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Estimation of induced mutation rates of four esterase genes in barley (*Hordeum vulgare* L.)

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Abstract. Induced mutation rate of barley esterase loci has been estimated. Results suggested that about 3% of investigated M_1 spikes had seeds which gave rise to M_2 seedlings mutated in one of four esterase loci. M_1 plants were obtained after chemical treatment of seeds from two spring barley cultivars Aramir and Bielik. The majority of mutants were reconfirmed in the M_3 generation.

Key words: *Hordeum vulgare*, isozymes, mutants, mutation frequency.

Introduction

In diploid self-pollinated plants, the mutation rate of a single gene is difficult to estimate. For morphological traits, usually inherited in a dominant/recessive manner, it is necessary to obtain hybrid seeds for large scale mutagenic treatment to make field observation of individual trait mutations possible (TSUNEWAKI 1983). The estimation of mutation rate in a specific locus is facilitated when the expression of a mutated gene is not obscured by the parental phenotype, which is the case for codominantly inherited characters. Isozyme alleles are generally codominant so all the gene combinations can be visualized in the first non-chimeric tissues formed after mutagenic treatment namely seed embryo (M_2) in M_1 spike. The presented investigation was planned to prove the usefulness of isozyme markers for estimation of the mutation rate of single genes of self-pollinated species and to compare the mutation frequency of specific loci in two spring barley varieties of different sensitivity to the mutagen. The inheritance of mutant allozymes in the next generation was also investigated.

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Material and methods

Presoaked seeds of two spring barley varieties Aramir and Bielik cultivated in Poland were subjected to mutagenic treatment with chemical mutagens: sodium azide (NaN_3) and N-methyl-N-nitroso urea (MNH). The mutagens were applied in double treatment, according to MALUSZYNSKA and MALUSZYNSKI (1983), in concentrations of 1.5 mM NaN_3 and 0.7 mM MNH.

The treated seeds were sown directly in the field. Mature M_1 plants were harvested separately. The individual spikes, except for apices, from 100 M_1 plants of each variety were sown on wet perlite. The upper halves of seven day old seedlings, at least 5 from each spike, were subjected to starch gel electrophoresis and then stained for esterase (SOLTIS et al. 1983). Spikes having less than 5 seedlings were discarded. For the majority of mutated Aramir spikes all seedlings were analysed, but as it affects the survival rates of developing plants from the investigated seedlings, it was decided to limit the number of analysed seedlings from each spike.

Results and discussion

The enzymatic system of esterase was chosen for two reasons. The first is that on a single gel it is possible to monitor the activity of four isoenzymatic loci, which are responsible for the very clear and reproducible banding pattern. The second is that in a previous study KUCHARSKA and MALUSZYNSKI (1991a) found that esterase is the most diverse system in the spring barley chemomutants collection. Three loci of esterases, *Est1*, *Est2* and *Est4* are located close to each other (ALLARD et al. 1970) in the terminal part of barley chromosome 3 (KONISHI, MATSURA 1987). *Est5* is located in chromosome 1S (KUCHARSKA, MALUSZYNSKI 1991b).

Allozymes different from those of the parent variety were considered as a consequence of the mutational event if the segregation in M_1 spike was observed. Table 1 summarizes data on frequency of esterase mutants induced in spring barley varieties Bielik and Aramir. Out of 257 Bielik spikes isozyme analysis revealed six (2.7%) carrying mutated seedlings while for Aramir it was nine mutated spikes (3.2%) out of 202 analysed.

Two out of nine mutated spikes of Aramir were derived from the same M_1 plant. Since identical allozymes segregated in both spikes in question, it was concluded that both developed from the same initial cell. The remaining 7 spikes represented different M_1 plants. In one Aramir spike segregation in two loci was detected. This can be interpreted as the occurrence of two sim-

Table 1. Frequency and description of M_1 spikes of two spring barley varieties having mutated seedlings

Variety/spike No.	Locus	Mutant allozyme	No. of seedlings		
			total	homozygote mutant type	heterozygote
Bielik					
1	*Est1*	fast	5	–	2
2	*Est1*	null*	5	1	?
3	*Est2*	slow	5	–	2
4	*Est5*	null	9	2	?
5	*Est1*	fast	9	2	3
6	*Est2*	null	9	2	?
Aramir					
1	*Est1*	fast	15	3	7
2	*Est1*	fast	5	1	–
3	*Est2*	null	7	1	?
4	*Est4*	slow	8	2	2
5, 6	*Est4*	slow	16	4	3
7	*Est4*	slow	5	1	1
8	*Est5*	null	5	1	?
9	*Est1, 4*	slow, slow	8	2	3

* for null mutants number of heterozygotes was not estimated

Table 2. Esterase genotyes of M_2 plants of two spring barley varieties

Variety	M_1 spike	No. of M_2 plants	Homozygotes mutant type	Heterozygotes	Homozygotes parent type	Locus
Bielik	1	9	–	1	8	*Est1*
	2	6	2	2	2	*Est1*
	3	2	–	2	–	*Est5*
	4	3	1	–	2	*Est2*
	5	6	2	2	2	*Est1*
	6	1	1	–	–	*Est2*
Aramir	1	5	–	2	3	*Est1*
	2	4	–	1	3	*Est1*
	3	2		–	2	*Est2*
	4	8	–	–	8	*Est4*
	5, 6	4	–	–	4	*Est4*
	7	0	–	–	–	*Est4*
	8	6		3	1	*Est5*
	9	2	–	2	–	*Est1, 4*

ultaneous mutations in one initial cell. Each segregating Bielik spike represented a different M_1 plant.

There was no significant difference in M_2 mutant frequency between progenies of both the treated varieties. For Bielik the *Est4* mutants were not observed, but more mutants were found in *Est2* instead.

In Table 1 the column 'Mutant allozyme' shows the relation of induced form to parent variety type in respect of isozyme mobility. Three independently induced 'slow' allozymes of *Est4* have probably the same mobility and they are coding for a commonly occurring band. They were run on various gels. Two independent 'fast' mutant forms of *Est1* from Bielik and two 'fast' forms from Aramir are respectively the same. Those from Bielik are commonly occurring allozymes, while those from Aramir are rare.

The relative mutation rates of particular esterase genes seem to be similar and also similar to the rates found by TSUNEWAKI (1983) for specific wheat genes responsible for plant morphology. In spite of the fact that only a limited number of M_2 plants were investigated from each spike, it was possible to prove that most of the mutants bred true in M_3 (Table 2). Nevertheless, the Aramir *Est4* mutants and *Est2* mutant were not recovered in the M_3 generation. As for the mutants derived from spikes Nos. 3, 5, 6, it was possible to investigate the progeny of only a portion of the planted seedlings, so there is a chance that there were mutants among those which were not investigated. In the case of spike No. 4, plants were obtained from all seedlings but none showed an isozyme pattern different from the parent variety.

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