Genetic Analyses of Isozyme in Lettuce, *Lactuca sativa*, and its Relatives

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Summary

Polymorphisms were observed in 14 enzyme systems in the following *Lactuca* species, lettuce, *L. sativa*, and its relative wild species, *L. serriola*, *L. saligna*, *L. virosa* and *L. indica*; 6 loci of the 3 enzymes, GOT, PGI and 6-PDH, were found to be dimeric. The segregation analysis of the F₂ progeny between lettuce and *L. serriola* revealed 4 loci, *Got-1*, *Got-2*, *Pgi-2* and *Pdh-2* and a linkage among 3 loci, *Got-2*, *Pgi-2* and *Pdh-2*. Band intensity of the somatic hybrid (hexaploid) between lettuce and *L. indica* showed that the hybrid is composed of 4 genomes of lettuce and 2 genomes of *L. indica*. Thus, the genes in the 6 loci are considered to be useful genetic markers for the breeding of lettuce.

Key Words: genetic analysis, hybrid, isozyme, lettuce, wild *Lactuca* species.

Introduction

Lettuce, *Lactuca sativa* L., is one of the most important salad crops in the world. Lindqvist (1960) reported the cross-compatibility and relationships between *L. sativa* and its relatives. *Lactuca serriola* L., *L. saligna* L. and *L. virosa* L., endogenous to the Mediterranean littoral, were found to be very closely related to *L. sativa*, belonging to the same section *Lactuca*. These species have important agricultural characteristics, such as mosaic-resistance and downy mildew resistance in *L. serriola* (Ryder, 1970; Gustafsson, 1989), and cabbage looper resistance in *L. saligna* (Ryder and Whitaker, 1976). Both species are interfertile with *L. sativa*, so that they can be important genetic resources for improving the cultivated lettuce. Although *L. virosa* of the same section is intersterile with *L. sativa*, Matsumoto (1991) tried to produce a somatic hybrid between them. Zohary (1991) summarized the available taxonomic information on the section *Lactuca* with some other wild species.

Furthermore, some species of other sections in the genus *Lactuca* have useful genetic resources for the breeding of lettuce. Although *L. indica* L. is indigenous to Eastern Asia, it possesses some important traits that may potentially improve lettuce. Hybrids between *L. sativa* and *L. indica* could not be produced by conventional crossing. However, Mizutani et al. (1989) produced the somatic hybrids between *L. sativa* cv. Bronze and *L. indica*, utilizing the wild species for the breeding of lettuce.

Because reliable genetic markers are prerequisite for practical breeding, isozyme genes are occasionally used as genetic and DNA markers. An isozyme is a direct

product of a gene (one gene – one enzyme hypothesis); it behaves as a co-dominant allele. The segregation analysis of an isozyme reveals the genes more readily than does any other phenotypic trait. These markers have been used to make linkage maps of higher plants. Landry et al. (1987) constructed a linkage map of lettuce using 41 RFLP markers, 4 isozyme loci, 5 downy mildew resistant genes and 3 morphological markers. Kesseli et al. (1994) also made a detailed genetic map of lettuce, using 7 isozyme loci, 152 RFLP markers and RAPD markers. These studies were carried out by an intraspecific hybridization that should minimize genetic distortion, though more polymorphisms of isozyme loci are observed in the interspecific crosses. Kesseli and Micheltore (1986) found several allozyme variations by starch gel electrophoresis among 31 accessions of *L. sativa* and wild *Lactuca* species. However, they could not use the isozyme genetic markers obtained from interspecific analysis in their genetic map, because they could not analyze the distortions observed in the F₂ generation of the interspecific hybrids. Cole et al. (1991) also studied the isozyme variation in lettuce by using polyacrylamide gel electrophoresis and identified many isozyme loci with multiple alleles.

Isozymes are useful genetic markers for the identification of hybrids and for the study of introgression of important traits from related species. Furthermore, it is possible to distinguish polyploidy by the expression of isozymes. In this study, genetic analyses of isozymes of lettuce and its relatives were performed by polyacrylamide gel electrophoresis to locate genetic markers of lettuce.

Materials and Methods

The followings were used; eight cultivars from 5 different groups of lettuce, *Lactuca sativa*, 3 wild

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Table 1. Source of materials used in this experiment.

Species	Cultivar name, accession no. or cross combination	Source
<i>L. sativa</i>	Crisp head type	
	cv. Great lakes 366	Takii Seed Co., Ltd.
	cv. Olympia	Mikado Seed Growers Co., Ltd.
	Butter head type	
	cv. Okayama-saradana	Yae Noge Co., Ltd.
	cv. Syusui	Tokita Seed Co., Ltd.
	Leaf type	
	cv. Bronze	Tokita Seed Co., Ltd.
	cv. Leaf lettuce green	Sakata Seed Co., Ltd.
	Stem type	
	cv. Stem lettuce	Takii Seed Co., Ltd.
	Cos type	
	cv. Cos lettuce	Takii Seed Co., Ltd.
<i>L. serriola</i>	KT93101	Nagano Vegetable and Ornamental Crops Experimental Station
<i>L. saligna</i>	KT93201	Nagano Vegetable and Ornamental Crops Experimental Station
<i>L. virosa</i>	KT93301	Nagano Vegetable and Ornamental Crops Experimental Station
<i>L. indica</i>	KT95511	Yatsushiro, Kumamoto
	KT95521	Aso, Kumamoto
	KT95551	Ishikawa, Okinawa
Hybrid	<i>L. sativa</i> cv. Bronze × <i>L. serriola</i> F ₁	Crossed in 1995
	<i>L. sativa</i> cv. Bronze × <i>L. serriola</i> F ₂	Self-pollinated in 1996, 1998
	<i>L. saligna</i> × <i>L. sativa</i> cv. Bronze	Crossed in 1995, 1997, 1999
	<i>L. sativa</i> cv. Bronze + <i>L. indica</i> , SH ₁	Somatic hybrid in 1995

species of section *Lactuca*, *L. serriola*, *L. saligna* and *L. virosa*, and one species of section *Indica*, *L. indica* (Table 1). Cross-pollinations between *L. sativa* cv. Bronze and wild species of section *Lactuca* and somatic hybridization between *L. sativa* cv. Bronze and *L. indica* were conducted. As the species in the genus *Lactuca* have a strong self-pollination system, F₂ generations were obtained easily by protecting the F₁ hybrids from insect pollinators in a netted greenhouse.

Enzymes were extracted from young expanding leaves and separated electrophoretically on vertical polyacrylamide gels according to Hirai's method (1988). Activity staining for 14 enzymes was carried out according to Hirai (1988) and Wendel (1983) (Table 2).

In the F₁ and F₂ generation between *L. sativa* cv. Bronze and *L. serriola*, 4 isozyme loci were analyzed for a single locus, Mendelian segregation, using the chi-square goodness-of-fit values (Table 4). Furthermore, 6 pairs of 4 loci were examined for linkage analysis (Table 5).

Results and Discussion

F₁ and F₂ hybrids between *Lactuca sativa* cv. Bronze and *L. serriola* and between *L. saligna* and *L. sativa* cv. Bronze were obtained by controlled open- and cross-pollinations, but efforts to obtain F₁ hybrids between *L. sativa* and *L. virosa*, and between *L. sativa* were not

Table 2. List of enzymes used in this experiment.

Enzymes	Enzyme code	Abbreviation
Alcohol dehydrogenase	E.C.1.1.1.1	ADH
Esterase	E.C.3.1.1.1	EST
Diaphorase	E.C.1.6.4.3	DIA
Formate dehydrogenase	E.C.1.2.1.2	FDH
Glutamate dehydrogenase	E.C.1.4.1.3	GDH
Glutamate oxaloacetate transaminase	E.C.2.6.1.1	GOT
Isocitrate dehydrogenase	E.C.1.1.1.41	IDH
Malate dehydrogenase	E.C.1.1.1.37	MDH
Malic enzyme	E.C.1.1.1.40	ME
Phosphoglucisomerase	E.C.5.3.1.9	PGI
Phosphoglucomutase	E.C.2.7.5.1	PGM
6-Phosphogluconate dehydrogenase	E.C.1.1.1.44	6-PDH
Shikimic acid dehydrogenase	E.C.1.1.1.25	SADH
Super oxide dismutase	E.C.1.15.1.1	SOD

successful. However, the protoplast fusion method between *L. sativa* and *L. indica* was successfully carried out resulting in a somatic hybrid, but the hybrids did not produce any seeds for the F₂ generation.

No intraspecific polymorphisms were detected in the 14 enzyme systems of 8 cultivars of cultivated lettuce, whereas numerous interspecific polymorphisms were found between *L. sativa* and other wild species (Table 3). Among the 14 enzyme systems studied, lettuce could

Table 3. Isozyme variations in lettuce, *L. sativa* and its 4 related wild species, *L. serriola*, *L. saligna*, *L. virosa* and *L. indica*.

Species and Accessions	ADH	EST	DIA	FDH	GDH	GOT	IDH	MDH	ME	PGI	PGM	6-PDH	SADH	SOD
<i>L. sativa</i>														
cv. Great lakes 366	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Olympia	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Okayama-saradana	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Syusui	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Bronze	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Leaf lettuce green	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Stem lettuce	I	I	I	I	I	I	I	I	I	I	I	I	I	I
cv. Cos lettuce	I	I	I	I	I	I	I	I	I	I	I	I	I	I
<i>L. serriola</i>	II	I	II	II	I	II	I	II	II	II	II	II	I	I
<i>L. saligna</i>	III	II	III	III	I	III	I	III	III	III	III	III	I	II
<i>L. virosa</i>	IV	III	IV	IV	I	IV	I	II	IV	II	IV	II	I	III
<i>L. indica</i>														
KT95.511	V	IV	V	V	I	V	II	I	V	IV	V	IV	II	III
KT95.521	V	V	V	V	II	VI	I	I	V	IV	V	IV	II	III
KT95.551	V	IV	V	V	I	V	I	I	V	IV	V	IV	II	III

Table 5. Linkage analysis of the 4 isozyme loci between *L. sativa* and *L. serriola*.

Pair of gene loci	Parental genotypes	Segregation of progeny genotypes										No.of plants	Chi-square analysis	P	Recombination value
		ll/ll:	ll/ls:	ll/ss:	ls/ll:	ls/ls:	ls/ss:	ss/ll:	ss/ls:	ss/ss					
		1	2	1	2	4	2	1	2	1					
<i>Got-1/ Got-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	5	14	3	12	32	9	3	11	6	95	7.063	0.70-0.50	-	
<i>Got-1/ Pdh-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	6	13	2	12	30	8	5	8	7	91	7.374	0.50-0.30	-	
<i>Got-1/ Pgi-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	6	14	2	12	29	7	4	9	8	91	8.385	0.50-0.30	-	
<i>Got-2/ Pdh-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	16	1	0	7	48	2	0	6	14	94	88.638	<0.01	8.704	
<i>Got-2/ Pgi-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	14	1	2	8	49	1	0	6	14	95	81.379	<0.01	10.829	
<i>Pdh-2/ Pgi-2</i>	<i>ls/ls</i> × <i>ls/ls</i>	16	1	1	2	48	2	1	2	16	89	108.124	<0.01	6.286	

Table 4. Chi-square analysis of the segregation at 4 isozyme loci between *L. sativa* and *L. serriola*.

Isozyme locus	Genetic type of F ₂ population ^z (no. of individuals)	Chi-square analysis (1:2:1)	P
<i>Got-1</i>	<i>ll</i> (28) : <i>ls</i> (72) : <i>ss</i> (30)	1.569	0.50 - 0.30
<i>Got-2</i>	<i>ll</i> (34) : <i>ls</i> (64) : <i>ss</i> (22)	2.933	0.30 - 0.20
<i>Pgi-2</i>	<i>ll</i> (21) : <i>ls</i> (52) : <i>ss</i> (19)	1.652	0.50 - 0.30
<i>Pdh-2</i>	<i>ll</i> (21) : <i>ls</i> (52) : <i>ss</i> (19)	1.652	0.50 - 0.30

^z: l and s represent genes of *L. sativa* and *L. serriola*, respectively.

be distinguished from *L. serriola*, *L. saligna*, *L. virosa* and *L. indica* by nine, 11, 11 and 13 enzyme systems, respectively. These enzymes may be useful genetic markers for the study of introgression of novel traits from these wild species. Intraspecific polymorphisms were also observed in the 4 of the 14 enzyme systems in *L. indica*. Although 8 cultivars of lettuce did not show any intraspecific polymorphisms in these 14 enzyme systems, they were representative of 5 morphologically different groups, suggesting that the differentiation or evolution of cultivars in the species of *L. sativa* is still small or limited. The homozygosity of the loci observed

in *L. sativa* might be due to its inbreeding system, whereas the heterozygosity observed in *L. indica* might be due to its partially outbreeding system. *L. indica* is not a cultivated species, but is occasionally collected as wild, local, edible herb. The intraspecific diversity of the *L. indica* that grows vigorously in Japan is preserved morphologically characteristics as well as by isozyme variations. Therefore, it could be an important resource for the breeding of lettuce.

The band patterns of *Glutamate-oxaloacetate transaminase*, GOT, isozyme showed an interspecific polymorphism among the species studied (Fig. 1 and 2). In the most cathodal region of the anodal site, *L. serriola* and *L. saligna*, were represented by two types of single-banded phenotypes, different from *L. sativa* (Fig. 2; band 1, 3). The F₁ hybrids between lettuce and these wild species showed three-banded phenotypes in this region (bands 3, 4 and 5 or bands 1, 2 and 5), with the additional double intensity bands having mobility intermediate to the parent bands (band 4 or 2), indicating that the bands represent a dimeric enzyme. The F₂ hybrids between *L. sativa* cv. Bronze and *L. serriola* segregated as expected (1:2:1 ratio; Table 4), demonstrating that the GOT enzyme in *Lactuca* species is controlled by a single gene locus designated as *Got-1*. Although the

segregation of the isozymes of the hybrids between *L. saligna* and *L. sativa* cv. Bronze does not fit the expected ratio at *Got-1* locus, the second anodal region from the origin showed polymorphism between *L. sativa* and *L. serriola* (Fig. 2; band 6 and 9); their F_1 hybrids possessed three-banded phenotype (band 6, 7 and 9), whereas the F_2 progeny segregated in a 1:2:1 ratio (Table 4). Therefore, this region is considered to be controlled by a single locus, designated as *Got-2*. Bands 8 and 9, in Fig. 2 are common and stable in the genus, because they are located at independent monomorphic

Fig. 1. GOT zymograms of *L. sativa*, *L. saligna*, *L. serriola*, *L. indica* and their hybrids.

Lanes 1: *L. sativa* cv. Bronze, 3: *L. saligna*, 8: *L. serriola*, 9: *L. indica*, 2: F_1 hybrid between *L. saligna* and *L. sativa* cv. Bronze, 4-7: F_2 hybrids between *L. sativa* cv. Bronze and *L. serriola* and 10: Somatic hybrid between *L. sativa* cv. Bronze and *L. indica*.

loci, *Got-3* and *Got-4*, respectively. Hirai et al. (1986), and Tashiro et al. (1995) reported that GOT isozyme loci of *Citrus* and *Allium* are dimeric isozymes controlled by multiple alleles, respectively. Isshiki et al. (1995) reported that relative band intensities of the triple-banded phenotype observed in GOT zymogram of Taro, *Colocasia esculenta*, are referred to as dosage effects of the genes. Theoretically, a relative band intensity of the heterogenous phenotype of the dimeric isozyme shows a 1:2:1 ratio in the three bands; the distortion of band intensity is derived from the dosage effects of the genes. In this study, the somatic hybrid between *L. sativa* cv. Bronze and *L. indica* showed a 4:4:1 band intensity ratio based on visible observation, similar to the triploid taro in *Got-1*. Because, the somatic hybrid was found to be a hexaploid plant by chromosome counts, they are considered to be composed of 4 genomes of *L. sativa* cv. Bronze and two genomes of *L. indica*.

Zymogram patterns of *Phosphoglucosomerase*, PGI, of *Lactuca* species (Fig. 3, Table 3) reveal that all of 5 species studied had 4 bands, 2 of which showed inter-specific polymorphisms. The most cathodal region of PGI zymogram showed two phenotypes; one is represented by *L. sativa*, *L. serriola* and *L. virosa* and the other by *L. saligna* and *L. indica*. All of the F_1 hybrids between *L. sativa* and *L. saligna* showed a three-banded phenotype, suggesting that it is a dimeric enzyme and designated as *Pgi-1*. The second region next to *Pgi-1* also revealed that the enzyme is a dimeric enzyme (Fig.

Fig. 2. Schematic illustration of GOT zymograms of *L. sativa*, *L. serriola*, *L. virosa*, *L. indica* and their hybrids.

Fig. 3. Schematic illustration of PGI zymograms of *L. sativa*, *L. serriola*, *L. virosa*, *L. indica* and their hybrids.

Fig. 4 Schematic illustration of 6-PDH zymograms of *L. sativa*, *L. serriola*, *L. virosa*, *L. indica* and their hybrids.

3). The F_2 generation between *L. sativa* and *L. serriola* segregated in a 1:2:1 ratio (Table 4), so that this gene locus was designated as *Pgi-2*, in which 3 multiple alleles exist. The two other anodal bands in the 5 species did not show any polymorphism in their F_1 hybrids; their loci are designated as *Pgi-3* and *Pgi-4*.

Zymogram patterns of 6-Phosphogluconate dehydrogenase, 6-PDH, (Fig. 4) reveal that all 5 species possess 2 bands, showing polymorphisms. The most anodal band in the zymogram of *L. sativa* is distinguishable from those of *L. saligna* and *L. indica*, and all of their F_1 hybrids by the three-banded phenotype, suggesting that the enzyme is also dimeric. The F_2 hybrid between *L. sativa* cv. Bronze and *L. serriola* segregated into 3 phenotypes in a 1:2:1 ratio (Table 4), which indicates that the enzyme is controlled by a single locus, *Pdh-2*. The segregation analyses of *Pgi-1* and *Pdh-1* in the F_2 generation will be discussed in the next paper based on the distortion observed in the F_2 progeny between *L. sativa* and *L. saligna*.

The results of contingency chi-square analyses of independent assortment for 4 pairs between *Got-1*, *Got-2*, *Pdh-2* and *Pgi-2* (Table 5) indicate that these enzymes in the 4 loci are co-dominant dimeric enzymes. The statistical analyses revealed not only the segregation of the alleles in a single locus (Table 4), but also the linkage of the loci. The evidence for linkage was not detected between *Got-1* locus and other loci, whereas the 3 loci, *Got-2*, *Pdh-2* and *Pgi-2* were considered to be linked strongly, having high recombination values of 6.3, 8.7 and 10.8 among the 3 pairs.

The analyses of the genus *Lactuca* by molecular and isozyme techniques, were found to be an excellent method for the reconfirmation of hybrid plants in *Lactuca* species and the DNA analyses. In this study, the genomic construction of polyploid was detected only by the band intensity in isozyme analysis. The somatic hybrids between *L. sativa* cv. Bronze and *L. indica* had an abnormal band intensity in two other loci, *Pgi-2* and *Pdh-1*, which showed a banding pattern, with a 4:4:1 intensity ratio as well as the locus for *Got-1*. Thus, the 3 loci indicate that the somatic hexaploid hybrid is consid-

ered to have originated from the two protoplasts of lettuce and one from *L. indica*.

Thus, 6 loci in the 3 enzymes allowed us to identify hybrids between lettuce, *L. sativa* and other *Lactuca* species. We confirm that isozyme analysis is as useful as DNA analysis for the genetic study in breeding lettuce.

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レタス *Lactuca sativa* および近縁野生種のアイソザイムの遺伝分析

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摘 要

レタス *Lactuca sativa* およびその近縁野生である *L. serriola*, *L. saligna*, *L. virosa* およびアキノノゲシ *L. indica* を供試して, 14種類のアイソザイムの分析を行い, それらの種間に多型が見られることを明らかにした. また, GOT, PGI および 6-PDH は共に 2量体の酵素で, これらの酵素の 6つの遺伝子座は *Lactuca* 属のアイソザイムの遺伝分析に有効であった. レタスと *L. serriola* の F_2 雑種におけるこれらの酵素の分離分析の結果, *Got-1*, *Got-2*, *Pgi-2* および *Pdh-*

2 の 4つの遺伝子座が明らかになり, それらのうち, *Got-2*, *Pgi-2* および *Pdh-2* の 3つの遺伝子座は互いに連鎖していた. レタスとアキノノゲシ間の 6倍体の体細胞雑種のバンドの濃さの分析はその雑種がレタスの 4つのゲノムとアキノノゲシの 2つのゲノムから構成されていることを示した. これら 6つのアイソザイム遺伝子座はレタスと近縁野生種の交雑を行ううえで有効な遺伝子マーカーとして利用できるものと思われる.