Investigating the effects of the sorghum *Bmr2* gene in sugarcane aphid reproduction

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Introduction

- The sugarcane aphid (*Melanaphis sacchari*) is one of the most important insect pests of sorghum (*Sorghum bicolor*) and grain in the United States
- Aphids insert their stylets into the plant tissues and feed on phloem sap. Aphids secrete honey dew on the plants which further promote the growth of sooty mold, overall affects the photosynthetic efficiency of plants.[1]
- 4-coumarate-CoA ligase (4CL) is an enzyme that catalyzes the chemical reactions responsible for the synthesis of various phenolic compounds and flavonoids.[2]

Objective

- To determine the effects of the *Brown midrib 2* gene (*bmr2, 4CL*) on sugarcane aphid reproduction

Materials and Methods

- **Plants and Insects:** We used two-week-old RTx430 (WT) and *bmr2* sorghum plants and wingless adult sugarcane aphids**.**
- **Gene expression studies**: Sorghum plants with and without SCA infestation were collected at specific time points and flash frozen in liquid nitrogen immediately. The samples were further processed for RNA isolations, cDNA synthesis, and qRT-PCR using 4CL and Tubulin (housekeeping) gene primers.
- **Choice assay:** Twenty adult SCA were placed in the middle of a pot containing RTx430 and *bmr2* plants, and then were allowed to choose which plant to feed on. After 6h and 24h, the amount of aphids on each plant were counted in each pot.
- **No-choice assay:** Each plant was infested with 5 adult SCA and covered with plastic cages. Total number of aphids including nymphs and adults were counted after 7 days of infestation.

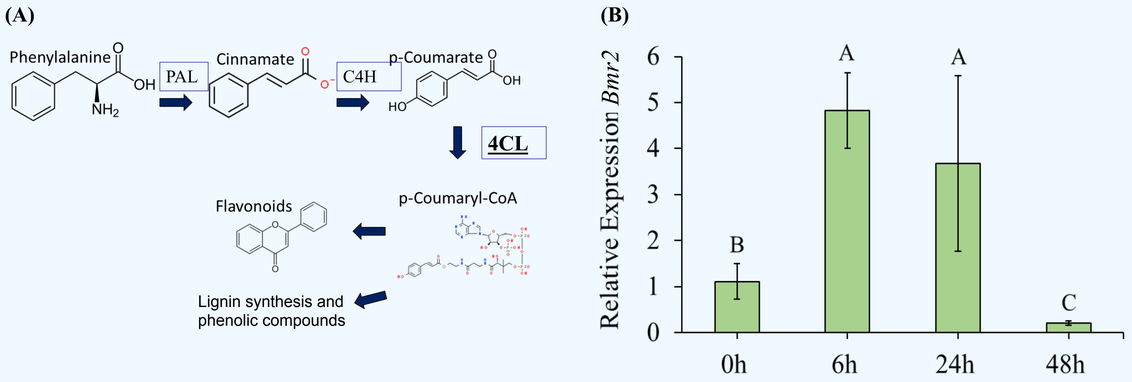

Figure 1: (A) Phenylpropanoid pathway in sorghum plants.[3] Enzyme abbreviations: PAL, phenylalanine ammonia lyase; C4-H cinnamate 4-hydroxylase; 4CL , 4-coumarate:CoA ligase
(B) qRT-PCR analysis of *Bmr2* expression in leaves of sorghum RTx430 plants after SCA feeding for 0, 6, 24, and 48 hours. (n=4) Different letters above the bars indicate values that are significantly different from each other (P<0.05). Error bars represent ± SE

Choice Assay Setup:

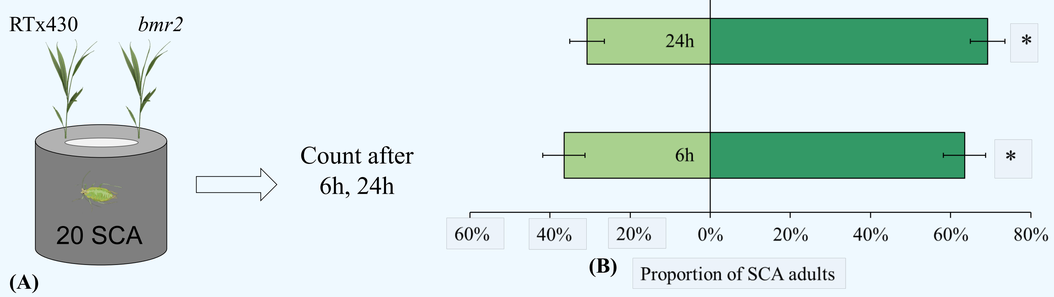

Figure 2: (A) Choice assay bioassay setup
(B) Choice assay comparison of aphid preference for RTx430 v. *bmr2* plants by releasing 20 adult SCA at the center of a pot containing 1 plant of each indicated sorghum line. Proportion of adult SCA that settled on different plants were monitored after 6 and 24 hours post-aphid infestation. (n=15). *indicates values that were significantly different from each other. (P<0.05). Error bars represent ± SE

No-choice Assay Setup:

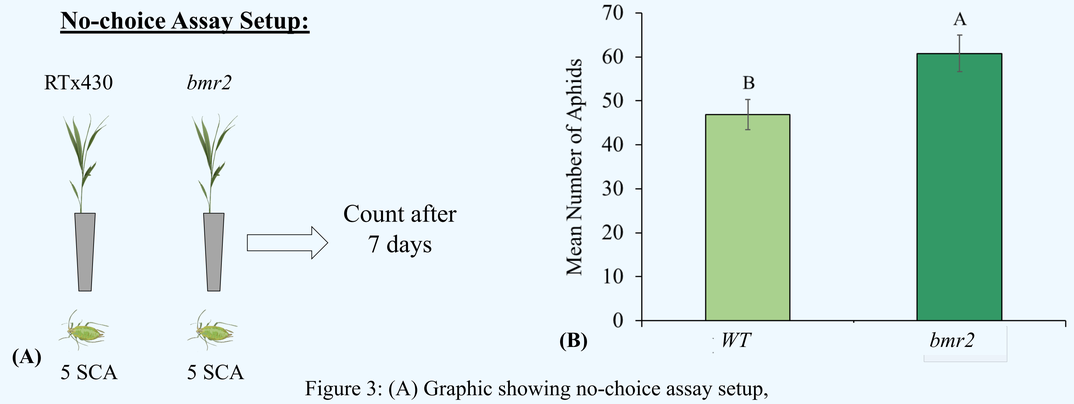

Figure 3: (A) Graphic showing no-choice assay setup,
(B) Total number of SCA adults and nymphs recovered 7 days after 5 SCA adult infestation, (n=15). Different letters indicate significant difference between sorghum plants. (P<0.05). Error bars represent ± SE

Results

- SCA feeding increased the expression level of the *4CL* gene at 6 and 24 hours. Then, at 48 hours, *4CL* expression dropped drastically (Fig 1B).
- In the choice assay, SCA preferred to settle over *bmr2* plants over the WT plants at both 6 and 24 hours (Fig 2B).
- In the no-choice assay, SCA count (adults and nymphs) was significantly higher on *bmr2* plants as compared to WT plants after 7 days of infestation (Fig 3B).

Conclusions

- **Our results clearly show that the *bmr2* gene plays an important role in plant defenses against sugarcane aphids**

Significance

- Our data will help to identify novel sources of insect resistance in sorghum and will help us to develop new targets for pest control. This will significantly reduce our dependence on toxic and harmful insecticides.

Future Research

- Basic study to further explore the role of key genes, signaling networks, and defense pathways that underlie sorghum resistance to sugarcane aphids

Acknowledgements

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References

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3. Scully *et al*., 2016. Overexpression of *SbMyb60* impacts phenylpropanoid biosynthesis and alters secondary cell wall composition in *Sorghum bicolor*. *Plant Journal*, 85: 378–395.