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RESEARCH OF STS MARKERS FOR VARIETAL IDENTIFICATION OF RYEGRASS

Document prepared by experts from France

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RESEARCH OF STS MARKERS FOR VARIETAL IDENTIFICATION OF RYEGRASS

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Introduction

In many forage crops, markers available for distinctness are very few and heterogeneity of synthetic varieties make them not easy to use. Additionally, genetic background of the varieties, presently registered is narrow. These reasons result in making distinctness between varieties difficult to establish. Even if isozymes bring additional help for distinguishing varieties and studying genetic diversity of ryegrass new markers are needed.

Molecular markers represent a not limited source of markers. DNA markers revealed by amplification - based on PCR methods with specific primers have been preferred to RFLPs because they are more easily carried out. Moreover, most of PCR specific markers follow simple mendelian inheritance and furthermore are codominant. Sequence-tagged site (STS) markers meet these requirements.

Very few genes of ryegrass have been described in sequence data bases. Only cDNAs encoding histones have been published in other *Lolium* species. No gene sequence in closely related species, such as *Festuca* and cocksfoot have been reported. The objective has been to search sequences in further related species of *Gramineae*, such as rice, maize, wheat, barley, oat, sugarcane and pearl millet.

As the concern is to detect intronic polymorphism which is much higher than exonic polymorphism sequences containing at least one intron have been sorted out.

The present work is to test the possibility to define consensus zones on both sides of an intron from various species of *Gramineae* along with the varietal polymorphism.

Material and Method

5 test DNAs of diploid ryegrass cultivars (one individual per cultivar): Aramo and Tribune (Italian ryegrass varieties), Repell (perennial ryegrass variety) and 2 perennial ryegrass populations P1 and P2 have been studied.

Gene candidates for a specific amplification have been searched in 'Entrez'. Secondly, each gene sequence has been compared with other sequences scored in the data base sequences EMBL. At 80% of sequence homology for about 100 base pairs with sequences of one or several *Gramineae,* a consensus zone is determined. Then, these consensus sequences have been used to design specific primer pairs with 'OLIGO'.

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Total DNA was isolated from leaves, using a method based on CTAB (Rogers and Bendich, 1988). Amplification conditions used were : 4 min at 94°C (pre-denaturation); 35 cycles of 1 min at 94°C (denaturation), 1 min at 52-62 °C according to the set of primers (annealing), and 2 min at 70°C (extension); and for the last extension cycle at 70°C for 4 min. The PCR products were resolved by 5% acrylamide gel and with silver staining (Pharmacia).

Results

Among the 250 genes containing at least one intron sorted out, 45 have been chosen with respect to the greatest number of introns. In addition, it is checked that this zone is located on both sides of one intron.

Matching of the candidate genes with other sequences in EMBL results in fourteen genes for which a specific PCR amplification can be expected.

The table 1 shows the fourteen genes selected from which primer pairs of about 20 nucleotides have been defined. Nine of them allow to detect size polymorphism. Two (OSR1/2 and RIC1/2) have not yet been tested with respect to polymorphism. Two to four different profiles can be observed for each polymorphic genes on the five test DNAs.

Size of the bands differs from the expected size for some locus. As shown for the gene OSRB1/2, the size of the intron varies in a maximum range of 60 base pairs, in the matching of six different sequences from barley and wheat.

In most cases, each test DNA displays one-banded or two-banded profiles, which is consistent with the assumed genetic control of co-dominant genes, for homozygous and heterozygous diploid individuals. Some primer pairs display more than two bands reflecting probably that other genes are expressed. It will be, therefore, necessary to elucidate genetic control by offspring analyses.

Conclusion

Later on, all the primer pairs already defined and displaying polymorphism will be tested on more cultivars and on more individuals per cultivar for cultivar characterization.

These first results are satisfactory and the use of consensus sequences in *Gramineae* species is a promising way to obtain new markers in ryegrass.

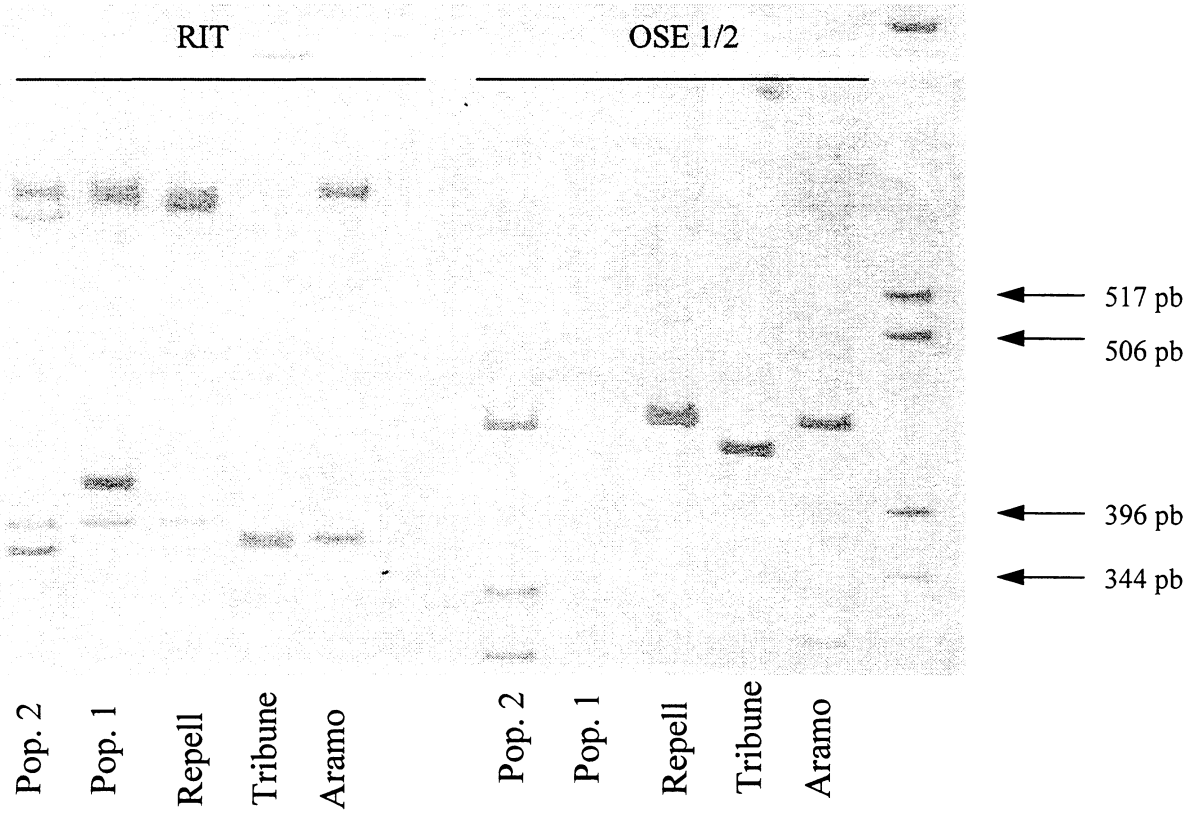
The objective of this survey is to get 20-30 polymorphic STS markers for establishing distinctness of ryegrass varieties. For breeding purposes, it would be *a priori* interesting to find out agronomically important genes in sequence data bases.

PRIMERS	LOCUS	GENE ORIGIN	EXPECTED PRODUCT SIZE	SIZE POLYMORPHISM❶
ADH 3/4	Alcohol Deshydrogenase	ADH of maize, barley and pearl millet	401 bp.	+ 3 or 4 profiles
ZMS 1/2	Sucrose Synthase	Maize gene.	431 bp.	-
MZE 1/2	Triosephosphate Isomerase	Maize gene.	1202 bp.	+ 4 profiles
ZEA 1/2	Actin 1	Maize gene.	558 bp.	-
OSW 2/3	ADP(UDP)-Glucose Glycosyl Transferase	Rice gene.	414 bp.	+ 4 profiles
LP1 c/d	The major allergenic protein, sequence of LOLP I	Rye-grass gene .	526 bp.	+ 3 or 4 profiles
OSO 1/2	Orysacystatin II	Rice gene.	595 bp.	❷
RIT 1/2	Endochitinase	Rice gene.	430 bp.	350-400 bp: 3 profiles 650-1000 bp : 4 profiles
OSE 1/2	Em protein I = Lea (late embryogenesis abondant)	Rice gene.	311 bp.	280-325 bp: 4 profiles ~400-450 bp: 2 profiles
SCF ½ --- **OSR 1/2**	Ribulose-1,5 biphosphate carboxylase/oxygenase small subunit gene.	Sugarcane gene. --- Rice gene .	402 bp. --- 217 bp.	3 or 4 profiles . --- ❷ not tested
RIB ½	α Amylase 1	Rice gene.	143 bp.	❷
OSRB ½	α Amylase 3	Rice gene.	759 bp.	2 profiles
RIC 1/2	ADP Glucose phosphorylase	Rice gene.	988 bp.	❷ not tested
HVM 1/2	Promoter MYB 3	Barley gene.	306 bp.	-

❶ + = Polymorphic. - = Monomorphic. ❷ Under way.

Table 1. Different STS Markers on Ryegrass

2 STS MARKERS ON RYE GRASS



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