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Changes in Transposable Element Fingerprint Profiles of Seladon Wheat Cultivar Under the Micronutrient Malnutrition

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Retrotransposons are natural parts of plant genomes. Under normal growth conditions most retrotransposons are transcriptionally silent or minimally expressed. However, certain stress conditions can lead to reactivation of retrotransposons at the level of transcriptionor transposition.Many monocot retrotransposons are transcriptionally active under abiotic stress conditions. In wheat, different abiotic stress was reported to be stimuli for retrotransposon activation. Here, IRAP fingerprints of Bare-1 retrotransposon and Cassandra TRIM element were anylysed analyzed in the drought susceptible *Triticum aestivum* L. cultivar Aladin under the stress of malnutrition. Wheat plants were grown under aseptic culture medium conditionswith reduced macronutrients and no micronutrientsand the IRAP (Inter Retrotransposon Amplified Polymorphism) technique was subsequently applied to obtain retrotransposons specific fingerprints. Both of the analysed retrotransposon showed polymorphic profiles. A total of 86 amplicons were generated in Bare-1 IRAP and 95 amplicons in Cassandra IRAP. Three unique fragments were obtained in control plants and two in the variant of 21 days of malnutrition for Bare-1 IRAP fingerprints. One unique fragment was obtained in control plants, two in the variant of 7 days of malnutrition, two in the variant of 14 days of malnutrition and five in the variant of 21 days of malnutrition for the Cassandra retrotransposon. IRAP fingerprints of Bare-1 and Cassandra retrotransposons are here reported for the first time under ther stress of malnutrition. In both of them, changes in generated amplicons were obtained what point the activity of analysed retrotransposons.

Keywords: Bare-1, Cassandra, retrotransposon, abiotic stress, *Triticum aestivum*

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Introduction

Transposable elements (TEs) are DNA sequences capable of moving (or copying) within a genome. Among TEs, retrotransposons replicate via an RNA intermediate ("copyandpaste" mechanism) and are particularly abundant in plant genomes. In plants, retrotransposons can comprise a major fraction of the genome; in some species they represent more than half of nuclear DNA (Kumar and Bennetzen, 1999; Schulman, 2013). From an evolutionary perspective, plant retrotransposons have played key roles in genome expansion, gene regulatory innovation (e.g., providing promoter/enhancer sequences), chromatin structure, and adaptation (Defraia and Slotkin, 2014). Retrotransposon insertions near genes can modulate neighboring gene expression, or create new regulatory networks (Ohtsu et al., 2007).

Undernormalgrowthconditions,mostretrotransposons are transcriptionally silent or minimally expressed. However, certain stress conditions can lead to reactivation of retrotransposons – either at the level of transcription (increased RNA expression) or even transposition (new insertions). These triggers often involve various stress conditions, including abiotic stresses (heat, cold, drought, salt), biotic stresses (pathogen infection), tissue culture/meristematic activation, hybridization, and also nutrient or starvation stresses (i.e., malnutrition) (Grandbastien, 2015; Vicient and Casacuberta, 2017; Ito, 2022). While many studies emphasize abiotic stresses like heat and drought (Nie et al., 2019; Theieme et al., 2022; Niu et al., 2024), there is growing evidence that nutrient deprivation/malnutrition, such as nitrogen starvation, can also trigger retrotransposon activity. In *Arabidopsis thaliana*, nitrogen starvation induced genomewide transcriptional activation of TEs, without a major change in DNA methylation levels, suggesting that nutrient availability can regulate TE silencing (Wang et al., 2022). Nutrient starvation may:

a) alter pools of metabolites required for epigenetic modifications (e.g., SAM for methylation),
b) trigger reactive oxygen species (ROS) or hormone responses that crosstalk with epigenetic regulation,
c) activate stressresponsive transcription factors whose binding sites may exist in LTRs of retrotransposons.

For example, many LTRs contain ciselements typical of stress (heat shock elements, ABA response elements, etc) that may recruit transcriptional machinery under stress conditions (Grandbastien, 2015; Ito 2022).

Wheat (*Triticum aestivum*) is a hexaploid species with one of the largest and most TE-rich genomes among crop plants. Over 85% of its (~16 Gb) genome is composed of transposable elements, most of which are retrotransposons. Among the retrotransposons, LTR elements dominate, and many families/subfamilies show subgenomespecific activity or legacy of waves of amplification (Wicker et al., 2021). The distribution of retrotransposons in wheat is nonrandom, centromeric and pericentromeric regions are enriched in certain retrotransposons (e.g., centromeric retrotransposons of wheat, CRWs), which play structural roles in chromosome architecture and centromere identity (Liu et al., 2008). Studies using evolutionary and population genomics have shown that different retrotransposon subfamilies were active at different times, and that the A, B, and D subgenomes of wheat harbour distinct TE subpopulations reflecting polyploidization history and chromosomal evolution (Wicker et al., 2021). Also, retrotransposon insertions serve as useful DNA based markers for genetic diversity in wild progenitors (e.g., wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*) via IRAP (Inter Retrotransposon Amplification Polymorphism)/REMAP (Retrotransposon Microsatellite Amplification Polymorphism) strategies (Vuorinen et al., 2018). Based on this, wheat retrotransposons are not just passive "junk" but integral to genome structure, evolution, plastically responding to past genome shocks (polyploidization, hybridization) and possibly to environmental cues. While nutrient deprivation per se (e.g., nitrogen or phosphate starvation) is well documented to induce TE activation in model species such as *Arabidopsis* (Wang et al., 2022), there is no specific data in wheat linking nutritional stress (malnutrition) with retrotransposon activation. However, given the shared mechanisms of epigenetic regulation and stress response, it is plausible that nutrient stress could also release TE silencing in wheat.

DNA based fingerprinting was proved to be an effective method to analyse the polymorphism based on the insertion of transposable elements (Biswas et al., 2010; Abdollahi Mandoulakani et al., 2012; Balážová et al., 2014). It is based on the amplification of genomic regions among individual retrotransposons using the primers that match sequences of their long terminal repeats (Schulman et al., 2012).

The aim of this study was to analyse IRAP fingerprints of Bare-1 retrotransposon and Cassandra TRIM element in the drought susceptible *Triticum aestivum* cultivarAladin under the stress of malnutrition.

Material and Methodology

Biological Material and Growth Conditions

Triticum aestivum cultivar Aladin (breeded in Germany) was used in the analysis. This cultivaris drought susceptible (Ražná et al., 2023). Biological material was obtained from GeneBank of Slovak Republic, Piešťany. Plants were grown in pentaplicates consistent of 5 plants in container under aseptic culture medium conditions (Murashige and Skoog, 1962) with half the macro- and zero microelements. Light regime was 16 hour light and 8 hours dark. Temperature regime was 20 °C during the phase of light and 11 °C during the phase of dark.Biological material for bulked DNA isolation was obtained from leaves of five plants (one from each container) after 7, 14 and 21 days of growth.

DNA Extraction and IRAP Analysis

Isolation of total genomic DNA of flax from fresh leaves of plants cultured under *in vitro* conditions was carried out by GeneJet Plant Genomic DNA Purification Kit (Thermo Fisher Scientific).

Polymerase chain reactions were performed in a buffer containing Combi PPP Master Mix (Top Bio) plus 1U of TopTaq DNA polymerase (Bioron). Bare-1 retrotransposon and Cassandra TRIM element sequences were used to perform IRAP analyse (Table 1).The time and temperature profile of the reactions were as follows: 1 min 94 °C; 40 cycles (15 s 94 °C; 15 s 54 °C/Cassandra or 59 °C/Barley-1; 1 min 72 °C) and a final 7 min 72 °C.

Table 1 Primers used in fingerprinting analyses

Retrotransposon	Sequence 5´-3´
Bare-1	GCAACGATGCACATATGGGAGAACACAA
Cassandra TRIM	TCTCCGTTGGTCGATGTGGGATGTTACA

Statistical Analysis

Amplified fragments were separated in 10% polyacrylamide gels stained by GelRed (Biotium) and analyzed online by GelAnalyser. Binary matrices were prepared according to the presence or absence of amplicons generated by the used IRAP markers and these were evaluated by iMEC software (Amiryousefi et al., 2018) to obtain basic descriptive genetic coefficients: H – heterozygocity index;PIC – polymorphism information content; MI – marker index; D – discriminating power and R – resolvingpower.

Results and Discussion

Individual fingerprint profiles were obtained for the length polymorphism of Bare-1 retrotransposon and Cassandra TRIM element in the Aladin wheat cultivar. The changes in the profiles were evaluated for amplicon insertions/deletions under the stress in the form of malnutrition of reduced nutrient medium. This factor has been reported to trigger processesthat activate transposable elements in plant genomes (Mansour, 2007), with the wheat genome being reported as active in retrotransposon dynamics (Kashkush et al., 2003; Ilman et al., 2025).

The wheat genome comprises from over 80% of repetitive sequences (Wicker et al., 2007) and it contains a diverse population of Ty1-copia retrotransposons, with a smaller subgroup related to Bare-1. LTR sequences of Bare-1 retrotransposon were previously successfully identified in the genome of wheat (Altıntaş et al., 2021) and used for the purposes of DNA fingerprinting.

In Bare-1 IRAP analysis, a total of 86 amplicons were generated for control and experimental plants (Table 2). Three unique fragments were obtained in control plants and two in the variant of 21 days of malnutrition. Both, insertions and deletions, of amplicons in generated fingerprint profiles were found (Figure 1).

Cassandra elements are small, non-autonomous LTR retrotransposons that lack protein-coding capacity and depend on autonomous retrotransposons for mobilization (Kalendar et al., 2008). They are widespread across vascular plants and have been found in many species. Because Cassandra sequences are conserved in parts and polymorphic in others,

Table 2 Amplicon numbers in individual experimental variants for Bare-1 IRAP profiles

Experimentalvariant	Number of amplicons	Range of the length of amplicons
Control plants	24	45–890 bp
7 days of malnutrition	23	45–890 bp
14 days of malnutrition	25	45–890 bp
21 days of malnutrition	24	45–810 bp

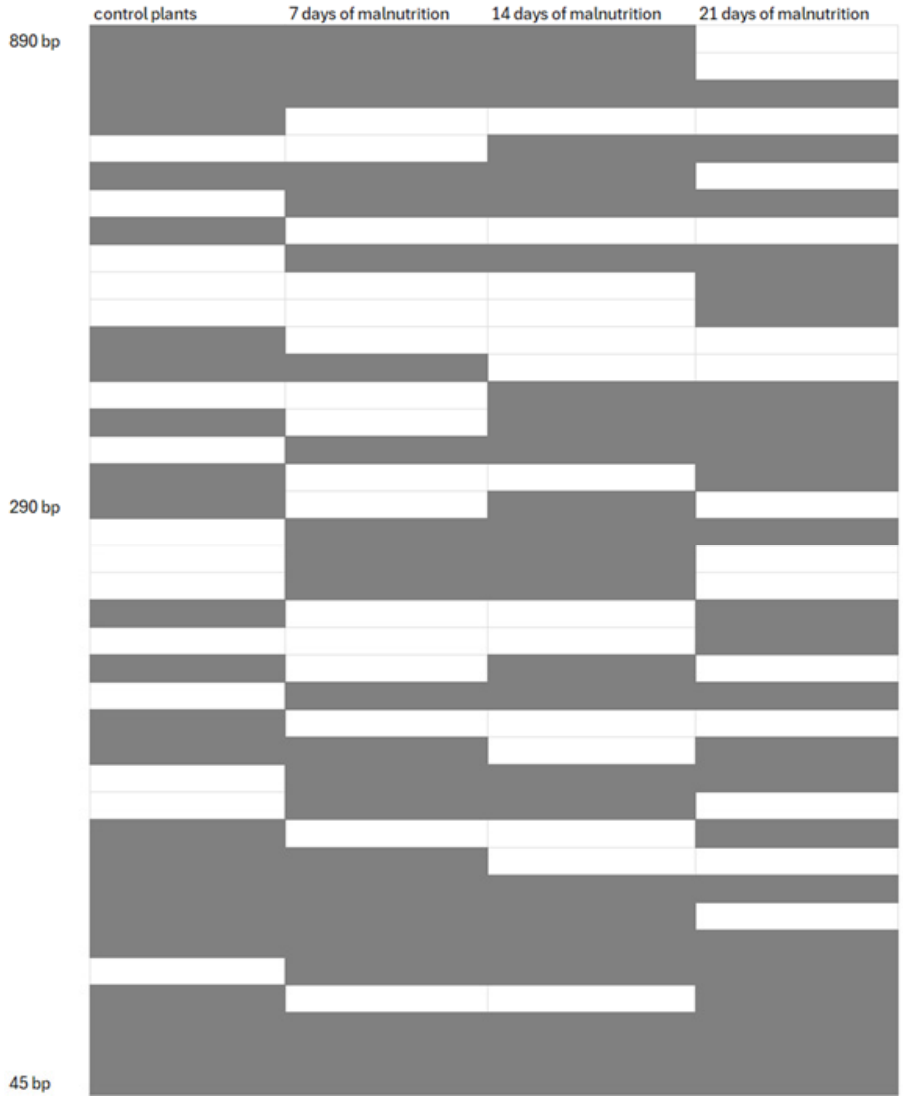

Figure 1 Obtained barcodes of Bare-1 IRAP profiles of wheat cultivarAladin for individual experimental variants

they have been used for marker development and comparative genomics in different plant groups (Žiarovská et al., 2022).

In the Cassandra IRAP analysis, a total of 95 amplicons were generated for control and experimental plants (Table 3). One unique fragment was obtained in control plants, two in variant of 7 days of malnutrition, two in the variant of 14 days of malnutrition and five in the variant of 21 days of malnutrition. Both, insertions and deletions, of amplicons in generated fingerprint profiles were found (Figure 2).

Comparing the used IRAP markers, both of them were very similar in calculated coefficients (Table 4). Despiteaverage discrimination power indices, in both of the used markers, unique amplicons were identified, although in Cassandra, unique amplicons were obtained on every one of the collecting days under the analysed stress factor.

Table 3 Amplicon numbers in individual experimental variants for Bare-1 IRAP profiles

Experimental variant	Number of amplicons	Range of the length of amplicons
Control plants	24	45–715 bp
7 days of malnutrition	30	45–775 bp
14 days of malnutrition	20	55–550 bp
21 days of malnutrition	21	65–750 bp

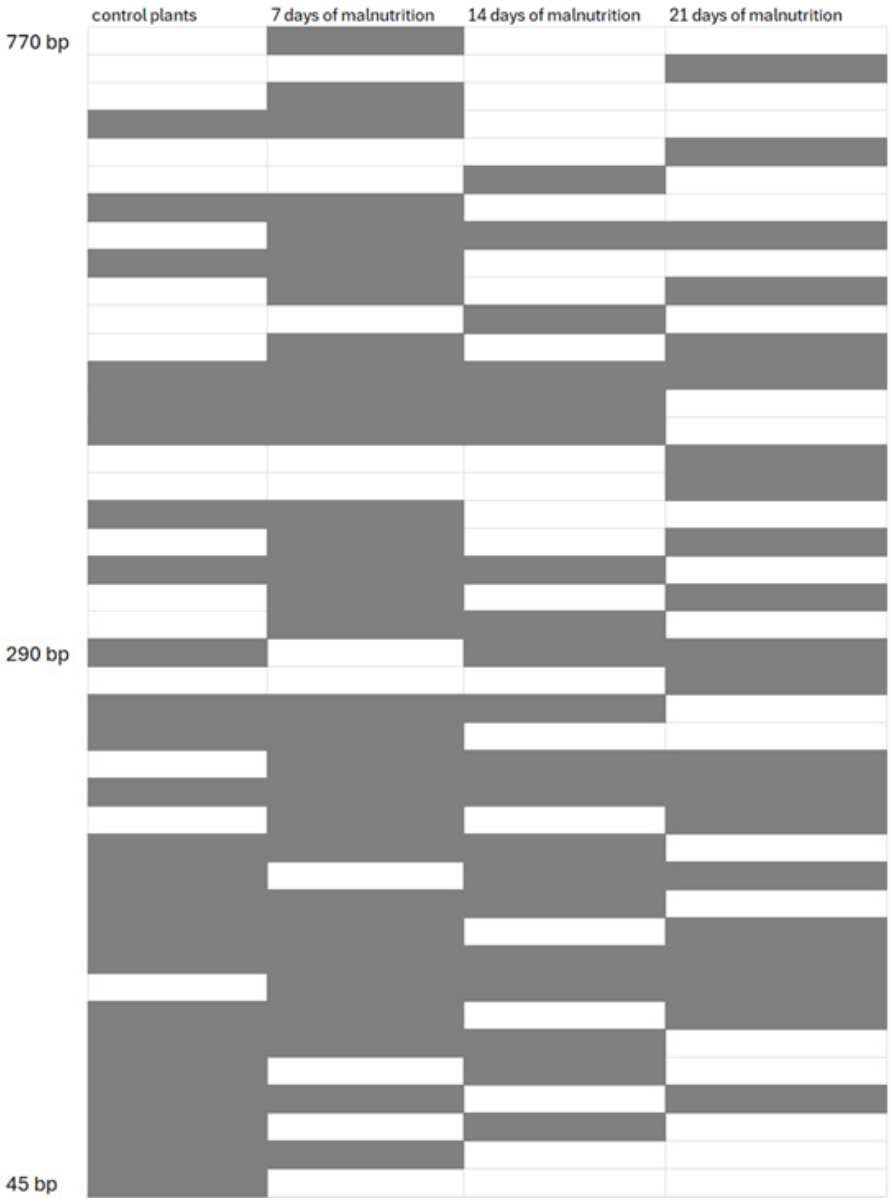

Figure 2 Obtained barcodes of Cassandra IRAP profiles of wheat cultivarAladin for individual experimental variants

Retrotransposons are major genome components in plants, typically silenced but capable of activation under stress. Nutrient deprivation or malnutrition is emerging as an important trigger of retrotransposon transcriptional activation (and possibly mobilization). Understanding this link is relevant to plant adaptation, genome dynamics, and could have implications for crop resilience in nutrientpoor soils. Interconnection between nutrient status and TE activation may reflect that malnutrition represents a "stress" to the plant genome, potentially loosening epigenetic control, altering chromatin context, and

Table 4 Characteristics of IRAP fingerprints obtained for Barley-1 and Cassandra markers for wheat cultivarAladin under malnutrition

Markér	Index				
	H	PIC	MI	D	R
Bare-1	0.48	0.36	0.07	0.64	24
Cassandra	0.49	0.37	0.07	0.67	26.5

Notes: H – heterozygocity index;PIC – polymorphism information content; MI – marker index; D – discriminating power; R – resolvingpower

activating stressresponsive promoters within LTRs, and nutrient starvation per se is among stimuli of retrotransposon transcriptional activation (Ito, 2022). Malnutritiontriggered retrotransposon activation may serve dual roles:

a) increasing genomic variability, potentially leading to novel adaptive insertions under nutrientlimiting conditions;
b) causing instability or detrimental insertions if unchecked. Some authors argue that stressactivated TEs may contribute to the adaptation and evolution of stresstolerant phenotypes (Grandbastien, 2015; Vicient and Casacuberta, 2017).

The unique biological and molecular characteristics of retrotransposon make them very good DNA markers. Direct comparisons of retrotransposon based marker techniques with other indicate that the retrotransposon markers are more informative and polymorphic in a cultivarof crops (Queen et al., 2004; Tam et al., 2005; Abdollahi Mandoulakani et al., 2012). IRAP has been in many studies of genetic diversity and phylogeny within several plant genera and species, including *Hordeum* L. (Kalendar et al., 1999), *Pisum* L. (Smykal, 2006), *Oryza* L. (Branco et al., 2007), *Aegilops* L. (Saeidi et al., 2008), *Citrus* L. (Biswas et al., 2010), *Medicago sativa* L. (Abdollahi Mandoulakani et al., 2012) or *Triticosecale* Wittm. ex A.Camus. (Balážová et al., 2014).

The distribution of retrotransposons in wheat is non-random: centromeric and pericentromeric regions are enriched in specific which play structural roles in chromosome architecture and centromere identity (Liu et al., 2008). Previously, individual groups of retrortransposon were used as DNA-based markers for different purposes in wheat. WIS2-1A, Wis, Wilma, Daniela, Fatima(originally identified in wheat genome) together with Bare-1 and Sukkula were utilized to detect integration events and activity of retrotransposon families isolated from wheat and barley in the Iranian bread wheat genome (Nasri et al., 2013). Transgenic wheat plants were screened by IRAP with Sukkula, Sabrina, Wham, Nikita and Wilma1 retrotransposons for the possible polymorphism (Morgun and Dubrovna, 2019). Screening of the genomic polymorphism of retrotransposons by IRAP was applied in the case of salinity stress, and the determination of the activity of retrotransposon polymorphism in both leaf and tissue culture of the wheat plant has been confirmed (Ilman et al., 2024). Similarly in our study, activity of retrotansposons was detected in wheat by IRAP method.

Conclusions

This study demonstrated changes in IRAP fingerprintsof Bare-1 and Cassandra retrotransposon in wheat under the nutritional starvation in *in vitro* culture for the first time. Based on the results of the research, the determination of the activity of the analysed retrotransposon polymorphism inthe wheat plant has been confirmed. Activation of retrotransposons in response to malnutrition stress may result in inducing specificgenetic and epigenetic changes, that shoul be the aim of further research as they have the potential to increase the adaptation of wheat to abiotic stress.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

This article doesn't contain any studies that would require an ethical statement.

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References

Altıntaş, S., Ilgar, B.A., & Karlık, E. (2021). Comparative retrotransposon analysis in wheat. *Journal of Advanced Research in Natural and Applied Sciences*, (3), 369–374.

Amiryousefi, A., Hyvönen, J., & Poczai, P. (2018). iMEC: Online marker efficiency calculator. *Applied Plant Sciences*. 6(6), e01159. https://doi.org/10.1002/aps3.1159

Balážová, Ž., Trebichalský, A., Gálová, Z., Kalendar, R., Schulman, A., Stratula, O., & Chňapek, M. (2014). Genetic diversity of Triticale cultivars based on microsatellite and retrotransposon-based markers. *Journal of Microbiology, Biotechnology and Food Sciences*, 3, 58–60.

Defraia, C., & Slotkin, R. K. (2014). Analysis of retrotransposon activity in plants. *Methods in Molecular Biology*, 1112, 195–210. https://doi.org/10.1007/978-1-62703-773-0_13

Grandbastien, M.A. (2015). LTR retrotransposons, handy hitchhikers of plant regulation and stress response. *Biochimica and Biophysica Acta*, 1849(4), 403–416. https://doi.org/10.1016/j.bbagrm.2014.07.017

Ilman, G. S., Heybat, M. M,Husni, T. O., Marakli, S., Ojaghi, J., & Mammad, I. H. (2025). Investigating retrotransposon dynamics in wheat genotypes under salinity stress: A comparative study of *in vitro* and *in vivo* cultivation. *Polish Journal of Environmental Studies*, 34 (5), 6203–6216.

Ito, H. (2022). Environmental stress and transposons in plants. *Genes & Genetic Systems*, 97(4), 169–175. https://doi.org/10.1266/ggs.22-00045

Kalendar, R., Tanskanen, J., Chang, W., Antonius, K., Sela, H., Peleg, O. & Schulman, A. H. (2008). Cassandra retrotransposons carry independently transcribed 5S RNA. *Proceedings of the National Academy of Sciences USA*, 105, 5833–5838. http://dx.doi.org/10.1073/pnas.0709698105

Kashkush, K., Feldman, M., & Levy, A. A. (2003). Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nature Genetics*, 33(1), 102–106. https://doi.org/10.1038/ng1063

Kumar, A., &Bennetzen, J. L. (1999). Plant retrotransposons. *Annual Reviews of Genetics,* 33, 479-532. https://doi.org/10.1146/annurev.genet.33.1.479

Liu, Z., Yue, W., Li, D., Wang, R. R., Kong, X., Lu, K., Wang, G., Dong, Y., Jin, W., & Zhang X. (2008). Structure and dynamics of retrotransposons at wheat centromeres and pericentromeres. *Chromosoma*, 117(5), 445–456. https://doi.org/10.1007/s00412-008-0161-9

Mansour, A. (2007). Epigenetic activation of genomic retrotransposons. *Journal of Cell and Molecular Biology*, 6, 99–107.

Morgun, B. V., & Dubrovna O. V. (2019). IRAP-analysis of transgenic wheat plants with double-stranded RNA suppressor of the proline dehydrogenase gene. *Cytology and Genetics*, 53, 384–391. https://doi.org/10.3103/S0095452719050116

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tabacco tissue cultures. *Physiologie Plantarum*, 15, 473–479.

Nasri, S., Abdollahi Mandoulakani, B., Darvishzadeh, R., & Bernousi, I. (2013). Retrotransposon insertional polymorphism in Iranian bread wheat cultivars and breeding lines revealed by IRAP and REMAP markers. *Biochemical Genetics*, 51(11–12), 927–493. https://doi.org/10.1007/s10528-013-9618-5

Nie, Q., Qiao, G., Peng, L., & Wen, X. (2019). Transcriptional activation of long terminal repeat retrotransposon sequences in the genome of pitaya under abiotic stress. *Plant Physiology and Biochemistry*, 135, 460–468. https://doi.org/10.1016/j.plaphy.2018.11.014

Niu, X., Ge, Z., & Ito, H. (2024).Regulatory mechanism of heat-active retrotransposons by the SET domain protein SUVH2. *Frontiers in Plant Science*, 15, 1355626. https://doi.org/10.3389/fpls.2024.1355626

Ohtsu, K., Smith, M. B., Emrich, S. J., Borsuk, L. A., Zhou, R., Chen, T., Zhang, X., Timmermans,M. C. P., Beck, J., Buckner, B., Janick-Buckner, D., Nettleton, D., Scanlon, M. J., & Schnable, P. S. (2007). Global gene expression analysis of the shoot apical meristem of maize (*Zea mays* L.). *The Plant Journal*, 52, 391–404.

Ražná, K., Vargaová, A., Kyseľ, M., Gálová, Z., & Žiarovská, J. (2023). Fingerprint characteristics and expression variability of miRNA based markers in wheat varieties with different susceptibility to drought. *Pakistan Journal of Botany,* 55,(1) 129–134.

Schulman, A. H., Flavell, A. J., Paux, E., & Ellis, T. H. (2012). The application of LTR retrotransposons as molecular markers in plants. *Methods in Molecular Biology*, 859, 115–153. https://doi.org/10.1007/978-1-61779-603-6_7

Schulman, A. H. (2013). Retrotransposon replication in plants. *Current Opinion in Virology*, 3(6), 604–614. https://doi.org/10.1016/j.coviro.2013.08.009

Thieme, M., Brêchet, A., Bourgeois, Y., Keller, B., Bucher, E., & Roulin, A. C. (2022). Experimentally heat-induced transposition increases drought tolerance in *Arabidopsis thaliana*. *New Phytologist*, 236(1), 182–194. https://doi.org/10.1111/nph.18322

Vicient, C.M., & Casacuberta, J.M. 2017. Impact of transposable elements on polyploid plant genomes. *Annals of Botany*, 120 (2), 195–207. https://doi.org/10.1093/aob/mcx078

Vuorinen, A. L. Kalendar, R., Fahima, T., Korpelainen, H., Nevo, E., &Schulman, A. H. (2018). Retrotransposon-based genetic diversity assessment in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*). *Agronomy*, 8, 107. https://doi.org/10.3390/agronomy8070107

Wang, Y., Liu, Y., Qu, S., Liang, W., Sun, L., Ci, D., Ren, Z., Fan, L. M., & Qian, W.(2022). Nitrogen starvation induces genome-wide activation of transposable elements in *Arabidopsis*. *Journal of Integrative Plant Biology*, 64(12), 2374–2384. https://doi.org/10.1111/jipb.13376

Wicker, T., Stritt, C., Sotiropoulos, A. G., Poretti, M., Pozniak, C., Walkowiak, S., Gundlach, H., & Stein, N. (2021). Transposable element populations shed light on the evolutionary history of wheat and the complex co-evolution of autonomous and non-autonomous retrotransposons. *Advanced Genetics*, 3(1), 2100022. https://doi.org/10.1002/ggn2.202100022

Žiarovská, J., Spevákova, I., Klongová, L., Farkasová, S.,& Rashydow, N. (2022). Transposable elements in the revealing of polymorphism-based differences in the seeds of flax varieties grown in remediated Chernobyl area. *Plants*, 11, 2567. https://doi.org/10.3390/plants11192567