Note

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Variations in the Gliadin Composition in Immature Embryo Culture-derived Somaclonal Lines of Durum Wheat

Naoko YOKOUCHI[1], Nobuaki ASAKURA[1,4], Sergei TSVETANOV[1,2], Naoki MORI[1], Atanas ATANASSOV[3] and Chiharu NAKAMURA[1,*]

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Abstract

Variations in the gliadin composition in the endosperm were studied in R_4 somaclonal lines that were derived from immature embryo callus cultures of durum wheat (*Triticum durum* Desf.). Electrophoregrams of gliadin polypeptides were analyzed in 10 somaclonal lines of cv. 'Zagorka' and seven lines of cv. 'Progress' and compared with the two parental cultivars. The analysis showed that the parental cultivars were both composed of individuals with different genotypes. Among the somaclonal lines, very high frequencies of variants which showed polypeptide patterns different from those of the parental cultivars were detected. The variations were characterized by the appearance and/or disappearance of particular polypeptides. Some variations were fixed in homozygous conditions but in a majority of somaclonal lines the variations remained in heterozygous conditions.

Because of the mutagenic nature, plant tissue culture has been expected to provide a potential system for generating novel types of genetic variations known as somaclonal variations that are beneficial to agriculture (Larkin and Scowcroft, 1981; Larkin, 1987 ; Vasil, 1987; Evans, 1989). In common wheat, a considerable number of research results on both qualitative and quantitative trait variations were reported in the 1980s (for a review, see Bajaj, 1990). In contrast, information on the somaclonal variations in durum wheat has been quite limited (Sagi *et al.*, 1990).

Wheat gluten is the major component of wheat endosperm proteins consisting of ethanol-soluble gliadins and insoluble glutenins. Particularly, gliadins provide excellent molecular markers for the assessment of genetic constitution of wheat plants because of their high resolution, high heterozygosity and selective neutrality. Gliadin variants showing different electrophoretic patterns from those of the parental donor cultivars were reported in embryo culture-derived somaclonal lines of common wheat (Larkin *et al.*, 1984; Maddock *et al.*, 1985; Cooper *et al.*, 1986). In addition to these, variants showing the increased amounts of particular groups of gliadin polypeptides were described in triticale regenerants (Jordan and Larter, 1985). In this communication, we report variations in the gliadin composition in 17 somaclonal lines derived from immature embryo callus cultures of two durum wheat cultivars.

A total of 221 self-fertile R_0 plants were obtained from immature embryo-derived callus cultures of two Bulgarian winter durum wheat (*Triticum durum* Desf.) cultivars, 'Zagorka' and 'Progres'. The donor cultivars for embryo cultures were propagated as 3 sets of standard seeds obtained after self-pollination of 3 single plants in each cultivar. After screening for several yield components (number of tillers, plant height, spike length, number of spikelets per spike and number of seeds per spike), a total of 45 somaclonal lines were selected to reach R_4 generation in the Institute of Cotton and Durum Wheat, Chirpan, Bulgaria. The two standard donor cultivars and 10 randomly selected somaclonal lines from 'Zagorka' standard and seven lines from 'Progres' standard were chosen for the present study. Total endosperm proteins were extracted from the mature seeds with Tris -HCl (pH6.8) containing 2% SDS and 5% β-mercaptoethanol. Gliadins were extracted from the total endosperm proteins by incubation with 70% ethanol for 40 min at 40°C after removal of 0.5 M NaCl-soluble fraction. The extracted gliadins were fractionated by Acid-PAGE according to the method by Cooke (1989). Gliadins were stained with Coomassie brilliant blue (R-250). Ten individual seeds were analyzed in each standard set of the two donor cultivars and each somaclonal line.

In cv. 'Zagorka', three different gliadin patterns were detected in the set 1 standard seeds (**Fig. 1-A**). A comparison of the electrophoretic patterns suggest-

[1] Laboratory of Plant Genetics, Faculty of Agriculture, Kobe University, 1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan
[2] Institute of Cotton and Durum Wheat, Chirpan 6200, Bulgaria
[3] Institute of Genetic Engineering, 2232 Kostinbrod-2, Bulgaria
[4] Present address: Laboratory of Biology, Faculty of Engineering, Kanagawa University, Yokohama 221-8686, Japan
* All correspondence should be addressed.
Contribution No. 117 from the Laboratory of Plant Genetics, Faculty of Agriculture, Kobe University.

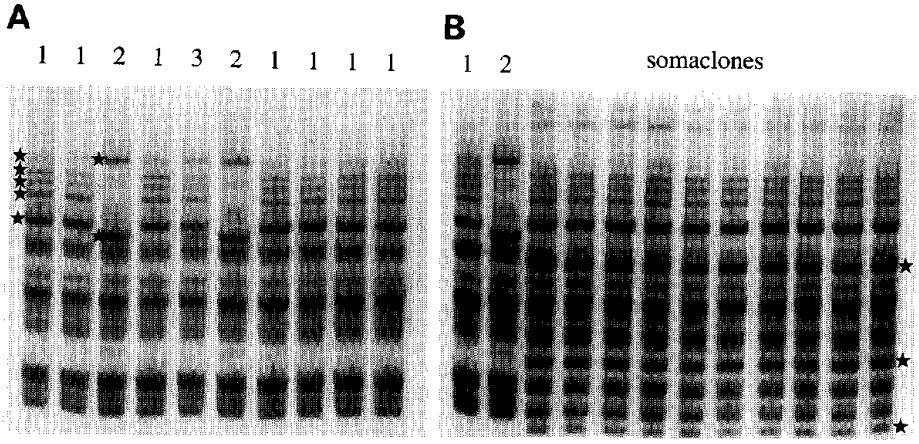

Fig. 1 Gliadin compositions in cv. 'Zagorka' and a somaclonal line.
A: 10 individuals of type 1, 2 and 3 in the set 1 standard, B: type 1 and 2 standards and 10 individuals of the somaclonal line 3. Variant polypeptides are indicated by asterisks. Numbers on lanes indicate gliadin types in the standard set and all other lanes are somaclones.

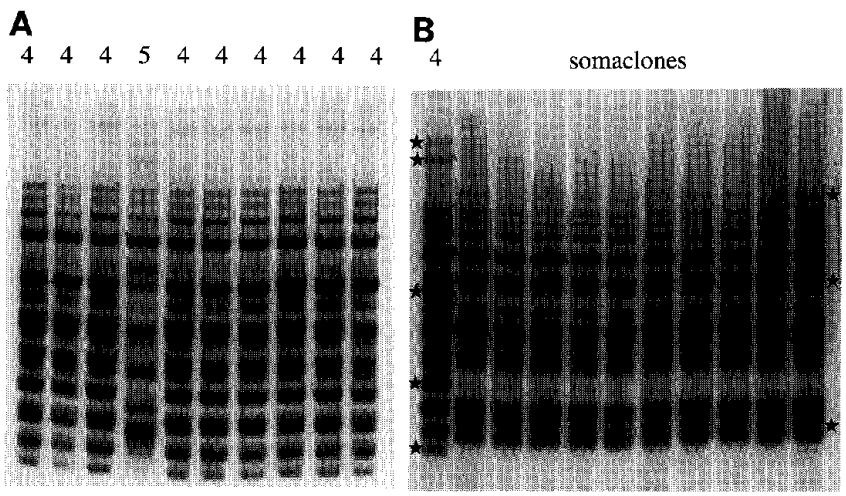

Fig. 2 Gliadin compositions in cv. 'Progres' and a somaclonal line.
A: 10 individuals of type 4 and 5 in the set 2 standard, B: type 4 standard and 10 individuals of the somaclonal line 4. Variant polypeptides are indicated by asterisks. Numbers on lanes indicate gliadin types and all other lanes are somaclones.

ed that five of the gliadin polypeptides in type 1 and two in type 2 were controlled by allelic genes. The results also suggested that type 1 and type 2 were homozygous for each of the alleles but type 3 was heterozygous for these alleles. Type 1 was found in seven out of 10 seeds analyzed and type 2 and 3 in the remaining two and one, respectively (**Table 1**). In the set 2 standard, eight individuals showed a type 1 pattern and two showed a type 2 pattern, whereas the set 3 standard was homozygous for type 1. In the 'Progres' standard also, two types (type 4 and 5, **Fig. 2-A**) were detected in set 2 and two other sets were homozygous for type 4 (**Table 1**).

In the somaclonal lines, variants showing different gliadin patterns from those in the standard cultivars were observed in 48 individuals in eight lines of 'Zagorka' and 57 individuals in all seven lines of 'Progress' (**Table 2**). These gliadin variants were characterized either by the possession of new polypeptide (s) or the absence of particular polypeptide(s) relative to the standard donor cultivars. In the somaclonal line 3 of 'Zagorka', *e.g.*, three new gliadin polypeptides appeared as compared with the standard type 1 (**Fig. 1-B**). In the somaclonal line 4 of 'Progres', five gliadin polypeptides were absent but three other polypeptides appeared or increased their stoichiometric amounts relative to type 4 of the standard (**Fig. 2-B**). Two lines of 'Zagorka' and one of 'Progres' were homozygous for the given variations, but all other variant lines were heterozygous (**Table 2**). The frequency of within-line variations varied considerably but the number of variant types was limited.

We observed considerably high frequencies of variations in the gliadin composition in the immature embryo-derived somaclonal lines of durum wheat. We also observed variations in the glutenin composi-

Table 1 The gliadin compositions in the standard sets of the two donor cultivars, 'Zagorka' and 'Progres'.

Standard set	'Zagorka'			'Progres'	
	type1	type2	type3	type4	type5
1	7	2	1	10	0
2	8	2	0	9	1
3	10	0	0	10	0
Total(%)	81.3	13.3	3.3	96.7	3.3

Ten seeds were analyzed in each standard set of the cultivars.

Table 2 Number of variant individuals and variant types in the gliadin composition in the somaclonal lines.

line	'Zagorka'		'Progres'	
	A[1]	B[1]	A[1]	B[1]
1	3	1	9	1
2	1	1	9	1
3	10	1	9	1
4	10	3	10	1
5	1	1	10	2
6	0	0	9	1
7	10	1	1	1
8	3	1	-	-
9	0	0	-	-
10	10	2	-	-
Total	48	4	57	2

[1]A: number of variant individuals; B: number of variant types.
Ten seeds were analyzed for each somaclonal line.

tions in the same somaclonal lines (data not shown). These results are in clear contrast to that by Sagi *et al.* (1990): they reported no variations in the glutenin composition in R_4 individuals of varying callus origins of three durum wheat cultivars. In common wheat, high frequencies of somaclonal variations in the gliadin composition were reported (Larkin *et al.*, 1984; Maddock *et al.*, 1985; Cooper *et al.*, 1986). Some of these variations were considered to be due to nuclear mutations, thus to be true somaclonal variations. However, Metakovsky *et al.* (1987) pointed out problems of interpreting the results obtained in such studies of somaclonal variation. One possible cause of differences observed in the gliadin composition between the somaclonal lines and the parental line is the presence of different biotypes and/or hidden off-types which can occur in a given cultivar (Appleyard *et al.*, 1979; Pogna *et al.*, 1982; Metakovsky *et al.*, 1986). To avoid such problems we used standard sets of seeds propagated by self-pollination as donor cultivars for embryo cultures and in the test for the gliadin compositions as controls. Even in such standard cultivars we observed the presence of different genotypes in the gliadin composition (**Table 1** and **Figs. 1-A** and **2-A**). Although the possibility cannot be ruled out that still additional variant types might have been present in the standard seeds, the frequency of variants in the somaclonal lines was much higher than that expected based on such hidden heterozygosity.

The gliadin variants detected in the somaclonal lines of durum wheat were characterized either by the presence of new polypeptides or by the lack of parental-type polypeptides (**Fig. 1-B** and **2-B**). Stoichiometric changes in the amount of some polypeptides were also observed. Gliadins as well as glutenins are known to be inherited in groups or blocks of polypeptides which are encoded by single alleles (Payne, 1987). The simultaneous occurrence of variations in different groups of gliadin genes suggests that modifications of the post-translational processing rather than activation and/or silencing (Larkin, 1987) of the gliadin alleles might be responsible for the observed variations.

The number of variant types detected was rather limited. It is however surprising that the frequencies of variants were extremely high among the somaclonal lines of durum wheat. No intentional selection has been applied on the endosperm protein quality and quantity in the present study. Although an exact pedigree of the somaclonal lines is not available, considering the selective neutrality of the endosperm proteins, the observed high frequency of variations suggests that these somaclonal lines might have been derived from a limited number of R_0 regenerants with the limited embryo origins. A further genetic analysis of allelic relationships of the variant polypeptides and molecular analyses of the coding genes and post-transcriptional/translational modifications are necessary to clarify the nature of these gliadin variations.

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