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Transposon insertion in *GA3-oxidase* gene causes dwarfism of *Ipomoea nil* strain Kidachi

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Introduction

Gibberellic Acid (GA) is an important phytohormone which controls a wide variety of plant developmental processes such as seed germination, stem elongation, leaf expansion and flower and fruit development. Bioactive forms of GA are synthesized by the action of GA3-oxidase (GA3ox). In this experiment, the model plant used was the Japanese Morning Glory or *Ipomoea nil*. *I. nil* has a variety of strains; however, two strains are commonly studied: Violet and Kidachi (Figure 1). Both Violet and Kidachi express highly different characteristics such as stem length and GA content levels. Kidachi is known as a dwarf variety and a mutant lacking in GA synthesis in comparison to Violet. Genetic cross-breeding experiment using Kidachi x Violet showed that the causal gene of dwarfism is a simple recessive locus: self-pollinated F2 population showed 3:1 segregation. In this experiment, I began from newly derived whole genomic DNA sequencing analysis data. Next Generation Sequencing (NGS) of genomic DNA data showed a difference in sequence within one of thirteen GA3ox genes in Violet vs. Kidachi. We hypothesized that a transposon insertion disrupted the GA3ox gene, now referred to as *GA3ox1*. We amplified the *GA3ox1* DNA region using PCR, sequenced it and discovered the existence of a transposon insertion in Kidachi.

Figure 1. *Ipomoea nil* st. Violet (left). *Ipomoea nil* st. Kidachi (right).

Materials and Methods

A high-fidelity PCR polymerase was used for PCR of Kidachi and Violet genomic DNA regions that correspond to the *GA3ox1* gene. A pair of primers was designed at each end of *GA3ox1* gene's two exons. Two amplification protocols were used (2-step and Step-down) to compare which one gave the best amplification results. The Step-down protocol displayed the best results along with nested PCR method used to increase sensitivity and specificity of amplification (Figure 2). Amplified DNA was extracted from a gel electrophoresis band and initially sequenced using Sanger sequence method followed by NGS for more detailed sequence information. Total RNA was extracted from young shoots of Violet and Kidachi and used for RT-PCR to display the differences in *GA3ox1* gene expression.

Results and Discussion

Sanger sequencing results showed that the transposon within Kidachi's *GA3ox1* gene belongs to the *Tpn1* family, one of the most extensively studied CACTA-type transposons. This transposon, now named *Tpn1*^{Kidachi} was inserted at the only intron of the *GA3ox1* gene. NGS data showed that *Tpn1*^{Kidachi} is 8,809 bp long. Furthermore, results from RT-PCR failed to detect mRNA from Kidachi's *GA3ox1* indicating that the *GA3ox1* gene is disrupted by this transposon insertion.

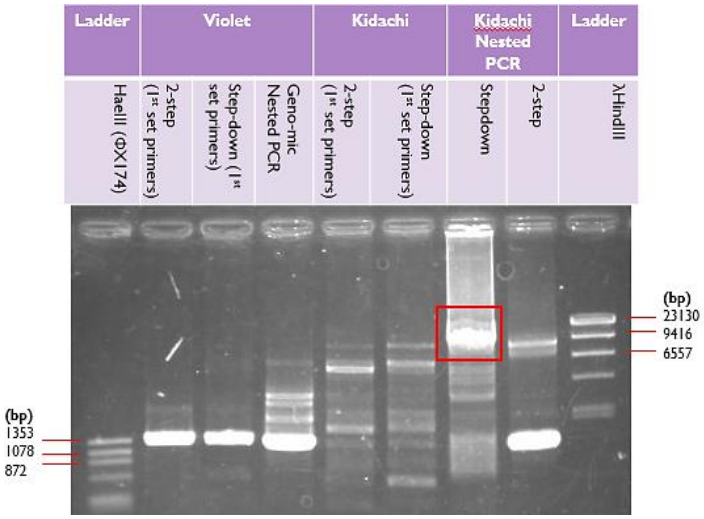

Figure 2. An agarose gel electrophoresis of PCR products. The sequenced DNA band (~10,000 bp) is marked with a square.

Conclusion

The large size of *Tpn1*^{Kidachi} may hinder its splicing resulting in a dysfunctional *GA3ox1* gene. This then induces the reduction of GA synthesis in Kidachi causing dwarfism. The future plan for this project includes the designing of CRISPR-Cas9 mediated knockout of *GA3ox1* gene in Violet to simulate the natural transposon mutation found in Kidachi.

References

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