Characterizing the Function of VICTR-like Genes and their Role in the Regulation of ABA in Response to DFPM in Arabidopsis Plants

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Abstract

The hormone abscisic acid (ABA) signal transduction is linked to plant drought responses including the regulation of stomatal closure. Through a chemical genetics approach, a synthetic small molecule "DFPM" ([5-(3,4-dichlorophenyl) furan-2-yl]-piperidine-1- ylmethanethione) was shown to negatively regulate ABA signaling and stimulate plant defense related genes by activating the Variation In Compound Triggered Root growth response (VICTR) locus in Arabidopsis (Kim et al, 2011; Kim et al, 2012). In order to study the role of the VICTR locus with regards to the ABA signaling pathway, we utilized Near Isogenic Lines (NILs) of Arabidopsis with accessions of the Bu-5 ecotype crossed into the background of Columbia-0 (Col-0). NIL-Col-0 is found to contain functional VICTR while NIL-Bu-5 does not. We first extracted DNA and genotyped leaf samples from Col-0, NIL-Col-0, and NIL-Bu-5 to verify their respective genotypes. We then performed a 9 and 5 hour chemical assay on Col-0, NIL-Col-0, and NIL-Bu-5 plants with control, ABA, DFPM, and ABA/DFPM chemical conditions followed by RNA extraction, complementary DNA (cDNA) synthesis, and quantitative reverse transcriptase-PCR (qRT-PCR) to compare levels of gene expression. We performed a similar chemical assay on NIL-Col-0 and NIL-Bu-5 lines containing the pRAB18:GFP bacterium and compared fluorescence when treated with ABA and DFPM. Lastly, we extracted protein samples from Col-0, NIL-Col-0, and NIL-Bu-5 after treating with DFPM under four time points and compared Mitogen-Activated Protein Kinase (MAP Kinase) activity using Western Blotting. Our results demonstrate the possibility that NIL-Col-0 expresses ABA better than NIL-Bu-5 due to the presence of the VICTR locus; this is seen through an increase in gene expression and fluorescence under ABA treatment and an increase in MAP Kinase activity under DFPM treatment when compared to NIL-Bu-5.

Introduction

Since its discovery in the early 1960s, ABA has been shown to regulate plant growth and development and play a role in stress response within plants. ABA can target specific guard cells for induction of stomatal closure as well as signal for adjustment in transpiration. Additionally, ABA is known as a stress hormone involved in stress induced synthesis.

Fig. 1. *DFPM structure (left) and proposed pathway (right) illustrating negative regulation of DFPM on ABA signaling. VICTR and plant immune signaling components involved in this negative regulation pathway are marked in pink (Kim, 2011, 2012).*

The VICTR region found within the Col-0 A. Thaliana plant wild type variant regulates the stress hormone biochemical pathway. Accordingly, other variants of the A. Thaliana that exclude the VICTR region such as the NIL-Bu-5 are severely stunted in plant development due to their inability to reduce transpiration. On the other hand, NIL-Col-0 contains the VICTR region allowing for the regulation of the ABA biochemical pathway involved in environmental stress responses. The small molecule DFPM triggers rapid inhibition of early ABA signal transduction and suppresses ABA.

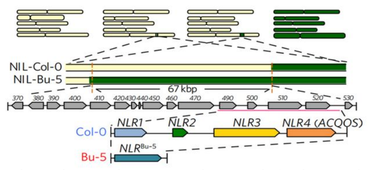

Fig. 2. *(from Ariga et al.) Genetic areas of interest between NIL-Col-0 and NIL-Bu-5. We are interested in the 67 kbp region which contains VICTR (NLR4) and its 3 homologous genes (NLR1, NLR2, and NLR3).*

We hypothesize that VICTR plays a role in the regulation of the ABA biochemical pathway.

Methods

A genomic DNA extraction and PCR was performed in order to verify the correct samples necessary to compare NIL-Col-0 and NIL-Bu-5 plants. The PCR was utilized to amplify regions inside and outside of the 67 kilo base pair (kbp), the location of genetic difference between NIL-Col-0 and NIL-Bu-5 plants. A gel-electrophoresis was then performed to compare DNA lengths of Col-0, NIL-Col-0, and NIL-Bu-5 plants.

Root growth assay was carried out to determine whether treatment of DFPM would affect root growth lengths or lead to root growth arrest in Col-0, NIL-Col-0, and NIL-Bu-5 plants. Root growth was inhibited in Col-0 and NIL-Col-0 but not in NIL-Bu-5 (data not shown).

A nine and five hour chemical assay of Col-0, NIL-Col-0, and NIL-Bu-5 plants was performed in order to compare levels of gene expression when exposed to 10 µM ABA, 30 µM DFPM, and both treatments together. The assay led to a RNA Extraction procedure and complementary DNA (cDNA) synthesis procedure. The cDNA synthesis allowed for further analysis of gene expression through qRT-PCR because the cDNA excluded the introns and non-coding regions of DNA and encoded for the necessary gene functions.

Additionally, a confocal microscope analysis was completed in order to obtain a visual image of the stomata and pavement cells of leafs sample of Arabidopsis lines of Col-0, NIL-Col-0 and NIL-Bu-5 with a pRAB18:GFP insertion (a bacteria that would cause the plants to fluoresce under UV light). The plants were treated with 0.002% ethanol, 10 µM ABA, and 10 µM ABA/ 20 µM DFPM and analyzed using a confocal microscope to determine whether DFPM inhibits ABA signalling as shown with a decrease in fluorescence compared to the ABA treatment.

Lastly, protein extraction of Col-0, NIL-Col-0, and NIL-Bu-5 plants with 0, 15, 30, 60 minute treatments of DFPM was performed and analyzed using an SDS gel running and Western Blot to compare the MAP Kinase activity.

Results

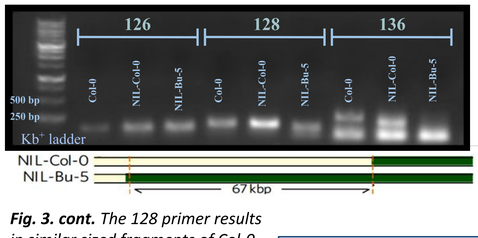

Fig. 3. *Gel Electrophoresis (left) allows for comparison of Col-0, NIL-Col-0, and NIL-Bu-5 DNA fragment sizes using primers corresponding to different DNA regions: 126 primer (left region), 128 primer (middle region), 136 primer (right region). The 126 primer results in fragments of similar sizes corresponding to the region left of the 67 kbp.*

Fig. 3. cont. *The 128 primer results in similar sized fragments of Col-0 and NIL-Col-0 but a smaller fragment than NIL-Bu-5 due to the difference in DNA strands within the 67 kbp region. The 136 primer results in similar band sizes between Col-0, NIL-Col-0 and NIL-Bu-5 when looking at the lower band, the presence of two bands in Col-0 and NIL-Col-0 could be due to their shared DNA of Col-0 in the region to the right of the 67 kbp. This could hint towards the incomplete amplification of the PCR.*

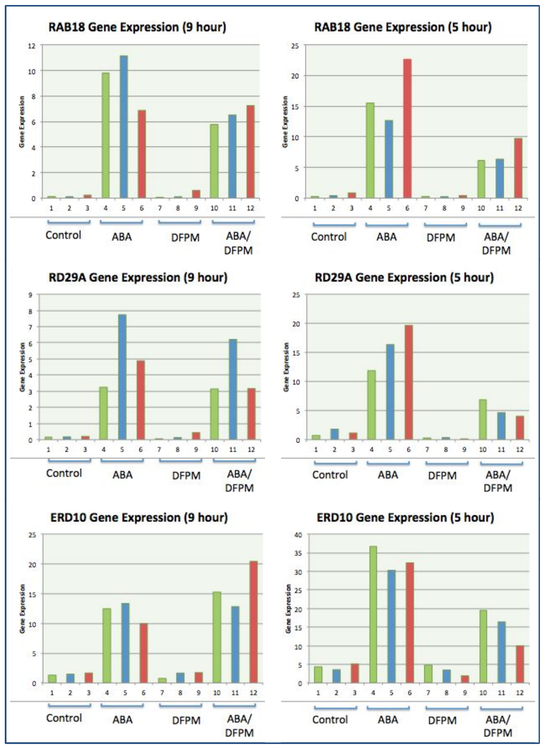

Fig. 4. *qRT-PCR results (right) showing gene expression levels of RAB18, RD29A, and ERD10 from Col-0, NIL-Col-0, and NIL-Bu-5 under 9 and 5 hour chemical treatments. The data shows that there are relatively low levels of gene expression under control (.002% Ethanol/ .006% DMSO) and DFPM conditions. Gene expression is increased under ABA treatment as well as in the ABA/DFPM treatment (except for the ERD10 9 hour treatment) but to a lesser degree due to the inhibition of DFPM in ABA signaling.* It can be seen that 5 hour chemical exposures lead to higher levels of gene expression.

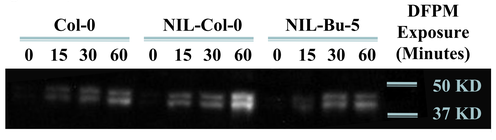

Fig. 5. *Western Blot results (left) showing MAP Kinase activity of Col-0, NIL-Col-0, and NIL-Bu-5 when exposed to DFPM under four time periods. Longer exposure to DFPM leads to an increase in MAP Kinase activity across the three genotypes.*

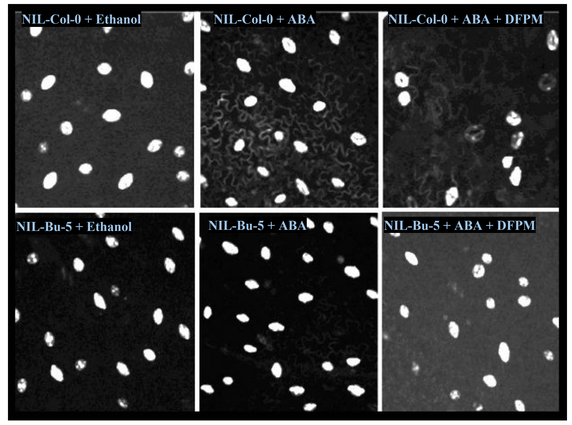

Fig. 6. *Confocal microscopy analysis of NIL-Col-0 and NIL-Bu-5 pRAB18:GFP insertion lines under control (Ethanol), ABA, and ABA/DFPM chemical treatments. Under the control condition, only stomata are shown to fluoresce. In the ABA condition, stomata and pavement cells are shown to fluoresce. In the ABA and DFPM condition, there is a weaker level of fluorescence in pavement cells than in the ABA treatment. NIL-Bu-5 shows weaker fluorescence than NIL-Col-0 in the ABA treatment.*

Conclusion

The results from the Gel Electrophoresis proved DNA extractions and PCR to be successful and confirmed the genotypes to be Col-0, NIL-Col-0, and NIL-Bu-5 when the fragments sizes under the three primer pairs were compared .

Data from the qRT-PCR demonstrate that the ABA and DFPM chemical treatments take effect on all three genotypes for both time periods. In the 9 hour ABA treatment, NIL-Col-0 has higher gene expression compared to NIL-Bu-5, while the 5 hour counterpart exhibits the opposite. This could mean that, over time, gene expression is lowered under ABA conditions in lines that are missing VICTR compared to lines containing VICTR. Additional biological replicates will be needed to better compare gene expression levels.

MAP Kinase data exhibits a higher level of activity in NIL-Col-0 60 minute treatment than activity in NIL-Bu-5 60 minute treatment. This could imply that the absence of VICTR is not required for MAP Kinase activation but results in a lower level of activity compared to lines that contain VICTR.

The data from the confocal microscopy analysis establishes that the absence of VICTR in NIL-Bu-5 leads to lower fluorescence in the epidermis when compared to NIL-Col-0 under the ABA treatment. This alludes that while VICTR may not be essential to the ABA pathway, the absence of VICTR may lead to a decrease in fluorescence.

In order to affirm the validity of our results, further experimentation with more biological replicates will be necessary.

References

Kim, Tau-Houn; et al. ; 2011. Chemical Genetics Reveals Negative Regulation of Abscisic Acid Signaling by a Plant Immune Response Pathway. Current Biology, Vol 21, No 11: 990-997

Kim, Tae-Houn; et al. ; 2012. Natural Variation in Small Molecule-Induced TIR-NB-LRR Signaling Induces Root Growth Arrest via EDS1- and PAD4-Complexed Protein VICTR in *Arabidopsis*. The Plant Cell, Vol. 24: 5177-5192

Ariga, Hirotaka; et al. ; 2017. NLR locus-mediated trade-off between abiotic and biotic stress adaptation in *Arabidopsis*. Nature Plants 3, 17072 (2017)

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