VARIABILITY OF DIHAPLOID LINES OF COMMON WHEAT EVALUATED BY SIGNAL GLIADIN AND HMW GLUTENIN GENES

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Electrophoresis of gliadins and high molecular weight (HMW) glutenins was used for evaluation of somaclonal or gametoclonal variability of 389 dihaploid lines of common wheat, generations A3 and A2, according to the changes of 7 gliadin and 3 glutenin loci. The rate of lines with the above-mentioned changes of signal protein genes was 6.17%. If variability to 3.890 evaluated signal genes was considered, 0.26% of these loci showed allelic changes and 0.41% of mentioned genes were in heterogeneous state. The rate of evaluated changes was statistically significantly lower in generations A3 or A2, respectively, compared with generations A2 or A1, respectively.

common wheat; dihaploid lines; somaclonal variability; gametoclonal variability; gliadin loci; glutenin loci; electrophoresis

INTRODUCTION

The use of *in vitro* technologies in induction of haploidy induces in dihaploid (DH) lines somaclonal or gametoclonal variability.

The aim of this study was to evaluate genetically conditioned variability of DH lines of common wheat, generations A3 and A2, by changes in 7 gliadin and 3 glutenin (HMW) loci. Set of 389 DH lines acquired in the years 1992–1994 was used in the evaluation when grant project Z 660 (DÚ 01) of ME CR "Dihaploidy – induction and use in genetic and breeding process of wheat" was solved.

MATERIAL AND METHOD

The total procedure of creation of dihaploid lines and its monitoring using signal genes and HMW glutenin genes was published (Černý et al., 1996).

In the field nursery of DH lines, generation A3 or A2 (growing season of 1993–1994), 178 spike progenies were evaluated in the site of University of Agriculture Praha-Suchdol and 211 progenies of seven tested hybrid combinations (A3 generation lines of combinations FLORIDA x BUSSARD x ILONA, FLORIDA x JANTAR 50, FLORIDA x ST 950-89, line of generation A2 of combinations Hana x ST 265-88 (= SIRIA) and ST 1393-87 (= SAMANTA) x APOLLO).

Two spikes were collected from each spike progeny during harvest for control electrophoretic analysis of gliadins and HMW glutenin subunits. 464 spikes of DH lines, generations A3 or A2, were analysed in total.

Control electrophoretic analysis tested electrophoretic composition of gliadins and HMW glutenin subunits accomplished in generation A1 of acquired DH lines.

RESULTS AND DISCUSSION

There is a few information on the task of reserve proteins in the process of induced pollen embryogenesis *in vitro*.

During ontogenesis it can be presupposed that except genes functionally connected with transition to sporophytic developmental programme, other numerous groups of genes are activated, typical for certain developmental stages of the germ (seed). Among these genes are, among others, such as Lea, also genes for reserve proteins whose regulation is significantly specifically dependent on developmental stage of seed (embryo, endosperm). Between endosperm and gametophyte a great overlapping of gene activity was described. Functional importance of overlapping gene activities was documented on the manifestation of endosperm mutations which disturb normal development of microspores and growth of pollen tubes and consequently they lead to segregation ratios (Ottaviano et al., 1988). The disturbance of segregation in DH wheat lines was recorded as for HSP (Ottaviano et al., 1991), as in reserve proteins (Bjorstad et al., 1993; Kučera et al., 1993).

In our study the variability of DH lines of generation A3 or A2, respectively, was considered by changes of allelic GLD and GLU (HMW) blocks of zones, i.e. according to changes of respective signal Gld and Glu (HMW) genes.

In total 389 DH lines of generation A3 or A2, respectively, were evaluated electrophoretically. In 9 lines substitutions of alleles of mutation character were found (i.e. 2.31%). Moreover, 15 lines showed heterozygosis in one gene (i.e. 3.86%). If both types of changes are evaluated as manifestations of somaclonal or gametoclonal variability, the rate of DH lines with both types of changes amounts is reached by 24 lines of 389 lines (i.e. 6.17%).

Variability of DH lines can be related also to analyzed Gld and Glu (HMW) signal genes. Of 3 890 evaluated signal Gld and Glu (HMW) genes, 10 genes showed allelic changes (i.e. 0.26%) and 16 loci were in hyterozygotic state (i.e. 0.41%). The total predicted somaclonal or gametoclonal variability reached the values of non-standard loci (i.e. 0.67%). (Tabs. I to III).

Observed predicted somaclonal or gametoclonal variability, respectively, of DH lines of generation A3 or A2, respectively, is significantly lower compared with similar values found in generation A2 or A1, respectively. According to t test of different ratios, the difference found is statistically significant what indicates rather genetic than epigenetic character of variability of DH lines, observed in generation A3 or A2, respectively. (Tabs. I to III).

Marburger and Jahour (1989) studied variability of DH wheat lines derived from the CHRIS variety in: agronomical traits, electrophoretic spectra of some isozymes and in some properties conditioning reduction division. They found that in DH lines significant changes occurred in evaluated traits and properties compared with an initial variety. The reason of observed changes were chromosome mutations which are generally considered as the main source of somaclonal or gametoclonal, respectively, variability.

Analogous knowledge on phenotypic variability of DH lines of triticale was obtained by Gonzáles (1993). Significant increase in comparison with parental forms of variability of prolamines in these DH lines are explained by aberrations or aneuploidy of chromosomes bearing prolamine genes.

The character of variability of prolamine genes induced in wheat by androgenesis is also testified by the knowledge of spontaneous mutability of gliadin genes (Metakovskij et al., 1993). These mutation changes are also manifested by the loss of all gliadins determined by one gliadin locus or by the loss of one zone of allelic gliadin block or by the change of electrophoretic mobility (REM) of some zone of block. The frequency of these spontaneous changes in wheat varieties reaches even 2%. The former changes are caused, as reported by the above-mentioned authors, by chromosome deficiency, while gene mutations induce changes of the second and third types.

In vitro technology used for induction haploidy indisputably induces mutations of prolamine genes. The rate of DH lines with mutated gliadin and glutenin (HMW) genes (6.17% of DH lines) or rate of mutated gliadin and glutenin (HMW) loci – 0.67% Gld and Glu (HMW) loci – confirm this prerequisite.

The knowledge found on variability of signal gliadin and glutenin (HMW) loci, induced during creation of DH lines of common wheat make more exact original ideas on efficiency of the androgenetic method in creation of homogeneous and homozygotic DH lines.

I. Testing (t-test) of different ratios of heterozygotic signal genes in generations A3 (A2) and A2 (A1)

Set A Heterozygotic state of loci DHL A3 (A2) 1993/1994	
Set B Heterozygotic state of loci DHL A2 (A1) 1992/1993	
Basic values	
Total number of individuals in set A	3 890
Number of individuals of the given genotype	16
Total number of individuals in set B	860
Number of individuals of the given genotype	16
Value of tested criterion t	–4.701

II. Testing (t test) of different ratios of mutated signal genes in generations A3 (A2) and A2 (A1)

Set A Mutation of alleles DHL A3 (A2) 1993/1994	
Set B Mutation of alleles DHL A2 (A1) 1992/1993	
Basic values	
Total number of individuals in set A	3 890
Number of individuals of the given genotype	10
Total number of individuals in set B	860
Number of individuals of the given genotype	16
Value of tested criterion t	–5.767

III. Testing (t test) of different ratios of heterozygotic and mutated signal genes in generations A3 (A2) and A2 (A1)

Set A Heterozygotic state of loci + mutation DHL A3 (A2) 1993/1994	
Set B Heterozygotic state of loci + mutation DHL A2 (A1) 1992/1993	
Basic values	
Total number of individuals in set A	3 890
Number of individuals of the given genotype	26
Total number of individuals in set B	860
Number of individuals of the given genotype	32
Value of tested criterion t	–7.376

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Variabilita dihaploidních linií pšenice obecné posuzovaná pomocí signálních gliadinových a gluteninových (VMH) genů.

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Cílem práce bylo posouzení geneticky podmíněné variability DH linií pšenice obecné generace A3 a A2 podle změn 7 gliadinových a 3 gluteninových (VMH) lokusů 389 DH linií kombinací FLORIDA x BUSSARD, FLORIDA x ILONA, FLORIDA x JANTAR 50, FLORIDA x ST 950-89, HANA x SIRIA a SAMANTA x APOLLO. Celkem bylo analyzováno 464 klasů DH linií generace A3, resp. A2.

Kontrolní elektroforetická analýza ověřovala elektroforetickou skladbu gliadinů a podjednotek gluteninů s VMH, uskutečněnou v generaci A1 získaných DH linií.

Variabilita DH linií generace A3, resp. A2 byla posuzována podle změn alelických GLD a GLU (VMH) bloků zón, tj. podle změn odpovídajících signálních Gld a Glu (VMH) genů.

Celkem bylo elektroforeticky hodnoceno 389 DH linií generace A3, resp. A2. U 9 linií byly zjištěny záměny alel mutačního charakteru (tj. 2,31 %). Navíc 15 linií

vykazovalo heterozygotnost v jednom genu (tj. 3,86 %). Pokud se posuzují oba typy změn jako projevy somaklonální či gametoklonální proměnlivosti, dosahuje četnost DH linií s oběma typy změn 24 linií z 389 linií (tj. 6,17 %).

Variabilitu DH linií je možné vztahovat rovněž k analyzovaným Gld a Glu (VMH) signálním genům. Ze 3 890 hodnocených signálních Gld a Glu (VMH) genů 10 genů vykazovalo alelické změny (tj. 0,26 %) a 16 lokusů se nacházelo v heterozygotním stavu (tj. 0,41 %). Celková předpokládaná somaklonální, resp. gametoklonální variabilita tak dosahuje hodnoty 26 nestandardních lokusů (tj. 0,67 %).

Pozorovaná somaklonální, resp. gametoklonální variabilita DH linií generace A3, resp. A2 je ve srovnání s obdobnými hodnotami zjištěnými v generaci A2, resp. A1 statisticky významně nižší.

pšenice obecná; dihaploidní linie; somaklonální proměnlivost; gametoklonální proměnlivost; gliadinové lokusy; gluteninové lokusy; elektroforéza

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