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Evaluation of Mutation Rate by Differences of Isozyme Band Patterns on M_2 Seedling Treated with Chemical Mutagen in Barley

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보리 化學突然變異劑 處理 M_2 유묘의 Isozyme band pattern差異에 의한 돌연변이율 검정

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ABSTRACT : Mutation rate of M_2 plants that were treated with three types of double treatments of chemical mutagens(1.5mol NaN_2 + 0.75mol MNH, 0.75mol MNH + 0.75mol MNH and 0.5mol MNH + 0.5mol MNH) were estimated on the rate of chlorophyll mutant, changes of isozyme loci ; esterase (Est), glutamate oxaloacetate transaminase(GOT ; AAT) and leucyl aminopeptydase(LAP ; AMP). Rate of chlorophyll mutants (3.3% = no. of seedling carrying mutant / all number of M_2 seedlings × 100) and rate of esterase isozyme loci mutants(3.5% = no. of plant carrying mutant / all number of M_2 plant) in Dema were higher than one of Sacheon 6, but no significant differences in GOT, LAP. Among isozymes, most of mutants in M_2 plant of two varieties were found in esterase (73% of total mutants were occurred in esterase loci). Although many of null bands were found in GOT 3, these were not repeatable and no real mutants. It might be due to qualities of starch, amount of extract buffer and degradation of isozyme during electrophoresis and staining.

Key words : Mutation, Isozyme, Mutation rate.

After mutagen treatment, evaluation of mutation rates and occurrences of target trait's modification in mutation breeding is the most important factor for the efficiency of selection. Useful and desirable variation can be distinguished and selected easily in mutation breeding programs if those characteristics have close relationship with phenotypic traits. But unfortunately, only a few phenotypic characteristics which were related with target characteristics could have been found in practical breeding program although many breeder have tried to get information for those relationships[1].

In mutation breeding, phenotypic traits which can be easily distinguished in visual were normally used to know degree of mutation and somatic effect. For example, the rate of chlorophyll mutants, the reduction rate of growth in culm, seedling length, root

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length, the reduction of fertility[6]. Evaluation of mutation rate and selection of good traits with molecular marker is more useful and efficient than the traditional selecting methods in size of pedigrees and expenses[7]. Especially Kahler[3], Kucharska[4] used isozymes to investigate mutation rate in barley. The purpose of this studies were focused to measure the degree of mutation rate with isozyme markers and the efficiency of those techniques.

MATERIALS AND METHODS

1. Plant material

Two varieties (Dema: two rows, spring barley in Poland; Sacheon 6 : two rows, winter barley in Korea) were used in mutagen treatment. Sodium azide and N-nitroso-N-methylurea(MNH) were chosen for chemical mutagen treatments. Three types of double treatment were composed(1.5mol NaN_2 + 0.75 mol MNH ; 0.75mol MNH + 0.75mol MNH ; 0.5mol MNH + 0.5mol MNH). Dry seeds had been pre -soaked in distilled water for 8 hours and treated with chemical mutagen. After washing, seeds treated with mutagens were kept in room temperature for inter incubation germination for 5 hours. Those seeds were treated by chemical mutagen again. This techniques were introduced from Malusynski[5] in order to increase mutation rate. The seeds treated were sown to the experimental field. Matured plants were harvested separately. It means that all spikes of one plant were cut and packed in same envelop.

Eighty plants of Dema, seven plants of Sacheon 6 were harvested in 1.5mol NaN_2 + 0.75mol MNH treatment, eighty four plants of Dema, ninety four plants of Sacheon 6 in 0.75mol MNH + 0.75mol MNH hundred plants of Dema, eighty plants of Sacheon 6 in 0.5mol MNH + 0.5mol MNH.

The most vigorous spike from each M_2 plant, most probably apex, was sown to wet perlite. If the mutant was found in apex, all the remaining spikes from the same M_2 plant were also sown. For plant number 2 and 3 from Dema, all of seedlings from apex were analysed. All seedlings from M_1 spikes containing mutant forms were transplanted to the greenhouse. Progeny of 6 harvested M_2 plants was also analysed. The remaining M_2 plants still grow in the greenhouse.

2. Isozyme analysis

For isoenzyme analysis 7~8 days old seedlings were used. From each spike at least 5 seedlings randomly chosen were analysed. The upper halves of leaves were crushed by glass stick with extraction buffer. Placing strips of Whatmann paper which had absorbed leaf extract, leaf extract was assayed using horizontal starch-gel(11%) electrophoresis described by Tankslay[10] and Shaw[8]. After 2~3hours electrophoresis (when front of gel moved 8cm from the start) gel was cut on three slices for three types of staining system (Est, GOT, LAP). Material was characterized for 9 isozymes loci (Est-1, 2, 4, 5, GOT-1, 2, 3, LAP-1, 2)[2]. Soltis's staining procedures[9] were used for staining.

RESULTS AND DISCUSSION

Table 1 and 2 show the results on frequency of chlorophyll and isozymes mutants induced in Dema and Sacheon 6 by each three doses of chemical mutagen. Out 264 M_1 plants from Dema, isozyme analysis revealed 11 carrying mutant seedlings in apex spikes,

Table 1. Types and frequency of mutants which were found in M_2 seedling treated with chemical mutagen

Variety	Total plant	Dose of mutagen	Plant No.	Locus		Allozyme types	No. of seedling analysed	Phenotype (segregation)			Note
								Induced homo	Hetero	Parental homo	
Dema	80	1.5 NaN_3	1	Est	4	fast	5	0	1	4	
		+0.75MNH		Est	5	fast	5	1	0	4	
			2	Est	1	slow	13	2	7	3	harvest[1)]
			3	Est	5	slow	12	1	1	10	harvest[2)]
				GOT	3	null	12	1	0	11	
				Est	4	null	12	1	0	11	
			4	LAP	2	null	5	1*	0	4	
			5	Est	5	slow	5	1**	0	4	harvest[3)]
	84	0.75MNH	6	Est	4	slow	5	1	0	4	
		+0.75MNH		Est	5	slow					
			7	Est	5	slow	5	1	0	4	
	100	0.5MNH	8	Est	5	slow	5	1	0	4	greenhouse
		+0.5MNH	9	Est	5	fast	5	1	0	4	greenhouse
			10	LAP	2	slow	5	1	0	4	greenhouse
			11	Est	2	null	5	3	0	2	greenhouse
Sacheon 6	7	1.5NaN_3 +0.75MNH					5	0	0	0	
	94	0.75MNH +0.75MNH					5	0	0	0	
	80	0.5MNH	12	GOT	1	null	5	1	0	4	greenhous
		+0.5 MNH		GOT	2	null	5	1	0	4	
				GOT	3	null	5	1	0	4	

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** Mutant was found again in another seedlings of same spike, but not analysed.

Greenhouse : seedlings which have mutants loci are growing in greenhouse.

1) harvested : got 2 seeds. After repeating electrophoresis, no mutant were found.

2) harvested : got 9 seeds (3 and 6 seeds from two plants) from rest of seedling in the same spike that didn't analyse with electrophoresis. No mutant were found.

3) harvested : got 7 seeds (2, 3 and 2 from three plants) from rest of seedling in the same spike that didn't analyse with electrophoresis. No mutant were found.

For Sacheon 6 out of 181 M_1 plants only 1 had apex with mutant seedling.

For plant number 5 from Dema, two spikes(one from apex and other from tiller) showed the same types of mutation, probably both developed from the same set of initial cells. For another M_1 plants which mutation was detected in apex, no mutated forms were revealed in remaining spikes.

For spikes from plants number 1, 3 and 6 simultaneous mutations in respectively two, three and two loci were detected. In each case the mutant form in multiple loci was identified in the same seedling.

Very few M_2 plants were recovered. From spikes 2, 3 and 5 it was recovered respectively 1 plant(2 seeds), 2 plants(9 seeds), 3 plants(4 seeds). From M_1 plants number 1, 4,

Table 2. The number of plants and chlorophyll mutants in different varieties

Variety	Treatment	Total plant	No. of spike analysed	No. of M_2 seedling	No. of chlorophyll mutant in M_2 seedling				Mutation rate	
					Albino	Xantha	Viridis	Total	Spike (%)	Seedling (%)
Dema	$1.5NaN_3$ +0.75MNH 0.75MNH +0.75MNH 0.5MNH +0.5MNH	80	108	864	21	2	6	29(11)*	10.2	3.3
		84	86	344	9	3	2	14(5)	5.8	4.0
		100	110	440	9	0	2	11(9)	8.2	2.6
	Total	264	304	1648	39	5	10	54(25)	8.1	3.3
Sacheon 6	$1.5NaN_3$ +0.75MNH 0.75MNH +0.75MNH 0.5MNH +0.5MNH	7	7	35	0	0	0	0(0)	0.0	0.0
		94	94	376	2	3	2	7(5)	5.3	1.9
		80	80	400	6	6	2	14(5)	6.3	3.5
	Total	181	181	811	8	9	4	21(10)	3.9	1.8

* ( ) : Number of M_1 spike which were analyzed. Five seedlings of that spike were randomly analysed.

6, and 7 we did not obtain M_2 plants. The M_2 plants from M_1 plants number 8~12 are still growing in green house.

Electrophoregrams from M_2 plants progeny seedlings did not show any mutant even though from a single M_2 plant developed from mutant seedling. Unfortunately, this particular seedling showed on the gel the heterozygous phenotype.

In this experiment, there was large varietal differences on the mutation rate of chlorophyll and isozyme esterase. Mutation rate of chlorophyll in Dema and Sacheon 6 were 8.1, 3.9% per spike, respectively, whereas those of mutation rate per plant were 3.3, 1.8%. When esterase was considered for mutation rate, the mean difference of mutation rate with three treatments between Dema(3.5%) and Sacheon 6(0.0%) per spike was less than one of chlorophyll and degree of mutation rate in Est loci was much less than mutation rate of chlorophyll. Kucharska[4] had got 2.7% of mutation rate of esterase loci in Bielik, 3.2% of mutation rate of esterase loci in Aramir. For Dema, the result of these trials was similar with those result. But in case of Sacheon 6, there were some differences among cultivars. When seeds were treated to mutagen, the germinating(activating) stage of seeds between two varieties seemed to be different. Therefore, the sensitivity to mutagen between two varieties might be different. After these works, difference of requirement of pre-soaking and inter incubation germination time in different varieties must be investigated.

Compared with other isozyme loci, esterase loci is the most sensitive one for chemical mutagen treatment. The percentage of mutant of esterase loci from total mutants was 73%. But Tsunewaki[11] discussed for frequency of mutation rate in specific loci, he concluded that there were no differences

Table 3. The mutation rate according to the different dose of mutagens

Variety	Treatment	Total plant	No. of spike	No. of M_2 seedling	Isozyme	No. of mutant	Mutation rate	
							Spike	Seedling
Dema	1.5NaN_3	80	108	864	Est	13(4)	5.0	0.7
	+0.75MNH				GOT	0	0.0	0.0
					LAP	1	1.3	0.1
	0.75MNH	84	86	344	Est	2	2.4	0.6
	+0.75MNH				GOT	0	0.0	0.0
					LAP	0	0.0	0.3
	0.5MNH	100	110	440	Est	5(3)	3.0	0.7
	+0.5MNH				GOT	0	0.0	0.0
					LAP	2(1)	1.0	0.2
	Total	264	304	1648		23(11)	2.2	0.3
Sacheon 6	1.5NaN_3	7	7	35	Est	0	0.0	0.0
	+0.75MNH				GOT	0	0.0	0.0
					LAP	0	0.0	0.0
	0.75MNH	94	94	376	Est	0	0.0	0.0
	+0.75MNH				GOT	0	0.0	0.0
					LAP	0	0.0	0.0
	0.5MNH	80	80	320	Est	0	0.0	0.0
	+0.5MNH				GOT	3(1)	1.3	0.9
					LAP	0	0.0	0.0
	Total	181	181	731		3(1)	0.1	0.1

* ( ) : Number of M_1 spike which were analyzed. Five seedlings of that spike were randomly analysed.

Table 4. Differences of mutation rate on isozyme loci

Isozyme	Mutation rate (%)			
	Dema		Sacheon 6	
	Spike	M_2 seedling	Spike	M_2 seedling
Est	3.5	0.7	0.0	0.0
GOT	0.0	0.0	0.4	0.3
LAP	0.8	0.2	0.0	0.0

among loci in mutation rate. In this point of view, some more detail work should be done.

Although many of null bands were found in GOT3, these were not repeatable and real mutants. According to the Soltis[9], it might be due to qualities of starch, amount of extract buffer and degradation of isozyme during electrophoresis and staining. When leaf extract had been made three times as much as normal buffer amount, no band was found at GOT3. If researcher want to get normal band for isozyme, It should be considered that exact amount of leaf and extract buffer, high quality of starch, careful electrophoresis and accurate staining procedure must be done.

摘 要

세 유형의 화학 돌연변이제 처리(1.5mol NaN_2 +0.75mol MNH, 0.75mol MNH +0.75mol MNH 및 0.5mol MNH +0.5mol MNH)를 한 M_2 유묘를 이용, Est 1, 2, 4, 5, GOT 1, 2, 3, LAP 1, 2 등의 isozyme band pattern에 대한 돌연변이율을 조사하였다.

1. 세 가지 돌연변이제로 처리된 M_2 식물체에 대한 돌연변이율 조사를 실시하였는데 엽록체에 대한 돌연변이율은 폴랜드 품종인 Dema에서

는 3.3%였고 사천6호에서는 1.8%로 나타났다.

2. Esterase유전자좌에 대한 돌연변이율은 Dema에서 3.5%로 사천 6호의 0%보다 높았으며 GOT와 LAP에서는 두 품종간 차이가 없었다.
3. 세 가지 isozyme중 대개의 돌연변이체가 Esterase에 관계된 유전자좌에서 발생하였으며 (75% 차지) 나머지에서의 돌연변이율은 극히 미미하였다.

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