グルコース・リン酸イソメラーゼ・アイソザイムからみた Lolium 属の種,フェストロリウムおよびメドーフェスクの属間関係

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Intergeneric Relationships among the *Lolium* species, *Festucalolium* and *Festuca pratensis* Huds. Based on Phospho-gluco Isomerase Isozyme Variation

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Introduction

At present, the classification of *Lolium* and *Festuca* species on a morphological and cytogenetic basis is adopted. However, it is not easy that the affinity between *Festuca pratensis* Huds. and *Lolium* species is assessed quantitatively. The isozyme analysis[2~6,7~9,12~15] may be one of the most useful methods to show the affinity between *Lolium* species and *Festuca* species. In the author's previous paper (1985 and 986), the affinity among the five *Lolium* species based on Phospho-gluco isomerase (Pgi—2) isozyme variation has been described. In the present experiment, in order to clarify the relationships among *Lolium* species, *Festucalolium* and *Festuca pratensis* Huds., leaf materials sampled from 140 plants were assayed by horizontal starch gel electrophoresis for an enzymic system, Pgi—2. In addition, the allelle frequencies obtained in the isozyme variation of outbred *Lolium* species, were quoted from the papers (1985 and 1986).

In the view of cytogenetic facts between the *Lolium* species and *Festuca* species, Borrill (1976) supposed that *Lolium multiflorum*, *Lolium perenne* and *Festuca pratensis* were derived from a common progenitor. However, such hypothesis has never been demonstrated. Therefore, in this study, the affinity between *Festuca pratensis* and *Lolium* species was assessed by the genetic distances (Nei, 1972).

This paper reports that *Festucalolium* is derived from *Festuca* species and *Lolium* species by means of Pgi—2 isozyme analysis in this experiment.

Table 1. *Festuca* species and *Festucalolium* used as materials

Species and intergenric hybrid	Cultivar and strain	(2 n)	Breeding country
Festuca pratensis Huds.	First	(2 x)	Japan
	Trader	(2 x)	Canada
Festucalolium	Z R/W	(—)	Netherlands
	Z 254	(—)	do
	Z 268	(—)	do

Note : The cultivar, 'First', was introduced from Snow Brand Seed Co., the cultivar, 'Trader', was introduced from Kyushu National Agricultural Experiment Station, and the three strains of *Festucalolium* were introduced from Hokuren Agricultural Cooperation Union in Japan.

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Materials and Methods

The materials consisted of two cultivars of *Festuca pratensis* Huds. and three strains of *Festucalolium* listed in Table 1. The individual plants except 'Trader', were grown in Wagner pots in a field, and were removed into a sunny room in November, 1985. The individual plants of 'Trader' were sown in the pots at the sunny room in February, 1986. Usually, young leaves picked from the individual plants were used for enzyme analysis from December of 1985 to March of 1986.

The same electrophoresis procedure (Scandalios, 1975 ; Hayward and McAdam, 1977) that has been described in detail in the privious papers (1985 and 1986), was adopted.

Results

In an analysis of zymograms obtained by horizontal starch gel electrophoresis, 7 bands were detected for leaf materials of the two cultivars of *Festuca pratensis* Huds., and such zymograms showed a single band and a three banded pattern (Fig. 1 and 2). And also, 7 bands were detected for leaf materials of the three Festucalolium strains, but such zymograms showed a three banded pattern, a five banded pattern, a six banded pattern and a seven banded pattern (Fig. 1 and 2). From the phenotypes contained four alleles, 'a', 'b', 'c' and 'd', proposed by Hayward and McAdam (1977) and Nielsen (1980), phenotypes, ab, bb, bc, cc, bd, cd and dd, were confirmed in *Festuca pratensis* Huds., and 14 phenotypes, abbc, abcc, abbd, aacd, accd, abcd, bbbc, bbcc, bccc, bbcd, bccd, bbbd, bbdd and cccd, were detected in *Festucalolium* used (Table 2 and Fig. 2).

For the intravarietal variation based on Pgi—2, 'First' in *F. pratensis*, contained the three phenotypes, bb, bc and cc, and 'Trader', cont ained the three phenotypes, bb, bc and cc, and 'Trader', contained the seven phenotypes, ab, bb, bc, cc, bd, cd and dd. This shows that 'First' contained lower intravarietal variation than 'Trader' (Table 2).

The three *Festucalolium* strains contained 14 phenotypes, that is, Z R／W, Z 254 and Z 268 contained six, nine and nine phenotypes, resp-

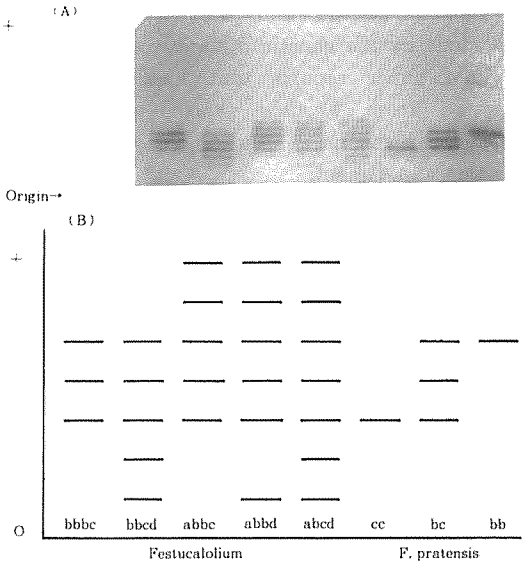

Fig. 1. The phospho-gluco isomerase (Pgi-2) phenotypes (A) and the diagram of the phenotypes in *Festucalolium* and *Festuca pratensis* Huds. (B)

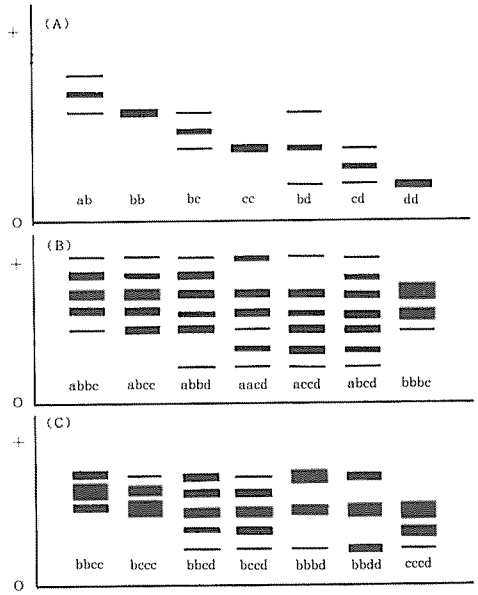

Fig. 2. Diagram of the phenotypes detected in *Festuca pratensis* Huds. (A) and *Festucalolium* (B) and (C)

Table 2. Number of individuals in the phenotypic classes

Pheno -type	*F. pratensis* First	Trader	*Festucalolium* Z R/W	Z 254	Z 268
ab	0	1	—	—	—
bb	8	9	—	—	—
bc	29	11	—	—	—
cc	9	4	—	—	—
bd	0	4	—	—	—
cd	0	10	—	—	—
dd	0	1	—	—	—
abbc	—	—	0	1	4
abcc	—	—	0	1	0
abbd	—	—	2	0	1
aacd	—	—	0	1	0
accd	—	—	0	0	1
abcd	—	—	2	2	1
bbbc	—	—	0	0	2
bbcc	—	—	1	5	3
bccc	—	—	0	1	1
bbcd	—	—	2	0	0
bccd	—	—	10	2	3
bbbd	—	—	0	1	0
bbdd	—	—	0	3	1
cccd	—	—	4	0	0
Total	46	40	21	17	17

Table 3. The allele frequencies of the cultivars of *Festuca pratensis* compared with those of out-bred *Lolium* species

Species (cultivar)	a %	b %	c %	d %	Total %
F. pratensis	0.5	46	43.5	10	100
(First)	0	49	51	0	100
(Trader)	1	43	36	20	100
L. multiflorum	9	38	12	41	100
L. perenne	31	50	14	5	100

Note: The allele frequencies of *L. multiflorum* and *L. perenne* were quoted from the author's prcvious papers (1985 and 1986).

Table 4. Genetic distance of three species based on 4 alleles at a locus

	L. multiflorum	*L. perenne*
Festuca pratensis	0.323	0.268
Lolium multiflorum	—	0.319

Note: Genetic distance was computed as follows; $D=-\ln I$, where I=the normalized identity of genes between two populations and is equal to; $I=J_{xy}/\sqrt{J_x J_y}$, where J_x, J_y and J_{xy} are the the arithmetic means of the probability of two randamly chosen genes in species x or y respectively whilst J_{xy} is that of a gene from x with that from y (Nei, M., 1972).

ectively, and the variation in Z R/W was lower than that in Z 254 and Z 268 (Table 2).

There was a certain intervarietal variation of Pgi—2 among the three strains of *Festucalolium*, but those differences were very slight (Table 2 and 5). The allele frequencies at the locus Pgi—2, were caluculated on the basis of number of individuals in the phenotypic classes (Table 2), and were presented in Table 3 and 5.

The alleles of the cultivars in *Festuca pratensis* used consisted of four alleles, 'a', 'b', 'c' and 'd'. The alleles, 'b' and 'c' occurred more frequently than the alleles, 'a' and 'd' (Table 3).

The genetic distances (Nei, 1972) among three species, *Festuca pratensis*, *Lolium multiflorum* and *Lolium perenne*, were computed on the basis of the allele frequencies in the Table 3, and were shown in Table 4. The distances show that *F. pratensis* has more close affinity with *L. perenne* than *L. multiflorum* does with *L. perenne*. But, the genetic distance between *F. pratensis* and *L. multiflorum* was a little greater than that between *Lolium perenne* and *L. multiflorum*.

Table 5 shows the allele frequencies of Pgi—2 in the three *Festucalolium* strains. Z 254 and Z 268 had the highest frequency of allele, 'b', and the lowest frequency of allele, 'a'. Z R/W had the highest frequency of allele, 'c', and the lowest frequency of allele, 'a'. Generally speaking, the three *Festucalolium* strains had higher frequencies of alleles, 'b' and 'c',

and lower frequencies of alleles, 'a' and 'd'.

To assess the relationships between *Lolium* species and *Festucalolium* and between *Festuca pratensis* Huds. and *Festucalolium*, chi square analysis was operated (Table 5). The results pointed out that the frequency of Z 254 had close affinity with the frequency expected in the case of *Lolium multiflorum* x *Festuca pratensis* Huds., and that of Z 268 was closely related to that supposed in the case of *L. perenne* x *F. pratensis*. However, there was a significant difference between the frequency of Z R／W and those expected in the case of *Lolium* species x *F. pratensis*.

Discussion

The cultivar, 'First', bred in Japan from 7 cultivars of Europe and Canada[17], was inferred to included the high allelic variation in *Festuca pratensis* Huds.. But, the only three phenotypes of 'First' were detected in Pgi－2 isozyme analysis. The cultivar, 'Trader', contained 7 phenotypes, and had more phenotypic variation than 'First'. However, it was confirmed that two cultivars of *F. pratensis* and *Lolium* species had the phenotypes of common.

There were some different frequencies of alleles between *F. pratensis* and *Lolium* species in spite of the common alleles between both species. That is, the high frequency of alleles, 'b' and 'c', characterized *F. pratensis*, while the high frequencies of alleles, 'a' and 'b' and alleles 'b' and 'd', characterized *L. perenne* and *L. multiflorum*, respectively.

Table 5 . The allele frequencies and the presumption of the parental species of the three strains in *Festucalolium*

Strain	Allele frequencies a %	b %	c %	d %	d.f.	χ^2	P
Z R／W							
Observed	5	26	45	24			
Expected							
(1) *F. pratensis* x *L. multiflorum*	5	42	28	25	3	16.45	<0.01
(2) *F. pratensis* x *L. perenne*	16	48	29	7	3	67.75	<0.01
Z 254							
Observed	9	40	34	18			
Expected							
(1) *F. pratensis* x *L. multiflorum*	5	42	28	25	3	6.54	0.1—0.05
(2) *F. pratensis* x *L. perenne*	16	48	29	7	3	22.54	<0.01
Z 268							
Observed	10	44	35	10			
Expected							
(1) *F. pratensis* x *L. multiflorum*	5	42	28	25	3	15.84	<0.01
(2) *F. pratensis* x *L. perenne*	16	48	29	7	3	5.11	0.9—0.1

Note : Chi square analysis was operated on the basis of the frequencies of the three species in Table 3 . Expected allele frequencies show the arithmetic means of the probability of two chosen genes in species, outbred *Lolium* species and *F.pratensis*, because of the codominant alleles of Pgi— 2 isozyme.

From the results of genetic distances, it was clarified that *Festuca pratensis* was similar to *Lolium perenne*. In addition, the genetic distance between *F. pratensis* and *L. multiflorum* was much the same as that between *L. multiflorum* and *L. perenne*.

The fact obtained in the present study, is not inconsistent with the fact confirmed by cytogenetic analysis. That is, the only slight differences were found in the size of chromosome and location of centromere between *L. perenne* and *F. pratensis*[6]. And also, the close genetic distances between *F. pratensis* and outbred *Lolium* species, support Borrill's hypothesis (1976) that such three species, *L. multiflorum*, *L. perenne* and *F. pratensis*, were derived from the common progenitor because the outbred *Lolium* species hybridized with 2 x *F. pratensis* to about the same as do the fescues with one another.

In general, the phenotypes in tetraploid plants of *Lolium* species have a maximum seven bands. As the seven bands of phenotypes were detected in the *Festucalolium* strains, Z R/W, Z 254 and Z 268, it seems that they are tetraploid[11]. It is certain that 15 phenotypes occurred by means of the gene dosage effects on the isozyme bands in *Festucalolium*.

Chi square analysis[2] points out that the strain, Z 254, may be hybrid between *F. pratensis* and *L. multiflorum*[10], and the strain Z 268, may be hybrid between *F. pratensis* and *L. multiflorum*. But, it could not be clarified that Z R/W might be hybrid between *F. pratensis* and outbred *Lolium* species. Therefore, in order to reach a certain conclusion, the isozyme analysis adopted many enzymic systems, must be conducted.

In conclusion, it was clarified that the outbred *Lolium* species and *Festuca pratensis* Huds. had the common alleles, 'a', 'b', 'c' and 'd', and they were the allied species because of their close genetic distance.

Festucalolium strains used, also contained the common four alleles of *Lolium* species and *F. pratensis*. Chi square analysis shows that two strains in the three *Festucalolium* strains used, possibly were the intergeneric hybrid between *F. pratensis* and outbred *Lolium* species. However, it was not ascertain whether Z R/W was hybrid between *F. pratensis* and outbred *Lolium* species.

Summary

This study was conducted to clarify the intergeneric affinity among the *Lolium* species, *Festucalolium* and *Festuca pratensis* Huds. based on the Phospho-gluco isomerase isozyme (Pgi— 2) variation. The leaf materials of the two cultivars of *Festuca pratensis* and of the three strains of *Festucalolium* were assayed by horizontal starch gel electrophoresis for an enzymic system, Pgi— 2 .

The results obtained were as follows.

1 . Seven bands in the two cultivars of *Festuca pratensis* Huds. and in the three strain of *Festucalolium* derived from the crossing between *Lolium* species and *Festuca* species were detected on the zymograms Pgi— 2 . So, seven phenotypes were confirmed in *Festuca pratensis* Huds., and 15 phenotypes were identified by means of the gene dosage effects on the isozyme bands in *Festucalolium*.

2 . It is sure that the alleles, 'a', 'b', 'c' and 'd' of the two cultivars of *F. pratensis* Huds. and of the three strains of *Festucalolium*, were included in the four alleles of the genus *Lolium*.

3 . Chi square analysis showed that the two strains in the three *Festucalolium* used were the hybrid types between *Lolium* species and *Festuca pratensis* Huds.

4 . Generally speaking, the relationships between *Lolium* species and *Festuca pratensis* Huds. obtained in the present study showed good agreement with those of phylogenetic relationships obtained by the morphological and cytogenetic basis (Terrell, 1966).

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グルコース・リン酸イソメラーゼ・アイソザイムからみた*Lolium*属の種，フェストロリウムおよびメドーフェスクの属間関係

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摘 要

Lolium 属の他殖性種，フェストロリウムおよびメドーフェスクの属間関係を明らかにする目的で，グルコース・リン酸イソメラーゼ（Pgi—2）アイソザイムを水平でんぷんゲル電気泳動法で分析した。なお，本試験では，メドーフェスク2品種およびフェストロリウム3系統を供用し，*Lolium* 属の他殖性種については，前報（1985および1986）の試験結果を引用した。

得られた結果はつぎのとおりである。

1．メドーフェスクおよびフェストロリウムの両草種とも，7本のバンドを確認した。メドーフェスクでは，7表現型が検出された。一方，フェストロリウムでは，15の表現型が検出されたが，これは，アイソザイムバンドの用量効果によるものと考えられる。

2．本試験で供試したメドーフェスクおよびフェストロリウムには，4つの対立遺伝子，すなわち，a，b，cおよびdが存在した。そして，それらの対立遺伝子は，*Lolium*属 の他殖性種と共通していることが明らかとなった。

3．対立遺伝子の出現頻度に基づき，カイ自乗検定を行った結果，フェストロリウムの供試系統のうち2系統は，*Lolium*属 の他殖性種とメドーフェスクとの中間的4倍体であることが推察された。

4．Pgi—2アイソザイムからみると，一般に，*Lolium*属 とメドーフェスクとは近縁種であることが明らかになった。このことは，細胞遺伝学的な分野から得られた属間関係とよく一致した。