Indian J. Genet., **63**(4): 295-298 (2003)

Assessment of somaclonal variations in two lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.]

Shivali Srivastav and S. L. Kothari

Department of Botany, University of Rajasthan, Jaipur 302 004

(Received: March 2003; Revised: September 2003; Accepted: October 2003)

Abstract

Somaclonal variation has great potential to enhance the advantages of conventional plant breeding, and increase the production and productivity of crops to meet the increasing demand for food and food products in the future. In pearl millet [*Pennisetum glaucum* (L.) R. Br.], plant morphogenesis has been achieved via somatic embryogenesis. Somatic embryos were isolated and regenerated into whole green plants on MS medium supplemented with indole-3-acetic acid (1 mgl^{-1}) and kinetin (0.5 mgl^{-1}). Ten R_0 plants of each cultivar were selected on the basis of 13 quantitative characters. Transmission of somaclonal variation was studied from callus derived (R_0) plants to R_1 and R_2 generations and were compared with those obtained from embryo culture without any callus formation (E_0-E_2). Significant variation was observed among regenerants for number of leaves/ plant, days to heading, length of panicle, average width of panicle and weight of panicle.

Key words: Pearl millet, agronomic performance, somatic embryogenesis, tissue culture

Introduction

It is well known that genetic changes occur in plant tissue culture and these changes expressed as variant traits, are transmitted to regenerated plants and their progeny through sexual or vegetative propagation [1, 2]. Genetic variation is an essential component of any conventional crop breeding programme. Plant cell and tissue culture provides increased genetic variability relatively rapidly. Somaclonal variation has been reported in a number of crops including rice [3], maize [4], wheat [5], barley [6] and pearl millet [7, 8]. There may be modifications in copy number of genes [9], activation of transposable elements [10] and changes in DNA methylation patterns [4] which may lead to variation for quantitatively inherited characters. The present study reports on the agronomic performance of plants derived from callus cultures of pearl millet and their progeny for thirteen quantitative characters.

Material and methods

Plants regenerated from embryos and callus derived from immature embryo culture of pearl millet [*Pennisetum glaucum* (L.) R. Br.] var. RIB 3135 and var. RIB 20K86 were investigated for somaclonal variation and its transmission in three successive generations.

Somatic tissue culture: Entire panicle containing immature embryos (10-15 days after pollination) of pearl millet var. RIB 20K86 and var. RIB 3135 were taken from plants grown in the fields of Agricultural Research Station, Durgapura, Jaipur, India. Initially the entire panicle was washed with 20% (w/v) liquid detergent (Extran) (Merck) and rinsed 3-4 times thoroughly with water. Panicle containing immature embryos was surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 3 min and rinsed thoroughly 3 times with sterile distilled water. Immature embryos were cultured on MS basal medium (11) with varying concentrations of 2,4-D (1-5 mgl^{-1}), 3% sucrose (w/v) and 0.9% (w/v) agar for somatic embryogenesis.

Plant regeneration: Approximately 200 mg embryogenic callus of each variety was transferred to regeneration medium consisting of MS medium supplemented with IAA (1 mgl^{-1}) and Kn (0.5 mgl^{-1}). All the cultures were incubated in a growth chamber. For rooting, plantlets were kept in tubes containing half strength MS medium. Regenerated wellrooted plantlets were transferred to pots containing 1:1 ratio of soil and compost at the Department of Botany, University of Rajasthan, Jaipur. The regenerated plants were referred to as the R_0 generation. Nineteen regenerants were obtained in var. RIB 20K86 and twenty three regenerants were obtained in var. RIB 3135. Plants were also numbered separately in both the varieties. Ten control plants (E_0) were also raised for each variety.

Morphological analysis: Ten R_0 plants of each variety were selected on the basis of 13 characters. From each of $20R_0$ plants 10 seeds of individual plants were collected and sown to raise $200R_1$ plants. All the plants were allowed to open pollinate. $200R_1$ plants progeny of $20R_0$ plants were compared with $20E_1$ plants progeny of ten control plants (E_0) of both varieties.

Seeds of 200R_1 plants were individually bulked and sown to raise 200R_2 plants. Ten plants have been used per progeny for recording data on morphological traits. R_1 and R_2 generations were evaluated in different seasons.

Data of each R_0, R_1 and R_2 progeny plants of each variety were recorded on maturity and after harvesting. Plants were evaluated for Plant height (cm), tillers / plant (nu.), internodes / plant (nu.), internode length (cm), number of leaves / plant, flag leaf length (cm), flag leaf width (cm), days to heading (nu.), number of panicles / plant (nu.), length of panicle (cm), average width of panicle (cm), weight of panicle (g) and average weight of 100 seeds (g).

Statistical analysis: Data analysis was performed using ANOVA. Somaclones (R_0, R_1 and R_2) and control plants (E_0, E_1 and E_2) were analyzed as independent experimental treatments. Each set of data was subjected to standard analysis of variance (ANOVA).

Results and discussion

R_0 generation: In var. RIB 3135 and RIB 20K86, R_0 generation showed significant variation for most of the 13 characters studied. Except for plant height in var. RIB 3135 (Table 1). In var. RIB 20K86, tillers/plant, number of panicles/plant and average weight of 100 seeds were significant only at $p = 0.05$.

Table 1. Mean performance of R_0 (Tissue culture raised plants) and E_0 (Seed grown plants) generation in pearl millet var. RIB 3135 & var. RIB 20K86

Character	Unit	Var. RIB 3135		Var. RIB 20K86	
		R_0	E_0	R_0	E_0
Plant height	(cm)	93.3^a	88.5^a	88.7^b	134.5^a
Tillers / plant	(nu.)	1.36^b	1.0^a	2.2^b	1.0^a
Internode number / plant	(nu.)	6.0^b	5.0^a	6.3^b	7.0^a
Internode length	(cm)	14.6^b	15.8^a	12.7^b	16.3^a
Number of leaves / plant	(nu.)	7.7^b	7^a	15.5^b	9.5^a
Flag leaf length	(cm)	26.4^b	29^a	19.8^b	31^a
Flag leaf width	(cm)	1.63^b	1.3^a	1.64^b	1.60^a
Days to heading	(nu.)	34.8^b	38.5^a	38^b	45^a
Number of panicles/ plant	(nu.)	1.36^b	1.0^a	2^b	1.0^a
Length of panicle	(cm)	11.45^b	9.1^a	10.7^b	15.5^a
Average width of panicle	(cm)	0.92^b	0.86^a	1.18^b	3.33^a
Weight of panicle	(g)	3.89^b	2.89^a	3.42^b	7.56^a
Average weight of 100 seeds	(g)	0.58^b	0.68^a	1.71^b	0.76^a

Means followed by the same letters are not significantly different at the 0.05 level of confidence (ANOVA)

R_1 and R_2 generation: In var. 3135, plant no. 2, 3, 4, 6, 9 performing better than control for five characters were selected for raising R_1 progeny and plant no. 10, 11, 12, 13 and 16 were selected on the basis of characters which were poorer than control (Table 2). Significant variations were observed for characters like plant height, number of leaves/plant, days to heading, flag leaf width, average weight of 100 seeds at $p < 0.05$.

In var. 20K86 plant no. 5,6,8,15 and 22 were selected on the basis of any five dominant characters and plant no. 4, 12, 13, 18 and 23 were selected on the basis of characters which were poorer. Significant variation were observed for characters like internode length, day of heading, length of panicle, average width of panicle and average weight of 100 seeds at $p < 0.05$. In R_2 generation, for var. RIB 3135 non-significant differences were observed for all the characters studied (Table 3) while for var. RIB 20K86 significant differences were observed for average width of panicle. As somaclonal variations are genotype dependent, these may account for differential response of var. RIB 3135 and var. RIB 20K86.

These data suggest that *in vitro* environment is mutagenic to pearl millet and that significant variations can be generated for several characters in the plant. This corroborates previous reports on wheat also (12). Retaining this variation through R_2 generation in both varieties suggests that the variation is genetic. Morrish *et al.* (13) in their studies on quantitative characters suggested the involvement of epigenetic factors and concluded that genetic fidelity of the progeny of embryogenic tissue culture is dependent on the genotype of the explant.

This work supports previous studies indicating that variation generated during tissue culture is heritable. Regardless of the mechanisms by which genetic variation is produced, evidence has accumulated that somaclonal variation includes few agronomically useful types [14, 15, 6]. Genetic variability in tissue culture can be perceived as a new source of variability or as an undesirable consequence of *in vitro* culture: Further research is needed on the nature and causes of this variation.

The application of this culture technique to adapted varieties in conjugation with appropriate screening and testing may provide useful variation to plant breeders.

Acknowledgement

We thank Dr. Srikant, pearl millet specialist, Agricultural Research Station, Durgapura, Jaipur for providing explants of *Pennisetum glaucum*.

References

1. **Larkin P. J. and Scowcroft W. R.** 1981. Somaclonal variation - A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., **60**: 197-214.

Table 2. Mean performance of R_1 (progeny of successively numbered R_0 plants) and E_1 (progeny of E_0 plants) generation in pearl millet (var. RIB 3135) (A) and (var. RIB20K86) (B).

Plant generation and Number		Plant height (cm)		Tiller/ plant		Internode number/ plant		Internode length (cm)		No. of leaves/ plant		Flag leaf length (cm)		Flag leaf width (cm)		Days to heading		No. of panicles plant		Length of panicle (cm)		Av. width of panicle (cm)		Weight of panicle (g)		Av. weight of 100 seeds (g)	
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
E_1	E_1	90.5^{a}	104^{ac}	1^{a}	1^{a}	8^{a}	6.5^{a}	15.5^{a}	15.9^{a}	5.5^{a}	9.5^{a}	28.5^{a}	30.6^{a}	1.4^{a}	1.55^{a}	38^{a}	43.5^{a}	1^{a}	1^{a}	8.8^{a}	15.9^{a}	0.93^{a}	3.4^{a}	2.7^{a}	5^{a}	0.68^{a}	0.7^{a}
$2R_1$	$4R_1$	155.9^{b}	95.1^{a}	1^{a}	1^{a}	8.7^{b}	6.9^{a}	15.6^{a}	12.8^{b}	9.6^{b}	7.9^{b}	35.6^{b}	30.5^{a}	2.5^{b}	2.29^{b}	33.7^{b}	36.9^{b}	1^{a}	1^{a}	15.7^{b}	10.8^{b}	1.21^{b}	1.1^{b}	7^{ah}	3.3^{b}	0.56^{b}	0.4^{b}
$3R_1$	$5R_1$	108^{c}	76.8^{b}	1^{a}	1.1^{b}	8.9^{ab}	6.8^{a}	12.3^{b}	10^{c}	9.1^{c}	9.3^{a}	21.5^{c}	24.8^{b}	1.6^{c}	1.7^{a}	30^{c}	33.9^{c}	1^{a}	1^{a}	10.5^{c}	9.7^{c}	1.06^{c}	0.9^{c}	3.2^{b}	2.2^{c}	0.6^{cb}	0.3^{cb}
$4R_1$	$6R_1$	101.1^{dc}	85.1^{cb}	1^{a}	1^{a}	8.4^{cb}	7.7^{b}	11.8^{cb}	10.6^{dc}	8.2^{d}	8.8^{a}	36.6^{db}	24.1^{cb}	2.6^{db}	1.6^{a}	27.4^{d}	39.2^{d}	1^{a}	1^{a}	11.3^{dc}	8.9^{dc}	1.03^{dc}	1.1^{db}	3.8^{c}	2.4^{dc}	0.51^{d}	0.3^{db}
$6R_1$	$8R_1$	110.3^{ec}	95^{a}	1^{a}	1^{a}	9^{db}	6^{a}	12.2^{db}	12^{e}	10.5^{e}	7.3^{cb}	27.7^{e}	29.9^{a}	2.2^{e}	1.6^{a}	30.4^{ec}	30.3^{e}	1^{a}	1^{a}	11.3^{ec}	11.1^{eb}	1.01^{ed}	1.3^{e}	2.2^{a}	5.9^{e}	0.36^{e}	0.5^{e}
$9R_1$	$12R_1$	29^{f}	93.3^{dac}	1^{a}	1.33^{c}	5.9^{e}	6.6^{a}	7.6^{e}	9.2^{fc}	9.1^{fc}	10.6^{d}	15.1^{f}	31.3^{a}	0.8^{f}	2.1^{c}	32.8^{fb}	41^{f}	1^{a}	1^{a}	12.8^{f}	11.1^{fb}	1.23^{fb}	1.2^{f}	4.2^{dc}	6.9^{f}	0.44^{f}	0.5^{f}
$10R_1$	$13R_1$	42.6^{g}	125^{e}	1^{a}	1.16^{d}	5.2^{f}	10^{c}	7.4^{fe}	12.4^{gbe}	8.1^{gd}	14.5^{e}	18.8^{g}	25.9^{db}	0.9^{gf}	1.9^{da}	46.9^{g}	32.3^{g}	1^{a}	1.16^{b}	10.4^{gde}	12.7^{g}	0.96^{a}	1.4^{ge}	1.5^{e}	3.9^{gb}	0.32^{ge}	0.4^{ge}
$11R_1$	$15R_1$	54.7^{h}	73.4^{fb}	1^{a}	1^{a}	8^{ajc}	7.7^{db}	9.6^{g}	10.3^{hc}	9.4^{hbc}	9.4^{a}	21.9^{hc}	27.4^{ed}	1.1^{h}	1.6^{a}	41.2^{h}	33.5^{hc}	1^{a}	1^{a}	9.3^{ai}	7.8^{h}	1.14^{g}	1.1^{hb}	1.4^{f}	1.6^{hc}	0.28^{hg}	0.3^{hc}
$12R_1$	$18R_1$	57.0^{ih}	110^{a}	1^{a}	1^{a}	7.4^{g}	7.2^{eab}	9.3^{hg}	13.7^{ib}	9^{ic}	10^{ad}	22.3^{ic}	29^{ae}	0.9^{ifh}	1.8^{ea}	49.1^{i}	37.8^{i}	1^{a}	1^{a}	10.3^{hc}	10.1^{ibc}	1.09^{hc}	1.2^{if}	2.1^{ae}	3.4^{ib}	0.31^{ieg}	0.2^{i}
$13R_1$	$22R_1$	102^{jc}	127.2^{ge}	1^{a}	1^{a}	11.3^{h}	8.9^{f}	12.2^{ib}	14.2^{ji}	10.5^{je}	11.1^{fd}	30.3^{ak}	26.5^{fbe}	2.7^{jb}	2.7^{f}	42.5^{jh}	37.1^{ji}	1^{a}	1^{a}	9.9^{ic}	11.7^{jbg}	1.12^{igh}	1.3^{jf}	1.6^{ge}	5^{a}	0.25^{jh}	0.5^{jef}
$16R_1$	$23R_1$	109.1^{kc}	121.1^{he}	1^{a}	1^{a}	10.2^{l}	8.9^{gf}	11.2^{jc}	13.8^{ki}	10.6^{ke}	11.5^{gd}	31.7^{k}	29^{ae}	1.6^{kc}	2.9^{g}	42.7^{kh}	47.8^{k}	1^{a}	1^{a}	14.7^{f}	10.8^{kb}	1.39^{j}	1.4^{k}	6.7^{h}	3.9^{jb}	0.46^{kf}	0.5^{kef}

Mean values with the same letters are not significantly different at the 5% level using ANOVA

Table 3. Mean performance of R_2 (progeny of R_1 plants) and E_2 (progeny of E_1 plants) generation in pearl millet (Var. RIB 3135) (A) (Var. RIB20K86) (B)

Plant generation and Number		Plant height (cm)		Tiller/ plant		Internode number/ plant		Internode length (cm)		No. of leaves/ plant		Flag leaf length (cm)		Flag leaf width (cm)		Days to heading		No. of panicles / plant		Length of panicle (cm)		Av. width of panicle (cm)		Weight of panicle (g)		Av. weight of 100 seeds (g)	
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
E_2	E_2	85.8^{a}	105^{a}	1^{a}	1^{a}	7.4^{a}	10^{a}	12.8^{a}	17.8^{a}	5.6^{a}	9.8^{a}	26.9^{a}	31^{a}	1.1^{a}	1.2^{a}	37^{a}	42.8^{a}	1^{a}	1^{a}	8.1^{a}	13.5^{a}	0.8^{a}	3.4^{a}	2.2^{a}	4.3^{a}	0.7^{a}	0.6^{a}
$2R_2$	$5R_2$	152.2^{b}	53.6^{b}	1^{a}	1^{a}	8.6^{a}	5.8^{b}	12.7^{a}	10.5^{b}	11.4^{a}	10.4^{a}	38.9^{a}	21.8^{b}	2.8^{a}	1.4^{a}	32.4^{b}	36.6^{b}	1^{a}	1^{a}	19.7^{a}	12.2^{a}	2.1^{a}	1.6^{b}	7.6^{a}	4.7^{a}	0.4^{b}	0.5^{b}
$3R_2$	$6R_2$	123.4^{c}	80.6^{cb}	1^{a}	1^{a}	0.9^{a}	6.8^{cb}	13^{a}	8.5^{cb}	9.6^{ab}	11.4^{a}	20.9^{b}	18.3^{c}	1.3^{ad}	1.4^{a}	29.2^{c}	33.4^{c}	1^{a}	1^{a}	10.7^{ab}	11.1^{ba}	1.7^{a}	1.6^{cb}	5.2^{ab}	4.9^{a}	0.8^{ab}	0.5^{cb}
$4R_2$	$8R_2$	100.8^{a}	98^{abc}	1^{a}	1^{a}	8.6^{a}	9.8^{ab}	11.9^{a}	11.2^{db}	8.6^{ac}	11.6^{a}	39.8^{ab}	23.1^{dbc}	2.6^{a}	2.1^{a}	26.8^{d}	38.6^{dbc}	1^{a}	1^{a}	12.7^{ac}	9.5^{c}	1.7^{a}	1.3^{db}	4.5^{ac}	5.7^{a}	0.6^{cb}	0.4^{d}
$6R_2$	$15R_2$	120.4^{ac}	109.5^{abc}	1^{a}	1^{a}	9^{a}	8^{db}	10.7^{b}	11.2^{eb}	10.8^{a}	11^{ac}	30.1^{agb}	16.1^{e}	2.2^{ae}	1.3^{ac}	28.8^{ecd}	30.6^{e}	1^{a}	1^{a}	11.3^{ad}	9^{dc}	1.9^{a}	1.7^{eb}	5.8^{ad}	15.6^{a}	0.2^{d}	0.6^{ebd}
$9R_2$	$22R_2$	30.8^{d}	90.4^{dbc}	1^{a}	1^{a}	4.8^{b}	8.6^{eb}	7.7^{c}	6.7^{fc}	8.8^{ad}	13.6^{a}	22.8^{cb}	19.5^{fc}	0.9^{b}	1.5^{ad}	31.8^{fbcd}	41.2^{abce}	1^{a}	1^{a}	10.3^{ae}	9.3^{ec}	1.4^{ab}	1.3^{fb}	5.6^{ae}	6.6^{ad}	0.4^{ebd}	0.9^{abd}
$10R_2$	$4R_2$	42.4^{e}	64.4^{abd}	1^{a}	1^{a}	5.2^{cb}	6^{fb}	7^{dc}	11.4^{gbf}	7.6^{ae}	10.6^{abd}	19.1^{d}	16^{g}	1^{afb}	0.8^{b}	47.8^{abcd}	38^{fbe}	1^{a}	1^{a}	9.2^{af}	9^{fc}	1.4^{acb}	1.4^{gbe}	5.8^{af}	7.8^{ae}	0.2^{fd}	0.4^{fd}
$11R_2$	$12R_2$	59.8^{fe}	188.4^{abc}	1^{a}	1^{a}	8.6^{ab}	10.6^{ab}	10.5^{ebc}	17.9^{abf}	9^{af}	15.8^{a}	21.7^{ebd}	36^{abcg}	1.2^{agb}	3.3^{ab}	40.4^{abcdg}	29.6^{ge}	1^{a}	1^{a}	11^{ag}	8.8^{gc}	1.4^{db}	1.5^{hbe}	8.3^{a}	5.9^{af}	0.4^{gbd}	0.9^{abd}
$12R_2$	$13R_2$	57.9^{ge}	14.6^{ec}	1^{a}	1^{a}	7.6^{adb}	6.6^{gb}	9.4^{fc}	12.6^{hbf}	9.2^{ag}	14.6^{af}	23.1^{fbd}	20.5^{hbcg}	0.9^{cb}	1.6^{aeb}	48.6^{abcd}	36.4^{hbce}	1^{a}	1^{a}	10.9^{ah}	9.6^{hbc}	1.3^{aeb}	1.4^{ibe}	9.4^{a}	6.4^{agf}	0.7^{abjd}	0.3^{g}
$13R_2$	$18R_2$	108^{aide}	158^{abcd}	1^{a}	1^{a}	11.2^{ab}	10^{ab}	13.1^{abc}	11.3^{ibf}	11.4^{a}	14.2^{a}	31.8^{ahbd}	31^{abcgj}	2.9^{ab}	1.9^{abf}	42.6^{abcdh}	35.2^{ibce}	1^{a}	1^{a}	9.3^{ai}	8.1^{ic}	1.4^{afb}	1.8^{ibe}	8.2^{a}	3.4^{ab}	0.4^{hbd}	0.5^{hbdg}
$16R_2$	$23R_2$	113.8^{he}	168^{abcd}	1^{a}	1^{a}	10.6^{ab}	10.4^{ab}	11.6^{gabc}	13.2^{ibf}	11.6^{a}	12.8^{ae}	32.4^{aibd}	28.9^{ibcg}	1.5^{ahb}	2.2^{abg}	42.8^{abcdi}	48^{abce}	1^{a}	1^{a}	8^{aj}	8^{jc}	1.3^{ag}	1.5^{kbe}	9.4^{a}	4.5^{acb}	0.5^{ibd}	0.7^{abdig}

Mean values with the same letters are not significantly different at the 5% level using ANOVA

2. **Phillips R. L., Kaeppler S. M. and Peschke V. M.** 1990. Do we understand somaclonal variation? *In*: Van Der Plas LHW, Van Aatrijk (Eds.) Progress in plant cellular and molecular biology. Kluwer Academic Publ., Dordrecht, pp. 131-141.

3. **Yang H., Tabei Y., Kamada H., Kayano T. and Takaiwa F.** 1999. Detection of somaclonal variation in cultured rice cells using digoxigenin-based random amplified polymorphic DNA. Plant Cell Rep., **18**: 520-526.

4. **Kaeppler S. M. and Phillips R. L.** 1993. Tissue culture induced DNA methylation variation in maize. Proc. Natl. Acad. Sci., USA, **90**: 8773-8776.

5. **Winfield M., Davey M. R. and Karp A.** 1993. A comparison of chromosome instability in cell suspensions of diploid, tetraploid and hexaploid wheats. Heredity, **70**: 187-194.

6. **Bregitzer P. and Poulson M.** 1995. Agronomic performance of barley lines derived from tissue culture. Crop. Sci., **35**: 1144-1148.

7. **Shenoy V. B. and Vasil I. K.** 1992. Biochemical and molecular analysis of plants derived from embryogenic callus cultures of napier grass (*Pennisetum purpureum* K. Schum.) Theor. Appl. Genet., **83**: 947-955.

8. **Lauzan J. R., Vento H., Herrera R. S., Martinez R. O., Monzoto M. and Cruz R.** 1989. A study of the green pigments and carotenoids in king grass (*Pennisetum purpureum*) somaclones 1. Establishment period Cuban J. Agri. Sci., **23**: 121-127.

9. **Kidwell K. K. and Osborn T. C.** 1993. Variation among alfalfa somaclones in copy number repeated DNA sequences. Genome, **36**: 906-912.

10. **Hirochika H.** 1993. Activation of tobacco retrotransposons during tissue culture. Embo. J., **12**: 2521-2528.

11. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia. Pl., **15**: 473-497.

12. **Mohmand A. S. and Nabors M. W.** 1990. Somaclonal variant plants of wheat derived from mature embryo explants of three genotypes. Plant Cell Rep., **8**: 558-560.

13. **Morrish F. M., Hanna W. W. and Vasil I. K.** 1990. The expression and perpetuation of inherent somatic variation in regenerants from embryogenic cultures of *Pennisetum glaucum* (L.) R. Br. (Pearl millet). Theor. Appl. Genet., **80**: 409-416.

14. **Karp A.** 1994. Origins, causes and uses of variation in plant tissue cultures. *In*: Vasil I. K. Thorpe T. A. (Eds.) Plant cell and tissue culture. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 139-151.

15. **Mohan Jain S.** 2001. Tissue culture - derived variation in crop improvement. Euphytica, **118**: 153-166.