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SHORT COMMUNICATION

SEQUENCING OF FLAX LIS-1 INSERTION SITE IN THE ALBIDUM GENOTYPE

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ABSTRACT

The paper presents a methodology of identifying the insertion site of LIS-1-1 (*Linum Insertion Sequence* 1) element in flax Albidum variety when growing under the *in vitro* combined with environmental stress conditions. Abiotic stress was induced by a reduced nutrient content in a growth medium. The LIS-1 insertion site amplification was reaLIS-1ed using the forward LIS-L: 5'-GGG CAG TTT AAC TGT AAC GAA - 3 'and revers LIS-R: 5'-GCT TGG ATT TAG ACT TGG CAA C - 3' primers by PCR. PCR product was sequenced by direct sequencing method to proove the nucleotide sequence for matching with database LIS-1 sequence. A comparison has been matched with the sequence of the amplified segment in the database for all nucleotides except the 11-position in the 5'-3 ' direction, where instead of the three adenine pair is a couple in the Albidum variety. Changes caused by mobile elements or insertion sequences result in common flax in variability that can be used for the purposes of development of effective marker identification or environment based markers development.

Keywords: common flax, LIS-1 insertion site, sequence, PCR identification

INTRODUCTION

Transponable elements are important components of eukaryotic cell genomes, served for recombination of specific areas and cell function changes, like protein translation control and gene transcription. Transponable elements have a great potential in pre-transcriptional gene regulation, like moving of transcription signals and making of new reading frames **(Thornburg, et al., 2006)**.

This phenomena was firstly described in the study of **Barbara McClintock (1984)** in maize. There was characterized an existence of autonomous and non-autonomous elements, moving of this elements within and between chromosomes, ability of gene expression change, and chromosomal rearrangement. Transponable elements can be within genome in silent or active state, and this state is connected with conditions of biotic or abiotic genomic stress **(Wessler, 2001)**.

Flax (*Linum usitatissimum L.)* served like model system for genetic factors research, which can effect on genome stability. These changes can be named like phenotypical, biochemical and molecular-biological alternations which are stable in progeny. Stable lines are called genotrophs. Characterization of single genotrophs **(Cullis, 1977)** is corelated with hight, weight, number of leafs, level of plant hormones, amount of nucleus DNA, number of RNA genes and repetitive sequences.

Specific polymorphism of flax is connected with LIS-1 (*Linum insertions sequence* 1). LIS-1 insertion is prooved in great amount of flax genotrophs, is inserted in specific area in genome. Activity of this element is identifield in conditions of environmental stress, an is inherited in next generations. LIS-1 is a single-copy 5,7 kb high specific fragment. Presence of LIS-1 is identical in small and large genotrophs. Structure of LIS-1 well-known, origin of this sequence is stil unknown **(Chen, et al, 2005)**.

The objective of the study was to identify a LIS-1-1 insertion sequence in common flax variety Albidum and subsequently the development of protocols for LIS-1 insertion site confirmation by the melting point analysis and the protocol for direct sequencing of the LIS-1 insertion site. LIS-1 sequence was identified in Albidum samples cultivated under the *in vitro* conditions when growing in stress presented by decreased amount of nutrientsin the growth medium.

MATERIAL AND METHODS

Plant samples of common flax variety Albidum were obtained from Gene bank in Piešťany. Plants were cultivated in *in vitro* conditions on MS medium **(Murashige, Skoog, 1962)** where the abiotic stress was simulated by using the half amount of micro and macroelements. After the two weeks of cultivation, plant material was homogenised with liquid nitrogen and DNA isolation was performed by Invisorb® Spin Plant Mini Kit, Invitek following the manufacturer´s protocol. Quantity end quality of extracted DNA was setted by Quibit ^{TM} Fluorometer. Primers used for amplification of LIS-1 insertion sequence site -stored in NCBI (National Center for Biotechnology Information) database with the accession of AJ131991- were designed using the Primer3 software. The amplified region determined by the used primers is located from 342 bp up to the 820 bp. of the LIS-1 element. Identification of LIS-1 was performed by real-time PCR and the confirmation of succesful amplification was done by melting point analysis.

Polymerase chain reaction (PCR) was performed in BIO-RAD C1000^{TM} Thermal cycler using the BIOLINE BIOTAQ^{TM} DNA Polymerase. Reaction conditions were set on 25 µl reaction volume. All the reaction were performed using 30 ng/µl DNA template, 1,5mM $MgCl_2$ and 0,3 mM dNTP, 400 nM of forward and reverse primers, and 1U of the Taq polymerase. The PCR was set up as follows: 95°C/3 min, folowed by 33 cycles (95°C/15 sec; 54°C/40sec; 72°C/120sec), and final extension 72°C/7 min. Control visualisation of amplified products were realised in the 2% agarose gel electrophoresis. Real - time PCR was performed in CFX96 Real-time system using 5 ng/ µl of template DNA in the PCR volume and PCR master mix with SYBR green. Concentration of both of the primers was 800nM. Real-time PCR was performed setted as follows: 95°C/3 min, followed by 45 cycles: 95°C/15 sec; 52°C/40 sec; 72°C/30 sec with final extension 72°C/10 min. Melt assay have ran with increasing of temperature (0,2 °C/ 1 sec.) and analyzed with CFX^{TM} Manager software. The following sequences of primers were used in PCR and sequencing reactions - LIS-L 5´- GGG TTT CAG AAC TGT AAC GAA - 3´ and LIS-R 5´- GCT TGG ATT TAG ACT TGG CAA C - 3´. Insertion sequence of Albidum was sequenced by direct sequencing method by GeXP Genetic Analysis System. The results of sequencing were used for comparing of Albidum and Pl line genotype in BLAST (Basic Local Alignment Search Tool) method under the NCBI database software to find out a similarity.

RESULTS

LIS-1 element was confirmed in all Albidum samples cultivated in *in vitro* conditions under the nutrition stress both, by end point PCR and real-time melt analysis, too. Before setting up a melt point analysis, the LIS-1 insertion sequence from NCBI database was ispected with CLC Workbench software for the amonut of purin and pyrimidine nucleotides presence in chain and its influence on melt temperature (figure 1).

Only a very short segments of GC rich regions were described and no effect on the melting peak was expected. This expectation was confirmed, as the figure 3 shows.

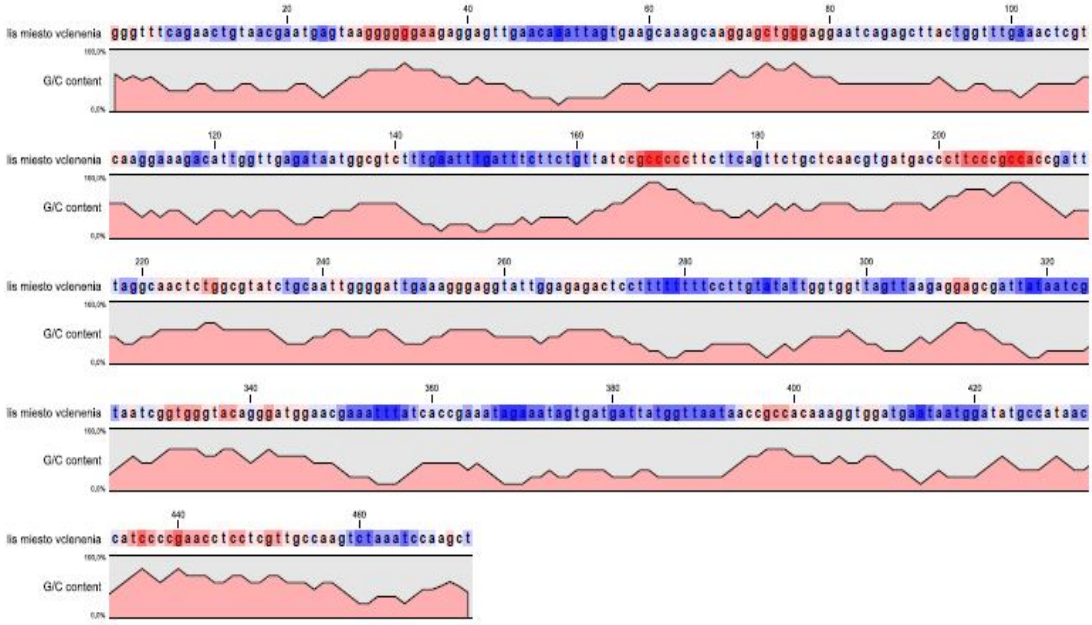

Figure 1 Influence of nucleotide presence on melt temperature of LIS-1 insertion sequence of common flax

Real-time PCR amplification of all Albidum samples gives sucessfull fuorescence increasing during the amplification of LIS-1. Increasing level of fluorescence was detected in 30th threshold cycle (C_t) and melting temperature during the melt assay was detected in 74 °C as is showed in figures 2,3.

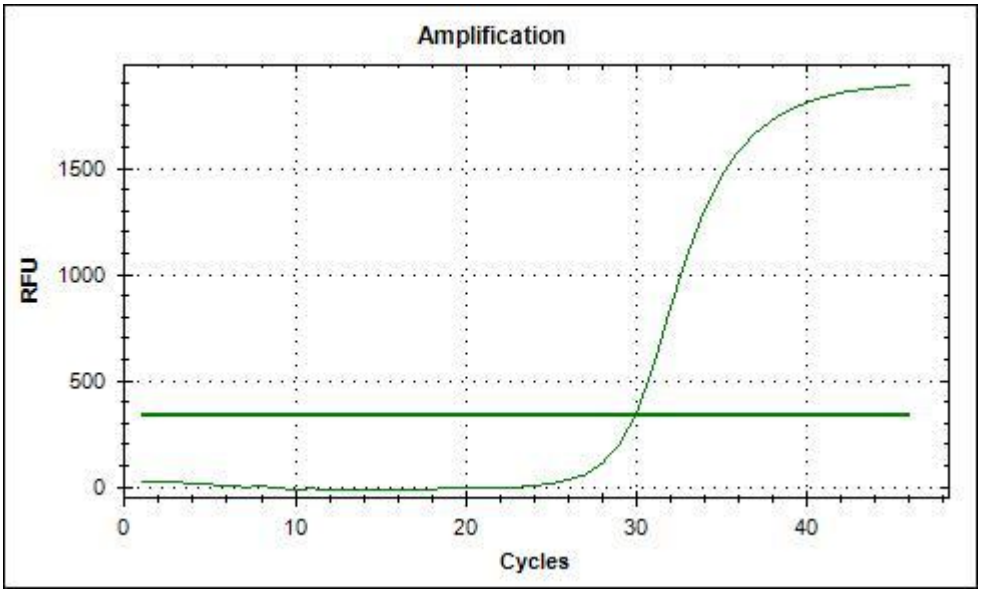

Figure 2 Albidum real-time PCR amplification

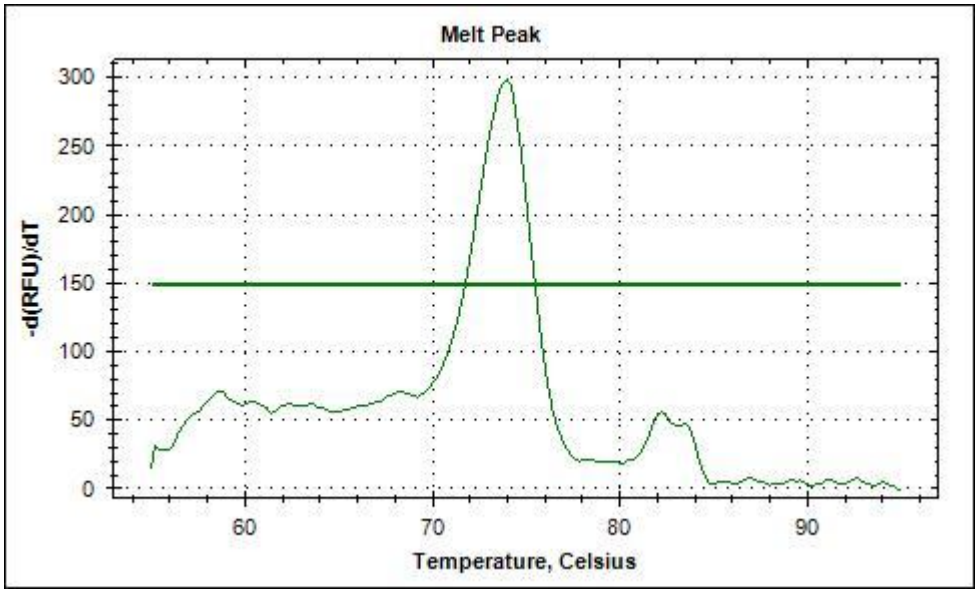

Figure 3 Albidum real-time PCR melt peak

The PCR amplified LIS-1 element of Albidum variety was sequenced by direct sequencing method by GeXP Genetic Analysis System. The nucleotide similarity of insertion sequences between Albidum obtained in this study and Pl AJ131991 from NCBI database was evaluated with a result of the 99% matching (Figure 4).

Figure 2 BLAST analysis of amplified sequence

All of the sequence is the same when comparing the database and our sequenced data. The only exception is the missing adenine in the position of the 11-th nucleotide and the non-identified nucleotide in the position of 263-th nucleotide of Albidum sequence. Such very high similarity shows the reliable identification of the LIS-1 insertion site.

DISCUSSION

Nowadays, different PCR and sequencing based method are used for the detection of specific parts of plants, animals or foodstuffs **(Balážová at al., 2007; Vivodík et al., 2011; Pochop et al., 2012)**. Transposable elements comprise much or most of plant genomes and their replication generates genomic diversity and makes them an excellent source of molecular markers **(Schulman et al. 2004)** and are very comfortable for PCR detection (Lopes et al., 2008). LIS-1 sequence in flax genome is the result of a targeted, highly specific, complex insertion event that occurs during the formation of some of the genotrophs, and occurs naturally in many flax and linseed varieties **(Chen et al., 2005)**.

Since the methods of gene expresion in flax has been described using of real-time PCR for identification of metabolic genes in flax **(Huis et al., 2010)**, the comparative studies of real-time PCR using in common flax DNA regions can be performed. The presence of LIS-1

was analyzed in other variants of flax, too. Research of LIS-1 was performed in Stormont Cirrus by **Chen et al. (2009)**. The authors described environmental based changes occuring in common flax plants, that were exposed of low nutrition conditions during the growth. Increased amount of whole DNA in nucelus, increased number of ribosomal genes and LIS-1 element was detected in Stormont Cirrus.

These two sequences were different in only two nucleotides, where one of this nucleotides can not be assigned unambiguously during the sequenation of Albidum sample, what can be a result of the changes caused by stress conditions, what was reported for the common flax previously **(Chen et al., 2005, 2009)**.

CONCLUSION

Since the common flax has been described as a model organism for genome unstability research, is widely analysed for its reaction to the stress conditions caused by low nutrient content or caused by transposable lements. The aim of this work was identification and sequencing of LIS-1 insertion sequence in Albidum flax variant. The comparison of sequences of Albidum and Pl line (AJ131991) gives result of similarity in 99%. Because the flax genome is documented as a sensitive one for stress reaction, changes caused by mobile elements or insertion sequences result in variability that can be used in development of effective marker identification or environment based markers development.

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