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Inheritance of Glutamate Oxaloacetate Transaminase Isozymes in Kekiri (*Cucumis melo* kekiri group)

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Glutamate oxaloacetate transaminase isozymes in kekiri (*Cucumis melo* kekiri group) were analyzed by polyacrylamide gel electrophoresis to demonstrate the usefulness of them as genetic markers in melon breeding. The GOT isozyme phenotypes in GOT-1 showed a simple Mendelian inheritance. The additional kekiri specific phenotype in GOT-l, which was not found in the previous study, was observed in the selfed progenies. As the phenotype did not have common band with other cultivars of *Cucumis melo* examined, this can be used as a genetic marker for testing the genetic purity of F_1 hybrids in commercial seed production. Cotyledonous leaves of the plants had identical phenotypes with those of mature leaves in GOT, indicating that this genetic marker can be employed in very early growth stage for melon breeding.

INTRODUCTION

Kekiri is a fruit-vegetable in Sri Lanka and India. Through morphological, genetical, cytological and chemotaxonomical investigations, kekiri has tentatively been classified into *Cucumis melo* kekiri group (Okubo *et al.*, 1990). With many useful characteristics such as heat tolerance, monoecy, small blossom scar, excellent flesh texture and good keeping quality, kekiri is expected to be used directly as a cooking vegetable or as a new genetic material for melon breeding (Okubo *et al.*, 1990).

Investigations of isozymes in 7 enzymes in kekiri and some other C. *melo* cultivars indicated that only the detectable bands of glutamate oxaloacetate transaminase (GOT) in kekiri will be useful as gene markers (Okubo *et al.*, 1990). There were two phenotypes of GOT isozymes, single-banded and triple-banded phenotypes, within the plants. Triple-banded phenotype was observed only in kekiri, while single-banded one was common in all of C. *melo* cultivars investigated (Okubo *et at.*, 1990).

In this study, we performed genetic analysis of GOT isozymes in kekiri to demonstrate the usefulness of them as genetic markers in melon breeding.

MATERIALS AND METHODS

Kekiri seeds were sown on April 1, 1987 and grown in a plastic-film greenhouse. Two hundred fifty mg of cotyledonous leaves of 3 to 4 weeks-old seedlings were collected and immediately used for GOT isozyme analysis. A half of the cotyledon pair of each seedling was remained to allow further growth. The plants which had the kekiri specific phenotype in GOT (Triple-banded phenotype) were selected and selfed to obtain S_1 seeds. Seedlings of S_2 generation obtained from the selfing of each S_1

having different phenotypes segregated for GOT isozymes were also analyzed. Seeds of S_1 and S_2 were sown on May 16, 1989 and on April 5, 1990, respectively in the plastic-film greenhouse.

The enzyme was separated by vertical polyacrylamide gel electrophoresis. Polyacrylamide gels were composed of a Tris HCl running gel at pH 8.9 (5.4% acrylamide) and a Tris HCl stacking gel at pH 6.9 (4.2% acrylamide). Electrode buffer was Tris glycine pH 8.3.

The sample was homogenized in 0.5 ml of extraction buffer with a small amount of sea sand and polyvinylpolypyrrolidone (PVPP) using chilled motar. The PVPP was washed with 35% HCl and dried with an oven before use. The homogenate was centrifuged for 10 min at 10000 rpm and 20 μl of the supernatant was used for electrophoresis. Electrophoresis was conducted under constant voltage of 100 V for the first 0.5 h and 250 V for the next 2.5 h at 2-3°C in an incubator. After running, gel staining was performed at 37°C for 1 h in the dark. Compositions of extraction buffer and staining solution were the same as those described by Wendel and Parks (1982) and Wendel (1983).

RESULTS

The activity of GOT isozymes was observed in 2 regions on gels. In this report, the region in which the fast anodally migrating isozyme set appeared was designated GOT-1 and that in which the slow anodally migrating isozyme set appeared was named GOT-2(Fig. 1). Since there was no variation in GOT-Z, inheritance of only GOT-1 was analyzed. Selfed progenies (S_1) from selected kekiri plants, which had a kekiri specific triple-banded phenotype of GOT-1(T), were segregated to three phenotypes (Fig. 1, Table 1). The slow single-banded phenotype designated S was additionally observed in this study to the previous study (Fig. 1). The phenotype F

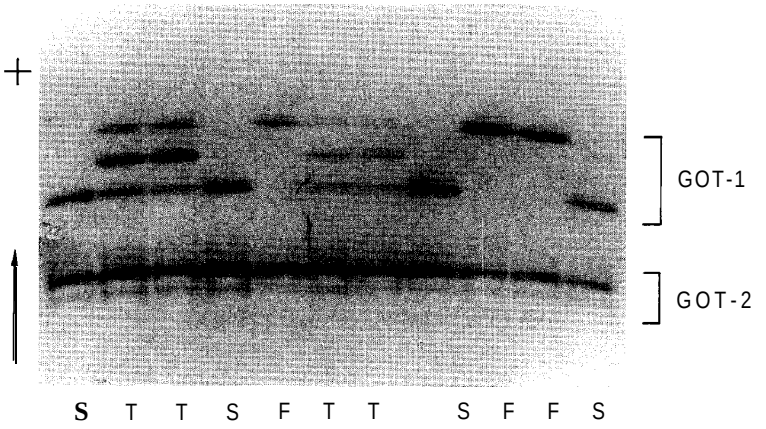

Fig. 1. Segregation of GOT isozyme phenotypes in selfed progenies of kekiri which had kekiri specific phenotype (Triple-banded phenotype in GOT-1). F, T and S indicates fast single-, triple- and slow single-banded phenotype, respectively.

Table 1. Goodness-of-fit test for segregation patterns of isozyme phenotypes in GOT-1 from self-pollinations in kekiri with phenotype T.

Parent (Phenotype)	Progeny phenotype*			Expected ratio	χ^2	P
	F	T	S			
Kekiri (T)	8	12	10	1: 2: 1	1.47	0.30 < P CO.50

* F, T, S ; see Text.

Table 2. Goodness-of-fit test for segregation patterns of isozyme phenotypes in GOT-1 from self-pollinations in S_1 of kekiri (T).

Parent (Phenotype)	Progeny phenotype*			Expected ratio	χ^2	P
	F	T	S			
S_1** (F)	20	0	0			
S_1 (S)	0	0	20			
S_1 (T)	12	16	12	1: 2: 1	1.60	0.30 < P < 0.50

* F, T, S; see Text.
** S_1 indicates selfed progeny of kekiri (T).

stands for the fast single-banded phenotype (Fig. 1). The phenotypes of S_2 obtained from S_1 with phenotypes S and F, were all S and F, respectively. Selfed progenies of S_1 with phenotype T were segregated to three phenotypes F, S and T in S_2 (Table 2).

DISCUSSION

Isozyme phenotypes of some enzymes are generally recognized to be useful as genetic markers, since most isozymes are stably controlled by codominant alleles and are not influenced by environment (Arus, 1983). The results that the phenotypes of S_2 obtained by selfing of the S_1 with single-banded phenotypes were single-banded and that the S_2 progenies of S_1 with phenotype T were segregated to three phenotypes indicate that GOT isozymes in GOT-1 have a dimeric subunit structure. Chi-square values for goodness-of-fit to a 1 : 2 : 1 ratio which were not significant for S_1 and S_2 whose parents having T phenotype for GOT-1 (Tables 1 and 2) clearly indicate that such isozyme phenotypes in GOT-l follow single locus Mendelian inheritance. As only F phenotype was observed in GOT-l in C. *melo* cultivars investigated except kekiri in previous report (Okubo et al., 1990), S and T phenotypes observed in GOT-l in kekiri progenies are kekiri specific ones. Particularly, the S phenotype, which has not common band with the F phenotype, might be worthwhile for using as a genetic marker. Varietal seed purity is one of the essential quality in commercial seed lots (Arus, 1983). In melon production, use of F_1 hybrids has been extended world-wide. To take the gene which controls the phenotype S into one of the F_1 hybrid parents would make it easy to confirm purity of commercial hybrid seed.

In this study, we employed polyacrylamide gel electrophoresis to separate leaf

GOT isozymes, while starch gel electrophoresis was used in the previous study (Okubo *et al.*, 1990). Although polyacrylamide gel method could obtain clearer zymogram than starch gel method, no difference was observed between these two methods as to relative band intensity and location on gels. Glutamate oxaloacetate transaminase zymogram of cotyledonous leaves was identical with that of mature leaves, indicating that this genetic marker can be employed in very early growth stage of the species for melon breeding.

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