Mitochondrial Genome Polymorphism in Lolium perenne

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MITOCHONDRIAL GENOME POLYMORPHISM IN *LOLIUM PERENNE*

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Abstract
The restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA) of perennial ryegrass (*Lolium perenne* L.) were investigated to elucidate the genetic relatedness among the 128 cultivars including diploid and tetraploid. Many patterns of RFLPs were observed and allowed assigning of the cultivars into the main eight haplotypes of mitochondrial genome relatedness. The American cultivars were classified into haplotype I and VIII which were remote at the mitochondrial genome from each other, the European ones were distributed to all haplotypes and the tetraploid ones were mostly assigned into the haplotype V. The assessment of mtDNA RFLPs may be a valuable method in analyzing a cytoplasmic differentiation among the perennial ryegrass cultivars. Further investigations are required to elucidate mtDNA diversity in relation with the maternal effects on the agronomic traits of perennial ryegrass.

KEYWORDS
perennial ryegrass, cultivars, RFLPs, mitochondria, classification, mtDNA

INTRODUCTION

Many perennial ryegrass cultivars have been released that could be distinguished from each other by some agronomic traits. These traits are governed not only by nuclear genes but to some extent are under the control of maternal inheritance (Yamashita and Shimamoto, 1993). The cultivars of perennial ryegrass as germplasm may show some cytoplasmic variabilities among them.

This paper presents the manners of the cytoplasmic genome polymorphism, classification and phylogeny among perennial ryegrass cultivars based on mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs).

MATERIALS AND METHODS

Analyses were performed on a cultivar base of the 128 seed stocks of perennial ryegrass, including 111 diploids and 17 tetraploids originating from various nations.

Total cellular DNA was isolated from leaf tissues of a few 1-yr old plants raised from seed stock with cetyltrimethylammonium bromide (CTAB), as described in Ishikawa et al. (1992). DNA was digested with the restriction enzymes, *BamHI, EcoRI* or *HindIII* according to the instruction of the supplier and subsequently was subjected to electrophoresis through a 0.8% agarose gel. The resulting gel was Southern-blotted onto a nylon membrane (Hybond N+, Amersham). The hybridization was carried out using an Enhanced Chemiluminescence (ECL) Gene Detection System (Amersham). Probe labelling, hybridization (in the presence of 0.5 M NaCl), washing and signal detection were performed following the manufacturer's instructions. Six probes containing known mitochondrial genes were tested preliminarily. Only four probes, three clones from sugarbeet and one clone from wheat, could detect some polymorphisms among the cultivars used:viz. *coxI* (Senda et al., 1991); *coxIII* and *nad9* (Kubo et al., 1993); and *nad1* (Chapdelaine and Bonen, 1991).

RESULTS AND DISCUSSION

The feature of plant mtDNA polymorphism is a result of recombination across dispersed repetitive sequences and is subjected to its structural change (Andre et al., 1992). It may be a reliable information for analysis of maternal lineage, phylogenic variation and differentiation in plant organisms. In this study, the mtDNA polymorphism of 128 perennial ryegrass cultivars could be investigated by Southern hybridization of four heterologous mitochondrial gene probes with three restriction enzyme digests of total DNA. The RFLPs results are compiled in Table 1. *BamHI* and *HindIII* fragments with four probes were polymorphic at their lengths, respectively. In particular the *BamHI* fragment lengths probed by *coxIII* and nad9 were highly diverse among the cultivars and moreover, the same RFLPs between the two probes were observed in 102 cultivars. Also the *HindIII* fragments with these two probes were common in 121 cultivars. This may indicate that these two genes on mitochondrial genome can be located closely to each other. The EcoRI fragments were highly polymorphic in hybridization with the *coxIII*, but those with the other three probes were low or negligible in their diversities among the cultivars.

In consideration for all RFLPs with a polymorphism an attempt could be made to characterize the cultivars by their mitochondrial genomes. The 118 cultivars, without the 10 cultivars which were different individually in some RFLPs, were grouped into eight haplotypes of mitochondria genome, as shown in Table 2. This reveals that the perennial ryegrass cultivars have been improved using the eight groups of mitochondrial genome as mother parent. Haplotype I to VII formed a group which was different in several RFLPs from the haplotype VIII. All genetic differences between haplotype I and II, IV and V, and VI and VII were small at mitochondria genome. Haplotype III was identical and moreover close to the IV or V. Of 24 American cultivars assayed, 17 and 3 were classified into the haplotype I and VIII, respectively. This may reveal that American cultivars have been raised by using the I or VIII as mother parent. Since genetic distance between the I and VIII was large, the American cultivars may be composed of two sources of mitochondrial genome. The haplotype V regrouped most of the tetraploid cultivars and thus has been used as the source of mitochondrial genome when doubling chromosome.

The assessment of mtDNA RFLPs may be a valuable method in analyzing maternal or cytoplasmic differentiation among perennial ryegrass cultivars. Further investigations are required to elucidate mtDNA diversity in relation with the maternal effects on the agronomic traits which have been detected frequently in perennial ryegrass.

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Table 1
The fragment lengths (kb) of DNA digested by the three restriction enzymes hybridized with the four mitochondrial gene probes.

Probe	Restriction enzyme					
	Bam HI	(No.)	*Eco* RI	(No.)	*Hind* III	(No.)
coxI	13.5	(122)	3.4	(124)	4.4	(92)
	13.8, 1.8	(3)	5.4, 4.6	(2)	3.8	(32)
	13.5, 4.8	(1)	2.4, 1.0	(2)	6.4, 5.4, 2.4	(2)
	13.5, 2.8, 1.8	(1)			6.4, 5.4, 2.4	(2)
	4.8	(1)				
coxIII	10.0, 9.0	(62)	9.4	(76)	2.7	(118)
	9.0	(45)	9.4, 3.0	(37)	5.2	(4)
	10	(11)	9.4, 3.8	(9)	3.8	(4)
	6.2	(4)	10.0, 2.7	(4)	8.2, 6.4, 3.8	(2)
	13.0, 11.0	(2)	9.4, 3.8, 2.7	(1)		
	9.0, 8.0	(2)	3.8	(1)		
	12.0	(1)				
nadI	2.9	(93)	3.4	(127)	5.0	(92)
	2.9, 1.4	(30)	4.0	(1)	5.4, 5.0	(30)
	2.0	(5)			1.7	(2)
nad9	10.0, 9.0	(46)	9.4	(128)	2.7	(117)
	9.0	(43)			5.2	(4)
	10.0	(28)			1.0	(4)
	6.2	(4)			10.0	(2)
	3.2	(3)			5.2, 2.7	(1)
	2.5	(3)				
	10.0, 9.0, 6.2	(1)				

(No.): number of cultivar observed

Table 2
The haplotype groups of perennial ryegrass cultivars based on RFLPs of mitochondrial DNA.

Haplo. Group	No. of Culti.	a) *Bam* HI			*Eco* RI	*Hind* III			
		b) *nad9*	*coxIII*	*nadI*	*cox III*	*coxI*	*nadI*	*nad9*	*coxIII*
I	32	9.0	9.0	2.9	9.4	3.8	5.0	2.7	2.7
II	10	9.0	9.0	2.9	9.4	4.4	5.0	2.7	2.7
III	11	10.0	10.0	2.9	9.4	4.4	5.0	2.7	2.7
IV	24	10.0, 9.0	10.0, 9.0	2.9	9.4, 3.0	4.4	5.0	2.7	2.7
V	9	10.0, 9.0	10.0, 9.0	2.9	9.4, 3.8	4.4	5.0	2.7	2.7
VI	17	10.0	10.0, 9.0	2.9, 1.4	9.4	4.4	5.4, 5.0	2.7	2.7
VII	11	10.0, 9.0	10.0, 9.0	2.9, 1.4	9.4, 3.0	4.4	5.4, 5.0	2.7	2.7
VIII	4	6.2	6.2	2.9	9.4	4.4	5.0	5.2	5.2
c) Ht	.833	.677	.596	.362	.512	.395	.362	.098	.098

a): Restriction enzyme
b): Probe
c): Gene diversity