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Induced isozyme polymorphism in spring barley mutants

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Abstract. The usefulness of mutagenic treatment to enlarge isozymic variability of barley and the use of induced mutants for genetic analysis were evaluated. N-methyl-N-nitroso urea, sodium azide and gamma rays were employed as mutagenic agents. Electrophoretic assays of 3848 M_2 seedlings obtained by chemical mutagenic treatment of the spring barley cultivars Dema, Aramir, Bielik and 3100 M_2 seedlings obtained by physical mutagenic treatment of the cv. Dema revealed 70 isozymic mutants, which represent 30 separate mutants in 25 M_1 plants. Most of mutations (27) were induced by chemical mutagen at polymorphic esterase loci. The occurrence of induced mutants at monomorphic loci, Got2 and Lap2, made it possible to perform genetic analysis of those loci in barley including mapping respective genes within chromosomes.

Key words: barley, induced mutants, isozymes.

Introduction

An effective and efficient mutagen must induce a high number of mutations and a low number of deleterious effects. More than 1700 officially released varieties of 154 different plant species have been developed by means of induced mutations (MAŁUSZYŃSKI et al. 1995). The high mutagenic effectiveness of MNH (N-methyl-N-nitroso urea) in inducing morphological variability, including "dwarfness" in spring barley, was proved by MAŁUSZYŃSKI (1982). Sodium azide is also considered to be an effective and efficient mutagen for barley that causes predominantly point mutations (KLEINHOFS et al. 1978).

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The use of genetic markers to select chromosome segments carrying beneficial agronomic genes is not new. A particulary good example is a close linkage between nematode resistance and the allozymic variant *Aps1* in tomato (RICK, FOBES 1974). Unfortunately, in barley an adequate number of useful markers have not been available to develop the "marker assisted selection" methods. First, the useful marker should not affect the agronomic phenotype, if we are seeking a "tag" for use in breeding, second, it should show variability between the lines which are employed in breeding programmes. The first requirement is generally met by isozymes while the second one is frequently a limitation due to a low level of isozyme variability among cultivars. TANKSLEY and RICK (1980) found that most cultivated tomato varieties have very similar genotypes. NEVO et al. (1979) examined isozyme diversity in the Composite Cross XXI of barley, synthesized from 6200 genotypes and recognized by barley breeders to be much more variable than any other variety, and found genetic variation in only 25% of the loci investigated.

The purpose of this work was: 1) to prove the usefulness of mutagenic treatment in obtaining isozyme mutants of barley; 2) to compare the effect of chemical and physical mutagens on mutation rates of 9 isozyme genes; 3) to exploit induced isozyme mutants for genetic study of barley.

Material and methods

Materials consisted of Polish spring barley cultivars, series of homozygous barley mutant lines, and barley chromosome markers.

Seeds of the three cultivars, Aramir, Bielik and Dema, were obtained from the Plant Breeding Station in Łagiewniki. Homozygous barley mutant lines (mutant locus in brackets): 394JK (*Est1*), 638D (*Est5*), 064AR (*Got3*), 673Q (*Got2*) and 843Q (*Lap2*) were developed at the Department of Genetics, Silesian University. Multiple Recessive Stock (MRS), containing markers *lk2* (short awn) and *n* (naked caryopsis) for chromosome 1L, and marker *o* (orange lemma) for chromosome 6L, was provided by Dr. J. D. Franckowiak from North Dakota University. Markers *uz* (semi-brachytic dwarf), *ert c* (dense spike), *ari a* (short awn) for chromosome 3L, marker *br* (brachytic dwarf) for chromosome 1S, and translocation lines involving chromosomes 1 (T1-7f, T1-3b), 3 (T3-7c), and 6 (T6-7i) were obtained from Svalöf. Markers of chromosomes 1, 3 and 6 were chosen on the basis of information suggesting a linkage group of the investigated loci (BROWN, MUNDAY 1982, NIELSEN 1982, SOLIMAN, ALLARD 1989).

For preliminary experiments with the use of the cultivars Aramir and Bielik (KUCHARSKA, MAŁUSZYŃSKI 1995) only a double chemical treatment with sodium azide (NaN_3) and N-methyl-N-nitroso urea, (1.5mM NaN_3 + 1.5mM MNH) was applied. In the next experiment seeds of the spring barley cv. Dema were subject to chemical and physical mutagenesis. Two methods of chemical mutagenic treatment were employed: a double treatment with MNH and NaN_3 or a double treatment with only MNH. The following treatment combinations were applied: 1.5mM NaN_3 + 1.5mM MNH, 0.75mM MNH + 0.75mM MNH, 0.5mM MNH + 0.5mM MNH. Each treatment lasted for three hours with six hour inter-incubation period. For physical treatment gamma rays were used in doses: 180Gy, 150Gy and 120Gy. Treated seeds were sown in the field and M_1 plants were harvested individually. In most cases 100 M_1 plants per treatment combination were analysed. All spikes, except the longest ones, from 100 M_1 plants of the cv. Aramir and from 100 M_1 plants of the cv. Bielik, numbering 202 and 257, respectively, were germinated and at least 5 seedlings from individual spikes were analysed. Moreover, 80, 84 and 100 M_1 plants of the cv. Dema from combinations with chemical mutagens were analysed. The longest spike from each M_1 plant was sown into wet perlite for germination. At least five randomly distributed seedlings from spike were analysed using starch gel electrophoresis. All the remaining spikes from each M_1 plant, found to have changes in isozymic patterns, were also analysed. It made 98, 92 and 116 spikes per combination, respectively. After physical treatment of the cv. Dema, all the spikes from 300 M1 plants (100 plants per dose) numbering 620, were analysed.

Starch gels were prepared and stained for esterases (*Est1*, *Est2*, *Est4*, *Est5*), aspartic acid transaminase (*Got1*, *Got2*, *Got3*) and leucyl aminopeptydase (*Lap1*, *Lap2*) according to SOLTIS et al. (1983).

The earlier selected mutants 638D, 064AR, 673Q and 843Q (KUCHARSKA, MAŁUSZYŃSKI 1991a) obtained after chemical mutagenesis with MNH and NaN_3 were crossed to chromosome markers and to each other. F_2 populations were morphologically and electrophoretically examined to estimate linkage relationships between isozyme loci and barley chromosome markers and to select a multiple mutant-isozyme recombinant plant.

Results

Isozyme mutants frequency

Table 1 summarises results on the frequency of isozymic chemomutants derived from the cvs. Dema, Aramir and Bielik. In total, 29 independent isozyme mutations were detected. There was no significant difference in mutant

frequency between the three cultivars after a double treatment with MNH and NaN_3. The percentage of M_1 plants carrying isozyme mutation was 5%, 8% and 6%, respectively. However, the frequency of mutations which occurred in the initial cells should be rather estimated on the basis of single spikes. The mutation frequencies expressed as the number of mutated loci per 100 spikes, were: 8.8% for the cv. Dema, 4.5% for Aramir and 2.3% for Bielik. The highest frequency of mutations observed for the cv. Dema was, probably, the effect of the longest spikes analysis. These spikes were apical in most cases, which means that they were developed from more initial cells than others.

The frequency of mutations of the cv. Dema after two doses of a double chemical treatment only with MNH was 2.3% and 3.4%. The majority of mutations, that is 8.8%, occurred after a double treatment with MNH and NaN_3.

Table 1. Isozyme mutants induced by a double chemical treatment for the spring barley cvs. Dema, Aramir and Bielik

Cv. / dose (mM)	M_1 plant No./No. of spikes with mutation	Loci/induced allozyme type	No. of M_2 seedlings with mutation spike	
			analysed	mutants
Dema / 1.5 NaN_3 + 0.75 MNH	1/1	*Est4, Est5* / fast, fast	5	1
	2/1	*Est1* / slow	13	9
	3/1	*Est4, Est5, Got3* / null, slow, null	12	2
	4/2	*Est5* / slow	10	2
Aramir / 1.5 NaN_3 + 0.75 MNH	5 /1	*Est1* / fast	15	10
	6/1	*Est1* / fast	5	1
	7/1	*Est2* / null	7	1
	8/1	*Est4* / slow	8	4
	9/2	*Est4* / slow	16	7
	10/1	*Est4* / slow	5	2
	11/1	*Est5* / null	5	1
	12/1	*Est1, Est4* /slow, slow	8	5
Bielik / 1.5 NaN_3 + 0.75 MNH	13/1	*Est1* / fast	5	2
	14/1	*Est1* / null	5	1
	15/1	*Est2* / slow	5	2
	16/1	*Est5* / null	9	2
	17/1	*Est1* / fast	9	7
	18/1	*Est2* / null	9	2
Dema / 0.75 MNH + 0.75 MNH	19/1	*Est4, Est5* / slow, slow	5	1
	20/1	*Est5* / slow	5	1
Dema / 0.5 MNH + 0.5 MNH	21/1	*Est5* / slow	5	1
	221	*Est5* / fast	5	1
	23/1	*Lap2* / slow	5	1
	24/1	*Est2* / null	5	3

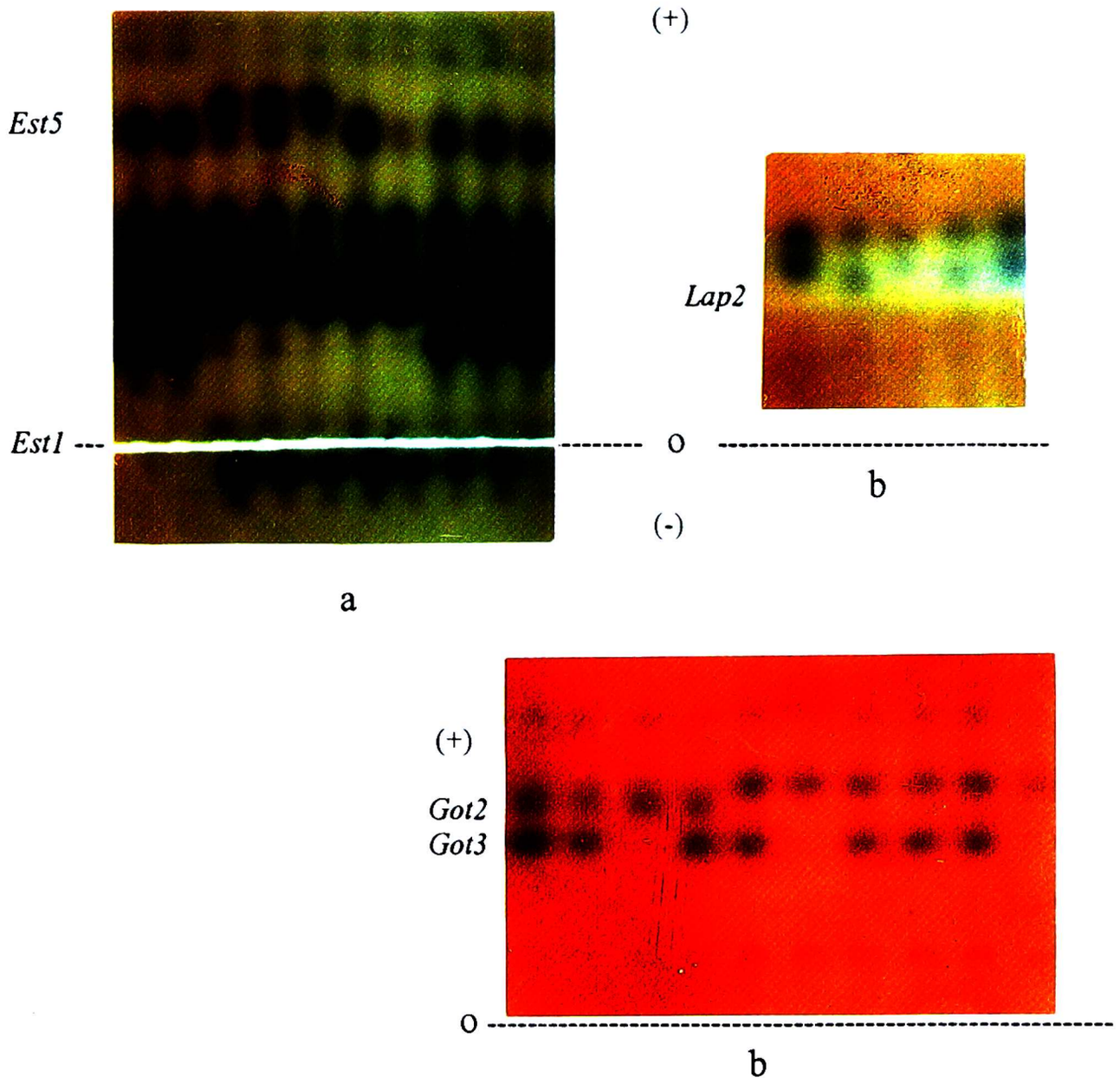

Fig. 1. Electrophoregrams of F_2 progenies

a – Esterases showing segregation for locus Est1: CC, CC, MM, MM, MM, MM, MM, MC, MC, CC and for locus Est5: MM, MM, MC, MC, CC, MM, MM, MM, MM, MM; b – Leucyl aminopeptydases showing segregation for locus Lap2: CC, MM, CC, MM, MC; c – Aspartic acid transaminases showing segregation for locus Got2: MM, MM, MM, MM, CC, CC, CC, CC, CC, CC, and for locus Got3: C., C., MM, C., C., MM, C., C., C., MM. M – mutant allozyme, C – common allozyme (genotype designation from left to right)

After physical treatment only one out of 620 spikes from M_1 plants had a seedling with a changed mobility of the band at one locus (*Est5* / fast). This makes the frequency of mutation events at nine isozyme loci as low as 0.16%.

In most cases isozyme changes occurred only in one spike per plant. However, in the case of plants No. 4 and 9 one more spike showed the same changes. In plants No. 1, 3, 8 and 19, changes were found to occur at more than one locus. Those multiple changes were always detected in the same seedling.

The column "Induced allozyme type" in Table 1, shows the relative mobility of the induced allozyme form as compared to parent variety type. Since the progeny of each mutant M_1 plant was analysed on separate gels, it was impossible to state whether all slow or all fast forms of *Est5* from different M_1 plants of the cv. Dema had identical mobility. For the cvs. Aramir and Bielik mutants from plants No. 5, 6 were identical and those from plants No. 13, 17 were identical as well. Majority of mutations detected for those two cultivars were confirmed in the next generation (KUCHARSKA, MAŁUSZYŃSKI 1995).

Most of esterase mutant allozymes seem to be the same as already identified in barley species (KAHLER, ALLARD 1981), while mutants in *Got3* from plant No. 3, *Lap2* from plant No. 23 and *Est1* from plants No. 5, 6 are probably unique.

Genetic analysis of isozyme mutants

Isozyme mutants detected earlier were used to perform genetic analysis including localization of isozyme loci within chromosomes (KUCHARSKA, MAŁUSZYŃSKI 1991b, KUCHARSKA et al. 1995) and to develop multiple isozyme marker line containing four unique allozymes for *Est1*, *Got2*, *Got3*, and *Lap2*.

All mutant allozymes (Fig. 1) showed mendelian segregation in F_2. It was consistent with 1:2:1 for *Est1*, *Est5*, *Got2*, *Lap2* (shift mutants) and with 3:1 ratio for *Got3* (null mutant). All five mutant isozymes segregated independently of each other.

Analysis of F_2 obtained from crosses between barley chromosome markers and mutants, introducing variability in the so-far monomorphic loci, made it possible to estimate linkage relationships of four isozyme loci with well known barley markers. The recombination values between the isozyme locus *Est5* and two markers localized on the short arm of chromosome 1, *Got3* and four markers localized on the long arm of chromosome 3, *Got2* and two markers localized on the long arm of chromosome 6, *Lap2* and one marker of the long

arm of chromosome 1 were determined (Table 2). Free segregation was shown between *Est5* and three markers of 1L (*n, lk2, ert a*). Free segregation was also shown between *Lap2* and markers *br, n,* and *lk2*, which exclude 1S and a distal part of 1L as a possible location of *Lap2*. On the other hand, preliminary analysis suggests linkage of this locus and *ert a*, marker of the central part of 1L.

Table 2. Linkage relationships between isozyme loci and spring barley chromosome markers

Isoenzyme locus	Marker / chromosome	Recombination value* (%)	Number of F_2 plants
Est 5	T1-7f / 1S	1.0±0.04	251
Est 5	br / 1S	15.7±3.1	165
Got 3	T3-7c / 3L	15.0±2.5	155
Got 3	uz / 3L	8.8±2.5	204
Got 3	*ert c* / 3L	11.6±1.9	152
Got 3	*ari a* / 3L	13.1±3.1	129
Got 2	T6-7i / 6L	17.8±3.0	136
Got 2	o / 6L	24.1±3.0	83
Lap 2	T1-3b / 1L	13.8±2.1	197

* Recombination values were calculated by Maximum Likelihood Method (ALLARD 1956, HANSON, KRAMER 1950)

Discussion

In total, 30 independent mutations were detected. Two different mutations out of them occurred in single spikes from three different plants, whereas three mutations occurred in one spike from the other plant. Only two mutations were expressed in more than one spike of the plant. This data shows that more than one mutation may occur in a single initial cell. At the same time, each mutation is expressed mainly in one spike.

There is a substantial difference in the mutant frequencies induced by optimal doses of chemical and physical mutagens at the isozyme loci of barley. It is in agreement with the statement of MICKE et al. (1987) that various chemical mutagens give a higher mutation rate than physical mutagens. Nevertheless, practical problems with chemical mutagens (e.g. safety of handling, poor reproducibility) cause that radiation was more often used to generate desirable traits in crops (MAŁUSZYŃSKI et al. 1994).

Chemical treatment should be regarded as an efficient source of isozyme mutants. Induced allozymes are predominantly of "shift" type which could be

interpreted as a result of point mutations. Most of the detected mutations among the investigated isozyme loci occurred in esterases. It can be inferred that either these loci are more vulnerable for mutagenic treatment or that cells with mutations in other loci were eliminated before they could develop into M_2 seedlings. The phenomenon of selectively high mutability of specific loci was also shown by LUNDQVIST and LUNDQVIST (1988).

Isozyme mutants proved to be useful in localization of relevant loci within chromosomes. So far, most of the isozyme loci in barley have been attributed to appropriate linkage groups with the use of wheat-barley addition lines (HART et al. 1980). The method of addition lines does not require intraspecific variability. On the other hand this method does not allow localization of a locus in question in a specific region of a chromosome. The precise localization can be achieved by multipoint linkage test, which requires variability in all loci involved. Due to a low level of variability only few isozyme loci have been integrated to RFLP map of barley (KLEINHOFS et al. 1993). A low level of variability in some of isozyme loci can be overcome with the help of mutagenesis.

It is worth pointing out that the isozyme systems which are currently applicable in breeding programmes, i.e. endopeptidase linked to eye spot resistance (Mc MILLIN et al. 1986) in wheat, are being used to "tag" chromosome segments rather derived from alien species than from bread-varieties with desirable agronomic variants. By the use of suitable mutagenic techniques it may be possible to induce an isozyme variant at already mapped locus in the parent carrying beneficial agronomic genes.

Conclusion

Mutagenic treatment is an efficient method to obtain isozyme mutants of barley. Chemical mutagenesis gives much higher mutation rates than physical at isozyme loci. Although, most of mutations occur at variable esterase loci, it is also possible to obtain mutants for other isozyme systems. Isozyme mutants are helpful in genetic study of barley species and possibly in breeding as "tags" of chromosome segments.

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