Phenotypic characterization of a novel *deregulated anthocyanin pigmentation* (*dap*) mutant in *Medicago truncatula*

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Background

Anthocyanins and proanthocyanins (PAs) are flavonoids that generate shades of red, blue, and purple in many flowers and fruit. Accumulation of these pigments in petals attract pollinators, and in seeds and fruits aid in seed dispersal[1]. Other roles include deterring foragers[2] and protection from biotic and abiotic stress[3]. In addition to the benefits conferred to plants, anthocyanins and PAs have agricultural and pharmaceutical applications. Dietary consumption of plants high in these pigments have been linked to numerous health benefits such as neuroprotective and anti-carcinogenic properties[4].

A large mutant population was created by inserting the tobacco retrotransposon *Tnt1* into the model legume *Medicago truncatula*[5]. By screening approximately 3,000 mutants, many mutants were identified that were defective in anthocyanin and PA pigment production. One of the mutants *deregulated anthocyanin pigmentation* (*dap*) shows increased numbers of reddish anthocyanin spots on both adaxial and abaxial sides of the leaves compared to the wild-type (WT), indicating the misexpression of anthocyanin pigmentation.

This project uses a forward genetics approach to describe the detailed phenotypic characterization and quantification of anthocyanin pigments in the novel *dap* mutant.

Methods

1. Isolation of *dap* mutant
   - ~3,000 *Tnt1* mutants were screened for phenotypes defective in anthocyanin pigmentation
   - *dap* mutant isolated due to novel display of pigment deregulation
2. Plant growth
   - Seeds were scarified → sterilized → vernalized
   - Seed coat removed prior to germination
   - Grown in soil media

Results

Leaf Phenotype

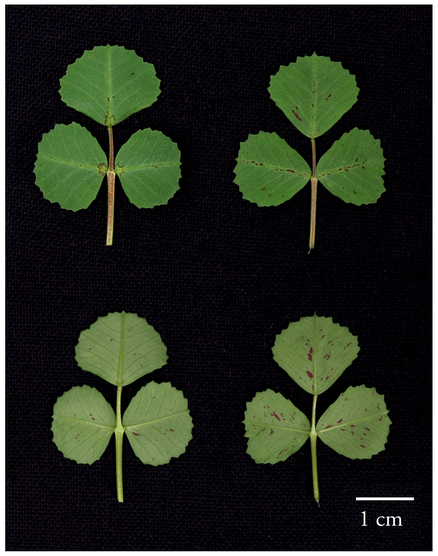

Figure 1. Adaxial (top) and Abaxial (bottom) view of wild-type (left) and mutant (right)

Quantification of Anthocyanin Spots

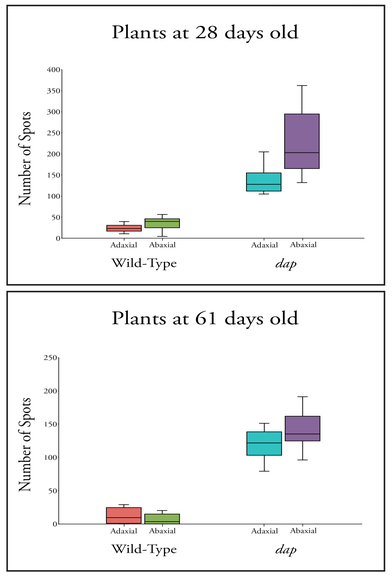

Figure 2. Expression levels of anthocyanin in wild-type versus *dap* mutant as quantified by counting number of spots (n = 10)

Seed Pod Phenotype

Figure 3. Dried seed pods of wild-type (left) and mutant (right)

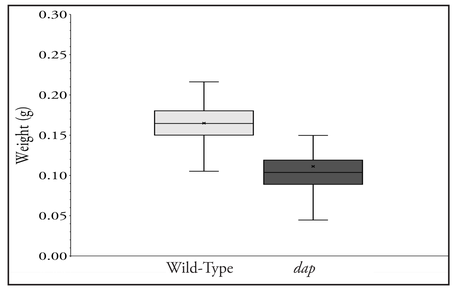

Figure 4. Seed pods from wild-type (n = 100) and mutant (n = 100) plants were weighed to determine differences in mass.

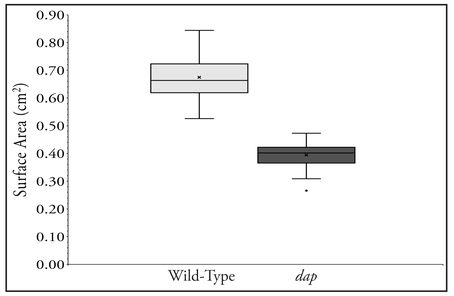

Figure 5. Surface area of wild-type (n = 50) and mutant (n = 50) seed pods were analysed using ImageJ.

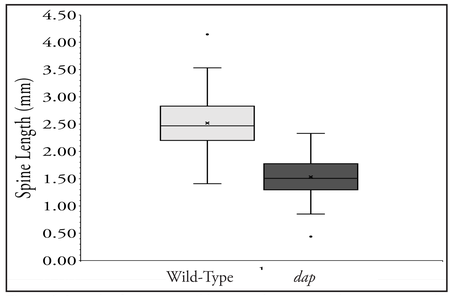

Figure 6. Length of spines on wild-type and mutant seeds were measured using ImageJ from SEM images of seed pods (n = 12).

Whole Plant Phenotype

Figure 7. Whole plant phenotype of young plants, wild-type (left) and mutant (right).

Figure 8. Whole plant phenotype of mature plants, wild-type (left) and mutant (right)

Conclusion

- Wild-type (WT) leaves contain few anthocyanin spots on adaxial and abaxial sides, with a small accumulation at base of leaflet. *dap* mutant displays a scattered pattern with many reddish spots on both sides of leaflets.
- WT seeds are lighter in color with longer, straighter spines on the pod exterior. *dap* seeds are darker with smaller, curved spines on pod exterior.
- WT seedpods are heaver and larger than *dap* seed pods
- WT plants are larger whilst *dap* plants are smaller
- *dap* mutants contain more anthocyanin than WT, however both decrease in anthocyanin content with senescence

Future Work

- Extraction and quantification of anthocyanin pigments
- qRT-PCR to study changes in mRNA expression of biosynthetic & regulatory genes
- Back-crossing of the mutant into the wild-type
- Analysis of whole genome DNA sequencing data to identify causative mutations
- Analysis of RNA sequencing data for differential gene expression between WT and mutant

References & Acknowledgments

[1]Petroni and Tonelli, 2011, Plant Sci 181:219-229
[2]Holton and Cornish, 1995, Plant Cell 7:1071-1083
[3]Lovinich et al., 2014, Planta 240:931-940
[4]Williams et al., 2004, Free Radic Biol Med 36:838-849
[5]Tadege et al., 2008, Plant J 54:335-347

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