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Somaclonal variation in high tannin sorghums

T. Cai, G. Ejeta, J.D. Axtell and L.G. Butler

Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA

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Summary. Genetic variants were found among over 6,000 primary plants (R_1) regenerated from embryogenic tissue cultures of eight high tannin sorghums [*Sorghum bicolor* (L.) Moench]. Field assessment of somaclonal variation has progressed to the R_2 population, with over 48,000 R_2 seedlings (27,000 plants) in 1,126 rows from 1,055 R_1 plants. A total of 43 variant phenotypes was recovered, including several types of chlorophyll deficiencies, dwarfism, short culm, sterility, narrow leaf, and several previously unreported variants, such as ragged leaf, multibranched heads, and *Hydra*, a developmental variant which produces large numbers of panicles. Variation production greatly depends on parent genotype and appears to increase with increasing time in cultures. The total average somaclonal variation rate (based per 100 R_1 plants) and somaclonal variant frequency (based per 100 R_2 plants) estimated in the tested population were 11.3 and 1.6, respectively. Chimerism was found in regenerants. The estimated size of the mutated sector carried by mutant regenerants ranged from the whole plant to less than 3% of a single head. The average proportion of mutated R_1 heads carrying large (80%–100%), medium (40%–80%), and small (<40%) mutated sectors was 38.7%, 26.0% and 35.3%, respectively. Some sector mutations do not appear until the R_3 generation. In order to avoid losing variants, the population for selecting somaclonal variation should be as large as possible. Some of these variants found may be useful for further study or for use in breeding programs.

Key words: Plant tissue culture – Somaclonal variation – *Sorghum bicolor*

Introduction

High tannin sorghums [*Sorghum bicolor* (L.) Moench] produce grain that is relatively resistant to herbivores and pathogens, but this tannin-rich grain has diminished nutritional value (Butler et al. 1986). This grain contains a wide variety of polyphenolic material (Butler 1989); in most cases it is not known which of these components is responsible for antinutritional effects or for agronomic benefits such as resistance to grain-eating birds. We are attempting to utilize in vitro culture to approach these problems. If it were possible to obtain from a well-characterized high-tannin cultivar a series of stable somaclonal variants lacking various polyphenolic components or groups of components, assignment of specific polyphenolic components to particular biological effects would be facilitated.

Somaclonal variation is considered to be a novel source of variability and a new tool for geneticists and plant breeders (Larkin and Scowcroft 1981; Evans and Sharp 1986). Heritable variations have been reported in progenies of the major cereals: wheat (Larkin et al. 1984; Maddock and Semple 1986), rice (Fukui 1983; Sun et al. 1983), corn (Lee and Phillips 1987; Zehr et al. 1987), and barley (Pickering 1989). Variants of grain quality of cereals have been reported from tissue-culture-derived plants (Hoffmann and Wenzel 1981; Jordan and Larter 1985; Larkin et al. 1984; Ryan et al. 1987; Maddock et al. 1985; Cooper et al. 1986). Ma et al. (1987) described variations in sorghum plant height, waxy midrib, and sterility found in progenies of regenerants derived from immature embryo culture. Bhaskaran et al. (1987) reported sorghum somaclonal variation in plant height, tiller number, shoot dry weight, grain yield, and days to flowering from a

population of 96 R_2 plants derived from eight R_1 plants (cv IS3620C). However, the traits of early flowering, enhanced yield, and increased shoot weight were not maintained over 2 years either in R_2 or R_3 generations. Selection of sorghum callus and regenerants tolerance to NaCl, Al, and drought has been reported (Smith et al. 1983; Bhaskaran 1983; Smith et al. 1985; TCCP 1987; Duncan 1989). Three regenerated lines of sorghum have been identified as having enhanced fall armyworm resistance, acid soil tolerance, or drought/salt tolerance (Hanning et al. 1989).

Even those sorghum cultivars that do not contain tannin tend to respond to the stress of in vitro culture by producing toxic pigmented phenolics (Brar et al. 1979). High tannin sorghums produce more of these materials (Oberthur et al. 1983). Establishment of long-lived cultures inevitably requires physiological resistance adaptations or selection against excessive polyphenol production. Because most of the 26,000 + entries in the world collection of sorghum do not contain tannin in their grain, tannin synthesis is clearly not essential for this crop. We therefore expected to obtain somaclonal variants that were no longer capable of synthesizing tannin and possibly other polyphenols.

The major purpose of the present study was to produce somaclonal variants of high tannin sorghums and to evaluate them for qualitative alterations in polyphenol production. Here we report on a wide range of morphological and developmental variations observed in field assessment of sorghum somaclones. The variants with altered levels of polyphenols in the grain will be described in a subsequent report.

Materials and methods

Nomenclature

We use R_1, R_2, R_3 ... to term the primary regenerants, the first selfed progeny, and successive selfed generations, respectively, in order to correspond with the F_1, F_2 ... and M_1, M_2 ... accepted genetic usage for selection of variations in cross-breeding and mutation breeding, respectively.

Establishment of somaclones

Sorghum cultivars IS0724, IS2830, IS3150, IS4225, IS6881, IS8260, and SCO167-14E are conventional high-tannin sorghums [Group III according to Price et al. (1978)], and IS8768 is a Group II sorghum relatively rich in tannin. Donor materials as sources of explants were seeds or plants grown at the Agronomy Farm, Purdue University, West Lafayette In. Cultures were established from shoot portions of mature embryo (as described by Cai et al. 1987) or from immature embryos or developing inflorescences (as in Cai and Butler 1990). Embryogenic calli were selected and subcultured every 30–40 days. Some calli that showed vigorous growth were separately transferred for long-term reproducibility. Over 6,000 plants were regenerated during 1 year. In the beginning, most plantlets died after transfer to pots in a growth chamber. With improved rooting media and transplantation techniques, a large proportion of the plants survived. Nearly 2,000 R_1 plants were grown to maturity in greenhouse or field. From each R_1 plant, one head was bagged and harvested. These self-pollinated seeds from individual R_1 heads were established as individual R_1 families, the somaclones. In order to test chimerism at the plant level, three to six selfed heads were collected from some of R_1 plants.

Field assessment

Seeds from individual R_1 heads were planted in single rows (henceforth called R_1 progeny rows) of 5-m length (20–35 R_2 plants), with parental controls planted every 12–40 rows. At various stages, visually detectable morphological variations were recorded. From each row the total number of R_2 plants, of variations, and of variants was recorded. Here the variants refer to individual plants that are different from the control in one or more characters, while the variations refer to individual mutant phenotypes. In the R_2 generation, 1,055 R_1 families were evaluated. These tested R_1 plants were regenerated from the eight genotypes after 120–300 days in culture, utilizing all three types of explants. Because fewer embryogenic calli were available for maintenance after 120–160 days in culture, most of these tested somaclones were derived from only a few (no more than three) selected callus lines for each parental genotype and explant source.

Somaclonal variation

Somaclonal variation rate (SVR) based per 100 R_1 plants and somaclonal variant frequency (SVF) based per 100 R_2 plants were calculated to determine the extent of genotypic variability of somaclones. SVR describes the probability of variation of given somaclones through tissue culture. SVF refers to the proportion of detectable variants in a given somaclonal population. The formula:

$$\text{SVR}(\%) = \frac{\text{No. of variations found in } R_1 \text{ families}}{\text{No. of total } R_1 \text{ families}} \times 100\%$$

$$\text{SVF}(\%) = \frac{\text{No. of } R_2 \text{ variants}}{\text{Total no. of } R_2 \text{ plants}} \times 100\%$$

If several plants of the same phenotypic variant were found in a single row, they were counted individually for SVF calculation but only once for SVR. SVR is a function of the rate of occurence of genetic events in vitro but, unlike mutagenesis, similar variations may result from independent mutation events as well as from proliferation of single mutated cell clones.

The size of a mutated sector (S) carried by a mutant R_1 panicle was estimated by the formula $S = Q/K$ where Q is the frequency of variants in individual R_1 panicle progeny rows. In order to avoid underestimating the size of all variations found here, K (the expected freqency) was assigned a value of 0.25, assuming recessive mutations to be preponderant. The methods used here for estimating SVR, SVF, and the size of the sector are similar to those used in plant mutation breeding (Gaul 1964).

A regenerant was designated as a chimera at the plant level when some but not all of the panicle rows from the R_1 plant segregated variations or when the rows segregated different variation phenotypes independently; i.e., when some but not all of the panicles are mutated. An R_1 panicle was designated as chimera at the panicle level when the number of variants of one phenotype counted in a progeny row from the panicle was less than the theoretical segregation frequency; i.e., when a mutated sector did not involve a whole R_1 panicle. In addition, if a

Table 1. Frequencies of chlorophyll-deficient variants in primary R_1 plants regenerated from ME, DI, and IE cultures of eight high-tannin sorghum lines

Explants / Genotypes	ME		DI		IE		Total	
	No. of R_1 plants	Frequency %	No. of R_1 plants	Frequency %	No. of R_1 plants	Frequency %	No. of R_1 plants	Frequency %
IS8260	–	–	599	0.17	689	1.89	1,288	1.09[a]
IS8768	226	0.44	290	0.35	775	0.26	1,291	0.31
IS4225	252	0.00	520	0.19	93	0.00	865	0.12
IS3150	349	0.00	948	0.11	348	0.86	1.645	0.24
IS0724	13	0.00	489	0.20	–	–	502	0.20
IS6881	45	0.00	516	0.19	4	0.00	565	0.18
IS2830	17	0.00	246	0.81	5	0.00	268	0.75
SC0167-14E	52	0.00	11	0.00	–	–	63	0.00
Total	954	0.11	3,619	0.22	1,914	0.94	6,487	0.42

ME = Mature Embryo, DI = Developing Inflorescence, IE = Immature Embryo. The number of R_1 plants was counted when the plantlets were transferred to culture bottles from petri dishes

[a] 12 albinos regenerated from a single albino callus were not included

mutation could be detected only in the R_3 generation and not in the R_2 generation, the R_1 plant to which the mutation could be traced was chimeric.

Results and discussion

Variants among R_1 plants

Previous reports on initial sorghum regenerants derived from calli indicated that most R_1 plants were fertile, normal in appearance, and had 20 chromosomes with normal meiotic pairing. Variations found in R_1 plants included albinism, sterility, tallness, waxy midrib as well as chromosomal abnormalities (Bhaskaran et al. 1983; Ma and Liang 1985; Ma et al. 1987; Brar et al. 1979). Similar results were found in our experiments. Almost all R_1 plants were uniform and similar in appearance to the controls. The variants most apparent were lethal types, some dwarfs, and chlorophyll-deficient variants. One dwarf variant had a height of 30 cm, 15 tillers, smaller heads, and was completely sterile. Several lethal seedlings had dark-green color with wide and creased, or narrow and thick leaves. These variants may be the result of chromosome aberration. Table 1 shows that the frequencies of chlorophyll variants in R_1 plants ranged from 0.00%–1.89%, with an average of 0.42%. Immature embryo cultures of IS8260 and IS3150 produced more chlorophyll variants than other cultures. Of 27 chlorophyll variants, 20 were albinos. Five virescent variants were normal green while growing in the culture medium. However, 2–4 weeks after transfer to soil they turned greenish-yellow or white and eventually died. These variants may have higher than normal requirements for nutrients. Two chimeric green and white striped plantlets also failed to survive transfer to pots. The frequencies of the albino, the virescent, and the chimeric striping variants ranged from 0.11%–1.16%, 0.26%–0.58% and 0.15%–0.20%, respectively, with an average of 0.31%, 0.08%, and 0.03%, respectively. The 27 chlorophyll variants were separately regenerated from different calli cultured 80–300 days. On subculture of the callus from which the variants regenerated, no albino or striping plantlets reappeared, except from one callus where 12 albinos regenerated. If those were included, the frequency of chlorophyll variants of IS8260 would be up to 3.6% for cultures established from immature embryos. This is close to the 4.4% produced by immature embryo cultures of sorghum genotype C401-1 (Ma et al. 1987).

Somaclonal variations can occur in homozygous form (Karp 1989). Larkin et al. (1984) and Maddock and Semple (1986) observed nonsegregating, nonparental variant families, suggesting homozygous change in their initial wheat regenerants. Previous studies on sorghum mutation indicated that the albino character is inherited as a simple recessive factor (Karper and Conner 1931). The emergence of R_1 albinos here appears to indicate occurrence of a homozygous sector of albino mutation. The homozygosity could result from mitotic crossing-over (Evans and Sharp 1986). Larkin et al. (1984) explained this phenomenon as a result of cycles of monosomy/disomy or trisomy/disomy and of non-reciprocal transfer of genetic information between repeated DNA sequences.

R_2 field assessment

Over 48,000 R_2 seedlings and 27,000 R_2 adult plants, progenies of 1,055 R_1 plants, were evaluated in 1,126 field rows (Table 2). Most of these tested somaclones were derived originally from several callus lines that had been maintained for 7–10 months and from three cultivars (IS8260, IS8768, and IS4225). R_2 plants were generally

Table 2. Number of R_1 families generated and R_2 seedlings and mature plants evaluated for somaclonal variations cultured from different genotypes

Genotypes	No. of R_1 families				No. of R_2 seedlings/and (no. of R_2 plants)			
	Total	ME	DI	IE	Total	ME	DI	IE
IS8260	480		240	240	20,317 (11,358)		10,676 (5,788)	9,641 (5,570)
IS8768	353	50	120	183	18,469 (9,793)	2,059 (1,840)	8,000 (3,081)	8,410 (4,872)
IS4225	112	24	40	48	3,950 (2,493)	1,739 (1,140)	812 (598)	1,399 (755)
Others	110	51	55	4	5,407 (4,014)	2,887 (2,221	2,397 (1,695)	123 (98)
Total	1,055	125	455	475	48,143 (27,658)	6,685 (5,201)	21,885 (11,162)	19,573 (11,295)

ME = Mature Embryo, DI = Developing Inflorescence, IE = Immature Embryo

Table 3. Somaclonal variations segregated from R_2 population

Phenotypes of variation	No. of R_1 families segregating variants						
		Segregation ratio (normal: variant)					
	Total	1:1–2:1	2:1–4:1	4:1–6:1	6:1–10:1	10:1–20:1	>20:1
Albino	18	1	5	3	5	4	
Yellow-green	9	1	4	2	1	1	
Longitudinal striping	9		1		2		6
Yellow	3		2		1		
Transverse striping	2		1	1			
Greenish-white	1						1
Red spotting	1		1				
Red seedling	1		1				
Abnormal lethal	4	1			3		
Total chlorophyll mutations	48	3	15	6	12	5	7
Super dwarf	5			1		3	1
Short culm	10	2	1		2	2	3
Short culm and sterile	7	1		1	2	1	2
Semisterile	8	5	3				
Sterile	6	2				1	3
Narrow leaf	5		1		2	1	1
Shoot or leaf necrosis	15	1	1	3	5	2	3
Ragged leaf	3				2	1	
Panicle variation	6	2	3	1			
Hydra	6	3	3				
Total viable variations	71	16	12	6	13	11	13
Total variations	119	19	27	12	25	16	20

uniform and phenotypically resembled their parental controls. However, a total of 43 variant phenotypes, which covered a wide spectrum of soghum variations, were found from five of eight cultivars tested.

In the R_2 seedling stage, various chlorophyll-deficient variants were found similar to those described in mutagenesis (Ramulu 1975), including albino (*albina*), lethal or viable yellow-green (*vividis*), yellow (*xantha*), greenish-white (*chlorina*), longitudinal striping leaf and/or stem (*striata*), red spotting (*maculata*), green and white transverse striping (*tigrina*), and red seedlings previously unreported (Table 3). Abnormal dark-green and purple lethal seedlings were also observed. The striping variants (Fig. 1) and some yellow-green variants matured and set seeds. No positive correlation in albino emergence was observed between the R_1 and R_2 generations. Of 689 R_1 plants from IS8260 immature embryo culture, 20 were albinos, including 12 derived from a single callus (2.9%).

Fig. 1. A variant with longitudinal striping leaf and stem derived from a 300-day immature embryo culture of IS8260

Fig. 3. Short culm variants derived from 210- to 270-day-old immature embryo cultures of IS8260, parent control (*extreme right*)

Fig. 2. A "super dwarf" that failed to head, derived from 120-day immature embryo culture of IS8768

However, no albinos appeared in 9,641 R_2 seedlings from 240 R_1 plants. In contrast, of 290 R_1 regenerants derived from IS8768 inflorescence culture, only one was albino (0.35%), while in the R_2 generation, 13.3% of the R_1 families (16 of 120) segregated albinos with an average SVF of 3.0% (93 albinos/3,081 seedlings). The result with IS8260 appears to demonstrate that albino variations are not necessarily caused by recessive nuclear gene mutation. The result with IS8768 may be due to proliferation of single mutated cell clones, because the R_1 plants with progeny segregating for albino were all derived from the same callus line, although from separate callus masses.

Variations found in mature R_2 plants (Table 3) included super dwarf (Fig. 2), various short culm (Fig. 3), sterile (Fig. 4), semisterile, narrow leaf, shoot or leaf necrosis and other variations previously unreported. The leaves of the ragged-leaf (Fig. 5) variant derived from IS8260 270-day inflorescence culture had a sawtooth appearance. They had shorter nodes than the parent and produced pollen but set few selfed seeds. Two kinds of inflorescence variants, one with multibranched panicles and another with long and helical rachilla with lower fertility (Fig. 6), appeared in R_1 progeny rows derived from 270 to 300 days IS8260 inflorescence culture. The two panicle variants were significantly shorter in plant height than normal plants. Another interesting variation is *Hydra*, derived from IS4225 mature embryo cultured for 120–150 days. *Hydra* first appeared as abnormal dwarfs (Fig. 7) in the R_2 generation. In the R_3 generation, *Hydra* developed multiple panicles and other abnormalities due to disordered growth and development (Fig. 8). This developmental variation may resemble an atavistic mutation reported in maize (Poethig 1988). Most of these variants identified were different from their parental controls in more than one character, such as narrow leaf with short culm, dwarf with sterilitiy, etc. This multicharacter change is consistent with previous reports on induced mutation (Gaul 1964). No variants were found in controls rows.

Fig. 4. Variant with sterility and short culm segregated in a R_1 progeny row derived from 300-day inflorescence culture of IS8260

Fig. 5. Ragged leaf variant derived from 270-day inflorescence culture of IS8260

Fig. 6. Panicle variant, branched head (*right*) and long and helical rachilla with lower fertility (*center*), segregated in a R_1 progeny row derived from 300-day inflorescence culture of IS8260 (*left*)

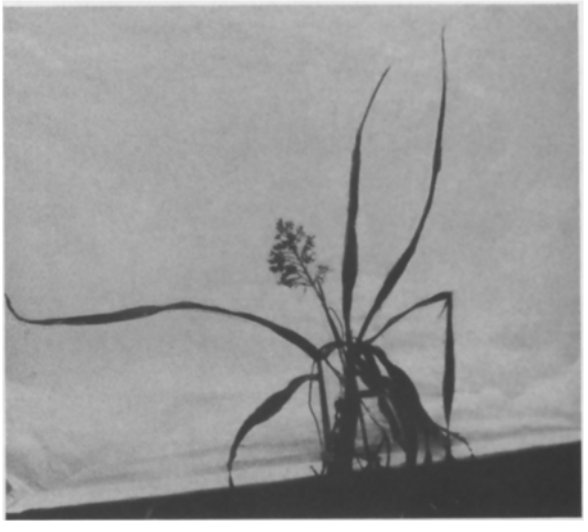

Fig. 7. *Hydra* as it appeared as an abnormal dwarf segregating in a R_1 progeny row derived from 120-day mature embryo culture of IS4225

Table 3 lists the phenotypes of variations observed and the number of each, all of which were detected in individual R_1 families, and the grouping of R_1 families with similar segregation ratios. About 25% of the variant R_1 plants segregated variants with a ratio close to 3:1 (normal:variant). Others gave higher or lower ratios, although the number of R_2 plants grown in R_1 progeny rows was limited, so the ratios cannot be regarded as definitive. Deviation of recessive mutations from 3:1 segregation ratio (recessive excess or deficiency) was previously reported in somaclonal variation (Sun et al. 1983; Zehr et al. 1987; Armstrong and Phillips 1988) and induced mutation (Gaul 1964; Ramulu 1975). Two panicle variants segregated not only independently but also simultaneously in R_1 progeny rows exhibiting multiple unlinked genetic changes. All variations reported here were found by segregating families, suggesting heterozygosity of these mutated sectors. However, chimerism in R_1 plants would hide non segregating phenomenon, for

which the homozygous sector is responsible. If an R_1 plant carrying a homozygous sector of a recessive mutation is a chimera, recessive excess would appear in R_2 progeny from the R_1 plant when the mutated sector is large, while the regenerant would be considered to be a nonsectored plant when the sector is small. Not until the R_3 generation could these possibilities be distinguished.

Possible mechanisms for somaclonal variation have been explained (Larkin and Scowcroft 1981; Evans and Sharp 1986) as change in chromosome structure and/or number, point mutation events, the mobilization of transposable elements, gene amplification and depletion, somatic crossing-over, and changes in cytoplasmic genome. The variations such as short culm, narrow-leaf, sterile, necrosis, and major chlorophyll variations described here could involve simple recessive gene mutations. Changes in chromosome structure or number are also possible for abnormalities such as dwarf, sterile, lethal seedling, etc., and the plastid mutations responsible for some chlorophyll deficiency, such as striping. Detailed genetic analysis of progenies would be required for elucidating the mode of inheritance of the variations.

Fig. 8. *Hydra* in the R_3 generation. This variant had abundant tiller and leaves, multibranched shoots, and numerous independent panicles. Its leaves showed various abnormalities such as small, short, narrow, wide, creased, and brittle. Its panicles were smaller than those of the parent and had normal fertility

The somaclonal variation rate (SVR) and somaclonal variant frequency (SVF) (Table 4) ranged from 1.8%–6.8% and 0.39%–1.54% for chlorophyll variations, 0.9%–25.0% and 0.08%–4.85% for viable variations, 2.7%–31.3% and 1.02%–6.39% for the total variations, respectively. Average SVR and SVF for the three were 4.6% and 0.72%, 6.7% and 0.87%, 11.3% and 1.59%, respectively. In a review on mutation breeding in sorghum (Ramulu 1975), the mutation rate (based on 100 M_1 plants) ranged from 4.0%–43.6% for chlorophyll mutations, and the mutant frequency (based on 100 M_2 plants) ranged from 0.4%–9.9% for chlorophyll mutants and 0.55%–1.37% for viable mutants. The SVR values calculated here do not represent the probability of mutation events occurring in somaclones through tissue culture, because similar mutation phenotypes can possibly result from a clone proliferated from a single mutated cell. However, for the purpose of contrasting recovery of detectable somaclonal variations with mutagenesis, the SVR and SVF values reported here are comparable with rates and frequencies in induction mutation. The SVR values we report are also comparable to those reported in rice (Sun et al. 1983), wheat (Maddock and Semple 1986), maize (Zehr et al. 1987; Armstrong and Phillips 1988), barley (Pickering 1989), and sorghum (Ma et al. 1987) somaclonal variation.

Sequential occurrence and accumulation of mutations have been described in rice (Fukui 1983) and maize (Zehr et al. 1987) somaclonal variation. Lee and Phillips (1987) reported that progeny of clonally related plants often segregated for phenotypically identical variants, and this phenomenon could lead to an overestimation of

Table 4. Somaclonal variation rate (SVR), somaclonal variant frequency (SVF), and number of variant phenotypes calculated in R_2 population

Genotypes	SVR (%)			SVF (%)			No. of variant phenotypes		
	CV	VV	Total	CV	VV	Total	CV	VV	Total
IS8260	3.1	6.9	10.0	0.39	0.83	1.22	8	11	19
IS8768	6.8	2.6	9.3	0.79	0.23	1.02	5	5	10
IS4225	6.3	25.0	31.3	1.54	4.85	6.39	4	7	11
Others	1.8	0.9	2.7	1.11	0.08	1.19	2	1	3
Total	4.6	6.7	11.3	0.72	0.87	1.59	19	24	43

CV = Chlorophyll Variations (for SVR) or variants, VV = Viable Variations (for SVR) or variants

Table 5. Distribution of 6 chlorophyll (CV) and 15 viable variations (VV) in a clonally related population derived from IS8260 immature embryo culture

Item	Culture source	Days in culture				
		210	240	270	300	Total[b]
No. of total R_1 families	Callus-1		16	16	20	52
	Callus-3		16	75	20	111
	Callus-12	11	16			27
	Multiple	29	12	9		50
CV[a]	Callus-1			C_4	C_2	2(2)
	Callus-3		C_2			1(1)
	Callus-12	C_3	C_1C_1			3(2)
	Multiple					0(0)
VV[a]	Callus-1		V_1	$V_1V_2V_5V_7$		5(4)
	Callus-3		V_7	$V_7V_2V_3V_5V_6$	V_6	7(4)
	Callus-12	V_4	V_3			2(2)
	Multiple			V_7		1(1)

[a] C_1, C_2 ... and V_1, V_2 ... represent different chlorophyll and viable variation phenotypes

[b] Number in brackets indicates the number of variation phenotypes

somaclonal variation. Most R_1 plants evaluated here were clonally related and often segregated similar variations in more than one R_1 family. Table 5 shows the distribution of 6 chlorophyll and 15 viable variations in clonally related R_1 populations derived from IS8260 immature embryo culture. It indicates that phenotypically similar variations can segregate in progeny of different callus lines or reppear later in the same callus line population. Among the three callus line populations, callus-3 population with large R_1 families produced relatively less variation phenotypes (5 of 111) than callus-1 with smaller R_1 families (6 of 52) and callus-12 (4 of 27). However, the R_1 families from multiple cultures gave the lowest incidence (1 of 50). Similar distributions were found in progeny populations from other genotypes or cultures except for the albino mentioned above.

We recovered a total of 43 variant phenotypes (Table 4) segregating from 119 R_1 progeny rows of five cultivars. This is a low estimate because some variants that were similar in a major character such as height, but different in another trait such as fertility, were calculated as a single variation. Moreover, variants not detected until the R_3 generation were not included. These results demonstrate that lethal chlorophyll-deficient as well as viable morphological and developmental variations can be recovered from sorghum somaclones derived from tissue cultures in some genotypes with a frequency comparable to that reported for induced mutations in sorghum and for somaclones of other cereal crops.

Factors affecting variation production

Somaclonal variation appears to occur at random, so variation production depends on the size of the base population. The failure to detect variations resulting from some cultivars or cultures may be due in part to the small number of R_1 progeny rows evaluated. However there are differences among parental genotypes on somaclonal variation production. As shown in Table 4, an R_2 population of IS4225 had two- to ten-fold greater SVR and SVF than other cultivars. IS8260 had lower SVR and SVF in chlorophyll mutations, but higher SVR and SVF in other morphological and developmental variations, as well as more (19) variant phenotypes. In contrast, IS8768 produced higher SVR and SVF in chlorophyll mutations but lower in others. Genotypic differences in incidence of somaclonal variation were previously described (Sun et al. 1983; Zehr et al. 1987) and were similarly observed also in mutagenesis work (Ramulu 1975). However, we report here that there were also qualitative genotypic differences in types of variants generated among the different cultivars used in this study.

The influence of time in culture on occurrence of variation has long been recognized (Karp 1989). Lee and Phillips (1987) demonstrated that the proportion of regenerated maize plants carrying qualitatively inherited variants and carrying two or more variations tends to increase with culture age. Our results also indicate that variation production appears to increase with increased time in culture. Table 6 shows that calli cultured 120–180 days, 210–240 days, and 270–300 days produced 7.0%, 14.7%, and 11.1% SVR as well as 10, 23, and 19 variant phenotypes, respectively. After 270 days, chlorophyll variations decreased but viable variations kept increasing. Cultivar IS8260 cultured for 270–300 days produced 10.1% SVR and 11 viable variant phenotypes, while from 210- to 240-day cultures, only 2.8% and 4, respectively, were obtained. Although the tissue source has also been considered to be an influential factor in variation production (Karp 1989), there seems to be no apparent difference among three explant types used here (Table 6). Fewer variant phenotypes were observed from mature embryo cultures, possibly due to shorter culture times or to a smaller population tested. More chlorophyll-deficient phenotypes were recovered from immature embryo cultures. Approximately 69% (33 of 48) R_1 plants carrying chlorophyll variations were regenerated from 210- to 240-day cultures, and 270- to 300-day inflorescence cultures of IS8260 produced the highest number of viable variant phenotypes (nine).

Besides the two striping R_1 variants mentioned above, sectored R_1 plants were also detected from progenies tested. Twenty-seven R_1 plants that had several self-pollinated panicles were tested for chimerism at the plant level. Of four showing the variation, two were chimeric at the

Table 6. Effect of culture age and explant source on somaclonal variation

		Total	Days in culture			Explant source		
			120–180	210–240	270–300	ME	DI	IE
No. of total R_1 plants tested		1,055	297	389	369	125	455	475
No. of mutated R_1 plants	Chlorophyll	48	7	33	8	2	27	19
	Viable	71	14	24	33	9	34	28
	Total	119	21	57	41	11	61	47
SVR (%)	Chlorophyll	4.6	2.4	8.5	2.2	1.6	5.9	4.0
	Viable	6.7	4.7	6.2	8.9	7.2	7.5	5.9
	Total	11.3	7.0	14.7	11.1	8.8	13.4	9.9
No. of variant phenotypes	Chlorophyll	19	5	12	5	2	6	14
	Viable	24	5	11	14	3	14	14
	Total	43	10	23	19	5	20	28

ME = Mature Embryo, DI = Developing Inflorescence, IE = Immature Embryo

Table 7. Proportion of mutated R_1 panicles carrying different sizes of mutation sectors

Item	No. of total R_1 panicles carrying mutation	Proportion of mutated R_1 panicles carrying different size of mutation sectors					
		Large (100–80%)		Medium (80–40%)		Small (<40%)	
		No.	%	No.	%	No.	%
Total	119	46	38.7	31	26.0	42	35.3
Mutations							
Chlorophyll	48	18	37.5	16	33.3	14	29.2
Viable	71	28	39.4	15	21.1	28	39.4
Explant sources							
ME	11	9	81.8	2	18.2	0	0.0
DI	61	25	41.0	14	22.9	22	36.1
IE	47	12	25.5	15	31.9	20	42.6
Cultured days							
120–180	21	11	52.4	8	38.1	2	9.5
210–240	57	23	40.3	16	28.1	18	31.6
270–300	41	12	29.3	7	17.1	22	53.6
Genotypes[a]							
IS8260	48	17	35.4	7	14.6	24	50.0
IS8768	33	10	30.3	13	39.4	10	30.3
IS4225	35	17	48.6	10	28.6	8	22.8

ME = Mature Embryo, DI = Developing Inflorescence, IE = Immature Embryos
[a] Not including three mutated R_1 panicles derived from other genotypes

plant level. One R_1 plant segregated albino only in one panicle row, while no albinos were observed in four other rows. In another R_1 plant, the dwarf sector occupied four of a total of five panicles. In the R_2 generation, segregation ratios (normal : variant) far greater than 3 : 1 may be the result of chimerism. Some chimeric R_1 panicles that segregated R_2 variants with a low frequency may be low-level chimeras. The size of mutated sectors estimated by segregation frequency of variants in individual R_1 progeny rows ranged from the whole plant to less than 3% (striping) of a single panicle. The average proportion of mutated R_1 panicles carrying large (80%–100%), medium (40%–80%), and small (<40%) mutated sectors that may have originated from a cell mass containing one, two, and three or more cells was 38.7%, 26.0%, and 35.3% respectively (Table 7). The range of the proportions varied from 25% to 80% for the large, 15%–40% for the medium and 0%–50% for the small sectors. No small-sectored regenerant from a mature embryo source was detected in R_1 progeny rows. Among 72 R_2 progeny

rows of 29 somaclones derived from mature embryo cultures tested in the R_3 generation, four rows of three somaclones segregated variants that did not appear in the R_2 generation. This demonstrates that chimera-carrying small sectors can also exist in mature embryo regenerants. The frequency of chimeric regenerants estimated in the present study ranged from a low of 10%–20% to a high of 60–70%. Mosaics may have a multicellular origin or may arise from a single cell, with the mutation occurring after division of pro-embryo cell, from a mutation event occurring at the chromatid or sub-chromatid level in the case of cytoplasmic differences or from culture-induced genetic instability.

Although the number of mutated regenerants observed here may be too small to be compared, the range of the proportions listed in Table 7 may reflect a potential difference caused by genotype, culture age, or explant source. In maize, Zehr et al. (1987) detected the existence of chimeric R_1 plants. Lee and Phillips (1987) found that 79% and 54%, respectively, of the R_1 plants regenerated from 3- to 4-month and 8- to 9-month immature maize embryo cultures were sectored. Armstrong and Phillips (1988) indicated that the frequency of chimerism in regenerated plants varies according to type and age of culture, and that it decreases with increasing culture age. These small-sectored R_1 variants and/or low level chimeras may lose their variations when a small number of panicles and seeds are taken from R_1 plants to produce their offspring. Even when the mutated sector only involves one of the male or female gametes, the recessive homozygotes fail to appear in the R_2 generation; nevertheless, the sector is large. Therefore, the chimerism of R_1 plants, the size of the mutated sector, and the procedure for selection of somaclonal variations also affect emergence of induced variations existing in the selected population. Because of the low SVR and SVF produced by some genotypes, as well as the chimeric R_1 plants and small mutation sector observed in this study, the population grown for selecting somaclonal variants should be as large as possible in order to avoid losing variations.

The somaclonal variations of sorghum produced in this study may be valuable for use in breeding programs and some, such *Hydra*, branched head, ragged leaf, semisterile, viable yellow-green mutation, etc., may be useful for basic studies in developmental biology, sorghum genetics, physiology, and biochemical investigations.

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