Genetic Variation in Several Isozymes of Indigenous Rice Varieties in Yunnan Province of China

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To investigate the magnitude of the genetic diversity of indigenous rice varieties in the Yunnan province of China, isozyme variations were analyzed. A total of 252 varieties were used compared with 82 varieties from other provinces of China, Japan and some Asian countries. Seven isozyme loci, *Est-1*, *Est-2*, *Pox-2*, *Sdh-1*, *Amp-1*, *Icd-1* and MDH, were assayed by starch gel electrophoresis. The Yunnan varieties showed a polymorphism of six isozyme loci except MDH. Sixteen alleles were detected at seven loci in the varieties in total and the value of average heterozygosity was 0.27. The varieties from the northern part of the Yunnan province showed a monomorphic variation in isozymes. The value of average heterozygosity in the varieties from the southern part was 0.29 and the region exhibited the largest variations in the province in isozymes. Allele frequencies of *Est-1*¹, *Est-2*⁰, *Pox-2*⁰ and *Icd-1*¹ decreased from the northern part to the southern part. No significant differences in the value of average heterozygosity was found between the *hsien* type (*Indica*) and *keng* type (*Japonica*) of varieties. Unclassified varieties showed a high value of average heterozygosity and did not have predominant alleles at *Est-1*, *Est-2*, *Pox-2* and *Amp-1*.

KEY WORDS: *Oryza sativa*, isozymes, genetic variation, differentiation, Yunnan province of China.

Introduction

Yunnan province of China is one of the centers of genetic diversity of rice along with East Nepal, Bhutan, Assam in India, Myanmar and North Thailand (CHANG, 1976; NAKAGAHRA and HAYASHI, 1977). A China-Japan cooperative research project was initiated at the Yunnan Academy of Agricultural Sciences, Kunming, Yunnan province, China to improve rice varieties with high yield, cold tolerance and disease resistance in the province in 1982. Before the beginning of the project only 40 indigenous Yunnan varieties had been introduced to Japan. During the collaborative research several hundreds of varieties were mutually exchanged between China and Japan. Varieties in the province were recently known to be cold tolerant at the booting and flowering stages (HORISUE *et al.*, 1988), resistant to blast and other diseases (IWANO *et al.*, 1990) and to harbour new endosperm characters (NAKAGAHRA *et al.*, 1986). Therefore, they attracted the attention of rice breeders. To detect the extent of the genetic diversity of the rice varieties in the Yunnan province, NAKAGAHRA (1984) analyzed the three loci of esterase isozymes, *Est-1*, *Est-2* and *Est-3* in 40 varieties introduced from the province and reported the presence of large isozyme variations there. XIONG *et al.* (1987) also analyzed four loci of esterase; *Est-1*, *Est-2*, *Est-3* and *Est-4* of the Yunnan

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varieties in the 1st and 2nd rice germplasm exchanges during the project. They observed that 22 out of 24 possible allele combinations were located at the four loci in the varieties, and they reported that allele variations increased from north to south in the province.

The purpose of the present paper is to analyse the genetic variation of indigenous varieties in the Yunnan province of China based on different kinds of isozymes.

Materials and Methods

A total of 252 indigenous rice varieties from the Yunnan province of China were used. Of them, 102 varieties were the 3rd rice germplasm exchange in the project and 150 were the germplasm conserved in the Yunnan Academy of Agricultural Sciences. Furthermore, 19 varieties from other provinces of China (mainly from Guizhou province), 21 from Japan and 42 from other Asian countries (India, Laos, the Philippines), were also tested. Fully developed leaves were sampled for the extraction of the enzymes two weeks after germination. Horizontal starch gel electrophoresis was performed at 4°C. Six kinds of enzymes, esterase (loci *Est-1* and *Est-2* were analyzed), peroxidase (*Pox-2*), shikimate dehydrogenase (*Sdh-1*), leucine aminopeptidase (*Amp-1*), isocitric dehydrogenase (*Icd-1*) and malate dehydrogenase (MDH) were assayed according to the procedure of SECOND and TROUSLOT (1980), NAGAMINE *et al.* (1989) and MORISHIMA and GLASZMANN (1990). For malate dehydrogenase since the locus has not been identified yet, in the present paper the enzyme name was used. Isozyme nomenclature followed the recommendation of isozyme gene symbols proposed during the 2nd Rice Genetics Symposium (MORISHIMA and GLASZMANN, 1990). To compare isozyme variations, the allele frequencies and the number of alleles observed at isozyme loci and the value of average heterozygosity (NEI, 1972) were calculated. All the experiments were carried out at the Yunnan Academy of Agricultural Sciences at Kunming, Yunnan.

Results

Isozyme variations of indigenous varieties in Yunnan province

Large variations in the six isozyme loci were observed in the indigenous varieties of the Yunnan province. Almost all of the known alleles were observed in the Yunnan varieties except for the *Sdh-1*³, *Amp-1*³ and *Amp-1*⁴ alleles (Table 1), but were monomorphic for MDH. Only one variant in MDH was detected in the varieties from Guizhou province of China. In the varieties from other provinces of China, 13 alleles were detected in total, while alleles *Est-1*⁰, *Est-2*¹, *Sdh-1*⁴, *Amp-1*³, *Amp-1*⁴, *Icd-1*¹ and *Icd-1*³ were not found. Seven alleles were found at six loci in the Japanese varieties. Varieties from other Asian countries showed a total of 14 alleles except for alleles, *Sdh-1*³, *Sdh-1*⁴ and *Icd-1*⁴.

The value of average heterozygosity of the Yunnan varieties was 0.27, which was higher than the value of varieties from other provinces of China and Japan. It is, therefore, concluded that the indigenous rice varieties of the Yunnan province show large variations in the isozyme alleles.

Allele frequencies of isozymes in the varieties of three regions in Yunnan

To investigate the isozyme variations in the Yunnan varieties regionally, the province was divided into three regions; the northern, the central and the southern parts according to the

Fig. 1. Yunnan province and the three divided regions, the northern area (1), the central area (2) and the southern area (3).

division by XIONG *et al.* (1987) with a small modification as shown in Fig. 1. The northern part belongs to temperate climate, while, the mountainous area with higher elevation is rather cool. Japonica (keng type) varieties are predominantly cultivated in the field of higher altitude and Indica (hsien type) varieties is partly grown at the lower altitudes below 1,300m. The climate of the central part is sub-tropical to temperate, where a double cropping for rice is possible. Indica varieties are mainly cultivated there. The southern part belongs to the sub-tropical climate, where both Japonica and Indica varieties are cultivated. Varieties with glutinous endosperm and Indica varieties with sticky endosperm are grown besides.

Nine alleles were observed in the varieties from the northern part (Table 2) with the predominance of specific alleles, *Est-1*¹, *Pox-2*⁰, *Sdh-1*² and *Amp-1*¹ at the respective loci. A rather monomorphic isozyme variation was observed in the varieties in this region as in the case of the Japanese varieties. On the other hand, 15 and 16 alleles were found in the varieties from the central and the southern parts, respectively. The number of alleles increased from the northern part to the southern part.

Allele frequencies of *Est-1*¹ and *Est-2*⁰ decreased from the northern part to the southern part, whereas those of *Est-1*⁰ and *Est-2*¹ increased from the northern part to the southern part. Such a geographical cline on the allele frequencies was observed for the loci *Pox-2* and

Table 1. Allele frequencies at isozyme loci in indigenous rice varieties from the Yunnan province of China

Source of varieties	<i>Est-1</i>		<i>Est-2</i>			<i>Pox-2</i>			<i>Sdh-1</i>				<i>Amp-1</i>			<i>Icd-1</i>			Number of varieties	He ¹⁾
	0	1	0	1	2	0	1		1	2	3	4	1	2	4	1	2	3		
1	0.08	0.92	0.70	0.16	0.14	0.71	0.29		0.06	0.92	0.00	0.02	0.86	0.14	0.00	0.80	0.19	0.01	252	0.27
2	0.00	1.00	0.74	0.00	0.26	0.95	0.05		0.05	0.90	0.05	0.00	0.95	0.05	0.00	1.00	0.00	0.00	19	0.12
3	0.00	1.00	1.00	0.00	0.00	0.76	0.24		0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	21	0.05
4	0.19	0.81	0.45	0.44	0.11	0.65	0.36		0.33	0.67	0.00	0.00	0.78	0.01	0.22	0.98	0.02	0.00	42	0.32

1: Yunnan province, 2: Other provinces in China, 3: Japan, 4: Other Asian countries

¹⁾ Average heterozygosity as defined by Nei (1972)

Table 2. Allele frequencies at isozyme loci in indigenous rice varieties from divided regions of Yunnan province

Source of varieties	<i>Est-1</i>		<i>Est-2</i>			<i>Pox-2</i>			<i>Sdh-1</i>				<i>Amp-1</i>				<i>Icd-1</i>			Number of varieties	He ¹⁾
	0	1	0	1	2	0	1		1	2	3	4	1	2	4	1	2	3			
1	0.00	1.00	0.96	0.04	0.00	1.00	0.00		0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.02	27	0.02	
2	0.07	0.93	0.72	0.11	0.17	0.87	0.13		0.07	0.90	0.00	0.03	0.83	0.17	0.00	0.86	0.14	0.00	76	0.22	
3	0.11	0.89	0.64	0.21	0.15	0.58	0.42		0.07	0.92	0.00	0.01	0.85	0.15	0.00	0.72	0.24	0.04	149	0.29	
Total	0.08	0.92	0.70	0.16	0.14	0.71	0.29		0.06	0.92	0.00	0.02	0.86	0.14	0.00	0.80	0.19	0.01	252	0.27	

1: Northern part, 2: Central part, 3: Southern part

¹⁾ Average heterozygosity as defined by Nei (1972)

Table 3. Allele frequencies at isozyme loci in hsien and keng type varieties in Yunnan province

Types	<i>Est-1</i>		<i>Est-2</i>			<i>Pox-2</i>			<i>Sdh-1</i>				<i>Amp-1</i>			<i>Icd-1</i>			Number of varieties	He ¹⁾
	0	1	0	1	2	0	1		1	2	3	4	1	2	3	1	2	3		
1	0.06	0.94	0.79	0.14	0.07	0.78	0.22		0.02	0.94	0.00	0.04	0.79	0.21		0.89	0.07	0.03	103	0.20
2	0.06	0.94	0.66	0.14	0.20	0.71	0.29		0.08	0.92	0.00	0.00	0.95	0.05		0.71	0.29	0.00	135	0.24
3	0.50	0.50	0.36	0.57	0.07	0.36	0.64		0.21	0.75	0.00	0.04	0.50	0.50		0.92	0.08	0.00	14	0.41

1: Hsien type, 2: Keng type, 3: Unclassified group

¹⁾ Average heterozygosity as defined by Nei (1972)

Icd-1, too. The values of average heterozygosity were 0.22 and 0.29 in the varieties from the central and southern parts, respectively. Based on the present results, it is concluded that the varieties of the southern part in the province displayed the largest genetic diversity.

Difference in allele frequencies of isozymes between hsien and keng types

Varieties used for isozyme analysis were classified into the hsien and keng types, based on the evaluation of six morphological characters, glume hairiness, phenol color reaction, length between 1st and 2nd nodes of panicle axis, glume color at heading stage, leaf pubescence and length/width ratio of spikelets (CHENG, 1985; ZHOU *et al.*, 1988). Among the varieties tested, 103 were classified into the hsien type, 135 into the keng type while 14 were not classified definitely into either the hsien type or the keng type.

A significant differences was detected in the allele frequencies of the loci *Est-1*, *Pox-2* and *Sdh-1* (Table 3). Hsien type varieties showed a higher frequency of allele *Est-2*² than the keng type. Keng type varieties showed a higher frequency of allele *Pox-2*⁰. Three different alleles; 1, 2 and 4 were found at the *Sdh-1* locus in the keng type whereas two alleles 1 and 2 were found in the hsien type. At the *Amp-1* locus hsien type varieties showed a relatively monomorphic variation. While alleles 1, 2 and 3 were found in the keng type at *Icd-1* locus, alleles 1 and 2 were observed in the hsien type. The values of average heterozygosity of the keng and hsien types were 0.20 and 0.24, respectively, and a significant difference was not detected between the two values. However, unclassified varieties exhibited a different variation in isozyme alleles. Alleles 0 and 1 were found equally in the varieties at *Est-1* locus. Similarly, alleles 1 and 2 were observed equally at the locus *Amp-1*. Unclassified varieties did not have specific predominant alleles at the loci *Est-1*, *Est-2*, *Pox-2* and *Amp-1*. Moreover, the average value of heterozygosity was 0.41, which is significantly higher than the values of hsien and keng types.

Discussion

To analyse the extent of genetic diversity in the indigenous rice varieties in the Yunnan province, we assayed isozymes by starch gel electrophoresis, which enables to stain several kinds of enzymes simultaneously. The analysis of seven loci of the six isozymes, *Est-1*, *Est-2*, *Pox-2*, *Sdh-1*, *Amp-1*, *Icd-1* and MDH indicates that the Yunnan rice varieties display a large genetic variation. The extent of genetic diversity in the Yunnan varieties was investigated by NAKAGAHRA (1984) and XIONG *et al.* (1987). NAKAGAHRA (1984) analyzed three esterase isozyme loci; *Est-1*, *Est-2* and *Est-3* in 40 indigenous varieties which were introduced into Japan in 1979. He observed large variations in the genotypes compared with the varieties in other Asian countries. XIONG *et al.* (1987) analyzed four esterase loci, *Est-1*, *Est-2*, *Est-3* and *Est-4*, in 392 varieties collected in the province and found a large genetic diversity in the varieties. Their findings on the genetic diversity of the Yunnan varieties are confirmed with our results.

The varieties from the northern part of the province showed a monomorphic allele composition, while the varieties from the southern part exhibited a large number of alleles at the isozyme loci and the value of average heterozygosity was high. These results suggested that the varieties in the southern part of the Yunnan province display the largest

genetic diversity. XIONG *et al.* (1987) also reported that the varieties in the southern part showed larger variations than those in the northern part in esterase isozymes. Our findings confirmed the result by XIONG *et al.* (1987). Therefore, the indigenous varieties in the southern part of Yunnan province are expected to study the diversity by other agronomic characters in detail.

The frequencies of the alleles *Est-1*⁰ and *Est-1*¹ in 252 varieties were 0.92 and 0.08 in the present experiment, respectively. On the other hand, those of the alleles in 392 Yunnan varieties were 0.91 and 0.09, respectively (XIONG *et al.* 1987). Furthermore, the frequencies of the alleles *Est-2*⁰, *Est-2*¹ and *Est-2*² were 0.70, 0.16 and 0.14 in the present study, respectively. XIONG *et al.* (1987) reported that the frequencies of the alleles were 0.75, 0.105 and 0.145, respectively. Although the varieties used in the present study were different from those of XIONG *et al.* (1987), it is interesting to note that almost the same values of allele frequencies of the two loci were obtained in the two experiments, suggesting that the varieties used in the two experiments are representatives of the Yunnan varieties.

CHENG *et al.* (1984) proposed the use of the terminology, hsien and keng types instead of Indica and Japonica, based on the differences in morphological characters. Most of the varieties used in the present experiments were classified into both types, but 14 varieties could not be classified definitely into either hsien type or keng type. We could not find a significant difference in the value of average heterozygosity in the seven isozyme loci between the two types. However, the 14 varieties showed high variability. If these varieties represented an intermediate type between the hsien and keng types, it would be important to study not only their isozyme variations but also other characters.

Literature Cited

- CHANG, T. T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. *Euphytica*, **25**: 425~441.
- CHENG, K. S. 1985. A statistical evaluation of the classification of rice cultivars into hsien and keng subspecies. *Rice Genet. Newsl.* **2**: 46~48.
- , X. K. WANG, J. W. ZHOU, Y. X. LU, J. LOU and N. W. HUANG 1984. Studies on indigenous rices in Yunnan and their utilization, II. A revised classification of Asian cultivated rice (in Chinese). *Acta Agronomica Sinica* **10**: 271~280.
- HORISUE, N., Y. KUNIHIO, T. HIGASHI, Z. OYAMADA, H. Y. WANG, J. H. XIONG, S. Z. ZHANG, Z. Y. LI and Y. H. WANG 1988. Screening for cold tolerance of Chinese and Japanese varieties and selection of standard varieties. In the proceedings of International Symposium on Rice Breeding through the Utilization of Unexploited Genetic Resources. *Trop. Agric. Research Series* **21**: 76~92.
- IWANO, M., J. L. LEE, C. Y. LEE and P. KONG 1990. Distribution of pathogenic races and changes in virulence of rice blast fungus, *Pyricularia oryzae* Cav., in Yunnan province, China. *JARQ* **23** (3): 241~248.
- MORISHIMA, H. and J. C. GLASZMANN 1990. Current status of isozyme gene symbols. *Rice Genet. Newsl.* **7**: 50~57.
- NAGAMINE, T., J. P. CATTY and B. V. FORD-LLOID 1989. Phenotypic polymorphism and allele differentiation of isozymes in fodder beet, multigerm sugar beet and monogerm sugar beet. *Theor. Appl. Genet.* **77**: 711~720.
- NAKAGAHARA, M. 1984. Geographical distribution of esterase genotypes of rice in Asia. *Rice Genet. Newsl.* **1**: 118~120.
- and K. HAYASHI 1977. Origin of cultivated rice as detected by isozyme variations. *JARQ* **11** (11): 1~5.

- , T. NAGAMINE and K. OKUNO 1986. Spontaneous occurrence of low amylose genes and geographical distribution of amylose content in Asian rice. *Rice Genet. Newsl.* **3**: 46~48.
- NEI, M. 1972. Genetic distance between populations. *Am. Nat.* **106**: 283~292.
- SECOND, G. and P. TROUSLOT 1980. Electrophoresis d'enzymes de riz (*Oryza* spp.) Travaux et documents **120**, ORSTOM, Paris, pp. 88.
- XIONG, J. H., H. Y. WANG, M. INOUE and M. NAKAGAHARA 1987. Ecotype differentiation and geographical distribution of indigenous rice varieties in Yunnan province of China based on esterase isozyme loci. in *Crop Genetic Resources of East Asia, Proceedings of the International Workshop on Crop Genetic Resources of East Asia* edited by S. SUZUKI. 221~229.
- ZHOU, H., J. C. GLASZMANN, K. S. CHENG and X. Q. SHI 1988. A comparison of methods in classification of cultivated rice. *Chinese J. Rice Sci.* **2** (1): 1~7.

数種同位酵素からみた中国雲南省在来稻の変異

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中国雲南省は稲の遺伝子中心地の一部と推定されており, 耐冷性, いもち病抵抗性, 多収性の遺伝資源としてわが国の稲育種では期待するところが多い。著者らの一人, 熊とその共同研究者はエステラーゼ同位酵素 4 遺伝子座を調査し, 本省在来稲は遺伝的多様性が多いこと, 省内では南部産品種のほうが遺伝子多様度が高いことを明らかにした (XIONG *et al.* 1987)。本報では澱粉ゲル電気泳動法を用いて 6 種の同位酵素を分析し, 雲南省在来稲の遺伝的変異をより詳細に調査したので報告する。

日共同研究第三期提供品種のなかの在来品種 102 と雲南省農業科学院に保存されている 150 品種とを用いた。ほかに比較として中国他省産在来種 19, 日本品種 21, その他外国品種 42 を用いた。発芽後 2 週の苗を分析材料とした。エステラーゼ (*Est-1*, *Est-2*), パーオキシダーゼ (*Pox-2*), シキミ酸脱水素酵素 (*Sdh-1*), ロイシンアミノペプチダーゼ (*Amp-1*), イソクエン酸脱水素酵素 (*Icd-1*), リンゴ酸脱水素酵素 (MDH) の合計 6 酵素種 7 遺伝子座を分析した。

雲南省在来品種には MDH を除く 5 酵素種で変異があった (Table 1)。雲南省全体で対立遺伝子 *Sdh-1*³, *Amp-1*³, *Amp-1*⁴ を除くすべての対立遺伝子が見出された。7 同位酵素遺伝子座で観察された複対立遺伝子の総数は 16 で, その他外国品種で観察した数より多かった。遺伝子の平均多様度は 0.27 と大きく, 雲南省在来品種には大きな遺伝的変異が存在していると結論できた。雲南省在来稲品種の遺伝多様性の大きさを数種の同位酵素を用いて明らかにすることができた。MDH は貴州省の 1 品種にのみ変異を認めた。

雲南省を北部, 中央部, 南部の 3 地域に分けて複対立遺伝子の数を調べた。北部は粳稻 (ジャポニカ型) がおもに栽培されている地帯で, 中央部は粳稻と秈稻 (中国のインディカ型) の両者の栽培地帯で, 南部は秈稻が多く作付されている地帯である (Fig.1)。7 遺伝子座で見られた複対立遺伝子の総数は北部産品種が 7, 中央部産が 15, 南部産が 16 であり, 南部へいくほど複対立遺伝子の数が増えることが明らかとなった (Table 2)。また, 南部産品種の遺伝子の平均多様度は 0.29 で 3 地域のなかでもっとも高かった。すなわち, 雲南省のなかでは南部ほど遺伝的多様性が多いと結論できた。

複対立遺伝子 *Est-1*¹, *Est-2*⁰, *Pox-2*⁰, *Icd-1*² のそれぞれは北部から南部にいくにしたがって低下する定方向的な変化を示した (Table 2)。*Est-1*, *Est-2* 座の各複対立遺伝子の頻度は XIONG ら (1987) が寒天ゲルで調べた値とよく類似した。

同位酵素の変異を調べた品種をおもに形態的特徴から粳稻と秈稻とに分類した。遺伝子の平均多様度には両者間で差がなかった (Table 3)。粳稻とも秈稻とも分類できなかった 14 品種は同位酵素の変異が大きく, 遺伝子の平均多様度は 0.41 で高かった。

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