J. Biosci·, Vol. 22, Number 5, December 1997, pp 585–592. © Printed in India.

Micropropagation of selected somaclones of *Begonia* and *Saintpaulia*

S MOHAN JAIN*
University of Helsinki, Plant Production Department, FIN 00014, Box 27, Helsinki, Finland

MS received 17 June 1996; revised 5 May 1997

Abstract. ***Begonia* x *elatior* plantlets which regenerated from leaf disk callus showed variations in plant morphology, number of flowers per plant, and flower size. Variations in flowering period, number of flowers per plant, and flower morphology were observed in *Saintpaulia ionantha* L. plants directly regenerated from leaf disk explants. The cytokinins, benzylaminopurine and zeatin, tested in the culture medium did not affect the basic plant characteristics including flower colour which remained stable in both species. Micropropagation of selected somaclones having the desirable trait of high number of flowers per plant was stable in the MV2 and MV3 generations.**

Keywords. Somaclonal variation; *Begonia; Saintpaulia;* cytokinins; auxins; plant morphology; flower morphology.

1. Introduction

Somaclonal variation is wide spread among tissue culture-derived regenerants (Jain 1993a, b, 1997a, b; Jain *et al* 1997a, b; Nehra *et al* 1992). It is generally attributed to pre-existing genetic variation in somatic cells (Walbot 1985), single gene mutations aneuploidy and transposable elements (Jain *et al* 1997b). The extent of variation depends on genotype, age of the donor plant, cytogenetic changes, DNA methylation, explant type and plant hormones in the culture medium (Evans and Sharp 1983; Jain and Newton 1990; Jain and Pehu 1992; Puolimatka 1993; Arnholdt-Schmitt 1995; Arnholdt-Schmitt *et al* 1995; Gupta 1997; Jain *et al* 1997a). Unlike epigenetic changes, somaclonal variation which results from altered gene expression is usually irreversible (Karp 1991, 1995). The segregation pattern of mutations in the progeny is mostly Mendelian (Larkin *et al* 1984). Only alteration in the genetic information would give rise to genetically stable lines (Jain 1997a). Non-genetic changes (epigenetic) appear more frequently, and are reversible and predictable (De Klerk *et al* 1990; Jain 1997b). Plant regeneration via organogenesis or protoplasts often leads to more somaclonal variations (Jain and Newton 1988, 1989; Karp 1989, 1995); the length of culture period also influences somaclonal variation (Nehra *et al* 1992).

This study was undertaken to investigate the influence of two cytokinins on somaclonal variation in the ornamental plants *Begonia* x *elatior* and *Saintpaulia ionantha* L. The somaclones selected for desirable traits were micropropagated to study the stability of the chosen traits.

*Fax, 358-9-708 5582, Email, Mohan .jain@helsinki.fi.

2. Materials and methods

The parental micropropagated plants of both *Begonia* x *elatior* and *Saintpaulia ionantha* were carefully selected for uniformity in morphology· Excised leaves were sterlized and leaf disks were cut and cultured according to Jain (1993a). Data were collected on commercially important traits in the regenerated plants, grown in the greenhouse.

Leaf disks (diameter 5 mm) from well-expanded leaves of greenhouse-grown plants were cultured on MS medium (Murashige and Skoog 1962) supplemented with different concentrations of cytokinins and auxins. For callus production in *Begonia*, 0 60 μM naphthalene acetic acid (NAA), 1·0 μM 2,4-dichlorophenoxy acetic acid (2,4-D), and 0·50 μM benzylaminopurine (BA) were added in the MS medium. *Begonia* callus was subcultured five times on this fresh medium every 4-weeks. The shoots regenerated from the callus were rooted. In *Saintpaulia* direct shoot induction (i.e., without preceded by callus phase) was achieved by culturing leaf disks on MS medium containing different concentrations of BA (table 2). The shoots were maintained on MS + 0·50 μM BA under 35 $\mu molmm^{-2}$ s^{-1} light intensity at 25°C. Well-developed 3 to 4-week-old shoots of both plants were rooted on MS medium + 0·60 μm NAA at 25°C under 42 $\mu molm^{-2}$ s^{-1} light intensity for *Begonia* and 35 $\mu molm^{-2}$ s^{-1} light intensity for *Saintpaulia*· Initially all the plantlets were grown in plastic trays filled with sterile soil, under greenhouse conditions, and data collected on commercial traits, namely flowering time, number of flowers per plants, and flower size.

The cytokinin influence on somaclonal variation was studied by selecting BA for *Begonia* and kinetin and zeatin for *Saintpaulia.*

A total of 200 plantlets of *Begonia* on each MS-I and MS-II medium, and 500 of *Saintpaulia* plantlets on each MS-SII and MS-SIII medium were regenerated, and planted in plastic trays filled with sterile soil and maintained in the greenhouse for data collection on the height of plants at flowering, number of flowers per plant, and flower size. The selected somaclones with desirable traits (MV0) underwent four successive cycles of micropropagation by direct shoot formation from leaf disks. In each micropropagation cycle shoot cultures were subcultured five times on fresh shoot regeneration medium (for details see Jain 1993a). Different characters (in MV0 generation, as a control) of *Begonia* and *Saintpaulia* plantlets were evaluated in response to different levels of cytokinins in the culture medium during subsequent generations. In the first micropropagated generation (MV1) selected *Saintpaulia* somaclones were multiplied on MS medium +0·20 μM BA (MS-S), whereas multiplication of selected *Begonia* somaclones was done on MS medium + with 5·0 μM kinetin (MS-B). Of each ornamental species 200 somaclones each in MV1 generation were evaluated. In MV2 and MV3 generations, two selected somaclones of each plant were studied for the stability of their traits—*Begonia* somaclones 190 and 250 regenerated on MS-I medium, somaclones 20 and 30 regenerated on MS-II medium; *Saintpaulia* somaclones WV 28 and WV 186 regenerated on MS-SII medium, and somaclones UV 50 and UV 60 regenerated on MS-SIII medium.

For the control plants (MV0), 100 micropropagated plants (originally developed by direct shoot induction from leaf disks) of *Begonia* on MS medium + 5·0 μM kinetin and *Saintpaulia* on MS medium + 0·2 μM BA were used. For details see Jain (1993a). All possible care was taken to select uniform control plants so that somaclonal variation could be studied in the regenerated plants of both the species.

The mean and the standard deviation (SD) were calculated for assessing the somaclonal variation in MV1 to MV3 generations.

3. Results and discussion

3·1 *Begonia*

Of the concentrations of kinetin and zeatin tested, 5·0 μM kinetin + 0·5 μM zeatin induced multiple shoots in 84% callus cultures, with an average of eight shoots per callus (table 1). On medium MS-I, the highest number of shoots (8 per explant) differentiated, whereas on MS-II medium six shoots per explant differentiated. Different concentrations of the two used cytokinins used in MS-I and MS-II culture media did effect qualitative differences among the regenerants; however, the number of variants obtained on MS-I and MS-II differed (table 2). None of the regenerants showed any variation in pink flower colour; there were no albino somaclones either. However, frequent variation was noticed among noncommercial traits such as leaf variegation, non-flowering, dwarfing of plants, and slow growth (data not given). Usually, the slow growing regenerants failed to flower.

3·2 *Micropropagation of selected somaclones*

The mean of MV1 flower number in somaclones of MV1 generation was the highest for somaclone 190 (table 3). In the MV2 and M V3 generations, the flower number per plant did not vary drastically, and seemed to be stable within two micropropagation cycles regardless of the culture medium used. But the extent of variation in the two generations is reflected in the SD values.

3·3 *Saintpaulia*

On MS-SII medium, the highest number of leaf disks formed shoots (table 4). Multiple shoots differentiated on the entire leaf disk within 3-4 weeks from culture. The somaclones regenerated on MS-SII and MS-SIII media showed variations in commercially important traits such as flowering period, number of flowers per plant, flower

Table 1. Shoot differentiation from leaf disk callus of *Begonia* on MS + cytokinin Culture period: 4 week interval.

Cytokinin concentration (μM)	No. of callus subcultures	Total No. of shoots regenerated	Average No. of shoots/culture (SE)
Kinetin			
2·5	50	30	4(0·40)
5·0	50	35	5(0·38)
Zeatin			
0·5	50	35	5(0·37)
1·0	50	28	3(0·38)
Kinetin + Zeatin			
5·0 + 0·5	50	42	8(0·28)
5·0 + 1·0	50	37	6(0·40)

Table 2. Somaclonal variation (MV1) in *Begonia* plantlets regenerated on different shoot culture media.

	Culture medium			
	MS-I		MS-II	
Plant characterestic	Mean	No. of variants	Mean	No. of variants
Height at flowering (cm)				
Control	15	4	15	4
MV1	24	4	24	4
	18	114	18	100
	12	62	12	96
	7	20	—	—
No. of flowers/plant				
Control	20	5	20	5
MV1	53	4	—	—
	46	16	46	24
	36	30	36	60
	24	10	30	20
	12	16	20	70
	5	66	15	26
Flower diameter (MM)				
Control	50	3	50	3
MV1	60	6	60	4
	50	130	50	120
	38	44	40	68
	30	20	35	12

—, Absent

Table.3 Number of flowers per plant in selected somaclones of *Begonia* in the MV2 and MV3 generations.

	Number of flowers/plant			
	Plant regeneration medium			
Generation	MSI-190	MSI-250	MSII-20	MSII-30
MV1				
Mean	54·99	40·00	44·00	33·00
MV2				
Mean	53·48	40·82	44·52	34·00
SD	2·12	1·72	2·19	1·95
MV3				
Mean	53·92	40·98	40·35	34·38
SD	1·92	2·19	3·95	1·72

size, and number of petals per flower (table 5). *Saintpaulia* somaclones also did not differ in the quality of the traits on either culture medium tested, however, the differences were found in flowering period, number of flowers per plant, and flower size (table 5). The number of petals per flower remained uneffected. The somaclones that failed to flower

Table 4. Influence of BA on shoot regeneration from leaf disks of *Saintpaulia* on MS medium. Culture period: 4 weeks.

Medium number (MS-S)	BA concentration (μM)	No. of leaf disks cultured	No. leaf disks which formed shoots	Average No. of shoots/leaf disk (SE)
I	0·05	60	52	32(0·293)
II	0·22	60	50	35(0·245)
III	0·50	60	45	40(0·195)
IV	2·50	60	39	32(0·279)
V	5·00	60	32	22(0·263)
VI	10·00	60	27	13(0·418)

Table 5. Variation (M V 1) in *Saintpaulia* somaclones. A total of 500 somaclones were evaluated to compare with 100 control plants in the greenhouse.

	Medium MS-SII		Medium MS-SIII	
Plant characteristic	Mean	No. of variants observed	Mean	No. of variants observed
Flowering time				
Control	85	6	85	6
MV1	78	38	80	55
	83	108	83	—
	87	85	88	165
	91	65	92	65
	96	124	98	148
	102	70	104	52
	109	10	115	15
Number of flowers/plant				
Control	15	3	15	3
MV1	3	72	3	100
	7	132	7	80
	12	102	12	—
	17	120	17	188
	22	55	22	72
	27	15	27	50
	31	2	30	10
Flower diameter (in mm)				
Control	24	2	24	2
MV1	22	42	20	21
	26	302	26	248
	30	130	30	215
	32	26	32	16
Number of petals/flower				
Control	5	0	5	0
MV1	5	480	5	465
	6	14	6	35
	7	6	7	—

—, Absent

Table 6. Number of flowers per plant in *Saintpaulia* selected somaclones of MV2 and MV3 generations, grown in MS-SII and MS-SIII medium.

	Number of flowers/plant			
	Somaclones			
Generation	WV 28	WV 186	UV 50	UV 60
MV1				
Mean	22·00	35·00	30·00	27·00
MV2				
Mean	23·36	34·95	29·86	28·34
SD	1·29	0·32	0·95	2·95
MV3				
Mean	23·68	34·91	29·68	28·95
SD	0·60	0·42	1·35	3·39

were slow growing, and bore a rosette of leaves which were coriaceous, serratted and variegated, or light green. Such leaves were exclusive to the regenerants. Most of the regenerants developed rotund corolla, excepting 4% of them developed tubular corolla (which abcissed with a gentle touch). None of the regenerants showed any variation in violet flower colour.

3.4 *Micropropagation of selected somaclones*

Saintpaulia somaclones WV 186 had the highest flower number per plant regardless of the culture medium used (table 6). All the four selected somaclones showed only a minor variation in flower number per plant in the MV2 and MV3 generations (table 6). However, in the MV3 generation the SD value increased in the somaclones UV 50 and UV 60. The number of flowers per plant stabilized in the subsequent micropropagated generations. The SD value has been used for assessing the extent of somaclonal variation in micropropagated plants of *Begonia* x *hiemalis* (De Klerk *et al* 1990) and *Begonia* x *elatior* and *S· ionantha* (Jain 1993a). The SD values in MV2 and MV3 generations indicated that the variation in flower number per plant is low.

The flower colour in *Begonia* and *Saintpaulia* micropropagated regenerants was stable. The lack of variability in flower colour may be due to the type of explant and the genotype, and the concentration and the type of cytokinins used. Because no sexual crosses were performed among the somaclonal variants, recessive genetic changes in flower colour, if any, could not be detected in the micropropagated somaclonal variants. Somaclones of both species might have arisen due to epigenetic changes, which could not be determined. The different concentrations of the auxins and cytokinins tested cause variation in basic characteristics such as flower number and flower size (Jain 1993a, b). According to D'Amato (1986), it cannot be excluded that some hormones, at certain concentrations or in combination with other hormones and/ or particular constituents of a culture medium, may act as mutagens. In our studies, changes in cytokinin concentrations seem to affect the number of variants. The selected somaclones can be stabilized in the subsequent micropropagation cycles

(Jain 1993a, b). The number of flowers per micropropagated plant stabilized in the successive MV2 and MV3 generations. Because we have tested the stability of number of flowers per plant for two generations only, it is difficult to predict the number of micropropagated cycles when the genetic stability of this trait may be lost. Rancillac and Nourrisseau (1989) improved the performance of the micropropagated strawberry plants by decreasing the cytokinin concentration and limiting the number of subcultures to 10-15 cycles. The stability of the traits in the somaclones is very much dependent on whether the trait arises as a result of genetic changes rather than epigenetic changes (Jain 1997a; Jain *et al* 1997a); however, it is rather difficult to discover this in the micropropagated generations. By using molecular markers [restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), DNA amplification fingerprinting (DAF), microsatellities or short sequence repeats (SSRs)], early detection of somaclones resulting either by genetic or epigenetic changes could be accomplished (Caetano-Anolles *et al* 1992; Jack *et al* 1995; Roder *et al* 1995; Taylor *et al* 1995; Thomas *et al* 1995). Further work is progressing in this direction·

In conclusion our results suggest that it is possible to: (i) create and evaluate somaclonal variation, (ii) select desirable somaclonal variants, (iii) micropropagate the selected somaclones for their stability, and eventually (iv) develop a cultivar.

References

Arnholdt-Schmitt B 1995 Physiological aspects of genome variability in tissue culture. II. Growth phase-dependent quantitative variability of repetitive BstNl fragments of primary cultures of *Daucus carota; Theor. Appl. Genet.* **91** 816-823

Arnholdt-Schmitt B, Herterich S and Neumann K H 1995 Physiological aspects of genome variability in tissue culture. I. Growth phase-dependent differentiated DNA methylation of the carrot genome (*Daucus carota* L.); *Theor. Appl. Genet.* **91** 809-815

Caetano-Anolles G, Bassam B J and Gresshoff P M 1992 DNA amplification fingerprinting using very short arbitrary oligonucleotide primers; *Biotechnology* **9** 553-557

D,Amato F 1986 Spontaneous mutations and somaclonal variation; in: *Nuclear techniques and in vitro culture for plant improvement*, International Atomic Energy Commision, Vienna, Austria, IAEAA282/65 publication, pp 3-10

De Klerk G J, Brugge T J and Bouman H 1990 An assay to measure the extent of variation in micropropagated plants of *Begonia* x *hiemalis; Acta Bot. Neerl.* **39** 145-151

Evans D A and Sharp W R 1983 Single gene mutations in tomato plants regenerated from tissue culture; *Science* **221** 949-951

Gupta P K 1997 Cytogenetic basis of tissue culture-induced heritable variation in plants; in *Somaclonal variation and induced mutations in crop improvement* (eds) S M Jain, B S Ahloowalia and D S Brar (The Netherlands: Kluwer Academic Publications) (in press)

Jack P L, Dimitrijevic T A F and Mayes S 1995. Assessment of nuclear, mitochondrial and chloroplast RFLP markers in oil palm (*Elaeis guineensis* Jacq); *Theor· Appl. Genet*, **90** 643-649

Jain S M 1993a Somaclonal variation in *Begonia* x *elatior* and *Saintpaulia ionantha* L.; *Sci. Hortic.* **54** 221-231

Jain S M 1993b Studies on somaclonal variation in ornamental plants; *Acta Hortic.* **336** 365-372

Jain S M 1997a Creation of variability by mutation and tissue culture in improving plants; *Acta Hortic*. (in press)

Jain S M 1997b Somaclonal variation and mutagenesis for crop improvement; *Maatalouden Tutkimuskeskuksen Julkaisuja* **18** 122-133

Jain S M and Newton R J 1988 Proto-variation in protoplast derived *Brassica napus* plants; in *Progress in plant protoplast research* (eds) K J Puite, J J M Dons, H J Huizing, A J Kool, M Koornneef and F A Krens (Dordrecht: Kluwer Academic Publications) pp 403-404

Jain S M and Newton R J 1989 Evaluation of protoclonal variation versus chemically induced mutagenesis in *Brassica napus* L; *Curr. Sci.* **58** 176-180

Jain S M and Newton R J1990 Prospects of biotechnology for tea improvement; *Proc. Indian Natl. Sci. Acad.* **B56** 441-448

Jain S M and Pehu E 1992 The prospects of tissue culture and genetic engineering for strawberry improvement; *Acta Agric. Scand, Sec. B Soil and Plant Sci.* **42** 133-139

Jain S M, Buiatti M, Gimelli F and Saccardo F 1997a Somaclonal variation in improving ornamental plants; in *Somaclonal variation and induced mutation in crop improvement* (eds) S M Jain, D S Brar and B S Ahloowalia (The Netherlands: Kluwer Academic Publishers) (in press)

Jain S M, Brar D S and Ahloowalia B S (eds) 1997b *Somaclonal variation and induced mutation in crop improvement* (The Netherlands: Kluwer Academic Publishers) (in press)

Karp A 1989 Can genetic instability be controlled in plant tissue cultures?; *IAPTC Newsl.* **58** 2-11

Karp A1991 On the current understanding of somaclonal variation; in *Oxford surveys of plant molecular and cell biology* (*ed·*) B J Miflin (Oxford: University Press) vol 7, pp 1-58

Karp A 1995 Somaclonal variation as a tool for crop improvement; *Euphytica* **85** 295-302

Larkin P J, Ryan S A, Brettel R I S and Scowcroft W R 1984 Heritable somaclonal variation in wheat; *Theor. Appl. Genet.* **67** 443-455

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco callus cultures; *Physiol. Plant* **15** 473-497

Nehra N S, Kartha K K, Stusnoff C and Giles K L 1992 The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry; *Plant Cell Tissue Org. Cult.* **29** 257-268

Puolimatka M 1993 *Effects of genotype on tissue culture induced cytological and biochemical variation in outbred and inbred lines of rye* (*Secale cereale L.*), Ph. D, thesis, University of Helsinki, Finland

Rancillac N J and Nourrisseau J G 1989 Micropropagation and strawberry plant quality; *Acta Hortic.* **265** 123-128

Roder M S, Plaschke J, Konig S U, Border A, Soorrells M E and Tanksley S D 1995 Abundance, variability and chromosomal location of microsatellites in wheat; *Mol. Gen. Genet.* **246** 327-333

Taylor P W J, Geijskes J R, Ko H L, Fraser T A, Henry R J and Birch R G 1995 Sensitivity of random amplified polymorphic DNA analysis to detect genetic change in sugarcane during tissue culture; *Theor. Appl. Genet.* **90** 1169 -1173

Thomas C M, Vos P, Zabeau M, Jones D A, Norcott K A, Chadwick B P and Jones J D 1995 Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to *Cladosporium flavum; Plant J.* **8** 785-794

Walbot V 1985 On the life strategies of plants and animals; *Trends Genet.* **1** 165-169

Corresponding editor: SIPRA GUHA-MUKHERJEE