SSR- and RAPD Analysis of a New Agropyron Repens Genotype

G Gyulai
Agricultural University, Hungary, Purdue University

H Ohm
Purdue University

I Dweikat
Purdue University

E Kiss
Agricultural University, Hungary

H Sharma
Purdue University

See next page for additional authors

Presenter Information
G Gyulai, H Ohm, I Dweikat, E Kiss, H Sharma, J Janovszky, and L Heszky

SSR- AND RAPD ANALYSIS OF A NEW AGROPYRON REPENS GENOTYPE

G. Gyulai[1,2], H. Ohm[2], I. Dweikat[2], E. Kiss[1], H. Sharma[2] and J. Janovszky[3], L. Heszky[1]
[1]Department of Genetics and Plant Breeding, Agricultural University, Godollo, Hungary, H-2103
[2]Department of Agronomy, Purdue University, West-Lafayette, USA, IN 47906,
[3]Irrigation Research Centre, Szarvas, Hungary, H-3356

ABSTRACT
SSR-(single sequence repeat), and RAPD (randomly amplified polymorphic DNA) primer generated DNA fingerprints were used to distinguish a new genotype of quackgrass from its original type of *Agropyron repenes* L. Beauv. (= *Elytrigia repens*). Products of polymerase chain reactions (PCR) were separated by agarose (AGE) and denaturing gradient gel electrophoresis (DGGE). Although, both kinds of primers were found to be polymorphic, the microsatellite primer with sequence of 5í-AC(GACA)4-3í generated distinguishing fingerprints in the two types of quackgrasses. This result gives genetic evidence for the new genotype of quackgrass.

KEYWORDS
DNS fingerprint, microsatellites

Acronyms: AGE - agarose gel electrophoresis; AP-PCR - arbitrarily primed polymerase chain reaction; DAF - DNA amplification fingerprinting; DGGE - denaturing gradient gel electrophoresis; PCR - Polymerase Chain Reaction; RAPD - Randomly Amplified Polymorphic DNA; SSR - Simple Sequence Repeat of DNA; SPAR - single primer amplification reaction.

INTRODUCTION

Numerous kinds of new procedures have been evaluated on the basis of the Nobel-prize-winning method of PCR, polymerase chain reaction, (Saiki et al., 1985; Mullis and Faloona, 1987). Among these, the RAPD technique was developed for a generation of DNA fingerprints by the use of random sequenced primers (Williams et al. 1990). RAPD primers (10 mer) recognise both kinds of coding and non-coding sequences in the genome complementary to the primers. Due to the fact that a dominant part of the eucaryotic genome consists of non-coding tandem repetitive DNAs (rep-DNA), SSR-primers complementary to the core sequences of rep-DNAs (16 mer) began to be used (Gupta et el., 1994). In the case of dinucleotide core sequence of rep-DNA, a term VNDR (variable number of dinucleotide repeats) is used. In the case of longer core sequences, the term VNTR (variable number of tandem repeats) is used (Nakamura et al. 1987). In addition, synonyms of microsats, STR (short tandem repeats), and SSR (simple sequence repeats) are also used. All of these sequences are able to characterise individual differences among the organisms. The investigated new genotype of quackgrass has previously been characterised cytologically and morphologically (Mázik-T. et al., 1996). In the present study both RAPD and microsatellite (SSR) primers were used to characterise and distinguish this new genotype of quackgrass from its original genotype of *Agropyron repenes* L. Beauv. (= *Elytrigia repens*).

MATERIALS AND METHODS

Plant materials, DNA isolation: The new genotype of quackgrass was selected and raised in a breeding program of *Agropyrons* by J. Janovszky. For investigation, seeds of the new and the standard genotypes of quackgrass, A. *repens* L. Beauv. (= *Elytrigia repens*), were germinated in a greenhouse. Leaf tissues were harvested for genomic DNA isolation at the 5 leaf stage. One gram of leaves was squeezed in a Leaf Squeezer (Ravenel Specialities, Inc., Seneca, USA) with DNA extraction buffer (Dweikat et al. 1984). PCR reaction, gel electrophoresis: RAPD and SSR oligonucleotide primers were purchased from Operon Technologies (Alameda, Calif, USA). PCR reaction mixtures (50 ml total vol.) consisted of 10 mM of Tris-HCl (pH 9.0, at 25°C), 50 mM of KCl, 1.8 mM MgCl2, nucleotides of dATP, dTTP, dCTP, and dGTP (200 mM each), 0.2 mM of primer, 100 ng of template DNA, and 2.0 units of *Taq* DNA polymerase (Promega). Amplifications were carried out in an MJ Research PTC-100 thermocycler programmed for 35 cycles. Each cycle consisted of the following steps: (a) 1 min at 95°C, for DNA denaturing, (b) 1 min for annealing at Tm (melting temperatures) of the primers calculated by adding 2°C for A and T, and 4°C for G and C in the primer sequence (Berger and Kimmel, 1987; Miyada and Wallace, 1987), and (c) elongation at 71°C for 1 min. PCR products were separated by denaturing gradient gel electrophoresis (DGGE) with a denaturing gradient range of 10-50 % (Dweikat et al. 1994) followed by etidiumbromide treatment. In the case of AGE, the agarose concentration was 0.8 %. Sequences of the RAPD primers were as follows: (1) 212: GCTGCGTGAC; (2) M16: GTAACCAGCC; (3) 608:GAGCCCGAAA; (4) 691: AAACCAGGGG; (5) 195: CTAGAGTCGC; (6) 428: GGCTGCGGTA;. The SSR primers: (1) 77H: 5'-(AGAC)4GC; (2) 78H: 5'-AC(GACA)4 (3) 7021: -under test; (4) 104H: 5'-(GACA)4GT; (5) 105H: 5'-(GAGA)4CT; (6) 155H: 5'-(CA)7GA (Fig. 1). All experiments were conducted in three repetitions.

RESULTS AND DISCUSSION

PCR based techniques have recently become the most important method for generation comparative DNA fingerprints of procarytoic and eucaryotic organisms. Among these, three kinds of techniques based on single primer amplification reactions (SPARs) have simultaneously developed: (1) RAPD, randomly amplified polymorphic DNA, (Williams et al., 1990); (2) DAF, DNA amplification fingerprinting, (Caetano-A. et al., 1991) and (3) AP-PCR, arbitrarily primed polymerase chain reaction, (Welsh and McClelland 1990; Owen and Uyeda, 1991). The RAPD technique has been successfully applied in the genome analysis of cereals, *Populus*, *Malus*, strawberry, sunflower, *Oryza*, *Brassica*, *Avena*, alfalfa, and forage grasses (in Mösges and Friedt, 1994; Mázik-T. et al., 1996). On the basis of RAPD, Gupta et al. (1994) has developed SSR-PCR in which olygonucleotide primers (16 mer) are site specifically synthesized to the core sequence of genomic SSR.

In our preliminary experiments with SSR and RAPD primers, DGGE separation showed a more selective band pattern than the AGE, similar to the results of Dweikat et al. (1984). When the same primer-generated PCR product was separated on AGE or DGGE, the following total band numbers were observed: Primer 1 (SSR-77H) in *A. repens* on AGE: 4 bands, on DGGE:14 bands; in the new quackGrass on AGE: 5 bands, on DGGE: 10 bands. With primer 2 (SSR-8H) in *A. repens* on AGE: 3 bands, on DGGE: 17 bands; in the new quackgrass on AGE: 5 bands, on DGGE:23 bands.

In the main experiment using DGGE separation, all six RAPD and all six SSR primers generated DNA polymorphism in both quackgrasses (Fig. 1). Nevertheless, the primers of SSR-3 (721), RAPD-2 (M16) generated only a few total bands (Fig. 1). SSR primer-2 (78H) was found to be discriminative, resulting in different fingerprints in the two quackgrasses (Fig. 2). From these, certain

genetic evidence for the new quackgrass investigated was proved. To add phenological markers to the genetic markers obtained, the new form of quackgrass was found to possess a surprisingly uniform height (30 cm), more intensively waxy coloured leaves, and a seed sterility of 93 % (Mázik-T. et al 1996).

To conclude, SSR-, and RAPD primer-based evidence of a new genotype of quackgrass was proved. The advantages of DGGE separation over AGE, and the use of SSR primers over RAPD primers were also shown.

ACKNOWLEDGEMENTS

This work was supported in part by the Hungarian Grant OMFB No. 94-97-44-0657, by the OECD-95/Gyulai, and by the AKP grant of the Hungarian Academy of Sciences. The Authors wish to thank Mark Davies for his comments on the manuscript.

REFERENCES

Berger S.L. and A.R. Kimmel. (1987) Guide to molecular cloning techniques. Methods Enzymol **152**: 435-437**.**

Caetano-Anolles D., B.J. Bassam and P.M. Gresshoff. (1991**)** DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology **9:** 553-557**.**

Dweikat, I., H. Ohm, S. Mackenzie, F. Patterson, S. Cambron and R. Ratcliffe. (1984) Association of a DNA marker with Hessian fly resistance gene H9 in wheat. Theor Appl Genet**. 89:** 964-968**.**

Gupta M., Y.S. Chyi, J. Romero-Severson and J.L. Owen. (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Tehor Appl Genet. **89:** 998-106**.**

Mázik T.K., G. Gyulai, L.E. Heszky and T. Lelley. (1996) Meiotic and RAPD analysis of a new genotype of *A. repens*. Cereal Res Comm (in press).

Miyada, C.G. and R.B. Wallace. (1987) Oligonucleotide hybridization techniques. Methods Enzymol. **154:** 94-107.

Mösges, G. and W. Friedt. 1994. Genetic "Fingerprinting" of Sunflower Lines and F1 Hybrids using Isozymes, Simple and Repetitive Sequences as Hybridization Probes, and Random Primers for PCR. Plant Breeding **113:** 114-124.

Mullis, K.B. and F.A. Faloona. (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155**: 335-350.

Nakamura Y. et al. (1987) Variable numbers of tandem repeat (VNTR) markers for human gene mapping. Science **235**: 1616-1622**.**

Owen J.L. and C.M. Uyeda. (1991) Single primer amplification of avian genomic DNA detects polymorphic loci. Animal Biotech. **2**: 17-122.

Saiki, R., S. Scharf, F.A. Faloona, K.B. Mullis, G.T. Horn, H.A. Ehrlich and M. Arnheim. (1985) Enzymatic amplification of b-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230**: 135-1354.

Welsh J. and M. McClelland. (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18**: 7213-7218.

Williams J.G.K., A.R. Kubelik K.J. Rafalski and S.V. Tingey. (1990) DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res. **18**: 6531-6535.

Figure 1

Comparison of SSR- and RAPD primer generated band numbers (0 - 20) of DNA fingerprints of *Agropyron repens* L (*A. r.*) and the new genotype of *A. repens* (*A. cv.*) separated by denaturing gradient gel electrophoresis (DGGE). Sequences of RAPD primers are as follows: RAPD-1 (212): GCTGCGTGAC; RAPD-2 (M16): GTAACCAGCC; RAPD-3 (608): GAGCCCGAAA; RAPD-4 (691): AAACCAGGGG; RAPD-5 (195): CTAGAGTCGC; RAPD-6 (428): GGCTGCGGTA;. The SSR primers: SSR-1 (77H): 5'-(AGAC)4GC; SSR-2 (78H): 5'-AC(GACA)4; SSR-3 (7021): -under test; SSR-4 (104H): 5'-(GACA)4GT; SSR-5 (105H): 5'-(GAGA)4CT; SSR-6 (155H): 5'-(CA)7GA

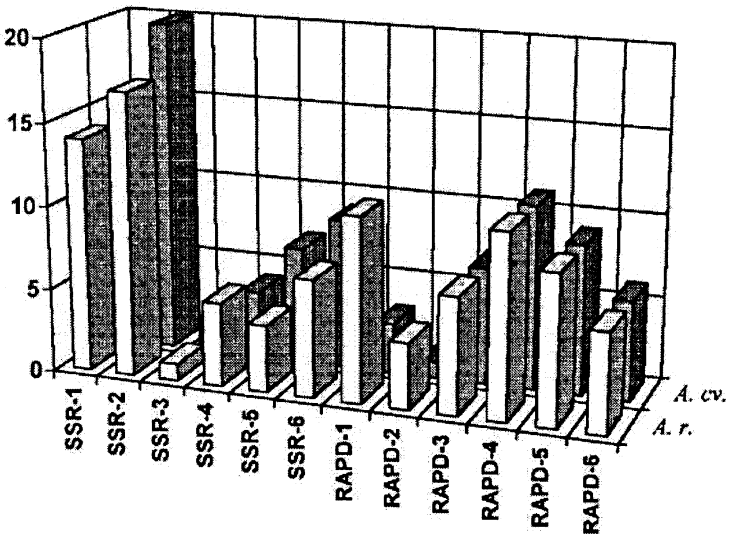

Figure 2

DNA fingerprints of *A. repens* L. Beauv. and the new genotype of *A. repens* generated by SSR primers of No. (1) 77H: 5í-(AGAC)4GC, and (2) - 78H: 5íAC-(GACA)4. *Lane 1:* l-DNA, digested by Pst I., *Lane 2:* DNA fingerprints of the new genotype of quackgrass generated by SSR primer No.1, *Lane 3:* fingerprints of the standard genotype of quackgrass generated by SSR primer No.1, *Lane 4:* fingerprints of the new genotype of quackgrass generated by SSR primer No.2, *Lane 5:* fingerprints of the standard genotype of quackgrass generated by SSR primer No.2.