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Molecular profiles of retrotransposon based length polymorphism in *Amarathus 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 growing under the stress of cadmium in the growth medium. Polymorphic 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 (**Kalendar *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 (ThermoScientific) according the instructions of manufacturer. The quantity and quality of extracted DNA was analysed using the Nanophotometer P360 (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.

Table 1 Characteristics of primers used in the study

name of primer	sequence	Copia-like lineage of retrotransposon	Ta
PBS-V1	5´TGGTATCAGAGCT3´	Maximus	55°C
PBS-V2	5´TGGTATCAGAGCC3´	Ivana	55°C
PBS-V3	5´TGGTATCAGAGCA3´	Ale-1	55°C
PBS-V4	5´GTGGCATCCGAGCC3´	Angela	56°C
PBS-V5	5´CGTTATCAGCAC3´	Bianca	55°C

Obtained amplicons were separated in 3% agarose gels stained by GelRed™ (Biotium) 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 sequencies. 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 accession the range from 200 bp up to the 1000 bp was obtained, too (figures 1 and 2).

Primer PBS-V1 provided 11 amplicons in both of the variants, control and Cd treated plants, but different length distribution was obtained thanks to three insertions 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 amplicons 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 amplicons 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 amplicon (figure 1).

Similar distinctive profiles were amplified in the case of the primer PBS-V3, too. A total of 11 generated amplicons were amplified withing 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 amplicons were of length 149 bp, 524 bp, 834 bp and 945 bp. Deletion of fragments 189 bp, 1220 bp and 1582 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 amplicons were of length 300 bp, 635 bp and 1149 bp. Deletion of fragments 205 478 bp and 888 bp were obtained (figure 2).

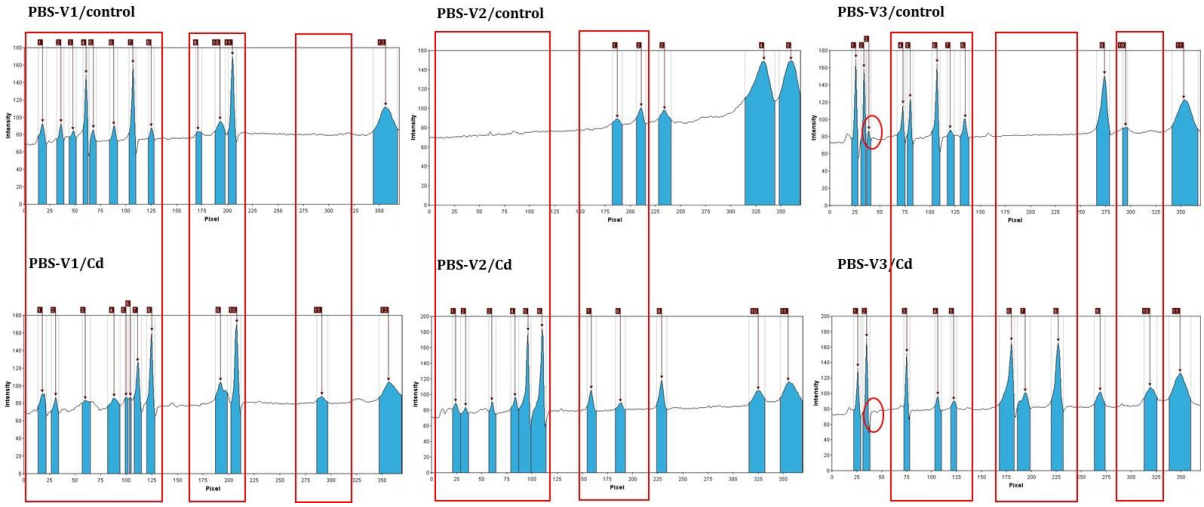

Figure 1 Fingerprints generated by PBS-V1, PBS-V2 and PBS-V3 markers for control and Cd treated plants of Pribina variety of *Amaranthus cruentus* L.

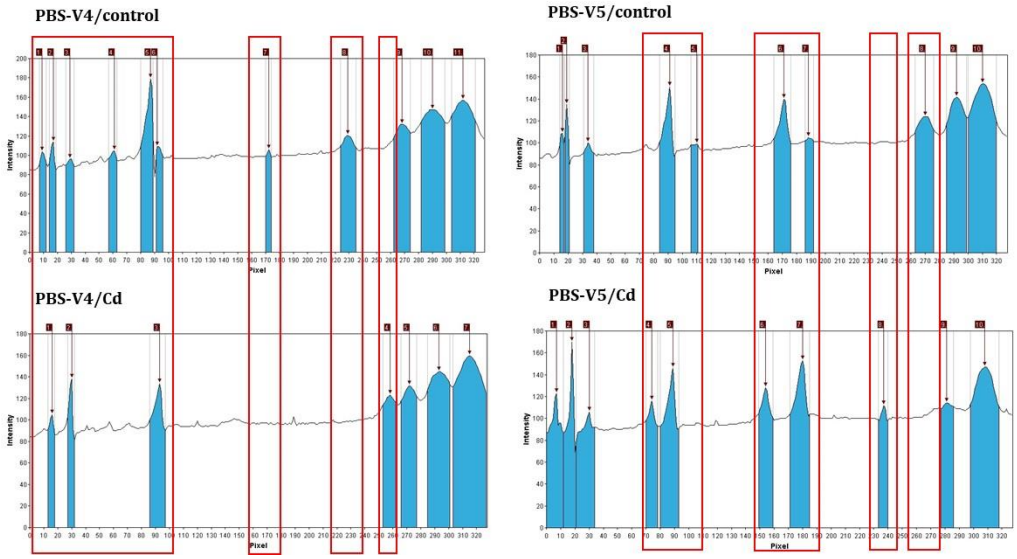

Figure 2 Fingerprints generated by PBS-V4 and PBS-V5 markers for control and Cd treated plants of Pribina variety of *Amaranthus cruentus* L.

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

1. Amiteye, S. 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon*. 7, e08093. https://doi.org/10.1016/j.heliyon.2021.e08093
2. Al-Qurainy, F. 2010. Application of inter simple sequence repeat (ISSR marker) to detect genotoxic effect of heavy metals on *Eruca sativa* (L.). *Afr. J. Biotechnol*. 9. ISSN 1684–5315
3. Angulo-Bejarano, P.I., Puente-Rivera, J., Cruz-Ortega, R. 2021. Metal and Metalloid Toxicity in Plants: An Overview on Molecular Aspects. *Plants*. 10, 635. https://doi.org/10.3390/plants10040635
4. Bigaliev, A., Boguspaev, K., Znanburshin, E. 2013. Phytoremediation potential of Amaranthus sp. for heavy metals contaminated soil of oil producing territory. In Proceedings of the 10th Annual International Petroleum Environmental Conference, Houston, TX, USA, 12–14 November 2013.
5. Boyko, A., Kovalchuk, I. 2008. Epigenetic control of plant stress response. *Environ. Mol. Mutagen*. 49,61-72. https://doi.org/10.1002/em.20347
6. Derakhshani, B., Jafary, H., Maleki Zanjani, B., Hasanpur, K., Mishina, K., Tanaka, T., Kawahara, Y., Oono, Y. 2020. Combined QTL mapping and RNA-Seq profiling reveals candidate genes associated with cadmium tolerance in barley. PLoS One. 16, e0230820. https://doi.org/10.1371/journal.pone.0230820
7. El Sherbeny, E. A., Morsy, A. A., Hassan, A. H. 2017. Impact of heavy metals pollution on molecular genetics of some medicinal plants. *J. Agr. Vet. Sci*. 10, 66-71.
8. Fasani, E., Giannelli, G., Varotto, S., Visioli, G., Bellin, D., Furini, A., Dal Corso G. 2023. Epigenetic Control of Plant Response to Heavy Metals. *Plants*. 12,3195. https://doi.org/10.3390/plants12183195
9. Galindo-González, L., Sarmiento, F., Quimbaya, M.A. 2018. Shaping plant adaptability, genome structure and gene expression through transposable element epigenetic control: focus on methylation. *Agronomy*. 8,180. https://doi.org/10.3390/agronomy8090180
10. Ito, H., Gaubert, H., Bucher, E., Mirouze, M., Vaillant, I., Pazskowski, J. 2011. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature*. 472, 115-119. https://doi.org/10.1038/nature09861
11. Jamla, M., Khare, T., Joshi, S., Patil, S., Penna, S., and Kumar, V. 2021. Omics approaches for understanding heavy metal responses and tolerance in plants. *Curr. Plant Biol*. 27,100213. https://doi.org/10.1016/j.cpb.2021.100213
12. Jonnalagadda, S., Nenzou, G. 1997. Studies on arsenic rich mine dumps. II. The heavy element uptake by vegetation. *J. Environ. Sci. Health*. 32, 455–464. https://doi.org/10.1080/10934529709376554
13. Kalendar, R., Antonius, K., Smýkal, P., Schulman, A.H. 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. *Theor. Appl. Genet*. 121, 1419–1430. https://doi.org/10.1007/s00122-010-1398-2
14. Kashino-Fujii, M., Yokosho, K., Yamaji, N., Yamane, M., Saisho, D., Sato, K., Ma, J.F. 2018. Retrotransposon Insertion and DNA Methylation Regulate Aluminum Tolerance in European Barley Accessions. *Plant Physiol*. 178, 716–727. https://doi.org/10.1104/pp.18.00651
15. Küpper, H., Andresen, E. 2016. Mechanisms of metal toxicity in plants. *Metallomics*. 8, 269–285. doi: 10.1039/c5mt00244c
16. Lancíková, V., Tomka, M., Žiarovská, J., Gažo, J., Hricová. A. 2020. Morphological Responses and Gene Expression of Grain Amaranth (Amaranthus spp.) Growing under Cd. *Plants*. 9, 572. https://doi.org/10.3390/plants9050572
17. Memon, A.R., Aktoprakligil, D., Özdemir, A., Vertii, A. 2001. Heavy metal accumulation and detoxification mechanisms in plants. *Turk. J. Bot.* 25, 111–121. https://journals.tubitak.gov.tr/botany/vol25/iss3/1
18. Mengoni, A., Gonnelli, C., Galardi, F., Gabbrielli, R., Bazzicalupo, M. 2001. Genetic diversity and heavy metal tolerance in populations of *Silene paradoxa* L. (Caryophyllaceae): a random amplified polymorphic DNA analysis. *Mol. Ecol*. 9, 1319-1324. https://doi.org/10.1046/j.0962-1083.2001.01336.x
19. Pan, X., Li, Y., Liu, W., Liu, S., Min, J., Xiong, H., Dong., Z., Duan, Y., Yu, Y., Li, X. 2020. QTL mapping and candidate gene analysis of cadmium accumulation in polished rice by genome-wide association study. *Sci. Rep*. 10:11791. https://doi.org/10.1038/s41598-020-68742-4
20. Prasad M. 2003. Phytoremediation of Metal-Polluted Ecosystems: Hype for commercialization. *Russ. J. Plant Physiol*. 50, 686–700. https://doi.org/10.1023/A:1025604627496
21. Sarma, H. 2011. Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *J. Env. Sci. Technol*. 4, 118-138.
22. Singh, R. et al. 2019. Chapter 13-Adaption Mechanisms in Plants Under Heavy Metal Stress Conditions During Phytoremediation. In Phytomanagement of Polluted Sites; Pandey, V.C., Bauddh, K., Eds.; Elsevier: Amsterdam, The Netherlands pp. 329–360. ISBN 978-0-12-813912-7. https://doi.org/10.1016/B978-0-12-813912-7.00013-2
23. Słomka, A., Sutkowska, A., Szczepaniak, M., Malec, P., Mitka, J., Kuta, E. 2011. Increased genetic diversity of *Viola tricolor* L.(Violaceae) in metal-polluted environments. *Chemosphere.* 83, 435-442. DOI: https://doi.org/10.1016/j.chemosphere.2010.12.081
24. Sorrentino, M.C., Giordano, S., Capozzi, F., Spagnuolo, V. 2022. Metals Induce Genotoxicity in Three Cardoon Cultivars: Relation to Metal Uptake and Distribution in Extra- and Intracellular Fractions. *Plants*. 9, 475. doi: https://doi.org/10.3390/plants11040475
25. Tchounwou, P. B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J. 2012. Heavy Metals Toxicity and the Environment. *Exp. Suppl*. 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6
26. Yan, A., Wang, Y., Tan, S.N., Yusof, M.L.M., Ghosh, S., Chen, Z. 2020. Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. *Front. Plant Sci.* 11. doi: https://doi.org/10.3389/fpls.2020.00359
27. Yin, M., Zhang, S., Du, X., Mateo, R.G., Guo, W. , Li, A., Wang, Z., Wu, S., Chen, J., Liu, J., Ren, G. 2021. Genomic analysis of *Medicago ruthenica* provides insights into its tolerance to abiotic stress and demographic history. *Mol. Ecol. Res.* 21, 1641-1657. https://doi.org/10.1111/1755-0998.13363
28. Žiarovská, J.; Speváková, I.; Klongová, L.; Farkasová, S.; Rashydow, N. Transposable Elements in the Revealing of Polymorphism-Based Differences in the Seeds of Flax Varieties Grown in Remediated Chernobyl Area. Plants 2022, 11, 2567. https://doi.org/10.3390/plants11192567