Molecular profiles of retrotransposon based length polymorphism in *Amaranthus cruentus* L. variety Pribina grown under cadmium treatment

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**Abstract**

During evolution, plants have developed complex mechanisms to overcome biotic and abiotic stresses. Studying the interactions between heavy metals and plants, especially at the molecular-genetic level, helps to understand the accumulation of heavy metals in plants and their resistance to heavy metal-induced stress. The impact of cadmium toxicity of genomic instability and polymorphism generated by retrotransposons on *Amaranthus* spp. is unknown. Here, five different markers of primer binding sites of retrotransposons were used to analyse the fingerprints generated by them for the changes in *Amaranthus cruentus* L. of the Pribina variety grown under the stress of cadmium in the growth medium. Polyorphic profiles were obtained for all of them and both, deletions and insertions, were obtained in the fingerprints of treated plants.

**1. Introduction**

Heavy metals occur naturally in nature as part of the environment. However, their excessive use leads to unbearable amounts entering the soil and air. With their excessive amount in the soil, they can enter the food chain through plants and thus endanger not only the plant but also the health of animals and people (Tchounwou et al. 2012). Heavy metals and metalloids have several adverse effects on plants (Sarma, 2011). The differences between plants and the reason why some plants are more sensitive to the presence of metals than others or are better accumulators of heavy metals are still not fully known. These differences are believed to be evident in the way heavy metals are deposited and transported from plant roots to aerial parts, as well as in the different antioxidant activity of plants (Singh et al., 2019). On the contrary, when comparing the molecular background of plants, it can be observed that hyperaccumulator plants have genes responsible for the accumulation of heavy metals, which are also present in plants that are not able to accumulate large amounts of heavy metals and are more susceptible to this type of stress. Nevertheless, the expression and regulation of these genes varies (Memon et al., 2001). The function of mechanisms in plants after exposure to heavy metal has been extensively studied (Kupper and Andresen, 2016). To date, several genes involved in the plant response to heavy metals have been identified (Yan et al., 2020). There are also GWAS (Genome-wide association study) analyses (Pan et al., 2020, Derakhshani et al., 2020), RNA-seq analyses (Derakhshani et al., 2020) which provide interesting results at the molecular level (Angulo-Bejerano et al., 2021). Modern research uses a variety of omics techniques. These include genomics, miRNAomics and transcriptomics to investigate the interaction between genes and heavy metals in plants (Jamla et al., 2021) as an addition to marker-based techniques such as ISSR (Sorentino et al., 2022), AFLP (Sherbeny, Morsi, Hassan, 2017) or RAPD (Random amplified polymorphic DNA) (Mengoni et al., 2003). Retrotransposon based DNA markers (Kalender et al., 2010) were reported as functional in fingerprinting the changes caused by heavy metal stress in plants. Transposable elements have a role in Al stress responses of barley and three Al-tolerant barley cultivars were characterized by the insertion of an LTR retrotransposon in the promoter of HvAACT1, (Kashino-Fujii et al., 2018).

In the case of *Amaranthus* spp. several studies focused on the specifics of growth, transportability and accumulation of metals as well as the ability to remove them (Jonnalagadda et al., 1997; Bigaliev et al., 2013; Prasad et al., 2003) have been carried out. However, the possibilities of marking changes on DNA in relation to polymorphism caused by the influence of heavy metals for the *Amaranthus* spp. are not well defined. While there is information about cadmium uptake by *Amaranthus cruentus* L., the molecular mechanisms of this process and genome stability have remained poor. The objective of our study was to analyse the fingerprints generated by iPBS based on different retrotransposon lineages for the changes in *Amaranthus cruentus* L. of the Pribina variety growing under the stress of cadmium in the growth medium.

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2. Material and methods

Plant material

The roots of young plants of *Amaranthus cruentus* variety Pribina were used. The plants grown in hydroponic regime in Hoagland media. Biological triplicates were used in the variants of control plants and plants under the treatment of Cd in the media with the concentration of 15 mg/L based on the analysis of expression of selected genes (Lancíková et al., 2020).

DNA isolation

Total genomic DNA was extracted as a bulk from biological triplicates by GeneJet Plant Genomic Purification Kit (Thermo Scientific) according the instructions of manufacturer. The quantity and quality of extracted DNA was analysed using the Nanophotometer P (Implen) and the final dilution of 25 ng/µL was prepared for individual samples.

Retrotransposon PBS based fingerprints amplification and products separation

Individual markers of copia like retrotransposons were designed according their evolutionary conserved lineages based on the comparison reported by Wicker and Keller (2007). Primers match the primer binding sites of different types of copia evolutionary lineages (table 1). PCR products were amplified by Dream Taq polymerase (Thermo Scientific) with 25 ng DNA and 500 nM of each primer. PCR thermal and time profile was as follows: 95 °C for 5 min; 40x (at 95 °C for 60 s; 55/56 °C for 45 s; 72 °C for 90 s); by final elongation at 72 °C for 10 min.

| name of primer | sequence | Copia-like lineage of retrotransposon | Ta
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<tr>
<td>PBS-V1</td>
<td>5’TGGTATCAGAGCT3’</td>
<td>Maximus</td>
<td>55°C</td>
</tr>
<tr>
<td>PBS-V2</td>
<td>5’TGGTATCAGAGCC3’</td>
<td>Ivana</td>
<td>55°C</td>
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<tr>
<td>PBS-V3</td>
<td>5’TGGTATCAGACGAC3’</td>
<td>Ale-1</td>
<td>55°C</td>
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<tr>
<td>PBS-V4</td>
<td>5’GTGGCATCCGAGGCC3’</td>
<td>Angela</td>
<td>56°C</td>
</tr>
<tr>
<td>PBS-V5</td>
<td>5’CGTTTATCAGAC3’</td>
<td>Bianca</td>
<td>55°C</td>
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Obtained amplicons were separated in 3% agarose gels stained by GelRed™ (Biotum) and analysed in GelAnalyzer software 23.1.1.

3. Results

A total of five specific primers were used that match the primer binding sites of copia like retrotransposons. Three of them (PBS-V1, PBS-V2 and PBS-V3) has only one difference in the last nucleotide with variants T/C/A. Two of other used primers has more different nucleotide sequences. When compared these two groups, primers PBS-V4 and PBS-V5 generated longer amplicons with most of them starting in around 1000 bp, whereas in the first group, in all accessions the range from 200 bp up to the 1000 bp was obtained, too (figures 1 and 2).

Primer PBS-V1 provided 11 amplions in both of the variants, control and Cd treated plants, but different length distribution was obtained thanks insertsions and three deletions in the fingerprint profile. In stressed plants, new insertion with length of 197 bp, 909 bp and 990 bp were amplified and deletion of the fragments 564 bp, 1268 bp and 1477 bp were obtained (figure 1). Compared to the two others similar PBS markers (V2 and V3), fingerprint profile generated by PBS-V1 was the most stable.

Primer PBS-V2 provided a total of 5 generated amplicons with the length range from 99 bp up to the 494 bp for control plants and 11 amplions with the length from 99 bp up to the 1772 bp for Cd treated plants. Here, distinctive profiles were amplified for control and treated plants with a total of 7 new insertions and 1 deletion in the fingerprint pattern of plant grown under the Cd stress. A large group of new amplicions with the length of 916 bp, 1021 bp, 1129 bp, 1348 bp, 1643 bp and 1772 bp was obtained as well as deletion of the 128 bp amplion (figure 1).

Similar distinctive profiles were amplified in the case of the primer PBS-V3, too. A total of 11 generated amplicons were amplified wihtin the range of 102 bp up to the 1746 bp, but their different distribution was obtained in control and treated plants. Here, three deletions and four insertions were in the fingerprint of plant grown under Cd stress. Inserted amplions were of length 149 bp, 524 bp, 834 bp and 945 bp. Deletion of fragments 189 bp, 1220 bp and 1502 bp were obtained (figure 1).

The second group of PBS primers that match the primer binding sites of retrotransposon lineages Angela and Bianca provided fingerprints that differ from the first group in the average length of amplicons where fragment longer than 1000 bp were obtained preferably.

Primer PBS-V4 provided a total of 11 generated amplicons with the length range from 107 bp up to the 1779 bp for control plants and 7 amplicons with the length from 107 bp up to the 1699 bp for Cd treated plants. For this primer, a total of 4 deletions and 1 new insertion were amplified in the fingerprint pattern of plant grown under the Cd stress. Deletions of the fragments 548 bp, 1012 bp, 1049 bp and 1257 bp and insertion of the fragment with length of 234 bp were obtained (figure 2).

Primer PBS-V5 provided a total of 10 generated amplicons withing the range of 115 bp up to the 1802 bp, but their different distribution was obtained in control and treated plants. Here, three deletions and three insertions were in the fingerprint of plant grown under Cd stress. Inserted amplions were of length 300 bp, 635 bp and 1149 bp. Deletion of fragments 205 478 bp and 888 bp were obtained (figure 2).
4. Discussion

In this research, we report the impact of cadmium treatment to retrotransposon polymorphism changes in *Amaranthus cruentus* L grown under cadmium stress. Previously, the impact of cadmium to amaranth Pribina variety was analysed on the level of gene expression and was proved, that Cd significantly increased the mRNA level of *Chit 5* in roots (Lancíková et al., 2020). Thanks to the identification and study of genetic diversity in plants, we are able to better understand the molecular basis of various biological phenomena.

Nowadays, a wide range of molecular marker techniques are available for genotyping plant genomes. DNA markers are increasingly used in basic genomic studies and applied plant breeding. The consideration of why to decide on a given technique is based on the type of plant that is the focus of the research, the goal of the research work and the availability of the necessary resources (Amiteye, 2021). The study of medicinal plants (El Sherbeny et al., 2017) with ISSR primers was able to differentiate populations grown near cement factory sites, which are usually polluted with Pb, Cu, Cr, Cd, Zn from non-polluted region-based populations. Comparable results were achieved for populations of *Viola tricolor* L. growing on Zn, Pb and Cd contaminated soils showed higher gene diversity and polymorphism than control populations (Słomka et al., 2011). ISSR analysis of rocket salad (*Eruca sativa* L.) with Zn, Pb and Cd treatment, detected a dose-dependent clustering and differentiation of HM treatments from control groups (Al-Qurainy, 2010).

Transposable elements are of great importance in changes of gene expression. This is actioned by methylation of a transposable element located in or near a gene involved in the methylation mechanism (Galindo-González et al., 2018). The activation of transposable elements and adaptation to stress are reported widely in literature by many authors previously (Boyko and Kovalchuk, 2008; Fasani et al., 2023). Long terminal repeat retrotransposon might be activated by abiotic and biotic stresses in different organisms what can pose a threat to the integrity of the host genome because of their movement. The remobilization of ONSEN LTR retrotransposons are increased in heat stress in Arabidopsis (Ito et al., 2011), and Gypsy and Copia LTR RTs in drought, low temperature, and
salinity stresses in some Medicago genotypes (Yin et al., 2021). Primer binding sites based markers of retrotransposon were previously applied for revealing the stress genomic answer, too. Two flax varieties growing under chronic exposition to ionizing radiation were used in screening of the length polymorphism generated by transposable elements insertions and unique amplicon additions were obtained in the fingerprints as well as and deletion (Žiarovská et al., 2022) what is in concordance with our findings reported in this study.

5. Conclusion

In our literature research, the effect of cadmium based stress on transposable elements based fingerprints in amaranth has not yet been reported. Here, the effectivity of this marker technique was proved by generating unique polymorphic profiles that mirror the genomic changes in Amaranthus cruentus L under the abiotic stress of heavy metals.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References