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Genetic Analysis of Isocitrate Dehydrogenase Isozymes in Cultivated and Wild Species of Section *Cepa* in *Allium*

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Summary

Isocitrate dehydrogenase (IDH) was studied to clarify the genetic system of the isozymes in section *Cepa* of *Allium*. Isozyme analyses using two cultivated species, *A. fistulosum* and *A. cepa*, four wild species, *A. altaicum*, *A. galanthum*, *A. oschaninii* and *A. vavilovii*, and hybrids between *A. fistulosum* and *A. cepa* and between the cultivated and wild species revealed that: 1) IDH was a dimeric enzyme; 2) the two cultivated and four wild species had a common gene locus *Idh-1* at which three alleles were identified; and 3) *A. cepa* Aggregatum group, *A. cepa* Common onion group and *A. vavilovii* had *Idh-1*^{A}, *A. fistulosum*, *A. altaicum* and *A. oschaninii* had *Idh-1*^{F}, and *A. galanthum* had *Idh-1*^{G}. The interspecific polymorphism of IDH isozymes in section *Cepa* is valuable for the genetic study and practical utilization of the wild species.

Introduction

Section *Cepa* of *Allium* includes the two economically important cultivated species, *A. cepa* L. and *A. fistulosum* L., and several wild species, *A. galanthum* Kar. et Kir., *A. oschaninii* O. Fedtsch., *A. vavilovii* M. Pop. et Vved., *A. altaicum* Pall., *etc.*[4] Some attempts have been conducted to introduce favorable genes of the wild species to the cultivated species[2),3),5),8),16)]. It is necessary to establish plenty of genetic markers for conducting such works efficiently. Isozyme genes were extensively utilized as genetic markers for studies on plant genetics and breeding[14)]. Before the isozyme markers are applied in practice, it is imperative to clarify their genetic systems.

In the section *Cepa*, genetic systems of three isozymes, glutamate dehydrogenase (GDH)[11)], glutamate-oxaloacetate transaminase (GOT)[15)], and phosphoglucoisomerase (PGI)[13)], were previously clarified. The chromosomal locations of four alleles *Gdh-1*^{A}, *Got-1*^{A}, *Got-2*^{A} and *Pgi-1*$^{A-1}$ in *A. cepa* Aggregatum group and an allele *Got-2*^{F} in *A. fistulosum* were determined by the isozyme analyses using alien monosomic addition lines of *A. fistulosum* with the extra chromosomes from *A. cepa* Aggregatum group[9),10),12)]. Peffley[6)] suggested that isocitrate dehydrogenase (IDH) isozymes were genetic markers available for identifying F_1 hybrids between *A. fistulosum* and *A. cepa*. Shigyo et al.[12)] reported that an IDH allele (*Idh-1*^{A}) was located on the chromosome 5A in *A. cepa* Aggregatum group.

In the present study, IDH isozyme analysis was performed to clarify the IDH genetic system in section *Cepa*, including wild species.

Materials and Methods

A total of 24 cultivars, strains and clones and 10 F_1 hybrids as presented in Table 1 were used; five clones of *A. cepa* Aggregatum group, seven cultivars of *A. cepa* Common onion group, three cultivars and three clones of *A. fistulosum*, two strains of *A. altaicum*, two strains of *A. galanthum*, two strains of *A. oschaninii*, one strain of *A. vavilovii*, two hybrids between *A. cepa* Aggregatum group and *A. fistulosum*, four hybrids between *A. fistulosum* and the wild species, and four hybrids between *A. cepa* Aggregatum group and the wild species.

Young expanding leaves were used for enzyme extraction. All processes of the extraction, starch gel electrophoresis and enzyme activity staining for IDH (EC 1.1.1.41) were conducted according to the method described by Wendel[17].

Results and discussion

Although no intraspecific polymorphism was observed on the IDH zymograms

Table 1. Plant materials used in this study.

Species	Cultivar name, accession No. or cross combination	Nuclear genome
A. cepa Aggregatum group	'Bangkok' 'Bawang Merah' 'Chiang Mai' 'Taiwan Odama' 'Myanmar'	AA
A. cepa Common onion group	'Hamasodachi' 'Satuki' 'Shippou Ama 70' 'Shippou Wase 7 go' 'KF-928' 'KF-930' 'Momizi 3 go'	CC
A. fistulosum	'Kujyo' 'Kunming' 'Shimonita' 'Omura' 'Tsai Tsung' 'Shanghai'	FF
A. altaicum	85003-12[z], 85003-17[z]	LL
A. galanthum	65447-6[z], 65447-11[z]	GG
A. oschaninii	78227-8[z], 78227-9[z]	OO
A. vavilovii	83010-1[z]	VV
F_1 hybrid	'Kujyo'× 'Chiang Mai'	AF
	'Kunming'× 'Myanmar'	AF
	85003-17 × 'Kujyo'	FL
	65447-6 × 'Kujyo'	FG
	78227-8 × 'Kujyo'	FO
	83010-1 × 'Kujyo'	FV
	85003-12 × 'Chiang Mai'	AL
	65447-11 × 'Chiang Mai'	AG
	78227-9 × 'Chiang Mai'	AO
	83010-1 × 'Chiang Mai'	AV

[z] Accessions from IVT (CPRO) of the Netherlands.

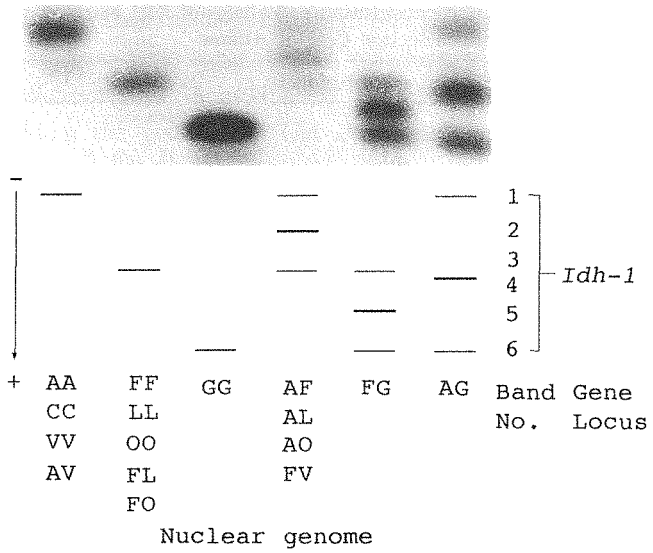

Fig.1. Isocitrate dehydrogenase zymograms of *A. cepa* Aggregatum group (AA), *A. cepa* Common onion group (CC), *A. fistulosum* (FF), *A. altaicum* (LL), *A. galanthum* (GG), *A. oschaninii* (OO), *A. vavilovii* (VV) and their F_1 hybrids (AF, AL, AG, AO, AV, FL, FG, FO, FV). A schematic illustration is shown in bottom half of the figure.

obtained, an interspecific polymorphism was detected (Fig. 1). The interspecific polymorphism was recognized by differential mobilities of bands. Three types of the bands (1, 3, 6) shifted gradually from cathode to anode. The slowest band (1) was observed in *A. cepa* Aggregatum group, *A. cepa* Common onion group and *A. vavilovii*, the intermediate band (3) in *A. altaicum*, *A. fistulosum* and *A. oschaninii*, and the fastest band (6) in *A. galanthum*. In F_1 hybrids between *A. fistulosum* and *A. cepa* Aggregatum group (AF), between *A. oschaninii* and *A. cepa* Aggregatum group (AO), between *A. altaicum* and *A. cepa* Aggregatum group (AL), and between *A. vavilovii* and *A. fistulosum* (FV), an additional band (2) as well as parental bands (1, 3) were observed. In F_1 hybrid between *A. galanthum* and *A. fistulosum* (FG), an additional band (5) as well as parental bands (3, 6) were observed. In F_1 hybrid between *A. galanthum* and *A. cepa* Aggregatum group (AG), an additional band (4) as well as parental bands (1, 6) were observed. The additional bands migrated to intermediate positions between the parental bands and were stained more intensely than the parental bands. In F_1 hybrids between *A. vavilovii* and *A. cepa* Aggregatum group (AV), between *A. altaicum* and *A. fistulosum* (FL) and between *A. oschaninii* and *A. fistulosum* (FO), the parental bands (1, 3) were only observed because of the identical band patterns in the parents. The results from the isozyme analyses of interspecific hybrids indicate the following: 1) The bands 1, 3 and 6 are homodimeric enzymes. 2) The bands 2, 4 and 5 are heterodimeric enzymes. 3) The two cultivated and four wild species used in this study have a common gene locus (*Idh-1*). 4) There are three kinds of subunits (α, β, γ) of the IDH enzyme proteins in the species of section *Cepa* used in the present study. 5) There are three alleles (*Idh-1*^{A}, *Idh-1*^{F}, *Idh-1*^{G}) at the gene locus

Table 2. Subunit constitution and alleles of IDH isozyme bands in section *Cepa* of *Allium*.

Band no.	Subunit constitution	Allele
1	$\alpha\alpha$	*Idh-1*^{A}
2	$\alpha\beta$	*Idh-1*^{A}, *Idh-1*^{F}
3	$\beta\beta$	*Idh-1*^{F}
4	$\alpha\gamma$	*Idh-1*^{A}, *Idh-1*^{G}
5	$\beta\gamma$	*Idh-1*^{F}, *Idh-1*^{G}
6	$\gamma\gamma$	*Idh-1*^{G}

Idh-1. 6) *A. cepa* Aggregatum group, *A. cepa* Common onion group and *A. vavilovii* have *Idh-1*^{A}, *A. fistulosum, A. altaicum* and *A. oschaninii* have *Idh-1*^{F}, and *A. galanthum* has *Idh-1*^{G}. Based on the above demonstrations, the subunit constitutions and alleles of IDH isozyme bands in Fig. 1 are summarized in Table 2. Further, genotypes of the species used in this study are shown in Table 3.

In the cultivated species, Peffley et al.[7] reported two alleles (*Idh-1*^{1}, *Idh-1*^{2}) at the gene locus *Idh-1* in *A. fistulosum* and *A. cepa* ; the former possessed *Idh-1*^{1} and the latter *Idh-1*^{2}. It seems that the alleles *Idh-1*^{F} and *Idh-1*^{A} recognized in the present study are identical with the alleles *Idh-1*^{1} and *Idh-1*^{2} described by them, respectively. It was suggested that IDH isozymes were useful in identifying F_1 hybrids between *A. fistulosum* and *A. cepa*[6]. The results of the present study demonstrate that IDH isozymes are also useful for identification of F_1 hybrids between the cultivated and wild species of section *Cepa*. Cryder et al.[1] analyzed IDH, PGI and alcohol dehydrogenase (ADH) in mixed populations of F_2 and BC_1 of hybrids between *A. fistulosum* and *A. cepa*. The gene locus *Idh-1* appeared to be linked to *Pgi-1* but not to *Adh-1*. Moreover, Shigyo et al.[9),10),12)] determined the chromosomal locations of several isozyme genes in *A. cepa* Aggregatum group using the alien monosomic addition lines of *A. fistulosum* with the extra chromosomes from *A. cepa* Aggregatum group. It was demonstrated that both the gene loci *Idh-1* and *Pgi-1* were located on chromosome 5A. To expand the usage of IDH isozymes as genetic markers, linkage tests and determination of chromosomal location of the isozyme genes identified in this study are under way in the wild species of section *Cepa*.

In section *Cepa*, the genetic systems of four enzymes, GDH[11)], GOT[15)], PGI[13)], and IDH

Table 3. Genotypes of gene locus *Idh-1* in the species of section *Cepa* in *Allium*.

Species	Genotype
A. cepa Aggregatum group	*Idh-1*^{A}/*Idh-1*^{A}
A. cepa Common onion group	*Idh-1*^{A}/*Idh-1*^{A}
A. fistulosum	*Idh-1*^{F}/*Idh-1*^{F}
A. altaicum	*Idh-1*^{F}/*Idh-1*^{F}
A. galanthum	*Idh-1*^{G}/*Idh-1*^{G}
A. oschaninii	*Idh-1*^{F}/*Idh-1*^{F}
A. vavilovii	*Idh-1*^{A}/*Idh-1*^{A}

(the present study) have thus far been clarified. Shigyo et al.[9] revealed that a glutamate-oxaloacetate transaminase gene locus, *Got-2*, was located on the homoeologous chromosomes 6F in *A. fistulosum* and 6A in *A. cepa* Aggregatum group. This indicates that a translocation including the gene locus *Got-2* did not occur between non-homologous chromosomes in both species during speciation and subsequent evolution. Further studies to determine the chromosomal locations of several isozyme gene loci including *Gdh-1, Got-1, Got-2, Pgi-1* and *Idh-1* in the wild species will elucidate the chromosomal evolution and process of speciation in section *Cepa*.

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ネギ属 *Cepa* 節の栽培種および野生種のイソクエン酸デヒドロゲナーゼアイソザイムの遺伝分析

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摘　　要

ネギ属 *Cepa* 節植物のイソクエン酸デヒドロゲナーゼ（IDH）の遺伝系を明らかにするために，アイソザイム分析を行った．シャロット，タマネギ，ネギ，野生種（*Allium altaicum, A. galanthum, A. oschaninii, A. vavilovii*），シャロットとネギの雑種およびネギ，シャロットと各野生種の雑種を分析した結果，IDH が二量体であることおよびこれらの栽培種および野生種は共通の遺伝子座 *Idh-1* を持ち，この遺伝子座上には三つの対立遺伝子（$Idh\text{-}1^{A}$, $Idh\text{-}1^{F}$, $Idh\text{-}1^{G}$）があることが確認された．シャロット，タマネギおよび *A. vavilovii* は対立遺伝子 $Idh\text{-}1^{A}$を，ネギ，*A. altaicum* および *A. oschaninii* は対立遺伝子 $Idh\text{-}1^{F}$を，*A. galanthum* は対立遺伝子 $Idh\text{-}1^{G}$をそれぞれ有していた．これらのことより，IDH アイソザイム遺伝子は *Cepa* 節植物における有用な遺伝的マーカーであることがわかった．