Increased Expression of Antifungal and Insecticidal Flavonoid Phytoalexins in Specialty Maize Lines

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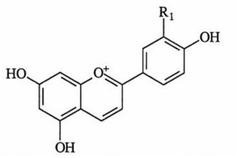
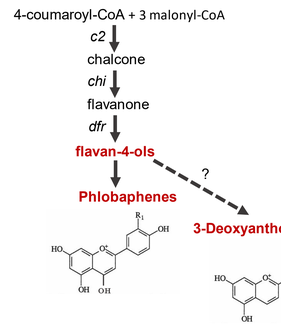
Introduction

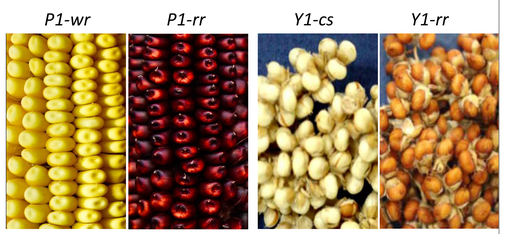


3-deoxanthocyanidins (3-DAs), Apigeninidin: R_1=H and Luteolinidin: R_1=OH

- During the past twenty years of flavonoid research in the Chopra Lab, a genetic pathway has been established to discover the role of flavonoids 3-deoxanthocyanidins (3-DAs) in host plant defense.
- Flavonoids are a ubiquitous group of polyphenols characterized by a three-ring flavone backbone.
- Apigeninidin and Luteolinidin are two 3-DAs

- 3-DAs also polymerize to form the phlobaphenes (red pigments seen at maturity).
- The 3-DF pathway genes are regulated by a *MYB* transcription factor *Pericarp color1* (*P1*) in maize and its sorghum orthologue *Yellow seed1* (*Y1*).
- *P1* and *Y1* share extensive similarity in their coding sequence, but diverge significantly in their promoters
- Further, presence of 3-DAs in maize silks and sorghum leaves were also detected

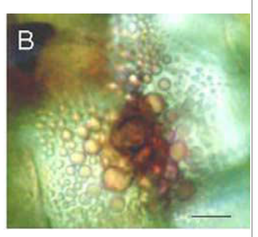




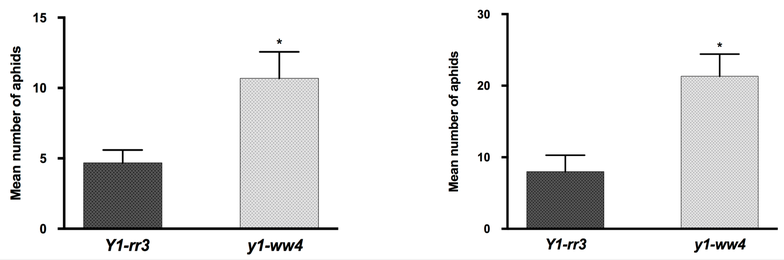
No phlobaphene vs. phlobaphene accumulation in Maize and Sorghum

3-DAs are toxic to foliar fungal pathogens

- Sorghum responds to the invasion of pathogenic and non-pathogenic fungi by inducing 3-DA phytoalexins
- The highest accumulation of these flavonoids is in the vicinity of fungal penetration.
- 3-DAs aggregating in an infected sorghum leaf. The surrounding plant cells are green while the aggregating 3-deoxyanthocyanidin compound produces amber to brown pigments.



CLA population growth is reduced in genotypes carrying functional *Y1-rr* allele



- (A) Juvenile seedlings and (B) adult flowering plants of *Y1-rr* and *y1-ww* genotypes analyzed with non-parametric Mann-Whitney test
- * denotes significance at P<0.05

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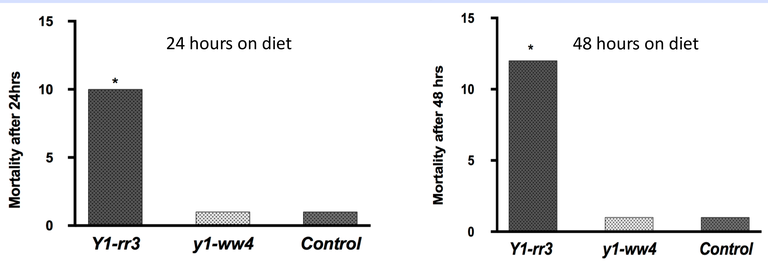
CLA mortality on artificial diet supplemented with flavonoid extracts from *Y1-rr3* and *y1-ww4*



- Non-parametric One-way ANOVA (Kruskal-Wallis) test
- * denotes significance at P<0.05

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Research Objective

- **Production of specialty maize lines for increased 3-DA production**
- **Assaying performance of increased 3-DA production lines against fungus & insects**
- **Application of extracted 3-DAs against fungus & insects**

Materials

Development of increased 3-DA producing maize lines

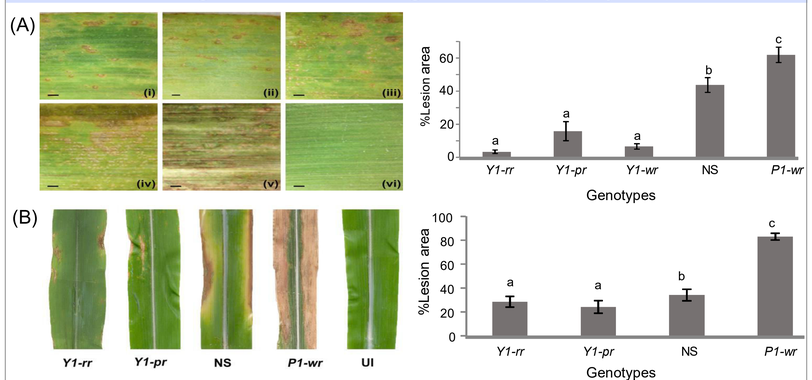
- We developed transgenic maize lines with sorghum *Y1* promoter::*Y1* gene to de novo induce 3-DAs in corn leaves.
- We characterized the response of independent maize transformation events (designated *ZmY1*) as compared to the control that is a negative segregant (NS) carrying no transgene

Maize mutant line for increased flavonoid production

- In maize, spontaneous overexpression mutant of *ufo1* was used. The mutant allele *Ufo1-1* is a dominant modifier of *P1* expression resulting in increased accumulation of flavonoids in pericarp, cob glume, tassel, husk, silk and different vegetative tissues. The stable expresser progeny of this mutant stock is denoted as *U-E*. The *U-E* plants are further used for this study. These plants carry *P1-wr* allele and control plants also carry *P1-wr* but wild type form of *ufo1*. Plants from the same progeny that show wild type phenotypes are denoted as *U-S*.

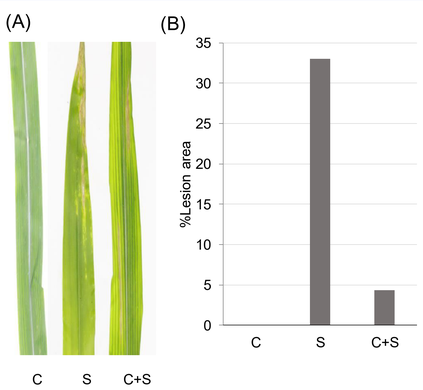
Results & Discussion

Sorghum *Y1* gene enhances resistance against *C. heterostrophus* and *C. graminicola* in transgenic maize (*ZmY1*)



(A). Detached leaf assay shows symptoms 4 dpi when *ZmY1* events were infected with *C. heterostrophus*. (i), *Y1-rr*; (ii), *Y1-pr*; (iii), *Y1-wr*; (iv), NS; (v), *P1-wr*; (vi), un-inoculated *Y1-pr*. Scale bar indicates 1 mm.
(B). Symptoms at 11 dpi when plants were infected with *C. graminicola*.
(C). Quantification of the lesion area 4 dpi with *C. heterostrophus*. Mean ± SE.
(D). Quantification of lesion area 11 dpi with *C. graminicola*. Mean of 44 replicates ± SE.

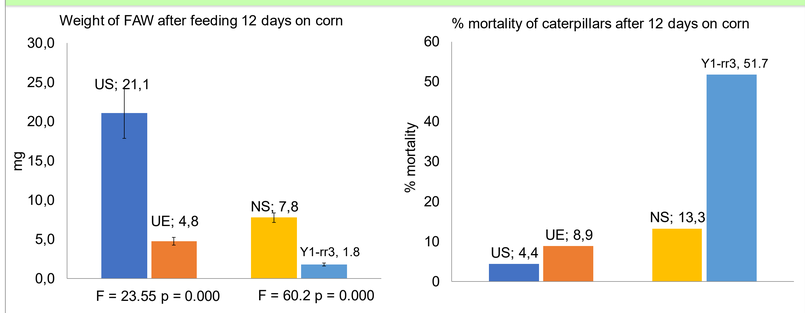
3-DAs can reduce disease incidence in B73 maize infected with *C. graminicola*.



A. Foliar symptoms;

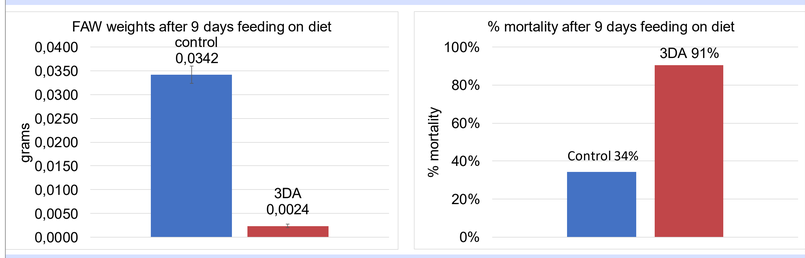
B. Relative leaf area covered by disease lesions. The treatments consisted of C, compound only, S, spores only, and C+S, compound followed by spraying of spores

Results & Discussion

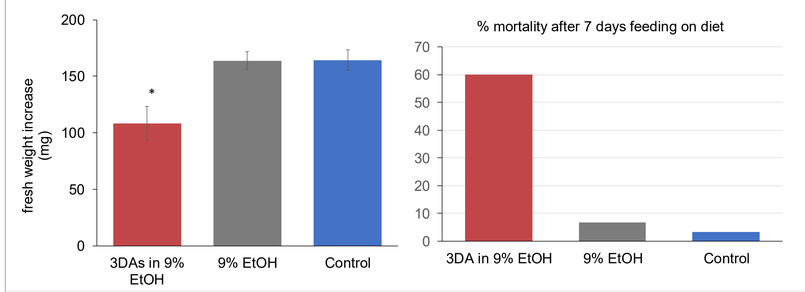


Larval mass (mg). At the end of 12 days is shown on the left y-axis and % mortality on the right y-axis (cross hatch bars).

FAW Larval Survival on Artificial Diet + 3-DAs



Effect of flavonoids from two sets of maize NILs on FAW larvae Detached leaf assay



- The 3-DAs extracted from plants were dissolved in 9% ethanol (EtOH)
- The neonates were fed on B73 leaves and transferred to treatments at third instar
- The three treatments are B73 leaves sprayed and subsequently dried in (1) 3-DAs in 9% EtOH, (2) 9% EtOH and (3) no solvent sprayed.
- Caterpillars were fed on sprayed leaf pieces everyday
- (A) Larval mass (mg) was calculated in the beginning of treatments and at the end of treatments at 7 days. The increase in mass in 7 days was calculated.
- (B) Larval mortality was recorded after 7 days of treatments

Future research direction

- Development of a spray formulation for greenhouse trials
- Evaluating the efficacy of 3-DAs as a botanical in the controlled condition in the greenhouse
- Evaluating the efficacy of 3-DAs in the field to control FAW infestation

References & Acknowledgements

Sorghum 3-Deoxyanthocyanidin Flavonoids Confer Resistance against Corn Leaf Aphid (2019) Rupesh R Kariyat, Iffa Gaffoor, Sampurna Sattar, Cullen W Dixon, Nadia Frock, Juliet Moen, Consuelo M De Moraes, Mark C Mescher, Gary A Thompson, Surinder Chopra, Journal of chemical ecology 45 (5-6), 502-514

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