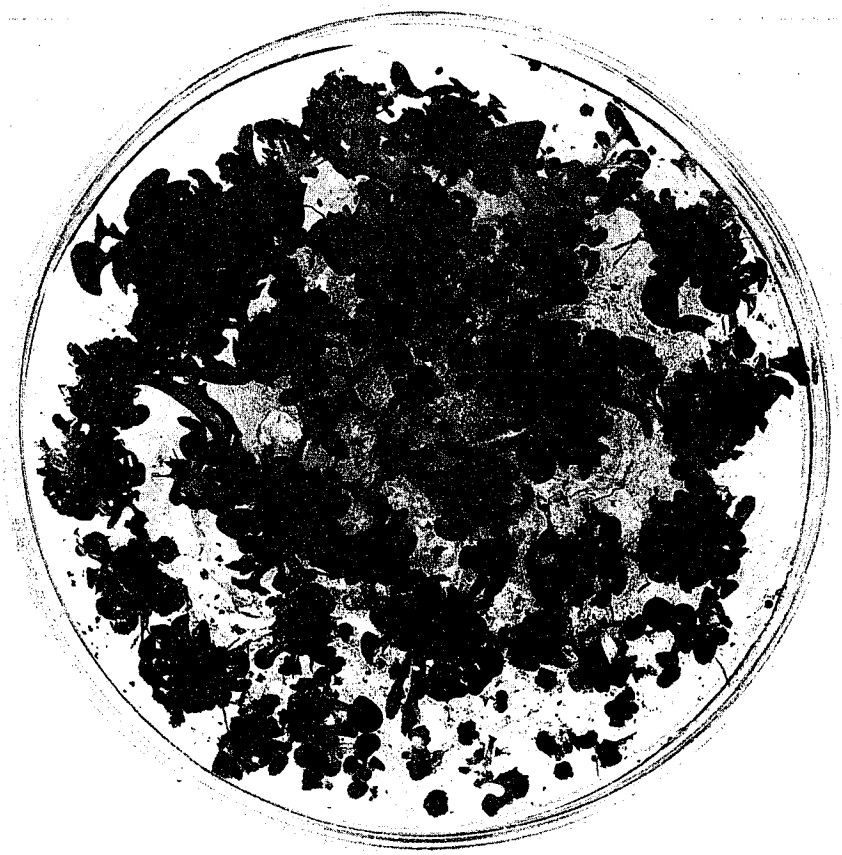

Fig. 3. Petri dish (10 cm) with shoots formed after homogenization of *S. ionantha* tissue culture.

TABLE 3

Observed number of shoots per 10 cm Petri dish 10 weeks after homogenization of *S. ionantha*. Genotype versus all combinations of initial and plating media, means of three replicates

Media (BA, NAA mg l^{-1})		Genotype					Mean
Initial	Plating	19	49	69	282	283	
0.5,0.1	0,0	38	1	2	1	0	10
0.5,0.1	0.5,0.1	248	30	19	23	2	57
0.5,0.1	1.0,0.5	136	33	26	17	0	43
0.5,0.1	2.0,0.5	54	19	8	6	4	14
1.0,0.5	0,0	10	4	1	3	9	5
1.0,0.5	0.5,0.1	152	27	12	11	15	48
1.0,0.5	1.0,0.5	–[1]	9	20	2	31	15
1.0,0.5	2.0,0.5	4	19	2	0	2	6
2.0,0.5	0,0	1	0	3	0	3	1
2.0,0.5	0.5,0.1	2	25	1	7	6	8
2.0,0.5	1.0,0.5	3	0	7	1	8	4
2.0,0.5	2.0,0.5	2	2	0	0	0	1
Mean of genotype		53	14	8	6	8	18

[1]Missing value.

cant. There were less significant interactions between genotype and plating media, and between initial media and plating media, for both clump and shoot formation.

The main effects of initial and plating media are shown in Fig. 2. The optimum combinations of hormones were 0.5 mg l^{-1} BA+0.1 mg l^{-1} NAA and 1.0 mg l^{-1} BA+0.5 mg l^{-1} NAA in the initial and plating medium. The absence of both hormones from the plating medium severely restricted shoot formation, as did increased concentrations of hormones.

The best combination of initial and plating media (0.5 mg l^{-1} BA+0.1 mg l^{-1} NAA in both) produced on average 57 shoots per Petri dish (Fig. 3) 10 weeks after plating (it should be noted that this combination is not significantly different from initial medium containing 0.5 mg l^{-1} BA+0.1 mg l^{-1} NAA in combination with 1.0 mg l^{-1} BA+0.5 mg l^{-1} NAA in the plating medium, or vice versa). As can be seen in Table 3, the number of shoots per Petri dish varied considerably among the genotypes, No. 19 being absolutely superior and No. 283 showing a somewhat divergent response. The shoots initiated rooting on the plating medium and were easily transferred to soil under greenhouse conditions directly from the culture plates. Frequencies of successful transplantation were above 95%.

Frequencies of 'off-type' plants (flower, leaf and whole-plant morphology) observed at the flowering stage were between 0 and 4% (Table 4). Genotype

TABLE 4

Frequencies of 'off-type' plants (%, numbers in parentheses) raised from homogenized cultures of *S. ionantha*

Genotype No.	Number of plants	Frequency of 'off-type' types for		
		Flowers		Leaf shape
		Colour	Double	
19	100	0	1 (1)	0
49	97	1 (1)	1 (1)	0
69	86	0	0	0
282	69	1 (1)	1 (1)	4 (3)
283	75	1 (1)	0	0

69 did not show any off-types among the 86 plants transferred to soil. This could be an indication of different stability of the genotypes in this multiplication procedure.

DISCUSSION

This is the first report of adventitious shoots being formed directly from homogenized tissue of an angiosperm. The experiment shows that shoot formation from the homogenates can be obtained reproducibly using the present method with a number of widely used cultivars of *S. ionantha*. However, the number of shoots produced strongly depends on the hormone concentrations in both the initial and the plating medium, and further studies are needed to establish the optimum concentrations and combinations of these hormones. The interactions between genotype and media observed indicate that the optimum concentrations are dependent on the genotype. It may still be possible, however, to construct a universal medium generally acceptable for all genotypes of *S. ionantha*.

The most expensive factor in traditional micropropagation is manual labour for the division and subculture of the tissue cultures. It has been estimated that 70–80% of the cost price of micropropagated plants is labour related (Pierik, 1988; Schulze, 1988). Somatic embryogenesis induced in cell suspensions is a possibility for reducing labour expenses for large-scale plant multiplication since the handling of suspensions may be automated. Reproducible methods for induced embryogenesis, however, are still restricted to species or genotypes within species and the cell cultures may change during initial multiplication, leading to large numbers of 'off-type' plants. The homogenization technique, if properly worked out, could be another possibility

for reducing the costs of micropropagation of *S. ionantha* and perhaps other species. Furthermore, the method has potential for automation. The small scale of this experiment does not permit any firm conclusions about the level of induced variation by the homogenization technique, but the percentages of deviations observed seem to be generally comparable with the variation induced by traditional propagation methods.

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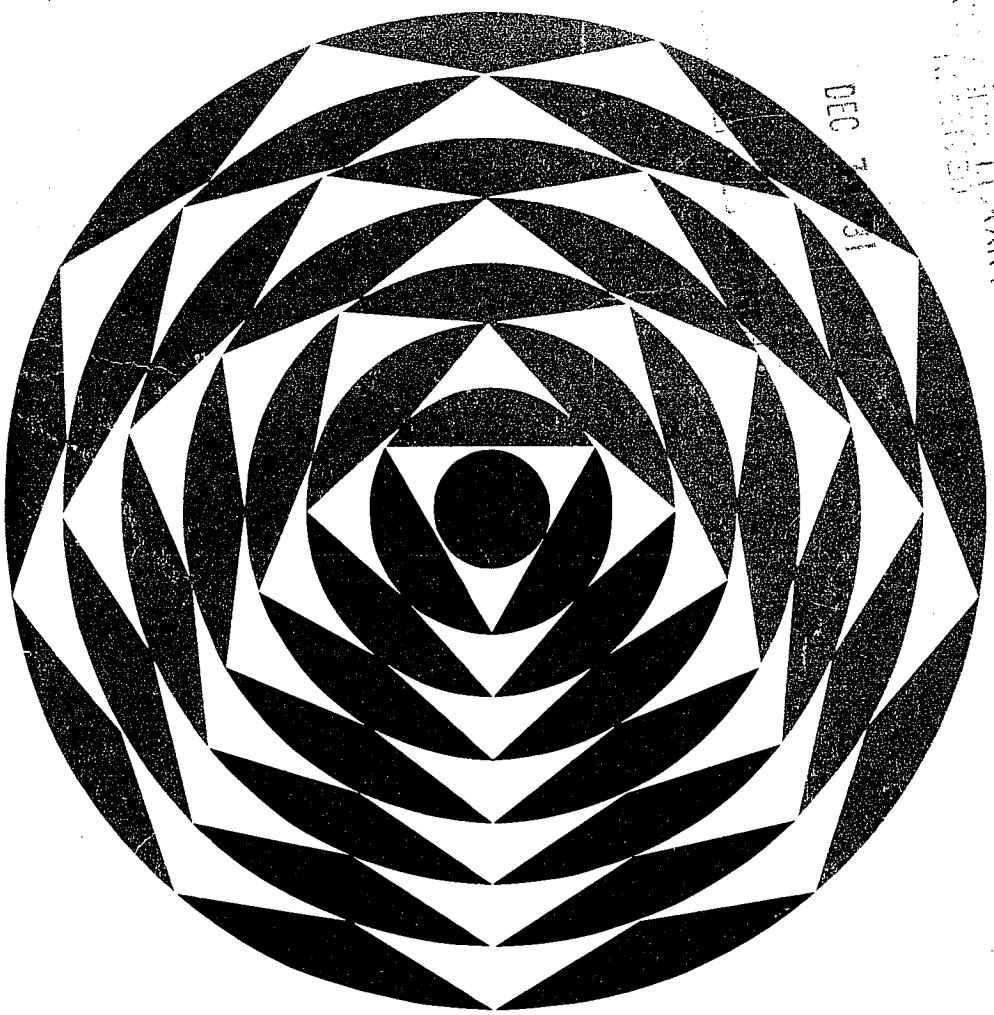

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