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Article *in* Plant Cell Tissue and Organ Culture · January 2004

DOI: 10.1023/B:TICU.0000009246.24216.ea

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Variations among somaclones and its seedling progeny in *Capsicum annuum*

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Received 17 May 2002; accepted in revised form 25 June 2003

Key words: paprika, somaclonal variation, two generations

Abstract

Genetic variation found in plants regenerated from any type of *in vitro* culture is termed somaclonal variation. The present study was initiated to study somaclonal variation in *Capsicum annuum* and the feasibility of using somaclonal variation for its improvement. Five genotypes of *C. annuum* were used for the present study (Round ornamental, PBC 535, PBC 375, PBC 385 and PBC 066). The morphological characters of the plants were recorded as per the IPGRI descriptor. The colour values were determined using the ASTA method. Selfed seeds of somaclones of these lines were germinated to obtain the seedling progeny of somaclones. Fifty plants of each of the lines were evaluated for their morphological, yield and colour characters. From the study, somaclonal variation was found to be genotype dependant and among the characters studied, fruit characters were most variable. There was variation in quality characters like fruit colour and it was also found that the variations were more among the seedling progeny of the somaclones.

Abbreviations: ASTA – American Spice Trade Association; BA – benzyl amino purine; IBA – indole-3-butyric acid; IPGRI – International Plant Genetic Resources Institute; MS – Murashige & Skoog

Introduction

Heritable genetic variation found in plants regenerated from any type of *in vitro* culture is termed somaclonal variation (Larkin and Scowcroft, 1981). In most cases, *in vitro* differentiation is a major cause of genetic variation (Swartz, 1991). According to De Klerk (1990), only random variations found in regenerated plants that are transmitted to the progeny through meiosis and are not reversible can be called as somaclonal variation. Such variation in callus regenerated plants has been documented in many plant species for a wide array of characters (Larkin and Scowcroft, 1981; Reisch, 1983; Vasil, 1986; Bajaj, 1990). Somaclonal variation has been successful in identification of new varieties in sugar cane, sorghum, tomato, wheat, celery, flax and *Pelargonium* (Skirwin and Janick, 1976; Compton and Veilleux, 1991; Sears et al., 1992; Duncan et al., 1995; Karp, 1995). Variation in morphological characters among callus regenerated plants were observed in rice for grain size, tiller number, leaf number, maturity (Sun and Zheng, 1990), panicle number, seed weight, mature plant height and culm height (Lal and Lal, 1990) and in potato for maturation time, shape, size, number and colour of tubers, leaf shape and size and yield (Karp, 1990; Lal and Lal, 1990).

The present study was initiated to study somaclonal variations in paprika and whether the variations found in somaclones of *Capsicum annuum* (S0) are heritable.

Materials and methods

Five genotypes of *C. annuum* L. were used for the present study (Round ornamental, PBC 535, PBC 375, PBC 385 and PBC 066). Seeds were germinated

Table 1. Morphological characters of somaclones and their seedling progeny in PBC 385

Characters	Expression	No. of plants (S0)	No. of plants (S1)	Control
Flower position	pendent	50	45	9
	intermediate	0	0	0
	erect	50	5	0
Fruit colour at intermediate stage	green	50	23	9
	dark green	0	27	0
	light green	0	0	0
	yellowish green	0	0	0
Fruit shape	elongate	50	32	9
	triangular	0	18	0
	round	0	0	0
	blocky	0	0	0

Table 2. Evaluation of seedling progeny of somaclones of PBC 385 for quantitative and qualitative characters

Character	Range (S0)	CV % (S0)	Range (S1)	CV % (S1)	Range (control)	CV % (control)
Plant height	40–78	11.9	50–85	12.0	58–70	13.3
Days to flower	34–72	10.7	39–47	4.8	38–40	3.8
Days to fruit	48–62	22.6	51–59	3.9	51–55	3.9
Fruit length	7.6–15.3	19.2	6.2–14.5	16.8	7.5–11	15.1
Fruit width	1–2.22	26.3	1–2.2	21.9	1.0–2.0	22.2
Fruit weight	1.3–2.91	25.5	1.12–2.68	25.3	1.8–2.5	16.4
Yield/plant	105–173	14.4	96–134	11.4	115–133	7.4
Seed size	0.21–0.38	10.1	0.20–0.41	7.7	0.25–0.31	10.7
Seed number	79–135	7.3	50–116	11.6	84–98	10.9
Colour value	70.2–205	28.06	84–170.3	17.3	145–205	14.3

on MS basal medium. Explants were collected from eight-week-old seedlings. The explants used included leaf, stem (2 cm), shoot tip (2 cm) and nodal segments (2.5 cm. MS medium containing sucrose (Qualigens, Bombay) 30 g ml^{-1} and 'Qualigens' bacteriological grade agar agar 7 g l^{-1} was used in all experiments. The cultures were incubated at $22 \pm 2\,^{\circ}C$ and were given a photoperiod of 12 h with light intensity $30\,\mu mol\,m^{-2}\,s^{-1}$, provided by 'Philips' cool white fluorescent tubes. For regeneration and elongation culture bottles (500 ml) containing 100 ml medium were used.

For callus regeneration BA at 3 mg l^{-1} plus IBA 1^{-1} mg 1 was found optimum and for elongation of regenerated shoots, MS containing IBA $1\,mg\,l^{-1}$ was used. For rooting plants MS medium with activated charcoal 2 mg l^{-1} was used. The rooted plants (10–15 cm long) were washed under running water to remove agar and transferred to plastic cups containing sand and potting mixture in the ratio 3:1. The cups were kept covered with polythene bags in a shade house and watered once a week. The plants that were covered were watered once in a week and those left uncovered were watered once in 2 days. The polythene cover was removed after one month. The callus-regenerated plants were transplanted to black polythene bags containing sterile potting mixture. A completely randomized experimental design was

Table 3. Morphological characters of somaclones and their seedling progeny in PBC 375

Characters	Expression	No. of plants (S0)	No. of plants (S1)	Control
Stem pubescence	dense	0	5	0
	intermediate	34	0	0
	sparse/nil	16	45	9
Flower position	pendent	50	31	9
	intermediate	0	19	0
	erect	0	0	0
Fruit colour at	green	50	35	9
intermediate stage	dark green	0	0	0
	light green	0	0	0
	yellowish green	0	15	0
		0	0	0
Fruit shape at blossom end	pointed	50	45	9
	blunt	0	5	0
	sunken	0	0	0
	sunken and pointed	0	0	0

Table 4. Evaluation of seedling progeny of somaclones of PBC 375 for quantitative and qualitative characters

Character	Range (S0)	CV % (S0)	Range (S1)	CV % (S1)	Range (control)	CV % (control)
Plant height	44–75	12.9	43–78	14.3	45–68	18.2
Days to flower	36–48	11.0	31–49	27.5	39–42	3.7
Days to fruit	42–63	8.1	49–63	5.7	50–56	5.6
Fruit length	7.8–16.2	22.7	10.2–16.2	13.3	8–12	18.8
Fruit width	1.08–2.6	28.0	1.14–2.31	22.14	1.6–2.2	7.9
Fruit weight	1.83–5.5	29.5	2.2–12.3	40.8	2.1–2.5	13.1
Yield/plant	92–192	17.7	122–178	9.8	162–175	4.0
Seed size	0.19–0.36	13.9	0.20–0.45	21.2	0.30–0.32	3.2
Seed number	82–143	11.7	60–100	14.8	104–140	14.7
Colour value	74–150	16.0	78–242	34.06	86–144	37.9

used and a population of nine seed germinated plants transplanted at the same size were used as control. The morphological characters of the plants were recorded as per the IPGRI descriptor. The colour values were determined using the ASTA method (Hort and Fischer, 1971). Selfed seeds of somaclones of these lines were germinated to obtain the seedling progeny of somaclones. Fifty plants of each of the lines were evaluated for their morphological, yield and biochemical characters.

Results and discussion

Morphological characters and colour values of somaclones and their seedling progeny in PBC 385 were observed (Tables 1 and 2). Variations that were not found in the somaclones (S0) such as dense stem pubescence, erect fruit bearing habit, triangular fruit shape and clustering of fruits were found in the seedling progeny (S1).

The morphological characters of somaclones and seedling progeny of PBC 375 were studied and S1

Table 5. Morphological characters of somaclones and their seedling progeny in PBC 066

Characters	Expression	No. of plants (S0)	No. of plants (S1)	Control
Fruit colour at intermediate stage	green	23	41	9
	dark green	27	6	
	light green	0	3	0
	yellowish green	0	0	0
Fruit shape at blossom end	pointed	50	46	9
	blunt	0	4	0
	sunken	0	0	0
	sunken and pointed	0	0	0
	present	0	0	0

Table 6. Evaluation of seedling progeny of somaclones of PBC 066 for quantitative and qualitative characters

Character	Range (S0)	CV % (S0)	Range (S1)	CV % (S1)	Range (control)	CV % (control)
Plant height	45–66	10.2	42–83	16.4	45–68	20.4
Days to flower	32–49	11.38	33–50	22.4	32–41	12.7
Days to fruit	43–63	9.0	46–63	7.5	45–55	9.6
Fruit length	8.3–14.1	28.8	12.1–16.0	11.1	9.5–13.1	14.9
Fruit width	0.8–4.0	4.0	0.88–2.56	27.8	2.2–4.3	33.5
Fruit weight	7.34–13.56	12.1	2.6–10.34	21.8	10.7–12.5	8.4
Yield/plant	138–238	14.0	143–256	16.2	225–230	1.1
Seed size	0.29–0.42	11.7	0.27–0.42	24.5	0.35–0.41	3.2
Seed number	76–120	10.8	76–129	12.3	95–110	7.8
Colour value	132–242	35.4	121–224	21.35	126–131	12.5

Table 7. Morphological characters of somaclones and their seedling progeny in PBC 535

Characters	Expression	No. of plants (S0)	No. of plants (S1)	Control
Flower position	pendent	50	45	9
	intermediate	0	0	0
	erect	0	5	0

showed more variation than S0 (Table 3). Dense stem pubescence was found in 5 plants in S1 (10%). Clustering of fruits was also observed among 5% of the plants. Colour values ranged from 74 ASTA to 150 ASTA in S0 and from 78 ASTA to 242 ASTA in S1 (Table 4).

Table 8. Evaluation of seedling progeny of somaclones of PBC 535 for quantitative and qualitative characters

Character	Range (S0)	CV % (S0)	Range (S1)	CV % (S1)	Range (control)	CV % (control)
Plant height	30–64	17.5	40–68	21.1	43–58	16.7
Days to flower	35–52	11	31–54	10.7	41–44	3.6
Days to fruit	50–70	16	44–65	8.5	54–56	1.8
Fruit length	6.8–13.4	20.2	10.3–16.2	11.0	7–12	26.3
Fruit width	0.7–2.5	21.4	1.0–2.3	21.8	1.5–2.5	21.3
Fruit weight	1.3–4.6	37.4	1.12–4.98	42.1	1.2–2.5	35.5
Yield/plant	47.5–142	28.4	59–148	27.4	126–130	5.6
Seed size	0.26–0.41	9.2	0.20–0.42	13.4	0.30–0.41	5.8
Seed number	89–146	10.6	55–114	19.5	115–142	9.7
Colour value	47.34–153.5	23.5	91.3–176.3	15.8	73–95	13.5

Table 9. Morphological characters of somaclones and their seedling progeny in Round Ornamental

Characters	Expression	No. of plants (S0)	No. of plants (S1)	Control
Stem pubescence	dense	0	0	0
	intermediate	0	2	0
	sparse/nil	50	48	9
No. of flowers/axil	1	13	50	9
	2 and more	37	0	0
Flower position	pendent	0	44	0
	intermediate	13	4	0
	erect	37	2	9
Fruit shape	elongate	36	41	0
	triangular	14	5	0
	round	0	2	9
	blocky	0	2	0
Fruit shape at blossom end	pointed	5	45	0
	blunt	45	5	9
	sunken	0	0	0
	sunken and pointed	0	0	0

Morphological characters of somaclones and seedling progeny of PBC066 were observed and not much variation in morphological characters was found (Table 5). Colour values ranged from 132 ASTA to 242 ASTA in S0 and from 121.35 ASTA to 224 ASTA in S1 (Table 6).

Morphological characters of somaclones and their seedling progeny in PBC 535 were observed and variation in plant growth habit and intermediate fruit colour was found in S1 (Table 7). Colour values ranged from 47.34 ASTA to 153.5 ASTA in S0 and from 91.3 ASTA to 176.3 ASTA in S1 (Table 8).

Table 10. Evaluation of seedling progeny of somaclones of Round Ornamental for quantitative and qualitative characters

Character	Range (S0)	CV % (S0)	Range (S1)	CV % (S1)	Range (control)
Plant height	25–73	29.6	30–64	30.0	44–48
Days to flower	32–60	15.6	38–64	4.8	42–55
Days to fruit	45–74	18.0	51–58	4.0	53–70
Fruit length	2.4–9.8	32.0	4.3–12.2	24.1	1.45–2.0
Fruit width	1.0–2.8	36.7	1.1–3.23	15.7	1.75–2.2
Fruit weight	1.6–7.9	64.0	1.42–5.8	27.0	1.6–2.1
Yield/plant	66–134	20.9	82–132	12.16	165–186
Seed size	0.21–0.36	36.7	0.28–0.46	14.1	0.30–0.35
Seed number	72–134	12.0	70–120	16.0	75–82
Colour value	46.4–123	26.07	64–132	23.3	95–122

Morphological characters of somaclones and seedling progeny of somaclones of Round Ornamental were observed (Table 9). Variation was found in stem pubescence, plant growth habit, flower position and stigma exsertion. Maximum variation was found in fruit characters like fruit shape and fruit position in both generations. Colour values ranged from 46 ASTA to 123 ASTA in S0 and from 46 ASTA to 132 ASTA in S1.

From this study, it was found that somaclonal variation was genotype dependent. Round Ornamental showed the maximum variation among the genotypes studied and PBC 066 showed the minimum variation. Among the characters studied, fruit characters were found to alter more compared to other characters. It was also found that variation among the seedling progeny was more than that found in the somaclones. Recessive characters such as clusterness of fruit and erect fruit habit were also expressed in the seedling progeny of somaclones of PBC 385. The recessive variation being heterozygous might not have been expressed in the somaclones, but it expressed in the next generation due to the homozygosity caused by selfing of the somaclones. Expression of primitive characters like dense stem pubescence and recessive characters mentioned above point that it may be possible to produce primitive characters such as disease resistance through somaclonal variation. Estimation of colour values also showed promising results, indicating that it may be possible to obtain a high colour line by somaclonal variation. Major criticisms against somaclonal variation are that, not all of the variation is useful; it is unstable and unpredictable. Accepting that not all of the variation is useful, or stable, the fact remains that some changes are useful. Somaclonal variation may not be the complete answer to the Capsicum breeder's problems. Realistic objectives, with careful choice of starting material and experimental procedure are required. There are numerous problems associated with the application of variation and extensive screening will be needed, particularly in the early stages, to remove unwanted variants such as aneuploids. However, judging from 'successes' in other Solanaceous crops like potato and tomato, a somaclonal programme is a procedure worth under taking.

Acknowledgement

We thank the University Grants Commission for providing the fellowship to the first author.

References

Bajaj YPS (1990) Biotechnology in Agriculture and Forestry. Vol 11. Somaclonal Variation in Crop Improvement I. Springer Verlag, Berlin

Compton ME & Veilleux RE (1991) Variation for genetic recombination among tomato plants regenerated from three tissue culture systems. Genome 34: 810–816

De Klerk GJ (1990) How to measure somaclonal variation. Acta Bot. Neerl. 39: 129–144

Duncan RR, Waskom RM & Nabors MW (1995) *In vitro* screening and field evaluation of tissue culture regenerated sorghum (*Sorghum bicolor* (L.) Moench) for soil stress tolerance. Euphytica 85: 373–380

Hort AM & Fisher JH (1971) ASTA method of analysis of colour in chillies. Modern Food Analysis (pp. 338–39). Springer Verlag, New York

Karp A (1990) Somaclonal variation in potato. In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry, Vol 11 Somaclonal Variation in Crop Improvement I (pp. 379–399). Springer – Verlag, Berlin

Karp A (1995) Somaclonal variation as a tool for crop improvement. Euphytica 85: 295–302

Lal R & Lal S (1990) Somaclonal variation in crop improvement. In: Crop Improvement Utilizing Biotechnology (p. 353). CRC Press, Florida

Larkin PJ & Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197–214

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol.Plant. 15: 473–482

Reisch B (1983) Genetic variability in regenerated plants. In: Evans DA, Sharp WR, Ammirato PV & Yamada Y (eds) Handbook of Plant Tissue Culture Vol. 1. Techniques for Propagation and Breeding (pp. 743–769). MacMillan Publishing Company, New York

Sears RG, Cox TS & Paulsen GM (1992) Registration of KS89WGRC9 stress-tolerant hard winter wheat germplasm. Crop Sci. 32: 507

Skirvin RM & Janick J (1976) Velvet Rose *Pelargonium*. A scented geranium. HortScience 11: 61–62

Sun ZX & Zheng KL (1990) Somaclonal variation in rice. In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry. Vol 11. Somaclonal Variation in Crop Improvement I (pp. 288–325). Springer- Verlag, Berlin, Heidelberg

Swartz HJ (1991) Post culture behaviour: genetic and epigenetic effects and related problems. In: Debergh PC & Zimmerman RH (eds) Micropropagation (pp. 95–121). Kluwer Academic Publishers, Dordrecht, The Netherlands

Vasil IK (1986) Cell Culture and Somatic Cell Genetics of Plants. In: Plant Regeneration and Genetic Variability. Academic Press, Inc. New York