The Genetic Instability of the Adh1 Locus Alleles in Sugar Beet Agamosperous Progeny

N.A. VINICHENKO, S.S. KIRIKOVICH and E.V. LEVITES

Institute of Cytology and Genetics of Siberian Department of Russian Academy of Sciences, Lavrentieva Av., 10, 630090, Novosibirsk, Russia

ABSTRACT

A method based on the specific and the microsatellite primers combination allowing reveal interallelic differences of enzyme marker genes was approbated. With the help of this method the instability in agamosperous progenies of the alleles of the locus Adh1, controlling sugar beet alcohol dehydrogenase was found.

Key words: Microsatellite, primer, alcohol dehydrogenase locus, isozymes, agamospermy, sugar beet

INTRODUCTION

It was shown that in some agamosperous progenies produced from heterozygous sugar beet plants an unusual segregation of marker enzymes was exposed (Levites et al., 1998). There were only two genotypic classes in these progenies: one heterozygous class and a homozygous one (Levites, 2002; Levites and Kirikovich, 2003).

The Adh1 locus, controlling the best investigated sugar beet marker enzyme alcohol dehydrogenase (ADH1) is most convenient for revealing molecular mechanisms of this phenomenon. The first stage of this work was studying molecular differences in the structural part of the Adh1 alleles. We compared nucleotide sequences of exon 4 and part of exon 5 of alleles F and S in the Adh1 locus. The Adh1-F and Adh1-S sequences of the examined fragment were shown to differ in two nucleotides (Vinichenko et al., 2004). These differences correlate with electrophoretic mobility differences of allelic ADH1 isozymes. The next stage was to develop the method allowing distinguish F- and S-alleles of the Adh1 gene without sequencing. For this purpose, we planned to use an ISSR-amplification technique (inter-simple sequence repeat). This technology is based on the amplification of regions (100-3000bp) between inversely oriented closely spaced microsatellites (Zietkiewicz et al., 1994). Single primers (16-18 bp) consisting of several simple sequence repeats (SSR), named as microsatellites, used for an amplification of this regions can be based on any SSR motif and be 5' or 3' anchored by 2-4 (usually) arbitrary selective nucleotides. However, unanchored primers have also been used. This method does not require DNA pre-sequencing and is used successfully for studying of DNA polymorphism in eukaryotic genomes. Our preliminary experiments in sugar beet have shown that PCR-profiles received with single microsatellite primers do not give enough information. The aim of this work is to modify an ISSR-amplification technique and to use a new method for revealing differences in DNA regions that contain sugar beet alleles of the Adh1 locus.

MATERIALS AND METHODS

Plant material

Sugar beet plants grown from seeds obtained by agamospermy were used in these experiments. Agamosperous seed progenies were obtained from individual plants with two different methods. Thus, agamosperous progeny E122c-9a was obtained by growing blooming pollen-sterile plant E122c-9 on an isolated plot in pollen-less regime. Agamosperous progeny 2-6a was obtained by growing of pollen-sterile plant 2-6 in stronger pollen-less regime: on an isolated plot and with covering each individual plant with a
coarse insulator. Both parental plants were heterozygous with respect to the Adhl gene. Preliminary analysis of isozyme patterns revealed all three phenotypic classes of ADH1 FF, FS and SS in experimental agamosperous progenies. Analysis of isozymes phenotypes was performed after preliminary induction of ADH1 by immersion of leaves in water at dark and room temperature during 24 hours.

Extraction of ADH1 after induction was performed by grinding leaf-stock in mortar at 40°C in 0.2V of extraction mixture consisting of 0.1M Tris-HCl (pH 8.3), 0.3% EDTA, 3.6% sucrose and 0.3% dithiotreitol. Electrophoresis in starch gel and gel staining were performed according to the methods reported earlier (Schwartz, 1966; Levites, 1986).

DNA extraction, amplification and electrophoresis conditions.

Genomic DNA of B. vulgaris was extracted from young leaf tissue by standard STAB-method (Doyle and Doyle, 1987).

Two ISSR primers, Mic1 (GATA)4 and Mic2 (GACA)4 were synthesized. Since single microsatellite primers did not give enough information, we decided to use the Mic2 microsatellite primer together with oligonucleotide specific to the Adh I gene: either with Adh 1 r (5'- act(ct)a-cagca-ag(ct)cc-(ct)ac(ct)g-ctcc-3') or with Adh 1 f (5'-agagt-gttgg-agagg-gtgtg-ac-3').

Amplification reactions were performed in volumes of 20 µl containing 10-200 ng genomic DNA, 65 mM Tris-HCl (pH 8.0), 16 mM (NH4)2SO4, 0.05% tween-20, 1.5 mM MgCl2, 0.2 mM of each dNTP, 1µM of each primer, and 2.5 unit of Taq DNA polymerase. The amplification procedure consisted of the first step at 94°C (4 min) followed with 30 cycles at 94°C (1 min)/42°C for Mic1, 52°C for Mic2, and for pairs Mic2-Adhr, Mic2-AdhF (1 min)/72°C (4 min), and 1 cycle at 72°C (7 min).

PCR-products were electrophoresed in 5% non-denaturing polyacrylamide gels and stained with ethidium bromide.

RESULTS AND DISCUSSION

Electrophoretic separation of ADH1 isozymes

Fig. 1 displays isozyme patterns of ADH1 for twenty investigated agamosperous descendants of plant E122k-9. ADH is a dimeric enzyme on its quaternary structure. Therefore, heterozygous plants have three-banded isozyme pattern containing two homodimeric and one heterodimeric isozyme, whereas homozygous ones have only one homodimeric isozyme, with fast (FF) or slow (SS) electrophoretic mobility.

Patterns 2, 11, 12, 13 and 17 correspond to FF-phenotypes; patterns 4, 6, 8 and 19 belong to plants of SS-phenotype, and the rest of patterns are typical of heterozygous phenotype FS. It is of interest that heterozygous patterns differ in band intensity obviously due to different relative activity of the Adhl alleles.

Molecular markers for F- and S-alleles of the Adhl gene

To make molecular markers, we supposed to use an ISSR-amplification with individual microsatellite primers Mic1 and Mic 2. These primers were selected because of their significant occurrence and relatively homogeneous distribution in sugar beet genome (Schmidt and Heslop-Harrison, 1996). But single microsatellite primers did not give enough information, so that we decided to use combinations of two primers: the oligonucleotide specific to the AdhI gene together with the microsatellite primer. We used two specific primers: Adhlf with binding site in exon 4 of the AdhI gene, and Adhlr with binding site in exon 5 (Fig. 2).

Fig. 2. The scheme of Adhf- and Adhlf-specific primer binding sites positioning in the AdhI gene.

The primer pair Adhlf (5'- act(ct)a-cagca-ag(ct)cc-(ct)ac(ct)g-ctcc-3') and Mic2 (5'-gacag-acaga-cagac-a-3'), permitting to analyze 5' -region of gene, was the most successful. Analyzing PCR products obtained with this primer pair, we can distinguish FF and SS homozygotes from one another and from FS heterozygotes. Furthermore, differences in PCR-profiles of plants with identical ADH1-phenotype were found. For instance, four different PCR-profile types for plants with FF-phenotype and three PCR-profile types for plants with SS-phenotype were revealed (Fig. 3, 4). These differences