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Research Article

Investigation of the Effect of Indole Acetic Acid Concentrations on Retrotransposon Movement in *Oryza sativa* L.

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Abstract: This study aims to investigate the effects of different Indole Acetic Acid (IAA) concentrations (0.25, 0.50, and 1 mM) on retrotransposon movement in the Kiziltan rice variety. This study chooses rice as it is a strategic agricultural product that serves as the primary food source for people worldwide. Analysis of the effects of the growth regulator hormone IAA at different concentrations on rice plants is of great importance for understanding hormonal regulation of plant development and for developing applications to increase yield. Our results reveal the relationships among genetic mobility, plant development, and hormone levels, with variable IAA concentrations regulating plant developmental processes and promoting root and shoot growth of rice. At specific doses, this study found that IAA application enhanced the *O. sativa* cv. Kiziltan rice yield. The rice grown under different IAA concentrations for 15 days showed distinct band profiles and polymorphisms. The Jaccard similarity index calculations revealed polymorphism rates ranged from 62% to 100% for *Hopi*, 0% to 100% for *Houba*, 53% to 100% for *Osr30*, and 62% to 100% for *Sukkula* in IRAP analyses. Pertinently, these study findings provide preliminary insights into the regulatory effects of IAA on mobile genetic elements.

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1. Introduction

Rice (*Oryza sativa* L.) serves as a key model crop for studying genome dynamics due to its relatively small but transposable element-rich genome, in which LTR retrotransposons constitute a significant genomic fraction, contributing to genome organization and regulatory variability (Li and Jiong, 2024; Chen et al., 2025; Fornasiero et al., 2025). IAA is the predominant endogenous auxin in higher plants and is known to regulate diverse growth and developmental processes (Feng et al., 2024; Wójcikowska, 2025). Recent evidence suggests that interactions between hormonal signaling pathways and epigenetic regulation may influence the activity of transposable elements (TEs), highlighting the importance of integrating hormone biology with retrotransposon behavior in crop genomes (Paul et al., 2025; Tossolini et al., 2025; Verma and Verma, 2025).

IAA is the primary auxin naturally produced in plants and plays a crucial role in regulating growth, development, and responses to environmental stresses (Raheem et al., 2018; EL Sabagh et al., 2022). IAA, which directly affects processes such as cell elongation, apical dominance, and lateral root development, is synthesized in plant tissues and transported to target areas via polar transport (Yu et al.,

2024; Sharma et al., 2025). Exogenous IAA applications are a strategic tool for increasing productivity by promoting plant development, particularly under stress conditions such as salinity and drought (Ranjan et al., 2024). In this respect, IAA is pertinent in modulating physiological and metabolic adaptation mechanisms. On the other hand, retrotransposons are elements that are sensitive to environmental stimuli and can move in the genome. It is known that these elements can be transcriptionally activated when epigenetic control mechanisms are weakened under stress (Ali et al., 2022; Perrella et al., 2022). However, the effects of hormones, such as IAA, on retrotransposons have been examined in only a limited number of studies. Therefore, studies investigating the effect of IAA on retrotransposon activity provide pioneering contributions to understanding the intersection of hormonal regulation and genomic mobility. This study aims to investigate the effects of varying IAA concentrations (0.25, 0.50, and 1 mM) on retrotransposon movement in the Kiziltan rice variety, with the goal of providing preliminary findings on the relationships among genetic mobility, plant development, and hormone levels. Thus, the regulatory effect of IAA on mobile genetic elements was tried to be understood. Retrotransposons are silent and mobile elements of plant genomes; they can be activated under stress and trigger genomic restructuring (Turkoglu, 2023; Hassan et al., 2024). This study, for the first time in the Kiziltan rice variety, evaluates the effect of the plant growth regulator IAA on retrotransposon mobility. Most importantly, examining how different IAA concentrations activate retrotransposons is a critical step in understanding how hormone-based regulators shape genetic diversity. In this respect, the study provides insight into the relationship between hormonal signals and genomic mobility, reveals the responses of mobile elements in the plant genome to hormones, and offers preliminary information for a potential genetic editing strategy in agricultural biotechnology applications.

2. Materials and Methods

This study conducted optimization studies in IAA conditions of the Kiziltan variety. IAA concentrations and examination periods to be applied to rice seeds were determined through optimization studies. Rice seeds were grown for 15 days in different IAA concentrations, as the control group (only water), 0.25 mM, 0.50 mM, and 1 mM. 7 seeds were germinated for each experimental group. The experiment was repeated 3 times to test the reliability and repeatability of the processes. IRAP retrotransposon analyses were performed on leaf samples from the planted rice.

2.1. Genomic DNA isolation

DNA isolation was performed from the leaf tissue of a single plant randomly selected from among the plants grown in each replicate, and no bulk DNA pooling was performed. Genomic DNA (gDNA) was isolated using a previously described method with minor modifications (Kidwell and Osborn, 1992). First, the leaves of each sample were crushed using liquid nitrogen to form a powder. For 50 mg of tissue, 500 µL of extraction buffer heated to 65°C was added, mixed, and incubated in a 60°C water bath for at least 1 hour. After being removed from the water bath, an equal volume of chloroform: isoamyl alcohol was added to the tube, which was then kept on ice for 5 minutes. The procedures specified in the protocol were followed. At the end of the procedure, the DNA pellet was washed with cold 70% ethanol. Thus, gDNA was obtained. The purity and quantity of the genomic DNA samples were determined using the NanoDrop 2000 (Thermo Scientific, ABD) device. Since the results were appropriate and at the desired purity level, PCR was performed for IRAP analyses.

2.2. IRAP Analyses

The isolated DNAs were used for IRAP analysis. The amount of isolated DNA was measured in Nonedrop and diluted to equal concentrations (20 ng µl⁻¹). IRAP-PCR was performed for *Houba*, *Hopi*, *Osr30*, and *Sukkula* retrotransposons using equal concentrations of DNA.

In IRAP analyses, four primers obtained from Arvas et al. (2022) and Shirasu et al. (2000) were used (*Hopi*, *Houba*, *Osr30*, and *Sukkula*). The primer sequences for IRAP-PCR are shown in Table 1 (Shirasu et al., 2000; Arvas et al., 2022a; Arvas et al., 2022b).

Table 1. Primer sequences to be used in the IRAP-PCR study

Primer sequences	NCBI Accession numbers of reference sequences
<i>Osr30-F</i> : 5' GCAGCCGATTCTCGCTCTGTTTCCG 3'	AC078891.2
<i>Osr30-R</i> : 5' GGCACGCTCACACATCCGAAGGCGA 3'	
<i>Hopi-F</i> : 5' CGCGAACCTTCCACACACAGACTAG 3'	AF537364.1
<i>Hopi-R</i> : 5' GGCCACGTGGGTGATCGTGTCTGCC 3'	
<i>Houba-F</i> : 5' CTTGAGTGGGCTAAGGCCC 3'	AF537365.1
<i>Houba-R</i> : 5' GTTTCGACCAAGCAGCCGGTC 3'	
<i>Sukkula</i> 5'GGAACGTCGGCATCGGGCTG 3'	AF254799.1

2.3. PCR conditions

In this study, four retrotransposon primers used for IRAP analyses were manually designed and obtained from Arvas et al. (2022). Details regarding the primer sequences and their accession numbers are provided in Table 1. Each IRAP-PCR reaction mixture contained 3 µl of DNA sample (20 ng µl⁻¹, 2.5 µl of MgCl₂ (3.125 mM µl⁻¹), 1.75 µl of primers (0.875 mM µl⁻¹), 10 µl of premixed master mix (Ampliqon, A180303), and 1 µl of nuclease-free water, making a total reaction volume of 20 µl. The PCR amplification was carried out under the following conditions: an initial denaturation at 95 °C for 3 minutes, followed by 30 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 52 °C for 3 minutes, and extension at 72 °C for 3 minutes, with a final extension at 72 °C for 10 minutes (Arctic Thermal Cycler, Thermo Scientific).

2.4. Assessment of PCR products

Next, IRAP analyses were conducted using genomic DNA from each sample, with three biological replicates per sample. The PCR-amplified fragments were separated by electrophoresis on a 2% (w/v) agarose gel at 120 V for 90 minutes, employing the GeneRuler 100 bp DNA marker (Thermo, 100 bp #SM0321) as a size reference. Following electrophoresis, the PCR products were stained with ethidium bromide (EtBr), then visualized and photographed under UV illumination. The resulting banding patterns were categorized as monomorphic or polymorphic and subsequently quantified. Genetic similarity among the tissue samples was determined using the Jaccard similarity coefficient, calculated according to the following equation:

$$\text{Jaccard similarity coefficient (J)} = \text{MAB} / (\text{MA} + \text{MB} + \text{MAB}) \quad (1)$$

where MA denotes the number of bands unique to sample A, MB represents the number of bands unique to sample B, and MAB indicates the bands shared by both samples (Jaccard, 1908).

3. Results

Retrotransposon movements in the leaf tissues of 15-day-old rice plants exposed to varying concentrations of IAA were assessed through the IRAP molecular marker technique. For this purpose, Kiziltan seeds were cultured in pots containing peat and perlite, as well as a control group (only water), with IAA concentrations of 0.25 mM, 0.50 mM, and 1 mM in four different treatments (a Total of 12 samples (4 treatments x 3 biological replicates)). IRAP studies were carried out using three primers (*Hopi/Osr27*, *Houba/Tos5/Osr13*, and *Osr30*) that are still active in Kiziltan plants and have many copies, as well as *Sukkula* primers specific to barley (*Hordeum vulgare* L.) plants and retrotransposons. Here, triplicate preparations comprising all 4 gDNA samples were used in the study. IRAP-PCR products were run on a 1% agarose gel at 120 V for 90 min, visualized on a UV transilluminator, and photographed. The resultant PCR products were analyzed using the GeneRuler 100 bp marker. DNA was separated using an Agarose gel with a 100 bp ladder (Thermo, #SM0321) and visualized with ethidium bromide (EtBr) staining. Jaccard similarity indices were calculated between different tissues based on the amplification results.

3.1. Evaluation of *Hopi* IRAP-PCR analysis results

Hopi IRAP-PCR analysis revealed 108 bands ranging from 100 bp to 1500 bp in 15-day-old leaf samples, comprising 73 monomorphic and 35 polymorphic bands (Figure 1). The polymorphism rates among the samples ranged from 61% to 100% (Table 2).

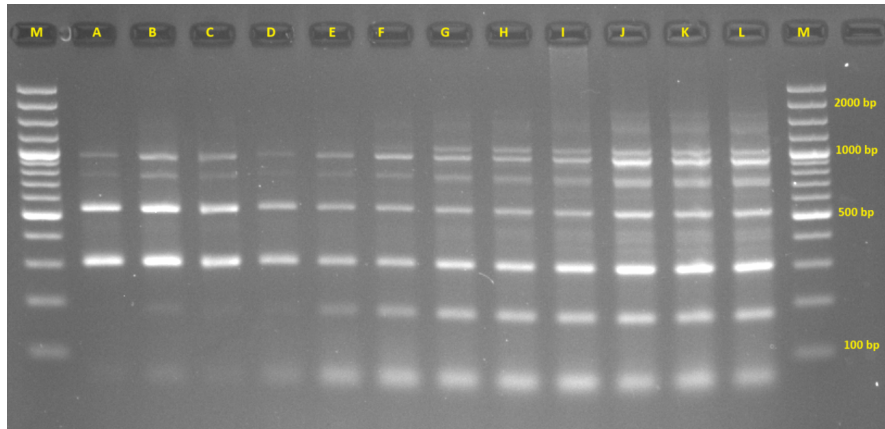


Figure 1. *Hopi* IRAP-PCR results for 15-day-old leaf samples.

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments $\times$ 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group, 1st replicate; B: Control group, 2nd replicate; C: Control group, 3rd replicate; D: 25 mM IAA, 1st replicate; E: 25 mM IAA, 2nd replicate; F: 25 mM IAA, 3rd replicate; G: 50 mM IAA, 1st replicate; H: 50 mM IAA, 2nd replicate; I: 50 mM IAA, 3rd replicate; J: 100 mM IAA, 1st replicate; K: 100 mM IAA, 2nd replicate; L: 100 mM IAA, 3rd replicate; M: DNA marker, GeneRuler 100 bp DNA Ladder (Thermo Scientific, #SM0321).

Table 2. Polymorphism rates determined for *Hopi* IRAP retrotransposons in 15-day-old rice leaves

	A	B	C	D	E	F	G	H	I	J	K	L
A	-											
B	100	-										
C	100	100	-									
D	92	92	92	-								
E	92	92	92	100	-							
F	92	92	92	100	100	-						
G	77	77	77	83	83	83	-					
H	77	77	77	83	83	83	100	-				
I	77	77	77	83	83	83	100	100	-			
J	61	61	61	67	67	67	80	80	80	-		
K	61	61	61	67	67	67	80	80	80	100	-	
L	61	61	61	67	67	67	80	80	80	100	100	-

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments $\times$ 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group 1st Repeat; B: Control group 2nd Repeat; C: Control group 3rd Repeat; D: 25 mM 1st Repeat; E: 25 mM 2nd Repeat; F: 25 mM 3rd Repeat; G: 50 mM 1st Repeat; H: 50 mM 2nd Repeat; I: 50 mM 3rd Repeat; J: 100 mM 1st Repeat; K: 100 mM 2nd Repeat; L: 100 mM 3rd Repeat; M: Marker, GeneRuler 100 bp. DNA Ladder (Thermo, 100 bp #SM0321)

3.2. Evaluation of *Houba* IRAP-PCR analysis results

In the *Houba* IRAP-PCR analysis of 15-day-old rice leaves, a total of 192 bands were detected within the size range of 100–2000 bp, including 117 monomorphic and 75 polymorphic fragments (see Sup. File 1). The polymorphism rates across the samples ranged from 0% to 100% (Table 3).

Table 3. Polymorphism levels of *Houba* IRAP retrotransposons in 15-day-old leaf samples

	A	B	C	D	E	F	G	H	I	J	K	L
A	-											
B	100	-										
C	100	100	-									
D	0	0	0	-								
E	0	0	0	100	-							
F	0	0	0	50	50	-						
G	0	0	0	37	37	75	-					
H	0	0	0	37	37	75	100	-				
I	0	0	0	37	37	75	100	100	-			
J	0	0	0	40	40	80	94	94	94	-		
K	0	0	0	40	40	80	94	94	94	100	-	
L	0	0	0	40	40	80	94	94	94	100	100	-

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments × 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group 1st Repeat; B: Control group 2nd Repeat; C: Control group 3rd Repeat; D: 25 mM 1st Repeat; E: 25 mM 2nd Repeat; F: 25 mM 3rd Repeat; G: 50 mM 1st Repeat; H: 50 mM 2nd Repeat; I: 50 mM 3rd Repeat; J: 100 mM 1st Repeat; K: 100 mM 2nd Repeat; L: 100 mM 3rd Repeat; M: Marker, GeneRuler 100 bp. DNA Ladder (Thermo, 100 bp #SM0321)

3.3. Evaluation of *Osr30* IRAP-PCR analysis results

In the *Osr30* IRAP-PCR analysis of 15-day-old rice leaves, 180 bands were detected within the size range of 100–1500 bp, including 141 monomorphic and 39 polymorphic bands (see Sup. File). Polymorphism rates across the samples ranged from 53% to 100% (Table 4).

Table 4. Polymorphism rates calculated for *Osr30* 15-day-old leaf IRAP retrotransposons

	A	B	C	D	E	F	G	H	I	J	K	L
A	-											
B	100	-										
C	100	100	-									
D	57	57	57	-								
E	57	57	57	100	-							
F	57	57	57	100	100	-						
G	71	71	71	67	67	67	-					
H	71	71	71	67	67	67	100	-				
I	71	71	71	67	67	67	100	100	-			
J	93	93	93	53	53	53	79	79	79	-		
K	93	93	93	53	53	53	79	79	79	100	-	
L	93	93	93	53	53	53	79	79	79	100	100	-

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments × 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group 1st Repeat; B: Control group 2nd Repeat; C: Control group 3rd Repeat; D: 25 mM 1st Repeat; E: 25 mM 2nd Repeat; F: 25 mM 3rd Repeat; G: 50 mM 1st Repeat; H: 50 mM 2nd Repeat;

I: 50 mM 3rd Repeat; J: 100 mM 1st Repeat; K: 100 mM 2nd Repeat; L: 100 mM 3rd Repeat; M: Marker, GeneRuler 100 bp. DNA Ladder (Thermo, 100 bp #SM0321)

3.4. Evaluation of *Sukkula* IRAP-PCR Analysis Results

Sukkula IRAP-PCR analysis revealed a total of 156 bands ranging from 100 bp to 1500 bp in 15-day-old leaf samples, consisting of 129 monomorphic and 27 polymorphic fragments (Figure 2). The polymorphism rates among these samples ranged from 62% to 100% (Table 5).

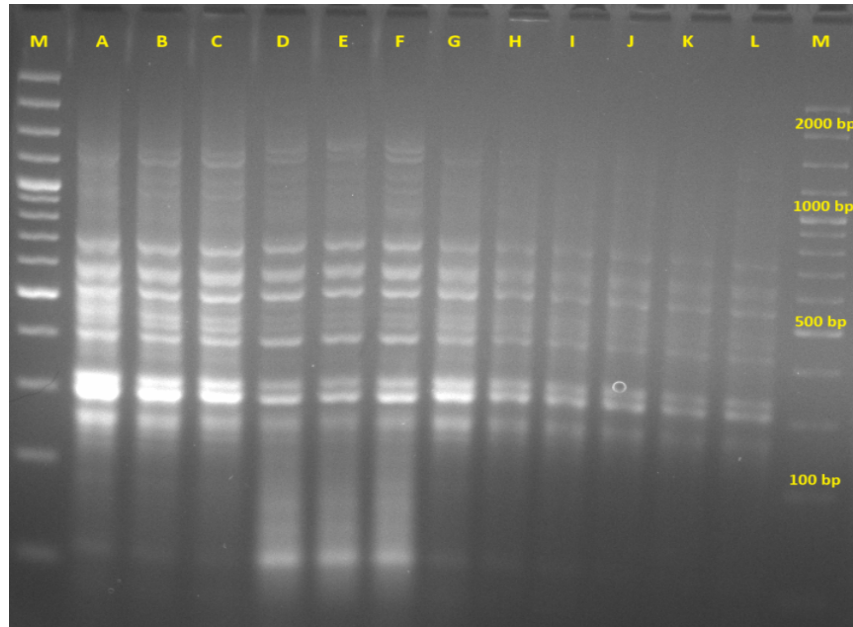


Figure 2. *Sukkula* IRAP-PCR results for 15-day-old leaf samples.

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments $\times$ 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group 1st Repeat; B: Control group 2nd Repeat; C: Control group 3rd Repeat; D: 25 mM 1st Repeat; E: 25 mM 2nd Repeat; F: 25 mM 3rd Repeat; G: 50 mM 1st Repeat; H: 50 mM 2nd Repeat; I: 50 mM 3rd Repeat; J: 100 mM 1st Repeat; K: 100 mM 2nd Repeat; L: 100 mM 3rd Repeat; M: Marker, GeneRuler 100 bp. DNA Ladder (Thermo, 100 bp #SM0321)

Table 5. Polymorphism rates calculated for *Sukkula* 15-day-old leaf IRAP retrotransposons

	A	B	C	D	E	F	G	H	I	J	K	L
A	-											
B	100	-										
C	100	100	-									
D	92	92	92	-								
E	92	92	92	100	-							
F	92	92	92	100	100	-						
G	77	77	77	83	83	83	-					
H	77	77	77	83	83	83	100	-				
I	77	77	77	83	83	83	100	100	-			
J	62	62	62	67	67	67	80	80	80	-		
K	62	62	62	67	67	67	80	80	80	100	-	
L	62	62	62	67	67	67	80	80	80	100	100	-

The experimental design comprises four treatments (control and three doses), of which one is the control. Each treatment was performed in triplicate. Accordingly, a total of 12 genomic DNA (gDNA) samples (4 treatments $\times$ 3 biological replicates) were used in the IRAP-PCR analyses. The wells labeled A–L in the gel electrophoresis represent the independent gDNA samples from each biological replicate. A: Control group 1st Repeat; B: Control group 2nd Repeat; C: Control group 3rd Repeat; D: 25 mM 1st Repeat; E: 25 mM 2nd Repeat; F: 25 mM 3rd Repeat; G: 50 mM 1st Repeat; H: 50 mM 2nd Repeat; I: 50 mM 3rd Repeat; J: 100 mM 1st Repeat; K: 100 mM 2nd Repeat; L: 100 mM 3rd Repeat; M: Marker, GeneRuler 100 bp. DNA Ladder (Thermo, 100 bp #SM0321)

4. Discussion

There is increasing evidence that auxin (particularly IAA) signaling pathways may exert indirect effects on epigenetic regulatory mechanisms in plants, not through direct stress signal production, but rather through cellular plasticity and genomic reprogramming processes. While the auxin signal reprograms gene expression via the TIR1/AFB–AUX/IAA–ARF module, this process is closely related to chromatin remodeling (Wang and Estelle, 2014). In particular, histone modifications and DNA methylation patterns have been reported to change dynamically during auxin-dependent cellular reprogramming processes. The suppression of transposons in plants is primarily achieved through DNA methylation, histone modifications, and RNA-based silencing mechanisms (Ashapkin et al., 2019). This epigenetic balance is susceptible to environmental signals. Indeed, under stress conditions, the relaxation of epigenetic suppression can make genomic regions—particularly transposons—usually silenced by methylation and heterochromatin structure more transcriptionally accessible (Ramakrishnan et al., 2021). Current studies reveal that transposons are not merely passive targets in the stress response; instead, they are regulatory elements that interact with epigenetic mechanisms and phytohormone signaling networks (Wang and Estelle, 2014).

Hormonal regulators, particularly auxins, which directly affect plant growth and productivity, can cause significant changes not only in physiological processes but also at the genomic and epigenetic levels (Fort et al., 2021). IAA is a fundamental phytohormone that regulates cell growth and differentiation, and is frequently used in external applications to modulate plant responses. Therefore, monitoring the genetic and epigenetic responses to hormones such as IAA in modern plant biotechnology is crucial for understanding the molecular basis of growth regulation (Yu et al., 2024; Wójcikowska, 2025). In this context, although auxin is not defined as a stress hormone in the classical sense, it can function as a signal modulator integrated into epigenetic regulatory networks under stress conditions. It is believed that auxin-mediated cellular reprogramming processes may result in binding-specific changes in DNA methylation patterns by increasing chromatin accessibility. This situation may indirectly contribute to the temporary weakening of epigenetic repression under stress conditions, leading to the emergence of genomic flexibility processes, such as transposon activation (Temel et al., 2008; Lisch, 2009; Akakpo et al., 2020).

Therefore, auxin signaling pathways should be considered as an integrative regulatory component operating within the stress–epigenetics–genome plasticity axis rather than as a direct stress sensor (Marzi et al., 2024).

Studies have shown that a limited number of TE families are associated with stress-responsive expression in neighboring genes. Although some TE families have been linked to multiple stress types, it has been reported that different TE family subsets play a role in each abiotic stress evaluated. In most cases, these TEs themselves are also upregulated in response to stress applications; however, it has been observed that despite the strong activation of some TE families, they do not exert a significant regulatory effect on a significant portion of neighboring genes (Naito et al., 2009; Yasuda et al., 2013). Although a large portion of stress-responsive gene expression is not directly associated with TEs, previous studies have shown that approximately 20% of stress-upregulated genes and up to 33% of stress-activated genes are regulated by TEs. As an alternative explanation, it has been suggested that only a limited number of genes located very close to TEs are directly affected by these TE insertions; other upregulated genes may be secondary targets indirectly regulated through genes affected by TEs. However, in this case, secondary target genes would not be expected to be preferentially co-localized with specific TE families (Makarevitch et al., 2015).

Previous studies across various plant species have demonstrated that at least some transposon families can be transcriptionally activated in response to environmental stress. Tissue culture applications are known to activate transposons and retrotransposons in many plant species (Hirochika et al., 1996; Kaeppler et al., 2000). Furthermore, examples of transcriptional activation of TEs in response to specific abiotic stresses have been observed in tobacco (Grandbastien et al., 2005), rice (Naito et al., 2009; Yasuda et al., 2013), and Arabidopsis (Pecinka et al., 2010; Ito et al., 2011; Cavrak et al., 2014). The stress-responsive expression of these TEs is thought to involve local enhancer regions that upregulate the TE promoter under stress conditions. These local enhancers may also affect other nearby promoters. Indeed, there are a limited number of examples where transposon insertions are linked to the stress-responsive expression of neighboring genes, such as mPING insertions associated with cold stress-responsive expression in rice (Naito et al., 2009; Yasuda et al., 2013) and ONSN insertions associated with heat stress-responsive expression in Arabidopsis (Ito et al., 2011).

The IRAP findings from this study, consistent with the relevant literature, reveal that different retrotransposon families exhibit varying levels of genomic polymorphism in response to auxin (IAA) applications. IRAP profiles obtained from leaf samples grown for 15 days under different IAA concentrations in rice plants revealed a total of 108 bands and polymorphism rates ranging from 62% to 100% in the analyses of the *Hopi* retrotransposon. IRAP-PCR analyses of the *Houba* retrotransposon revealed 192 bands, with polymorphism rates ranging from 0% to 100%. For the *Osr30* retrotransposon, 180 bands were obtained, and polymorphism rates ranging from 53% to 100% were detected. Similarly, in IRAP-PCR analyses of the *Sukkula* retrotransposon, 156 bands were identified, with polymorphism rates ranging from 62% to 100%. These different polymorphism profiles observed among retrotransposon families suggest that auxin application does not produce a uniform effect across the genome; instead, different TE families may exhibit distinct sensitivities to auxin-mediated cellular reprogramming and epigenetic regulation. This supports the view that the auxin signal may play an integrative regulatory role, affecting genomic flexibility and retrotransposon mobility in a context-specific manner, rather than acting as a direct stress factor.

It should be emphasized that IRAP-PCR does not provide direct evidence of de novo retrotransposition events. Instead, changes in IRAP banding patterns reflect retrotransposon-associated genomic polymorphisms that may arise from insertions, deletions, recombination events, structural rearrangements, or epigenetic modifications affecting primer-binding sites. Therefore, the polymorphisms observed in this study should be interpreted as indicators of retrotransposon-related genomic dynamics rather than definitive proof of active transposition.

These findings are broadly consistent with previous IRAP-PCR studies reported under different stress and application conditions in rice plants. Indeed, a survey conducted by Arvas et al. (2022b) on rice under drought stress reported that *Hopi* IRAP-PCR analyses of leaf samples yielded a total of 405 bands in the 300–1500 bp range, with polymorphism rates ranging from 0% to 97% (Arvas et al., 2022b; Arvas, 2026). The same study also reported 405 bands in the 300–1500 bp range and polymorphism rates ranging from 0% to 97% in the *Osr30* IRAP-PCR analyses. Similarly, in a study conducted by Arvas et al. (2022a) on rice under salt stress conditions, 405 bands were detected in the *Hopi* IRAP-PCR analyses of 15-day-old leaf samples, spanning the 300–1500 bp range, and a polymorphism rate of 0–97% was observed (Arvas et al., 2022a). In the same study, IRAP-PCR analyses of the *Osr30* retrotransposon revealed 360 bands in the 300–3000 bp range, with 0–92% polymorphism, and 360 bands in the 300–2000 bp range, with 0–94% polymorphism. These results indicate that different types of stress can generate a wide range of polymorphisms within the same retrotransposon families.

On the other hand, in samples obtained from *O. sativa*, *Brachypodium distachyon* L., *Hordeum vulgare* L. (barley), and *Triticum aestivum* L. (wheat) species by Tufan et al. (2020), IRAP-PCR analyses were performed using the *Hopi*, *Houba*, *Osr30*, and *RIRE1* primers in IRAP-PCR analyses conducted on 10-day-old leaf samples grown in Petri dishes; however, it was reported that significantly high polymorphism was only observed in rice samples (Tufan et al., 2020). Similarly, Noormohammadi et al. (2020) investigated the effect of salicylic acid and NaCl applications on cotton plants (GT40 and NNC varieties) using IRAP analysis. Using the primers Nikita, LTR6149, LTR6150, 5'LTR1, 5'LTR2, and 3'LTR, the analysis revealed that salt stress and salicylic acid applications significantly altered the band profiles of retrotransposon activity (Noormohammadi et al., 2020).

In addition, in a study conducted by Yetgin (2019) on Turkish Agricultural and Bafra Star rice varieties under different boron concentrations, the mobility of the *Houba*, *Osr30*, *RIRE1*, *Hopi*, *Sukkula*,

and *Nikita* retrotransposons was analyzed using the IRAP molecular marker technique; Polymorphism rates ranging from 0–37%, 0–87%, and 0–60% were reported for the *Houba*, *Osr30*, and *Hopi* retrotransposons, respectively (Yetgin, 2019). Furthermore, Yüzbaşıoğlu et al. (2016) compared transgenic (GM) and non-transgenic (non-GM) rice samples to investigate the effects of genetic transformation on transposon mobility; Analyses of the mobility of *Hopi/Osr27*, *Houba/Tos5/Osr13*, *Osr30*, and *RIRE1* retrotransposons reported polymorphism rates of 0–75% for *Hopi*, 0–57% for *Osr30*, and 0–52% for *Houba*. These findings generally show a trend similar to that observed for the *Hopi*, *Houba*, *Osr30*, and *Sukkula* retrotransposons in the current study (Yuzbasioglu et al., 2016).

The *Sukkula* retrotransposon is an LTR-retrotransposon first identified in the *H. vulgare* genome and is associated with “Sukkula-like” sequences commonly found within the Poaceae family. The presence of Sukkula elements in the barley genome, their copy structure, and their distribution within the genome have been reported in detail previously. It was also shown that polymorphisms associated with these elements can arise under tissue culture and environmental conditions. However, subsequent studies have revealed that LTR retrotransposon elements structurally and sequentially similar to Sukkula are also present in Poaceae members, such as wheat and other cereal species, and may be associated with gene expression regulation in some cases. Indeed, it has been reported that Sukkula-like transposon insertions identified in wheat may be associated with stress-responsive or environmentally sensitive expression patterns of specific genes. It is also known that grass genomes are rich in LTR retrotransposons and that these elements can exhibit dynamic behavior in response to environmental stimuli under epigenetic control (Shirasu et al., 2000; Vicient et al., 2001; Kartal-Alacam et al., 2014; Yokosho et al., 2016).

In this context, the band profiles obtained using the *Sukkula* IRAP primer in the present study indicate the presence of retrotransposon sequences with LTR regions homologous or similar to *Sukkula* (“Sukkula-like” elements) and application-dependent polymorphism patterns in the rice genome, rather than the definite presence of “Sukkula” elements. It is known that the IRAP-PCR technique allows primers initially defined for retrotransposons in a specific species to target similar LTR sequences in related species. Therefore, the polymorphism patterns obtained in this study using the Sukkula primer suggest that Sukkula-like retrotransposon sequences in the rice genome can exhibit genomic flexibility in response to auxin treatment. However, further genome-wide (*in silico*) scans and sequencing-based analyses are needed to obtain more precise results regarding the exact identity, copy number, and autonomous/non-autonomous structures of these sequences in rice (Cakmak et al., 2017; Maraklı, 2018; Vuorinen et al., 2018).

When considered collectively, these studies indicate that IRAP-PCR-detected retrotransposon polymorphisms depend on both the retrotransposon family and the specific experimental conditions, which vary across different plant stress and hormone treatments. In line with this, the present study's observations reveal that IAA application induces retrotransposon polymorphism, likely reflecting plant genomic responses to hormonal changes, further supporting the potential use of retrotransposons as bioindicators in hormone-mediated stress models.

Conclusion

This study has provided essential data on how hormone-based epigenetic responses are shaped at the molecular level, revealing the effects of different IAA concentrations on retrotransposon mobility in Kiziltan rice (*O. sativa*). In analyses using the IRAP-PCR method, it was observed that the polymorphism rates of the *Hopi*, *Houba*, *Osr30*, and *Sukkula* retrotransposons varied. This change shows that different IAA doses can stimulate retrotransposon activity at certain levels and regulate genomic mobility. The findings indicate that environmental stress and hormonal stimuli can direct retrotransposon activity. Further analyses can be conducted to better understand responses to IAA by monitoring retrotransposon activity across different developmental stages and tissues. New studies can be designed to determine the effects of retrotransposons on gene expression. The long-term goal of this approach is to develop retrotransposon-based molecular markers for developing rice lines with high stress tolerance.

Ethical Statement

This study did not involve any experiments on humans or animals and fully complies with ethical research standards.

Conflict of Interest

The author declares that there is no conflict of interest.

Artificial Intelligence Declaration

This study is based entirely on original laboratory findings, and the results obtained have not been previously published, used, or replicated anywhere. Furthermore, Generative Artificial Intelligence (AI) tools were not used in the preparation of this article, except for basic grammar and spell checking. The author assumes full responsibility for the originality, accuracy, and scientific integrity of all data presented in this article.

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Author Contributions

The conceptualization of this study, the creation of the research design, the execution of all laboratory experiments, the collection and analysis of data, the interpretation of the results, the writing of the article, and the final review were all carried out by the author. The author reviewed the final version of the article and approved it for publication.

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