Auxin and brassinosteroid interdependent regulation of stomatal development in maize

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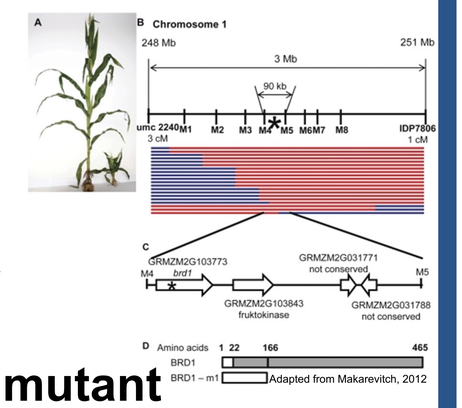
Abstract

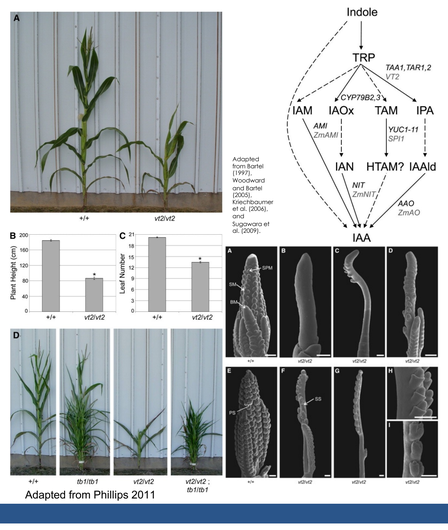
Zea mays (maize) contains pores in the epidermis known as stomates that are important for regulating gas exchange for photosynthesis. Maize, a C4 photosynthetic plant, thrives in warm temperatures by continuing photosynthesis via a spatial separated carbon mechanism within the bundle sheath cells with reduced stomatal aperture. Auxin (IAA) and brassinosteroids (BR) are hormones for growth and development, shown to inhibit the development of stomates in Arabidopsis. The *brassinosteriod deficant1* (*brd1*) mutant is a serve dwarf with low concentrations of BR. The *brd1* mutant is a BR C-6 oxidase, which functions in the final step of BR synthesis. BR regulates stomatal development through the Mitogen Activated Protein Kinase (MAPK) pathway. The *vanishing tassel2* (*vt2*) mutant contains low levels of IAA, exhibiting reduced height, reduced number of leaves, and altered inflorescence development. The *vt2* mutant is a co-ortholog of tryptophan aminotransferase (TAA1) in Arabidopsis. The *vt2* mutant exhibits a decreased stomatal index and density on the abaxial side of the leaf compared to the wild-type plants. The stomatal index also was decreased on the adaxial side of the leaf, although the density was higher compared to the wild-type siblings. The *brd1* mutant displayed a decreased stomatal index and an increased stomatal density on both sides of the leaf compared to the wild type. The double mutant displayed a higher density on the abaxial epidermis of the leaf. Stomatal index and density were higher on the adaxial side of the leaf in *brd1* mutants than in the wild type. A study to determine the cellular effects of BR reduction on IAA polar transport, and the effects of lowered IAA on BR signaling in developing stomata is in progress to further understand the initiation and development of stomata in maize.

The *brd1* mutant

The *Brassinosteriod deficient1* (*brd1*)
- Severe dwarf
- It's an ortholog of a brassinosteroid C-6 oxidase
- encodes the enzyme for the final steps in synthesizing brassinosteroid.
- Exhibits low BR
  - alters leaf and floral morphology compared to its WT sibling

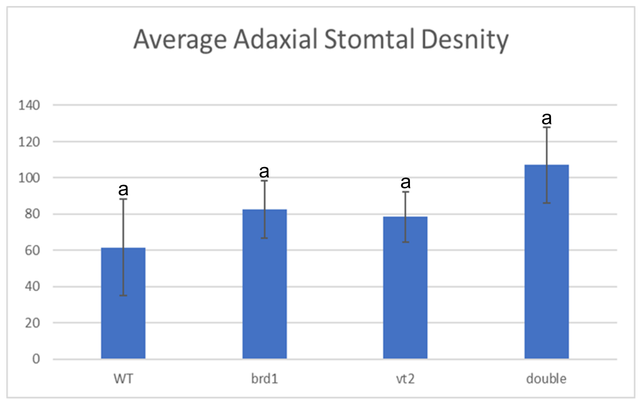


The *vt2* mutant



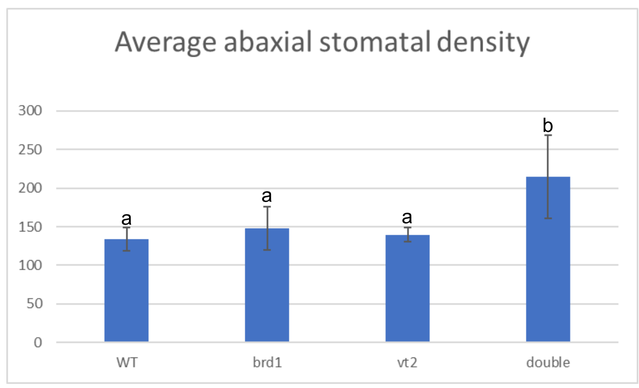
Vanishing tassel2 (*vt2*)
- Co-ortholog of TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1)
- Grass specific
- Reduced height and number of leaves
- Shorter tassels
- No lateral branches produced or functional spikelets
- Smaller ears and fewer kernels

Average stomatal density



P<0.0083

- No statistical significance in average adaxial stomatal index among genotypes



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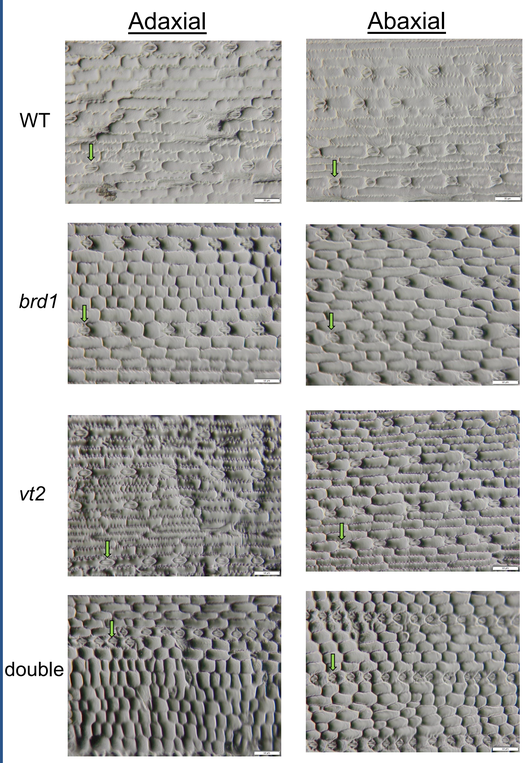
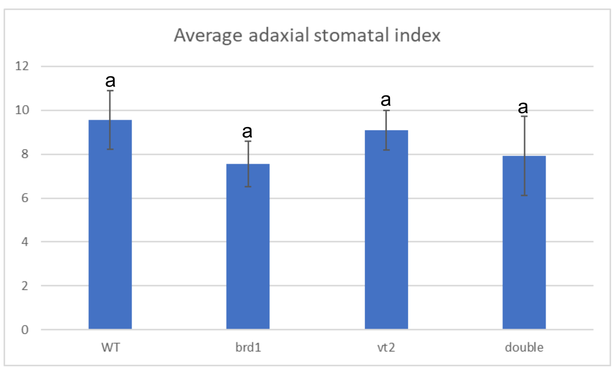
- The double mutant is not statistically different from the wild type sibling

Stomates in *Zea mays*



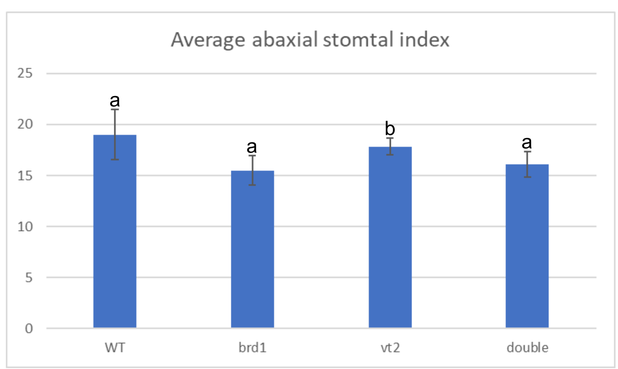
- Pores in the epidermis
- Exchanges CO2, O2 and H20
- Made of guard cells, subsidiary cells, and the cuticle
- Responsible for exchanging gases by changing the pore aperture
- Function in water conservation
- Water vapor leaves through the stomata
- Closed stomates save transpirational water loss in high temperatures
- Maize's (C4) stomates close:
- Utilizes the spatial separated carbon reaction mechanism to continue photosynthesis

Average stomatal index



P<0.0083

- No statistical significance in average adaxial stomatal index among genotypes



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- The *vt2* mutant is significantly different from the WT sibling

Future directions

Determine cellular effects of BR reduction on IAA polar transport

- *vt2* observed with the BES1-YFP marker for assessing BR signaling
- Expects BR pathway in *vt2* to be disrupted resulting in the BES1-YFP to be localized to the cytoplasm near developing stomata
- Determines if altering BR or IAA effects the transport or signaling of the respective hormones
- Test hormones functioning together in controlling stomatal development

The *brd1* mutant segregating in various populations

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Conclusion

Stomates are important for gas exchange which is critical for conservation of transpirational water loss affected by warm temperatures. IAA and BR interdependently regulating the inhibition of developing stomates in maize. There is no statistical significance in the average adaxial stomatal index nor average adaxial density. The double mutant was not significantly different from the WT sibling for average stomatal density. The *vt2* mutant was not significantly different from the WT sibling for average abaxial stomatal index. Further analysis will be conducted to understand the cellular effects of BR reduction on IAA polar transport.

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