Genetic Analysis of Glutamate-Oxaloacetate Transaminase Isozymes in Wild Species in Section *Cepa* of *Allium*

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Summary

Glutamate-oxaloacetate transaminase (GOT) was studied to establish the genetic system of GOT isozymes in the wild species in section *Cepa* of *Allium*. From the results of isozyme analyses using two cultivated species (*A. fistulosum*, *A. cepa* Aggregatum group), three wild species (*A. vavilovii*, *A. oschaninii*, *A. galanthum*), one hybrid between *A. fistulosum* and *A. cepa* Aggregatum group and six hybrids between the cultivated and wild species, it was revealed that 1) the three wild species possessed the two GOT gene loci (*Got-1*, *Got-2*) in common with *A. fistulosum* and *A. cepa*, 2) two alleles were detected at *Got-1* and three alleles at *Got-2*, 3) *A. fistulosum* possessed *Got-1^F* and *Got-2^F* and *A. cepa* Aggregatum group had *Got-1^A* and *Got-2^A*, and 4) *A. vavilovii* possessed *Got-1^A* and *Got-2^A*; *A. oschaninii* had *Got-1^A* and *Got-2^F*; *A. galanthum* had *Got-1^A* and *Got-2^G*. These conclusions indicate that the GOT isozyme genes are useful genetic markers in the wild species in section *Cepa*.

Introduction

Allium vavilovii M. Pop. et Vved., *A. oschaninii* O. Fedtsch. and *A. galanthum* Kar. et Kir. are known as the wild species in section *Cepa* of *Allium* (McCollum, 1976; Traub, 1968; Wendelbo, 1971). These wild species seem to be important genetic resources for improving the cultivated species in section *Cepa*. It is necessary to establish genetic markers in these species for their practical utilization.

Isozyme gene is one of the useful genetic markers because of the co-dominant expression (Moore and Collins, 1983). In the cultivated species of section *Cepa*, genetic analyses of isozymes of many enzymes have been carried out, especially on the glutamate-oxaloacetate transaminase (GOT) as follows. Halijah and Raniah (1990) reported intraspecific polymorphisms observed in four cultivars of *A. cepa*. From the results of BC₁ analyses of

GOT isozymes in the hybrids between *A. fistulosum* and *A. cepa* common onion group, Van der Valk et al. (1991) reported two gene loci (*Got-1*, *Got-2*) which appeared to be unlinked. There was no linkage between an acid phosphatase gene locus (*Acp-1*) and *Got-2* in *A. fistulosum* (Haishima and Ikehishi, 1992). Haishima et al. (1993) recognized that there were intraspecific polymorphisms in 13 cultivars of *A. fistulosum*. Shigyo et al. (1994) determined chromosomal locations of the GOT isozyme genes in *A. fistulosum* and *A. cepa* Aggregatum group by means of isozyme analysis of alien monosomic addition lines between these species. Consequently, gene locus *Got-2* was located on the homoeologous sub-telocentric chromosomes, 6F in *A. fistulosum* and 6A in *A. cepa* aggregatum group, and gene locus *Got-1* was on the other chromosomes. Although many observations mentioned above had been reported, the genetic analysis of the GOT isozymes in the wild species had not been conducted.

In the present study, GOT isozyme analysis was performed to establish the GOT genetic systems of the wild species in section *Cepa*.

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Materials and Methods

The following plants were used; Japanese bunching onion (*A. fistulosum*), shallot (*A. cepa* aggregatum group), *A. vavilovii*, *A. oschaninii*, *A. galanthum*, hybrid between *A. fistulosum* and *A. cepa* Aggregatum group, hybrids between *A. fistulosum* and each of the wild species, and hybrids between *A. cepa* Aggregatum group and each of the wild species (Table 1).

Table 1. List of plant materials.

Species	Cultivar name, accession no. or cross combination	genome ²
<i>Allium fistulosum</i>	'Kujyo'	FF
<i>A. cepa</i> Aggregatum group	'Chiang mai'	AA
<i>A. vavilovii</i>	83010 ^y	VV
<i>A. oschaninii</i>	78227 ^y	OO
<i>A. galanthum</i>	65447 ^y	GG
Hybrid	'Kujyo' × 'Chiang mai'	AF
	83010 × 'Kujyo'	FV
	78227 × 'Kujyo'	FO
	65447 × 'Kujyo'	FG
	83010 × 'Chiang mai'	AV
	78227 × 'Chiang mai'	AO
	65447 × 'Chiang mai'	AG

² F and A; Tashiro (1984), V, O and G; denoted in the present study for the genomes of *A. vavilovii*, *A. oschaninii*, *A. galanthum*, respectively.

^y Accessions from IVT (CPRO) of the Netherlands.

Young expanding leaves were used for enzyme extraction. All processes of the extraction, polyacrylamide slab gel electrophoresis and enzyme activity staining for glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1) were carried out according to the method suggested by Hirai (1988).

Results and Discussion

The band patterns of GOT isozyme clearly showed an interspecific polymorphism among the species used in this study (Figs. 1 and 2). From the results of GOT isozyme analysis in *A. fistulosum*, *A. cepa* and their hybrids by Van der Valk et al. (1991) and Shigyo et al. (1994), a polymorphism between these two species was already confirmed. In the wild species used in the present study, *A. vavilovii* showed the same band pattern as that of *A. cepa* Aggregatum group, while the band patterns of *A. oschaninii* and *A. galanthum* were different from both of *A. fistulosum* and *A. cepa* Aggregatum group. In *A. oschaninii*, the cathodal band (band 3 in Fig. 2) had the same migration position as the cathodal band of *A. cepa* Aggregatum group, and the anodal band (band 7) had the same migration position as the anodal band of *A. fistulosum*. In *A. galanthum*, the cathodal band (band 3) had the same migration position as the cathodal band of *A. cepa* Aggregatum group, and the anodal band (band 4) was

Fig. 1. GOT zymograms of *A. fistulosum* (FF), hybrid between *A. fistulosum* and *A. galanthum* (FG), *A. galanthum* (GG), hybrid between *A. cepa* Aggregatum group and *A. galanthum* (AG), *A. cepa* Aggregatum group (AA), hybrid between *A. cepa* Aggregatum group and *A. vavilovii* (AV), *A. vavilovii* (VV), *A. oschaninii* (OO) and hybrid between *A. cepa* Aggregatum group and *A. oschaninii* (AO).

Fig. 2. Schematic illustration of GOT zymograms of *A. fistulosum* (FF), *A. cepa* Aggregatum group (AA), *A. vavilovii* (VV), *A. oschaninii* (OO), *A. galanthum* (GG), and their hybrids (AF, FV, FO, FG, AV, AO, AG).

Table 2. Subunit constitution, gene locus and allele of GOT isozyme band in section *Cepa* of *Allium*.

Band no.	Subunit constitution	Gene locus	Allele
1	$\alpha\alpha$	<i>Got-1</i>	<i>Got-1^F</i>
2	$\alpha\beta$	<i>Got-1</i>	<i>Got-1^F, Got-1^A</i>
3	$\beta\beta$	<i>Got-1</i>	<i>Got-1^A</i>
4	$\gamma\gamma$	<i>Got-2</i>	<i>Got-2^G</i>
5	$\gamma\delta$	<i>Got-2</i>	<i>Got-2^F, Got-2^G</i>
6	$\gamma\epsilon$	<i>Got-2</i>	<i>Got-2^A, Got-2^G</i>
7	$\delta\delta$	<i>Got-2</i>	<i>Got-2^F</i>
8	$\delta\epsilon$	<i>Got-2</i>	<i>Got-2^F, Got-2^A</i>
9	$\epsilon\epsilon$	<i>Got-2</i>	<i>Got-2^A</i>

close to the cathodal band and was species specific.

In the hybrid (AF, FV), in addition to the four bands derived from parents (bands 1, 3, 7, 9) two more bands (bands 2, 8) were detected. In the hybrid (FO), in addition to the three parental bands (bands 1, 3, 7) an extra band (band 2) was detected. In the hybrid (FG), in addition to the four parental bands (bands 1, 3, 4, 7) two more bands (bands 2, 5) were detected. In the hybrid (AV), only the two parental bands (bands 3, 9) were detected. In the hybrid (AO), in addition to the three parental bands (bands 3, 7, 9) an extra band (band 8) was detected. In the hybrid (AG), in addition to the three parental bands (bands 3, 4, 9) an extra band (band 6) was detected. The band 2 migrated to an intermediate position between the bands 1 and 3, the band 5 between the bands 4 and 7, the band 6 between the bands 4 and 9, and the band 8 between the bands 7 and 9. Further, in all diploid hybrids, the additional bands (bands 2, 5, 6, 8) were stained more intensely than the parental bands (bands 1, 3, 4, 7, 9).

These results from isozyme analyses of interspecific hybrids indicate the following : 1) There are five kinds of subunits (α , β , γ , δ , ϵ) of the GOT enzyme proteins in the species of section *Cepa* used in the present study. 2) Bands 1, 3, 4, 7 and 9 are homodimeric enzymes. 3) Bands 2, 5, 6 and 8 are heterodimeric enzymes. 4) The two cultivated and three wild species used in this study have two common gene loci (*Got-1*, *Got-2*). 5) There are two alleles (*Got-1^F*, *Got-1^A*) at *Got-1* and three alleles (*Got-2^F*, *Got-2^A*, *Got-2^G*) at *Got-2*. 6) *A. fistulosum* has *Got-1^F* and *Got-2^F* at the two loci. 7) *A. cepa* aggregatum group and *A. vavilovii* have *Got-1^A* and *Got-2^A*. 8) *A. oschaninii* has *Got-1^A* and *Got-2^F*. 9) *A. galanthum* has *Got-1^A* and *Got-2^G*. Based on the above analyses, the subunit constitutions, gene loci and alleles of GOT isozyme bands in Fig. 2 are summarized in Table 2. Furthermore, genotypes of the species used in this study are shown in Table 3.

Previous studies on the GOT isozymes in *A. fistulosum* and *A. cepa* had suggested the existences

Table 3. Genotype of gene loci *Got-1* and *Got-2* in the species of section *Cepa* in *Allium*.

Species	Genotype	
	Gene locus	
	<i>Got-1</i>	<i>Got-2</i>
<i>A. fistulosum</i>	<i>Got-1^F/Got-1^F</i>	<i>Got-2^F/Got-2^F</i>
<i>A. cepa</i> Aggregatum group	<i>Got-1^A/Got-1^A</i>	<i>Got-2^A/Got-2^A</i>
<i>A. vavilovii</i>	<i>Got-1^A/Got-1^A</i>	<i>Got-2^A/Got-2^A</i>
<i>A. oschaninii</i>	<i>Got-1^A/Got-1^A</i>	<i>Got-2^F/Got-2^F</i>
<i>A. galanthum</i>	<i>Got-1^A/Got-1^A</i>	<i>Got-2^G/Got-2^G</i>

of the alleles *Got-1^F* and *Got-1^A* at *Got-1*, and *Got-2^F* and *Got-2^A* at *Got-2* (Van der Valk et al., 1991; Shigyo et al., 1994). In the present study, the allele *Got-2^G* was newly recognized in a wild species, *A. galanthum*. According to the different combinations of the two alleles at *Got-1* and the three alleles at *Got-2*, the interspecific polymorphisms were exhibited among the species of section *Cepa* used in this study. The GOT isozyme genes were available for the identifications of hybrids used in this study except the hybrid between *A. cepa* Aggregatum group and *A. vavilovii*. These isozyme genes are expected to serve as useful genetic markers for the genetics and practical utilization of the wild species in section *Cepa*.

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ネギ属 *Cepa* 節野生種のグルタミン酸-オキサロ酢酸トランスアミナーゼアイソザイムの
遺伝分析

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摘 要

ネギ属 *Cepa* 節野生種のグルタミン酸-オキサロ酢酸トランスアミナーゼ (GOT) の遺伝系を明らかにするためにアイソザイムの分析を行った。ネギ, シャロット, 両者の雑種, 野生種 (*A. vavilovii*, *A. oschaninii*, *A. galanthum*) およびネギ, シャロットと各野生種の雑種を分析した結果, これらの野生種はネギおよびシャロットと共通な GOT 遺伝子座 *Got-1* および *Got-2* を持ち, *Got-1* 上には二つの対立遺伝子 (*Got-1^F*, *Got-1^A*) が, *Got-2* 上には三つの対立遺伝子 (*Got-2^F*,

Got-2^A, *Got-2^G*) が観察された。ネギが *Got-1^F* および *Got-2^F* を持ち, シャロットが *Got-1^A* および *Got-2^A* を持っていたのに対し, *A. vavilovii* は *Got-1^A* および *Got-2^A* を, *A. oschaninii* は *Got-1^A* および *Got-2^F* を, *A. galanthum* は *Got-1^A* および *Got-2^G* を持っていた。これらのことにより, GOT アイソザイム遺伝子は *Cepa* 節野生種の有用な遺伝的マーカーであることが分かった。

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