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Changes of flax dLUTE marker profile in nutrition deprivation

Jana ŽIAROVSKÁ[1*], Adam KOVÁČIK[1], Veronika ŠTEFÚNOVÁ[1]

[1] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, e-mail: jana.ziarovska@uniag.sk; adam.kovacik@uniag.sk; veronika.stefunova@uniag.sk

* Corresponding author: jana.ziarovska@uniag.sk

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Abstract
DNA marker analyses are still relevant and indispensable in the current era of targeted applications of plant genetic technologies. The practical application of DNA marker techniques is mainly in the field of processing and development of genetic resources of individual plant species and in the field of application of markers *per se*. In this study, the polymorphism of the insertion element dLUTE of flax was analyzed under conditions of nutritional deprivation in *in vitro* conditions that was compared to fingerprint profiles from field conditions of growth. dLUTE transposon is represented in numerous copies in the flax genome and is active under the abiotic stress. A total of six varieties were analysed – Albidum, Svaloef, Flanders, La Plata 1938, Rembrant and Gisa. The obtained results of the analyses showed the variability of amplicon profiles in the different flax varieties also in relation to the tested abiotic stress conditions. Rembrant was evaluated as the variety with the most stable profile.

Keywords: *Linum ussitatissimum*, dLUTE transposon, polymorphism

Introduction

Domesticated throughout central Asia and the Mediterranean Sea region, flax (*Linum usitatissimum*, L.) is still utilized for its seed oil and stem fibers. Common flax is being used as a functional food to enhance these two basic uses [1,2]. Numerous methods, including RFLP [3], RAPD [4,5], ISSR [6,7], EST-SSR [8], IRAP [9], iPBS [10], BAC-end sequence analysis [2], or coding region analysis [11] have been reported to be employed in the field of molecular markers research of common flax. DNA based markers were successfully used in common flax genetic variability analysis as well as in identification of individual genotypes [3].

In phylogenic investigations, transposon-based molecular markers are effectively employed. It has been demonstrated that transposable elements affect both transcriptional regulation and changes in genomic structure that take place throughout evolution [12, 13]. Because transposons are found in many plant species, their high integration activity, conservative sequences, and huge copy numbers have all promoted their use in genetic diversity research and plant variety characterization [14,15]. Classical DNA transposons are grafted as mediator dsDNAs and reintegrated anywhere into genomic DNA [13]. Transposons of this group are referred to in the literature as 'cut-and-incorporate' and are represented by a mostly relatively simple structure composed of simple transposase-encoding ORFs flanked by Terminal Inverted Repeats (TIRs) and are typically less than 5 kb in length [16].

So far, the only known confirmed transposon of the flax genome was reported in 1991 [17], named dLUTE (defective *Linum usitatissimum* transposable element) and identified in two spontaneously mutant alleles of the flax rust resistance gene L6. The dLUTE transposon is 314 bp long, has 70% adenine and thymine base content, and contains 14 bp of terminal inverted-repeat sequences. It has no extended open reading frame, so it is probably non-autonomous. It contains 14 bp long imperfect terminal inverted repeats like the Ac group of plant transposons. Like the Ac transposon, dLUTE causes the formation of an 8bp duplication of the target site at the insertion position [17, 18].

It was the similarity of the dLUTE transposon and the Ac, Ds transposons of maize that was used to develop a transposon tagging system for localizing rust resistance genes [19]. Resistant backcrossed mutant plants were regenerated from the progeny of both mutants, and the return to gene function was accompanied by excision of dLUTE. The excision also restored the nucleotide order of the L6 gene to that found in the original genotypes. If the excision of the dLUTE was inaccurate, changes remained in the gene sequence that caused a change in the L6 protein (1-3 amino acids). No changes were observed in the phenotype of plants containing the functional L6 gene and the regenerated L6 gene after mutation [20, 21]. Numerous copies of the dLUTE-like transposon sequences are also represented in the flax genome [17].

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The aim of the study was to analyse the changes of dLUTE based fingerprint profiles od selected common flax varieties under the abiotic stress conditions of half dose of macroelements and null dose of microelements in growth medium when compare dto the field conditions of growth.

Material and Method

The length polymorphism among dLUTE nucleotide sequences of common flax was evaluated in a total of six genotypes (Table 1.) obtained from the gene bank in Piešt'any and from Agritec, research, breeding and services, s.r.o. Šumperk.

Table 1. Characteristics of common flax varieties used in the study

Name of variety	Origin	Type	Level of breeding
Albidum[a]	IND	Oil	3.1. X12[b]
Svaloef[c]	SWE	Fibre	3.1. X12[b]
Flanders[b]	CAN	Oil	5.4. X13[b]
Rembrant[b]	NDL	Fibre	5.4. X13[b]
Gisa[c]	Egypt	Oil	5.4. X13[b]
La Plata [b]	ARG	Oil	5.4. X13[b]

Note. Informations about genotypes origin in: [a] http://links.jstor.org; [b] Gene bank Piešťany, Slovak Republic; [c] Agritec, s.r.o Šumperk, Czech Republic 3.1.X12 – old land variety; 5.4. X13 – variety

Biological material was sampled under two variations of growth conditions. The first growth variant was field conditions with controlled irrigation, and plant material was collected in a number of 10 randomly selected upper leaves during the tillering and yellow maturity periods for dLUTE incorporation polymorphism analyses and during the yellow maturity period for incorporation stability analyses by comparison to artificially induced stress conditions. The second variant was the growth of plants under aseptic culture medium conditions [22] with half the macro- and zero microelements under UV doses for 20 min at weekly intervals and the material for DNA isolation was obtained from leaves of five plants after 30 d of growth. Isolation of total genomic DNA of flax from fresh leaves of plants cultured under *in vitro* conditions was carried out according to the protocol of Rogers, Bendich et al. [23].

Polymerase chain reactions were performed in a buffer containing 20 mmol.dm-3 Tris-HCl (pH 8.0), 50 mmol.dm-3 KCl, 30 ηg DNA, 4 nmol.dm-3 primer, 1 U Taq polymerase, 3 mmol.dm-3 MgCl2, and 0.2 mmol.dm-3 dNTPs. The time and temperature profile of the reactions were as follows: [2 min 94°C; 35 cycles (1 min 94°C; 1 min 54°C; 3 min 72°C) and a final 7 min 72°C. Individual primers were also added to the reaction mixture, the pipetted amounts and characteristics of which are given in the table 2.

Table 2. Primers used in fingerprint analyses

Name	Primer sequence	Final concentration in PCR
dLUTE-F	GCCCTGTGCTGAAATCTGA	400 nmol
dLUTE -R	CAGCACAGGTTATTGGGCGG	400 nmol

PCR products were separated in 2% (w/v) agarose gels in 1 × TBE buffer. Gels were stained by GelRed™ and digitally photographed. All the accessions were growth and sampled through the two seasons to ensure the stability of the markers and all the PCR amplifications were repeated at least twice to establish reproducibility of polymorphic fragments and scored independently by KODAK EDAS software.

Results and Discussion

The aim of the analysis of the length polymorphism of the dLUTE element incorporation in the flax genome was the evaluation of PCR profiles mapping the length polymorphism of dLUTE insertion after laboratory induction of stress conditions in the form of malnutrition of reduced nutrient medium and exposure to UV radiation. Both factors have been reported in the literature as capable of triggering processes that

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activate transposable elements in plant genomes [24], with the flax genome being reported as genomically unstable as early as 1962 by Durrant [25].

Retrotransposon-based techniques were utilized here to describe the flax variety variability under the stress previously, when individual iPBS markers were used to analyze the changes of flax varieties under the ionizing radiation [26]. Transposable elements were used for the developing of marker techniques for the flax analysis, too. Previously, an in silico approach aimed at developing dLUTE primers for length polymorphism analysis, and also at generating polymorphic profiles for 24 different flax varieties [27].

The results of the dLUTE length polymorphism analyses following abiotic stress showed variability in PCR profiles for the flax growth variants under field conditions and for the samples taken on the seventh, fourteenth and twenty-first day of exposure to the stress factors (Figures 1 and 2).

Rembrant was evaluated as the variety with the most stable profile, which is an interesting fact given the documented activity of the LIS-1 element in this variety in the literature, but, since any interconnection of the individual transpositionally active elements of the flax genome is not yet known, it is not a fact that can be excluded. The stability of the profile of Rembrandt consisted of only one change, which, however, was observed in all the flax varieties analysed. Comparison of the profile obtained from field growth conditions with that obtained after the first 7 days of growth after exposure to stress conditions revealed a 475 bp fragment loss. This was again synthesised for the varieties Svaloef, Flanders, La Plata 1938 and Gisa already in samples taken after 14 days of growth under stress conditions and for all varieties analysed after 21 days of growth.

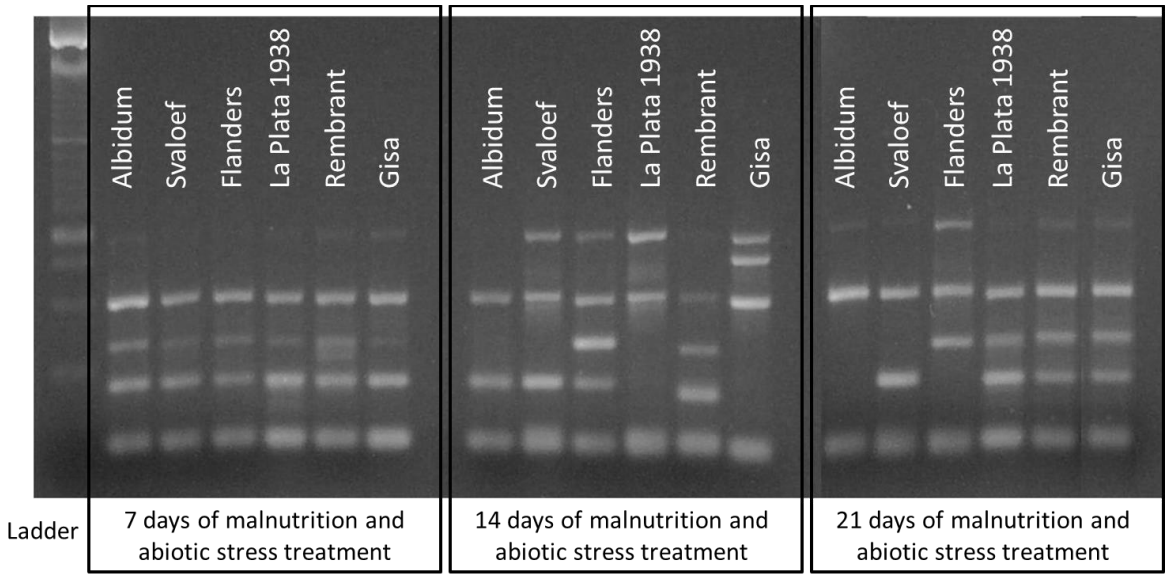

Figure 1. Electrophoregrams of the analysis of the length polymorphism of the dLUTE transposon insertion for stress conditions.

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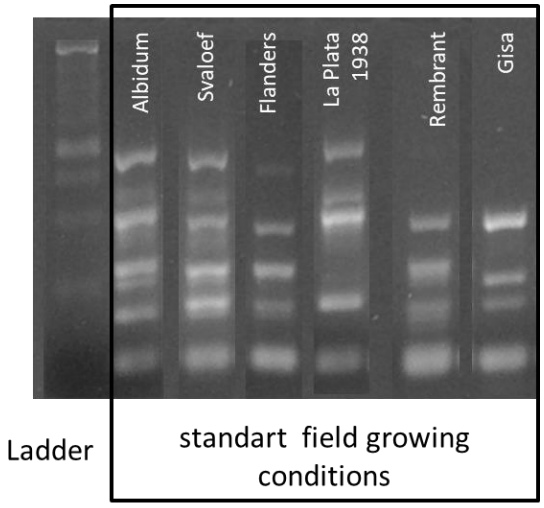

Figure 2. Electrophoregram of the analysis of the length polymorphism of the dLUTE transposon insertion for field conditions in flax

For Albidum and Svaloef, a 300 bp fragment loss was observed in the profile when sampled after 14 days, and for Albidum and Flanders, a 300 bp fragment loss was observed in the profile when sampled after 21 days, a fragment dropout of 250 bp was observed. As in the dLUTE profile under stress factors, the most variable cultivars evaluated were La Plata 1938 and Gisa, whose profiles changed most visibly. In the case of sampling after 14 days, both showed 250 bp and 300 bp fragment dropouts, but in addition, a 375 bp inclusion appeared in the profile compared to the other varieties analysed, and a 500 bp long fragment, which dropped out after 7 days but was present in the original field growth conditions, was also re-synthesised.

The previous study [26] confirmed that DNA marker systems based on transponable elements have a high discrimination ability for dLUTE. When a single marker was used to analyze the length polymorphism data, the results showed that there was 80% polymorphism among the 23 tested accessions. Discrimination of all analyzed acessions was at 72% using this one marker. Because transposable elements are prevalent in plant genomes, many polymorphic markers based on them have been documented in the literature. Transposable elements are excellent molecular markers because of the high genome variability caused by their replication and activity [28].

Genomic stress response mediated by transposable element-mediated genome restructuring and tracking of genetic adaptation to environmental change have been the subject of research across the eukaryotic domain since the work of Barbara McClintock [29, 30]. It is the active response of transposable elements that is the source of the wide genetic variation in their host genomes, and this is transferable to subsequent generations [31, 32]. Activation of transposable elements has been documented in the plant kingdom because of both abiotic and biotic stresses, e.g. suboptimal temperature, soil nitrogen deficiency, wounding of the cover crop, etc. In the case of the *Anthiridium majus*, the increased transcription levels of the Tam3 transposon were observed at temperatures below 10°C, and nuclear import of its associated transposase was also increased [33, 34]. Temperature-dependent Tam3 transposase-mediated DNA methylation has been described as the mechanism responsible for the increase in transcription [33]. Similarly, transcription of the Tnt1 retrotransposon is induced by low temperatures in tobacco and tomato [35-37]. Another of the transcriptionally active representatives of transposable elements, OARE-1 found in oat, is activated by several types of abiotic or biotic stresses, including UV irradiation, wounding, salicylic acid, and fungal pressure [38]. In the seaweed *Phaeodactylum tricornutum*, two retrotransposons, Surcouf and Blackbeard, have been found to be transcriptionally active and their transcription is activated under conditions of nitrogen deficiency or exposure to toxic concentrations of aldehyde [39, 40].

Since transposable elements (TEs) are said to be abundant in plant genomes and to have been crucial to their evolution, any TE-based methodology is highly beneficial when examining the diversity of TEs [41]. Exploring the diversity of TEs and their relationship to genes and gene expression is made possible by the availability of a genome assembly of flax (*Linum usitatissimum* L.) [42]. A major challenge in the markering of common flax remains the lack of knowledge on functional polymorphisms, because up to date, only a minimum

is known. For the further development mainly of MAS and genomic selection of flax, it is necessary to find efficient markers to identify DNA polymorphisms that determine valuable traits [43].

Conclusions

DNA marker analysis is being used today and is a very useful tool for managing plant genetic resources. Here, dLUTE-based marker approach was used to characterize the fingerprint profile changes for assessing common flax under the abiotic stress. The result proved this technique to be effective in the screening of genomic changes within the flax genome because of nutrition deprivation combined with UV radiation.

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