J. Appl. Genet., 39(1), 1998, pp. 59-72

Somaclonal variation in winter wheat (*Triticum aestivum* L.): frequency, occurrence and inheritance

X.Y. CHENG, M.W. GAO, Z.Q. LIANG, G.Z. LIU

Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University, Hangzhou, China

Abstract. Plants were regenerated from immature embryo cultures of 35 winter wheat genotypes. General responses of regenerated plants were investigated and a total of 7142 R_2 spike lines from 1593 R_1 plants were assessed in the field for somaclonal variants in 1985/86, 1986/87 and 1987/88. Selected variants were studied for their possible genetic inheritance. From regenerated plantlets, 81% survived and 63% produced fertile plants. Forms with reduced plant height, length of spike and other morphological abnormalities were found in this progeny. Populations of R_1 plants were highly variable due mainly to the physiological disturbances resulting from the in vitro process. Overall somaclonal variation frequencies were 14.2% per plant basis and 5.3% per R_2 spike basis. The variants were similar in the three different R_2 generations with predominant variants being negative in plant height, maturity, awns, spike type and plant type. Both uniform R_2 variant families and spike lines were found in addition to the segregating variants which constituted the majority. On average, in a variant family or line, 18% and 14% of their component lines and plants were variants, respectively. Inheritability was demonstrated for the uniform variant families and spike lines as well as segregated variants. Of those 134 selections, about 70% were classified as inheritable. Both recessive and dominant gene mutations at one, two or three loci were evident in some variants as suggested by the segregating data.

Key words: plant breeding, somaclonal variation, tissue culture, *Triticum aestivum*.

Introduction

Somaclonal variation derived from tissue culture has been reported in many plant species, including those of agricultural importance: such as rice (OONO

Received: June 1997.
Correspondence: X.Y. CHENG, Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University, Hangzhou, China.

1975, FUKUI 1983, CAI et al. 1989, 1993) maize (GOEBEL et al. 1986, ZEHR et al. 1987, NOVAK et al. 1988) sugarcane (LIU, CHEN 1976) and wheat (LARKIN, SCOWCROFT 1983, EVANS et al. 1984, LARKIN et al. 1984, 1985, MADDOCK, SEMPIE 1986, RYAN, SCOWCROFT 1987, CARVER, JOHNSON 1989). Extensive work with wheat has revealed that the somaclonal variation observed might result from point mutations, chromosomal translocations, chromosomal aberrations, alterations in gene expression, and mtDNA rearrangement (NAKAMURA, KELLER 1982, LARKIN et al. 1984, OONO 1985, DAVIES et al. 1986, TAO et al. 1989, MORERE-Le PAVEN et al. 1992) although little is known about the mechanisms regulating the processes.

Potential applications of somaclonal variation has been proposed as a supplementary tool to other well established breeding approaches such as cross-breeding and mutational breeding for crop improvement. Earlier studies showed that somaclonal variation could take place at high frequencies and might be stabilized in a few generations (EVANS et al. 1984, LARKIN et al. 1985). Success in improving wheat with somaclonal variation has not been as satisfactory as expected, although a few examples have been reported in which variants with improved agronomical traits have been obtained (RYAN, SCOWCROFT 1987, LAZAR et al. 1988, CARVER et al. 1989). On the other hand, a comprehensive survey by AHLOOWALIA (1986) indicated the frequency and type of somaclonal variation in a number of plant species.

Despite recent attempts to estimate the relative contributions from different sources of regenerants (CHEN et al. 1987, CARVER et al. 1989) an understanding of the occurrence and inheritance of somaclonal variants in wheat and other crops is not well understood. Such knowledge is essential if a breeding strategy using somaclonal variation as a useful donor source is to be designed.

In this paper we present results of analyses on general growth responses of plants regenerated from tissue culture. The frequencies, occurrence and segregation patterns of the variants in R_2 somaclonal families and spike lines, and inheritance of selected variants to R_3 and R_4 generations will be discussed.

Material and methods

Thirty five hexaploid winter wheat *Triticum aestivum* L. genotypes including commercial varieties and advanced breeding lines were used in the experiment conducted during 1985 to 1988 (Table 1). Field-grown plants were employed as the sources of immature embryos for the tissue culture.

The procedures used for callus induction: propagation and plant regeneration were the same as reported earlier (LIANG, GAO 1985, LIANG et al. 1988). Briefly, the embryos were excised 14 - 16 days after anthesis and placed onto a modified MS medium with 2 mg L^{-1} 2,4-D. Resultant callus cultures were subcultured every one to two months and transferred onto regeneration medium without 2,4-D. The cultures and regenerants from the same embryos were systematically numbered to ensure that origins of regenerants were recognizable.

Regenerants, referred to as R_1 plants, were transplanted to soil individually after 160 to 200 days of in vitro culture. Changes in morphological traits were monitored during the subsequent growing periods. At maturity, plants were harvested individually and the relevant parameters such as plant height, length and type of spike, number of spikelets per spike and tillers per plants and fertility were determined along with those of the seed-grown plants. Seeds of each spike were threshed separately to produce spike lines.

R_2 generation refers to plants derived from the selfed R_1 plants. Each R_2 family composed of all spike lines from single R_1 plants (Table 1). The R_2 plants were grown in standard field trials with seed-derived plants of corresponding genotypes as controls.

The R_2 plants were assessed throughout the growing season. Tentative variants were marked. Only plants with qualitative alterations were rated as variants and analysed. When more than one trait was altered simultaneously in a variant, only the most prominent one was recorded. Similarly, a spike line containing variant(s) was referred to as a variant line and a family as a variant family, irrespective of the number of variants contained.

The main characters evaluated for variants included plant height, days to maturity, plant and spike type and waxy character. Strict criteria were applied to identify the variants. For example, plants which were 10 cm taller or shorter and 5 days earlier or later than the control population without other abnormal growth, were rated as respective variant.

The R_2 variant plants were individually harvested along with the phenotypically normal plants appearing in the same variant line. In cases where the variants were sterile or too late to harvest, normal plants were collected. Seeds from individual R_2 plants were bulked and used for the R_3 progeny test.

In the R_3 generation, the field trials were arranged according to the types of R_2 variants and were basically divided into two categories. For the non-segregating families, 8 to 20 R_3 rows were grown using seed from 4 to 10 R_2 variant plants. All of the variant plants of the segregating R_2 families were grown to produce the R_3 generation, together with a number of phenotypically

Table 1. Wheat genotypes used in the somaclonal variation assessments in 1985/86, 1986/87 and 1987/88, and numbers of their regenerants, survival plantlets, fertile adult regenerants as well as numbers of R_1 plants and R_2 spike lines assessed in R_2 generation

Genotype	Description*	Regenerants			Fertile plants		R_1 plants no.	R_2 lines no.
		no.	survived		no.	%		
			no.	%				
1	2	3	4	5	6	7	8	9
1985/86								
Henong 1	C						31	45
Zhemai 2	C						33	121
Yangmai 3	C						55	127
Kangziu 2	C						31	59
908	C						80	232
Xiangmai 9	C						2	6
Shen 2	L						9	27
Wang 9	C						2	5
79 zhong	L						12	20
Xiang 1675	L						17	26
Shuwang 761	L						16	41
Wanpinzu 1	C						158	366
1910/525	L						34	71
Shumai 3	C						45	106
Xiaoyang 6	C						8	17
Shen 5/209	L						9	36
79S3384	L						17	43
Nin 7840	L						22	58
79P-17	L						27	44
Taishan 4	C						88	140
1986/87								
Feng 9	C	14	10	74.1	10	74.1	3	16
Hong 9	C	243	192	78.0	192	65.8	156	1550
Ningmai 6	C	39	26	66.7	15	38.5	14	95
Jia 25	L	4	3	75.1	2	50.0	2	8
Hezing 7495	L	26	17	65.4	14	53.8	14	94
Hejian 477	L	39	25	64.1	23	59.0	23	234
352	L	23	22	95.7	17	73.9	17	72
Henong 1	C	12	6	50.0	4	33.3	4	17
Kangziu 2	C	50	37	74.0	22	44.0	20	99
Jian 78-19	L	29	23	79.3	21	72.4	21	156
Yangmai 4	C	13	6	46.1	6	46.1	6	35
908	C	15	14	93.3	6	40.0	6	96
Shimai 3	C	9	8	88.9	6	66.7	6	32
79P-17	C	8	8	100.0	5	62.5	5	41
1987/88								
Mianyang 11	C	129	112	86.8	66	50.1	41	198
Kangxiu 2	C	149	125	83.9	75	50.3	35	175
Yangmai 4	C	116	101	87.1	81	69.8	52	260
Jian 78-19	L	99	87	87.9	67	67.7	50	250
Jia 6	L	74	63	85.1	51	68.9	41	205

Table 1 (cont.)

1	2	3	4	5	6	7	8	9
Jia 2	L	45	36	80.0	23	51.1	4	36
Feng 13	L	64	53	82.8	39	60.9	32	157
Mianshu	L	98	32	83.7	51	52.0	25	125
Enan	L	31	28	90.3	23	74.2	17	85
Shumai 3	L	61	58	95.1	43	70.5	35	172
79S3384	L	23	19	82.6	11	47.8	2	10
Shuwang 761	L	96	84	87.5	70	72.9	48	240
ND7532	L	50	45	90.0	38	76.0	21	105
908	C	406	356	87.7	265	65.3	197	985
Sum and average %		1965	1593	81.1	1238	63.0	1594	7142

*C – cultivars, L – adranced line.

normal sib-plants from the same lines. Control material was also grown. Data were collected for any altered traits. When segregation for the selected variant traits occurred in the R_3 generation, the segregants were again assessed in the R_4 generation.

Results

Many (1593) regenerants were obtained through callus cultures initiated from the immature embryos of 35 different winter wheat genotypes (Table 1). The responses of the genotypes to in vitro culture have been earlier described (LIANG, GAO 1985). Regenerated plants (R_1) were cultured for as long as 160 to 200 days and therefore displayed high degree of morphological abnormalities. The collected data indicate that on average, 81.1% of the regenerants survived an adaptation period of about two weeks prior to transplanting into the field, and 63.0% of those that survived produced fertile spikes, although considerable variation was seen in the two parameters among the genotypes and between years (Table 1). For instance, in 1985/86 and 1986/87 the former ranged from 46.1% in Yangmai 4 to over 90.0% in lines 908, 352 and 79P-17, and 82.6% in 79S3384 to over 90% in ND7532, Enan and Shumai 3, respectively, while the latter varied from 33.3% in Henong 1 to 74.1% in Feng 9, and 47.8% in 79S3384 to 76.0% in N07532, respectively.

In most of the adult R_1 plants, morphological changes could be easily detected (Table 2). The increased variations recorded in Table 2 were virtually observed in all other genotypes as well. Among the other alterations observed, an extended flowering period was seen in many genotypes, with some regenerants flowering soon after being transplanted to the soil, while others remained

in vegetative growth for one to two months beyond the normal flowering time. In the awnless materials, many tip- or half-awned R_1 plants appeared. For example, 127 and 4 out of 301 R_1 plants from the awnless cultivar 908 were tip- (40.9%) or half- (1.3%) awned, respectively. The results of progeny tests showed that most of the morphological variations observed in the R_1 generation disappeared in later generations (data not shown). Therefore, this variation was not recorded in a class of somaclonal variants.

Table 2. Comparison of morphological traits between the in vitro culture and seed-derived plants of three winter wheat genotypes

Genotype	Source of plant	Plant height (cm)		Length of spike (cm)		Number of spiklets		Fertility (%)	
		mean	range	mean	range	mean	range	mean	range
Hong 9	Seed	81	76–83	7.4	7.2–7.5	17	15–18	95	92–100
	in vitro	64	40–79	7.0	4.5–9.8	17	14–20	86	22–100
Kangxiu 2	Seed	87	81–90	9.4	9.1–9.7	19	17–22	97	95–100
	in vitro	58	49–76	8.9	5.0–9.4	18	13–21	83	10–100
908	Seed	102	96–106	6.7	6.2–6.9	17	15–19	96	93–100
	in vitro	63	23–97	6.1	4.4–7.0	16	12–19	89	0–100

A total of 7142 R_2 spike lines from 1593 R_1 plants of the 35 genotypes were assessed in three successive years for morphological somaclonal variations. The variations and the results related to qualitative traits are presented in Table 1. Somaclonal variants were identified in all genotypes studied, except Jia 25, which was comprised of only 8 spike lines. The overall frequency of the variants were estimated to be 14.2% of R_2 families and 5.3% of R_2 lines. Although considerable differences were seen among the genotypes in producing variants, precise analysis of the relative contribution of genotypic effect to the differences was not attempted because many of the genotypes did not produce the adequate R_2 population (Table 1). The data from 7 genotypes which had relatively large R_2 populations and very similar frequency of typical variants are presented in Table 3. These seven genotypes gave weighted frequencies of 13.5% (on R_1 plant basis) and 5.0% (on R_2 spike line basis). It is worthy noting that the frequencies calculated on R_2 lines basis were not significantly variable while those on R_1 plant basis differed from each other.

R_2 variants of different types recovered from the parental plants varied in a number of morphological traits such as plant height, flowering time, awnedness, waxy type, fertility, and plant stature (Table 4). Although different

Table 3. Frequency of R_2 morphological somaclonal variations in seven winter wheat genotypes

Genotype	No. R_1 plant		No. R_2 line		% of variant based on	
	observed	variant	observed	variant	R_1 plant	R_2 line
908	49	6	190	11	12.2	5.8
Hong 9	32	7	190	16	21.9	4.8
Kangxiu 2	64	9	260	15	14.1	5.8
Shumai 3	60	4	166	7	6.7	4.2
Zhemai 2	20	1	73	3	5.0	4.1
Mianyang 11	57	8	276	13	14.0	4.7
Jian 78-19	30	7	149	8	23.3	5.4
Sum and weight average	312	42	14448	73	13.5	5.0

Table 4. Representative spectra of morphological variations in the R_2 winter wheat somaclones

Trait altered and variant type		Variants[a] in 1986		1987		1988		Sum over years	Sum over traits	
		no.	%	no.	%	no.	%		no.	%
Plant height	+[b]	0	0.0	0	0.0	1	1.3	1		
	−	23	34.3	64	40.8	17	22.6	104	105	35.1
Maturity	+	6	9.0	21	13.4	15	20.0	42		
	−	10	14.9	15	9.6	4	5.3	29	71	23.7
Awnedness	+	0	0.0	0	0.0	7	9.3	7		
	−	12	17.9	2	1.3	2	2.7	16	23	7.7
Waxiness	+	1	1.5	1	0.6	0	0.0	2		
	−	1	1.5	1	0.6	4	5.3	6	8	2.7
Spike type		7	10.4	10	6.4	9	12.0	26	26	8.7
Sterility		4	6.0	4	2.5	5	6.7	13	13	4.3
Stunt plant		0	0.0	29	18.5	9	12.0	38	38	12.7
Tillering		0	0.0	4	2.5	0	0.0	4	4	1.3
Chlorophyll deficiency		0	0.0	4	2.5	0	0.0	4	4	1.3
Kernel colour		2	3.0	N.D.		N.D.		2	2	0.7
Leaf colour		1	1.5	2	1.3	2	2.7	5	5	1.7
Sum		67	100.0	157	100.0	75	99.9	299	299	99.9

[a] The same variants are counted only once for a R_2 variant family, regardless of how many individual variant lines or plants it had

[b] Alterations towards increase or decrease of the trait, respectively

N.D. – Not determined

genotypes were investigated for somaclonal variants between years, similarities could be seen that the most frequently occurring variants were those with reduced height, prolonged maturity, altered awness and spikes, and stunted plants, while chlorophyll deficient variants and tall-strawed variants were rare (Table 4). Specific genotype-dependence on the type of variants was not observed. The tendency to produce short-strawed somaclonal variants dominated. The majority of the variants recovered were not useful as breeding material but some variants with improved agronomic traits were also isolated (i.e., early maturing and short-strawed variants).

The types of variants were classified according to their most pronounced effect in order to simplify analysis, as more than one type of variant was often observed (Table 4). For example, about 85% of the variants isolated in 1985/86 were classified as short-strawed variants associated with late maturity, altered spike type, sterility or early maturity.

Table 5. Incidence of stunt variant families in different somaclonal family groups, each derived from the same embryos of six winter wheat genotypes

Genotype	Embryo	No. of resultant R_2 families	No. of stunt variant families
Yangmai 4	Y1	3	1
908	9-1	2	2
Jia 2	J1	3	2
Hong	H1	9	1
	H2	5	3
	H3	2	1
	H4	3	2
Ninmai	N1	3	1
Hejia	H5	2	1

Observations indicated that the R_2 families originating from the same embryos could contain the same type of variants. For example, stunted plants, which remain in vegetative growth with narrow and darker leaves beyond the tillering stage, were found in six genotypes and most of them could be traced back to a common embryo (Table 5). It was also clear that the proportions of R_2 families containing the stunted variants from the same progenitors varied considerably.

In most cases. only one type of variant appeared in the progenies of an R_1 plant. However, two presumably unrelated variant types were also found in some R_2 families. Among the 71 and 103 variant families isolated in 1986/87

Table 6. Inheritance of R_2 uniform variant family in R_2 generation of winter wheat

Variation	Number of R_2 uniform family in R_3			
	observed	bred true	not inherited	segregated
Late maturity	24	11	10	3
Short strawed	5	1	4	0
Stunt plant	8	8	0	0
Sum	37	20 (54.0)[a]	14 (37.9)	3 (8.1)

[a] Figures in brackets are percentages of the relevant groups

and 1987/88, 3 (4.8%) and 5 (4.2%) were found to segregate for variants of two different traits.

The progenies of R_2 plants from the families identified as uniform late maturity, short-strawed and stunt variants were analysed in R_3 generations (Table 6). Twenty out of the 37 families bred true, 3 segregated for the variant traits, while 14 reverted to normal phenotypes. The uniform stunt variant families were 100% inheritable, while one out the five short-strawed families transmitted the traits to the next generation.

Table 7. Inheritance of R_2 uniform variant line in R_3 and R_4 generation of three winter wheat genotypes

Genotype	R_1 plant code	Variant traits in		
		R_2	R_3	R_4
Feng 9	5-100	Reduced wax coating	Bred true	Bred true
	5-134	Late maturity (7 days later)	Segregated	Bred true (6 days later)
Hong 9	5-67	Late maturity (5 days later)	Not inherited	
	5-31	Darker leaf colour	Bred true	Bred true
Hexheng	5-21	Short-strawed	Not inherited	

Five uniform R_2 variant lines with altered wax coating, plant height. maturity and leaf colour were studied in the R_3 and R_4 generations. The results show that in 3 of them variant traits were inherited in the next two generations. Among them two were not segregating for the variant traits, while the other was stabilized after an additional cycle of selection in R_3 generation (Table 7).

Table 8. Inheritance of R_2 somaclonal variants to R_3 generation of winter wheat

Traits altered		No. of R_2 selections	No. of R_3 progenies			
			not inherited	bred true	simply segregated[a]	cimplex segregated[ab]
Awnedness	+[c]	6	2	3		1
	−	13		1	2	10
Plant height	+	2			2	
	−	29	7	15	7	
Maturity	+	16	2	14		
	−	9	6	1	1	
Spike	Compact	8		5	3	
	Bar	9	1	4	4	
	Sparse	2		2		
Waxness	+	3		2		1
	−	6	1	3	1	1
Stunt plant		15	1	9	5	
Others[d]		16	5	7	2	2
Sum		134	25 (18.7)[e]	66 (49.3)	27 (20.1)	16 (12.0)

[a]Segregated only for the selected variants and their normal counterparts
[b]Segregated also for other traits
[c]As indicated in Table 4
[d]Includes variants of unique culm abounded tillers and curied stem
[e]Percentages over total selections analysed

From the R_2 generations, 134 variants were tested the in R_3 generation for patterns of inheritance. The results indicated that 49.3% of the selections were stabilized for the variant traits and 32.1% were still segregating, while 18.7% did not show any variant traits in the R_3 generation (Table 8). Most of the segregating variants showed simple Mendelian patterns, while a few, particularly tip-awned or awnless variants showed multiple inheritance. Taking the variants of true-breeding and simple segregation together, proportion of inheritable variants would be near 70% or even higher in many variants (Table 8).

To understand the genetic basis of the somaclonal variation, segregation patterns were investigated in the R_3 generation for segregating variants. Data pooled for each family from progenies of single variant plants or normal sibling plants strongly suggested that the gene mutation at single locus, but also in two or three loci, could be involved in the observed phenotype alterations, and be recessive (6 out of 13), dominant (5 out of 13), and co-dominant (2 out of 13) (Table 9).

Table 9. Segregations of the selected somaclonal variants in R_3 winter wheat generation

Variant phenotype	R_1 number	No. of plants in R_3 progeny		Expected ratio	P
		observed	variant		
Stunt plant	139	46	4	9 : 1	0.75 – 0.90
	160	50	5	9 : 1	>0.95
	186	126	4	27 : 1	0.75 – 0.90
Tall-strawed	673	23	17	1 : 3	>0.95
	1105	40	9	3 : 1	0.75 – 0.90
Short-strawed	58	134	97	1 : 3	0.25 – 0.50
	113	17	5	3 : 1	0.50 – 0.75
	136	45	33	1 : 3	>0.95
	160	55	37	1 : 3	0.10 – 0.25
	216	26	19	1 : 3	0.75 – 0.90
	223	48	33	1 : 3	0.25 – 0.50
	241	57	38	1 : 3	0.25 – 0.10
	283	61	42	1 : 3	0.25 – 0.50
	319	65	40	1 : 1	0.05 – 0.10
	530	26	20	1 : 3	0.75 – 0.90
	652	35	9	3 : 1	0.25 – 0.50
	809	33	24	1 : 3	0.90 – 0.95
	824	23	18	1 : 3	0.90 – 0.95
	955	32	23	1 : 3	0.75 – 0.90
	981	39	21	1 : 1	0.75 – 0.90
	1026	26	18	1 : 3	0.25 – 0.50
	1210	24	19	1 : 3	0.50 – 0.75
Broad leaf	463	45	36	1 : 3	0.25 – 0.50
Sterility	958	46	11	3 : 1	0.75 – 0.90
Cured awn	1410	27	6	3 : 1	0.75 – 0.90
Compact spike	85	32	14	1 : 1	0.25 – 0.50
	1397	32	9	3 : 1	0.75 – 0.90
	1110	24	10	1 : 1	0.25 – 0.50

Discussion

The development in wheat tissue culture techniques has made possible mass production of regenerated plants by using immature embryos as explants. Recent studies in wheat have indicated that there is no genotype-dependence for callus induction, but for plant regeneration (CAI et al. 1989). Although there are some reports concerning morphological and chromosomal abnormalities in R_1 plants (HU 1983, KARP, MADDOCK 1984, YE, YU 1989), data on survival and subsequent growth of wheat regenerated plants are rather scattered. The present results indicated that there were genotype differences

in the regenerant's ability to grow into mature fertile plants and about 40% were unable to reach maturity. Both genetic and physiological disturbances following the in vitro processes might account for these abnormalities. As in previous studies (NAKAMURA, KELLER 1982, MADDOCK, SEMPLE 1986, YE, YU 1989), morphological variation among the regenerated plants is significant and generally not inherited. Presence of chimerical structures in the R_1 plants would also prohibit the expression of even dominant changes.

A primary concern in applying somaclonal variation in crop improvement is the amount of variation expressed in the R_2 generation. In earlier reports, LARKIN et al. (1984) showed a high degree of frequencies of somaclones derived from less than 10 embryos in many agronomic traits of a spring wheat Yaqui 50, which could not be repeated in later experiments (MADDOCK, SEMPLE 1986). In the present study, overall R_2 variation frequency in 1593 families and 7142 spike lines from 35 genotypes showed 14.2% variation on R_1 plants, and 5.3% on R_2 spike basis. However, since 10 traits were scored, the number of variants per individual trait would be much lower. The variants appearing in R_2 generations would be those escaping from meiotic selection pressures occurring in the long term culture steps. The frequency calculated on an R_1 plant basis might give a more precise estimation of the degrees of variation occurring in in vitro culture, while that on an R_2 spike basis might provide an estimation of the variation transmitted to the R_2 generation. It is clear that the R_2 spike based frequency depends not only on the R_1 plant variation, but also on the number of spike lines derived from each R_1 plant.

Many morphological and biochemical variants have been identified in regenerated wheat plants (LARKIN et al. 1984, COOPER et al. 1986, DAVIES et al. 1986, MADDOCK, SEMPLE 1986). The variant types depend on the selection method employed and thus do not reflect the entire range of variation. It should be pointed out that minor changes, although frequently seen, have not been included in the analysis. The majority of the variations noted were agronomically negative in value, which is consistent with other results (MADDOCK, SEMPLE 1986). However, variants showing improved maturity, plant stature and disease resistance were also selected.

As genetic changes are induced in callus they would rise to chimeric structure of regenerated plants. FUKUI (1983) made a detailed analysis on the occurrence of mutations in rice callus. Similar examples have been noted in wheat (LARKIN et al. 1984, MADDOCK, SEMPLE 1986) and in maize (NOVAK et al. 1988). In the present study, the average yield of regenerants per embryo was low and plant differentiation started early in culture. Furthermore, the coincidences of the variants suggested that variation occurred at an early stage prior to plant differentiation. In addition, the segregation ratio of individual

spike lines was also highly variable implying that mutations can be induced after formation of spike primordia.

Heritability of the somaclonal variants is a prerequisite to their utilization in breeding programs. Nearly 70% of the variants isolated in R_2 generation transmitted the traits to the next generation. A preliminary analysis based on the segregation of the selected variants or their phenotypically normal sibling plants in the variant lines indicated that these variations could be fitted to assortments of single, double, and triple locus mutations directed to both recessive and dominant action.

Notes. This work was performed in connection with the FAO/IAEA Coordinated Research Programme on "The Use of Induced Mutations in Connection with Haploids and Heterosis in Cereals". Scientific editing of the paper was undertaken by Perry GUSTAFSON (Columbia, Missouri, USA) and Miroslaw MALUSZYNSKI (FAO/IAEA, Vienna, Austria).

REFERENCES

AHLOOWALIA B.S. (1986). Limitations to the use of somaclonal variation in crop improvement. In: Somaclonal Variation and Crop Improvement (J. Semal, ed.). Martinus Nijhoff Publishers, Dordrecht: 14-27.

CAI Q., GAO M.W., LIANG Z.Q. (1993). Preliminary studies on in vitro mutation techniques applied to rice (*Oryza sativa* L.). I. Effect of gamma-rays on somatic tissue *in vitro* culture in rice. Acta Agricult. Zhejiangensis 15: 27-32.

CAI T.S., TIAN H.Q., LIN S.K., LI J.M. (1989). Effect of genotype and embryo age on wheat immature embryo response in in vitro culture. Acta Genet. Sinica 16: 81-88.

CARVER B.F., JOHNSON B.B. (1989). Partitioning of variation derived from tissue culture of winter wheat. Theor. Appl. Genet. 78: 405-410.

CHEN T.H.H., LAZAR M.D., SCOLES C.J., GUSTA L.V., KARTHA K.K. (1987). Somaclonal variation in a population of winter wheat. J. Plant Physiol. 130: 27-36.

COOPER D.B., SEARS R.G., LOOKHART G.L., JONES B.L. (1986). Heritable somaclonal variation in gliadin proteins of wheat plants derived from immature embryo callus culture. Theor. Appl. Genet. 71: 784-790.

DAVIES P.A., PALLOTTA M.A., RYAN S.A., SCOWCROFT W.R., LARKIN P.J. (1986). Somaclonal variation in wheat: genetic and cytogenetic characterisation of alcohol dehydrogenase 1 mutants. Theor. Appl. Genet. 72: 644-653.

EVANS D.A., SHARP W.R., MEDINA-FILHO H.P. (1984). Somaclonal and gametoclonal variation. Am. J. Bot. 71: 759-774.

FUKUI K. (1983). Sequential occurrence of mutations in a growing rice callus. Theor. Appl. Genet. 65: 225-230.

GOEBEL E., BROWN P.T.H., LÖRZ H. (1986). In vitro culture of *Zea mays* L. and analyses of regenerated plants. In: Nuclear Techniques and In Vitro Culture for Plant Improvement. IAEA, Vienna: 21-27.

HU H. (1983). Genetic stability and variability of pollen-derived plants. In: Plant Cell Culture in Crop Improvement. Anonymous, Plenum Press, New York: 145-157.

KARP A., MADDOCK S.E. (1984). Chromosome variation in wheat plants regenerated from cultured immature embryos. Theor. Appl. Genet. 67: 249-255.

LARKIN P.J., BRETTELL R.I.S., RYAN S.A., DAVIES P.A., PALLOTTA M.A., SCOWCROFT W.R. (1985). Somaclonal variation: impact on plant biology and breeding strategies. In: Biotechnology in Plant Science (P. Day, M. Zaitlin, A. Hollaender, eds.). Academic Press, New York: 83-100.

LARKIN P.J., RYAN S.A., BRETTELL R.I.S., SCOWCROFT W.R. (1984). Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67: 443-455.

LARKIN P.J., SCOWCROFT W.R. (1983). Somaclonal variation and crop improvement. In: Genetic Engineering of Plants (T. Kosuge, C.P. Meredit, A. Hollaender, eds.). Plenum Press, New York: 289-314.

LAZAR M.D., CHEN T.H.H., GUSTA L.V., KARTHA K.K. (1988). Somaclonal variation for freezing tolerance in a population derived from norstar winter wheat. Theor. Appl. Genet. 75: 480-484.

LIANG Z.Q., GAO M.W. (1985). Response of different wheat genotypes to somatic tissue culture. Scientia Agricultura Sinica 2: 42-48.

LIANG Z.Q., GAO M.W., CHENG X.Y. (1988). Study on *in vitro* technique of immature embryo culture for wheat breeding. Acta Agronom. Sinica 14: 137-142.

LIU M.C., CHEN W.H. (1976). Tissue and cell culture as aids to sugarcane breeding. I. Creation of genetic variation through callus culture. Euphytica 25: 393-403.

MADDOCK S.E., SEMPLE J.T. (1986). Field assessment of somaclonal variation in wheat. J. Exp. Bot. 37: 1065-1078.

MORÉRE-LE PAVEN M.C., HENRY Y., de BUYSER J., CORRE F., HARTMANN C., RODE A. (1992). Organ/tissue-specific changes in the mitochondrial genome organization of in-vitro cultures derived from different explants of a single variety. Theor. Appl. Genet. 85: 1-8.

NAKAMURA C., KELLER W.A. (1982). Callus proliferation and plant regeneration from immature embryos of hexaploid triticale. Z. Pflanzenzüchtg. 88: 137-160.

NOVAK F.J., DASKALOV S., BRUNNER H., NESTICKY M., AFZA R., DOLEZELOVA M., LUCRETTI S., HERICHOVA S., HERMELIN T. (1988). Somatic embryogenesis in maize and comparison of genetic variability induced by gamma radiation and tissue culture techniques. Plant Breeding 101: 66-79.

OONO K. (1975). Production of haploid plants of rice (*Oryza sativa*) by anther culture and their use for breeding. Bull. Natl. Inst. Agric. Sci. Ser. D. Physiol. Genet. 26: 139-222.

OONO K. (1985). Putative homozygous mutations in regenerated plants of rice. Mol. Gen. Genet. 198: 377-384.

RYAN S.A., SCOWCROFT W.R. (1987). A somaclonal variant of wheat with additional beta-amylase isozymes. Theor. Appl. Genet. 73: 459-464.

TAO Y.Z., HU H. (1989). Recombination of R-D chromosome in pollen plants cultured from hybrid of 6x *Triticale* × common wheat. Theor. Appl. Genet. 77: 899-904.

YE X.R., YU Y.J. (1989). Study on the variation in wheat regenerated plant. II. Cytologic and morphologic variations of R_1 generation. Acta Genetica Sinica 16: 111-117.

ZEHR B.E., WILLIAMS M.E., DUNCAN D.R., WIDHOLM J.M. (1987). Somaclonal variation in the progeny of plants regenerated from callus cultures of seven inbred lines of maize. Can. J. Bot. 65: 491-499.