Characterization of the Relationship Between *BODYGUARD2* and Abscisic Acid in Response to Osmotic Stress

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BACKGROUND

- *Arabidopsis thaliana* is a model organism often studied in plant science.
- *BODYGUARD2 (BDG2)* is one of five genes in the *BODYGUARD* gene family. These genes encode proteins that are a part of the α/β hydrolase superfamily.
- *BODYGUARD1 (BDG1)* is another member that has been characterized.
- *BDG1* and *BDG2* have ~80% similarity in amino acid sequence, indicating likely similar function.
- *BDG1* has been linked to water retention in two ways
  - Cuticle formation and polymerization
  - Abscisic Acid (ABA) synthesis and stomatal closure

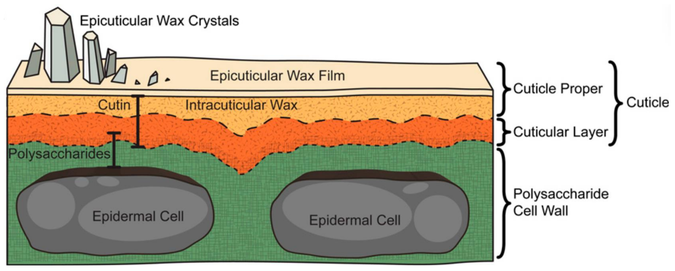


Figure 1.Model of the plant cuticle from Yeats and Rose, 2013.

Research Question

What are BDG2's effects on the cuticle and ABA biosynthesis, and how do these relate to osmotic stress?

Research Hypothesis

BDG2 regulates cuticle formation and the production of ABA or its signaling, all of which affect water loss under osmotic stress.

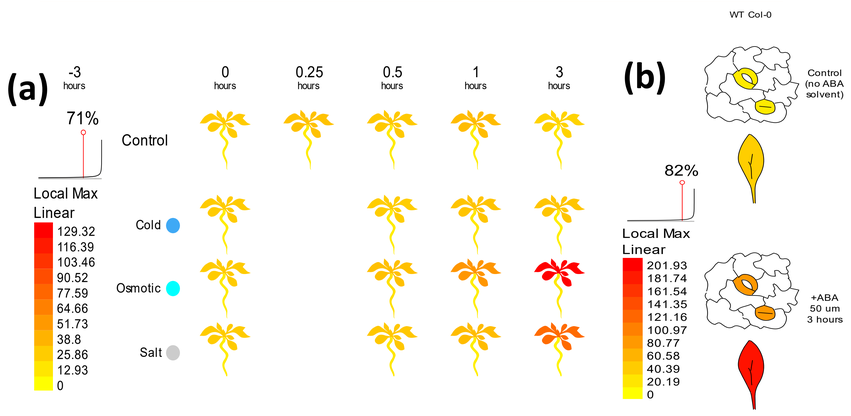
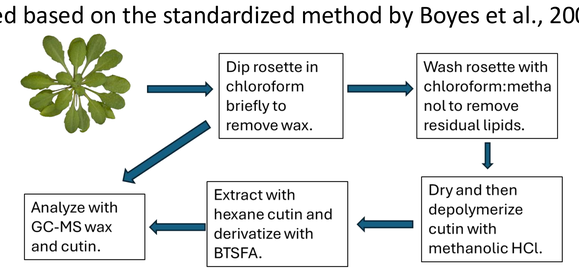


Figure 2.Expression pattern of *BDG2* mRNA under abiotic stress (a) and under ABA exposure. Data from Pandey et al., 2010 obtained through the ePlant website (Fucile et al., 2011).

METHODS

BDG2 mutants SALK_067478 (S067), SALK_076158C (S076), and CS860022 (CS86) were grown alongside WT on Murashige and Skoog Basal (MS) media-containing agar plates without (control) and with (treatment) mannitol. Two concentrations of Mannitol, 40mM and 100mM, were used for osmotic stress treatment. Phenotypes were recorded based on the standardized method by Boyes et al., 2001.



RESULTS

	AtBDG	AtBDG3	AtBDG4	AtBDG5
AtBDG2	82.6%/73.1%	64.3%/51.0%	46.8%/31.8%	48.4%/31.6%

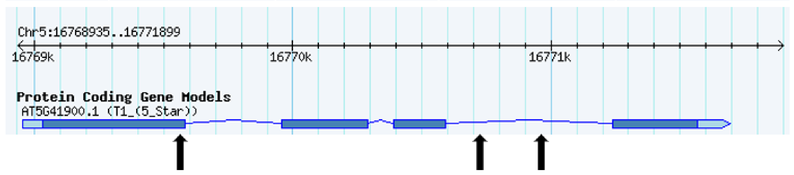


Figure 3. (top) Multi-alignment of *AtBDG2* with other *AtBDGs,* given by "consensus position/identity position" on protein level. (bottom) Gene sequence of *AtBDG2*, showing the locations of T-DNA insertions for the three mutants

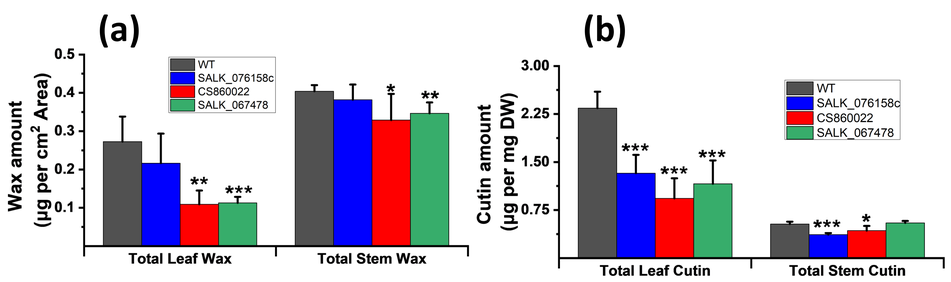
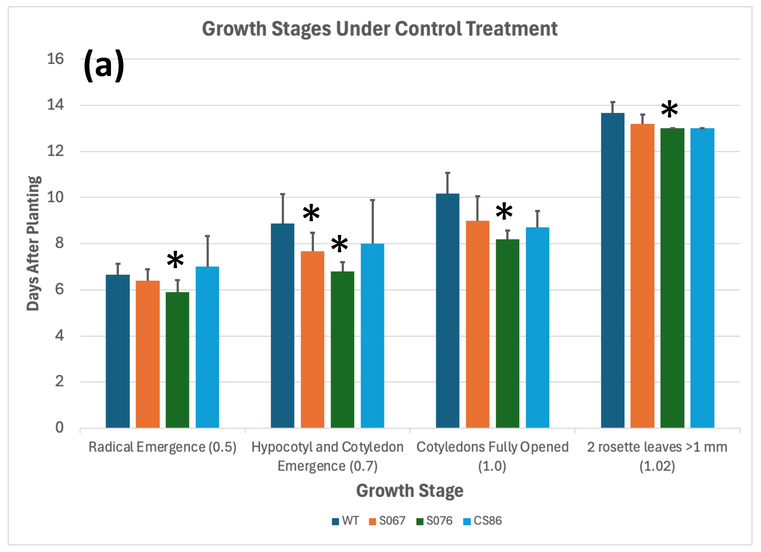


Figure 4. Total wax (a) and cutin (b) amounts in leaves and stems of wild-type and *BDG2* mutants. n = 3; Error bars = SD; * = p<0.05; ** = p<0.01; *** = p<0.001



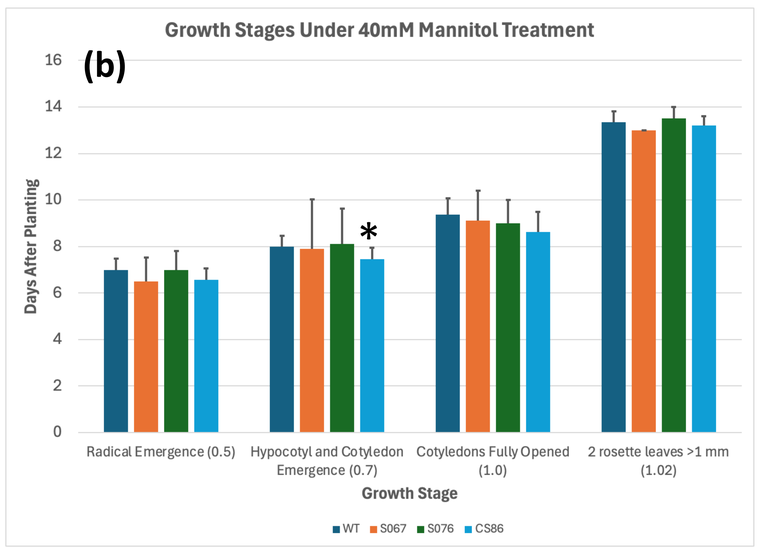


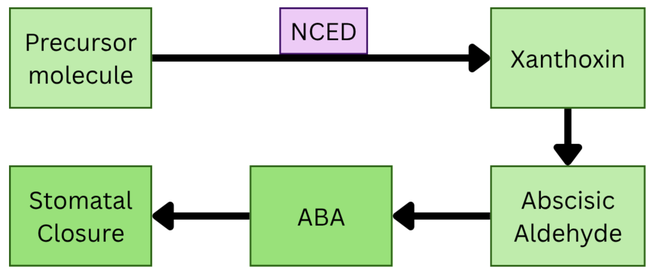
Figure 5. Growth stage phenotypes under control (a) and osmotic stress (40 mM mannitol, b) of WT and *BDG2* mutants. n = 2 to 10; Error bars = SD; * = p<0.05 compared to WT using t-test.

CONCLUSIONS

- BDG2 is required for cutin and wax biosynthesis (Figure 4).
- Osmotic stress induces the expression of *BDG2*mRNA (Figure 2a).
- *BDG2* expression (mRNA) is upregulated by ABA treatment in the leaves and guard cells (Figure 2b).
- Mutants of *BDG2* appear to have an early growth phenotype under control conditions (Figure 5a).
- Osmotic stress appears to inhibit the early growth phenotypes of *BDG2* mutants (Figure 5b).

FUTURE DIRECTION

- Repeat phenotyping experiment with additional levels of osmotic stress treatment to confirm this study's results.
- Compare expression levels in WT and mutant plants under control and osmotic stress of *9-cis-epoxycarotenoiddioxygenase* (*NCED*), which is critical for ABA production under osmotic stress using RT-qPCR.
- Compare ABA levels in mutant and WT plants under osmotic stress using Liquid Chromatography Mass Spectrometry (LC-MS).
- Measure stomatal kinetics in BDG2 mutants and WT:
  - When treated with ABA under control conditions
  - Under osmotic stress conditions
- Complement mutants with WT copy of *BDG2*.
- Determine subcellular localization of BDG2.



Acknowledgements

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