

# A Gem for the Queen of Forages: *RUBY*in Alfalfa

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# Betalain

- Red food dye
- Bio-available Antioxidant
  - Inflammation (cellular level)
  - Anti-diabetic properties
  - Lowers cholesterol



# Betalain

- Betacyanin and Anthocyanin are mutually exclusive
  - Tyrosine – betacyanin
  - Phenylalanine - anthocyanin
- Found only in Caryophyllales

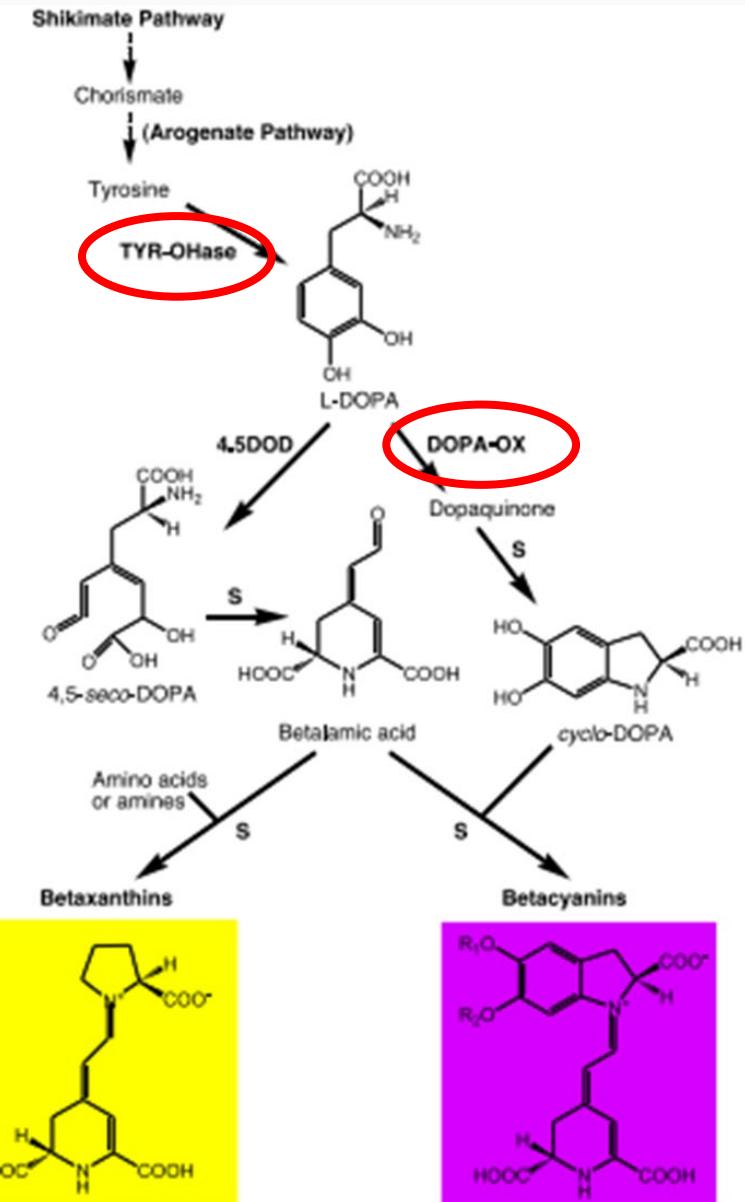


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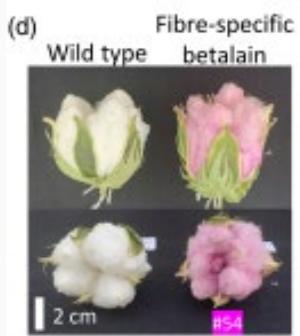
# Betalain production is possible in anthocyanin-producing plant species given the presence of DOPA-dioxygenase and L-DOPA

Nilangani N Harris<sup>1,4</sup>, John Javellana<sup>1</sup>, Kevin M Davies<sup>1</sup>, David H Lewis<sup>1</sup>, Paula E Jameson<sup>2</sup>, Simon C Deroles<sup>1</sup>, Kate E Calcott<sup>1,3</sup>, Kevin S Gould<sup>3</sup> and Kathy E Schwinn<sup>1\*</sup>



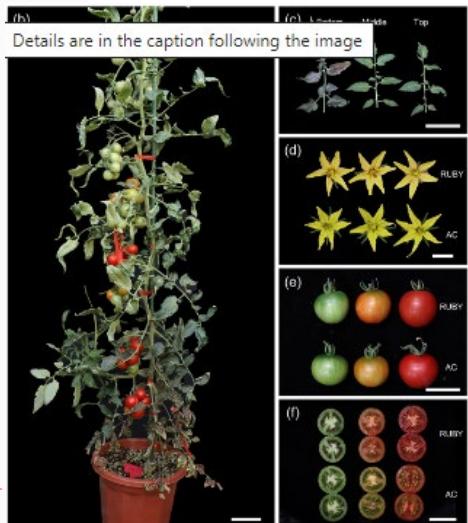
# RUBY-

## Cotton

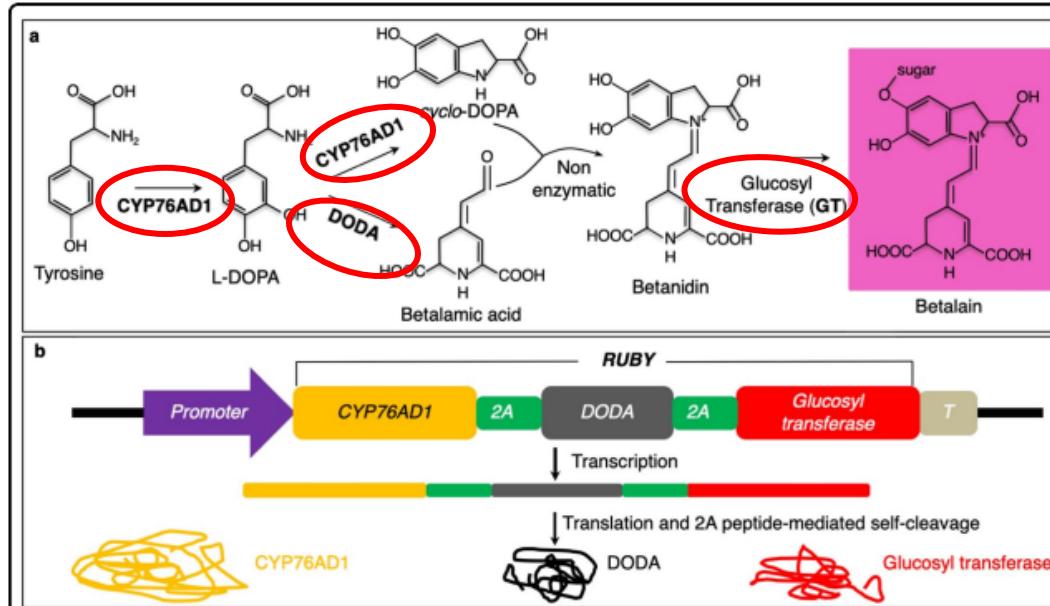


Li et al. 2023 Plant Biotech J.

## Tomato



Wang et al. 2023 Plant Biotech J.



**Fig. 1 Components required for betalain biosynthesis. a** Chemical reactions for converting tyrosine into betalain, which has a red color. Tyrosine is first oxidized by the P450 CYP76AD1 into L-3,4-dihydroxyphenylalanine (L-DOPA), which can be further converted into cyclo-DOPA by P450 CYP76AD1. In the presence of L-DOPA 4,5-dioxygenase (DODA), DOPA is oxidized and circularized into betalamic acid. Cyclo-DOPA condenses with betalamic acid, a non-enzymatic reaction, to produce betanidin. Glycosylation of betanidin generates the red color betalain. **b** A strategy for expressing the whole betalain biosynthetic pathway in a single cassette. The three betalain biosynthetic genes were fused into a single open reading frame, which can be expressed using a single promoter and terminator. Between the genes, sequences that encode 2A peptides were inserted. The 2A peptides undergo self-cleavage to produce functional proteins. The CYP76AD1 gene can be placed under the control of a promoter or terminators. The DODA gene's unit can be placed under the control of 2A-linked betalain biosynthesis genes name



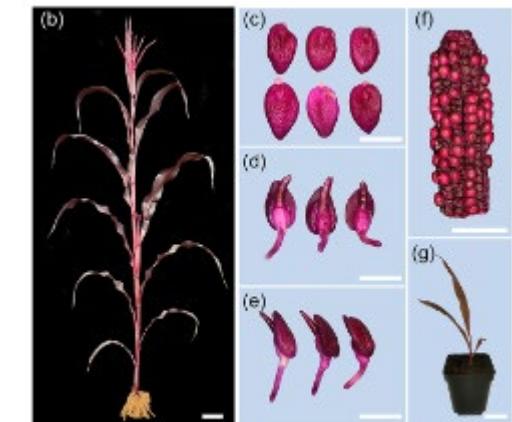
Deng et al. 2023 Hort. Research

## Rice



Tain et al. 2020 New Phyto.

## Corn



Wang et al. 2023 Plant Biotech J.

## Carrot

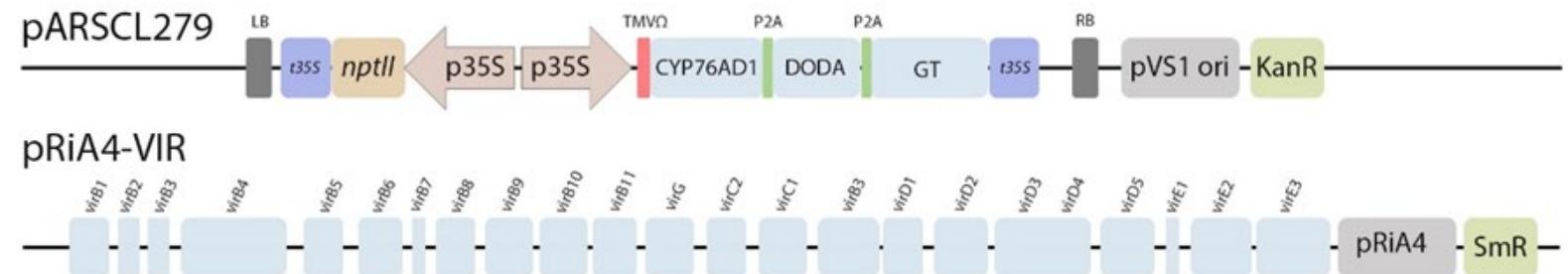


# RUBY

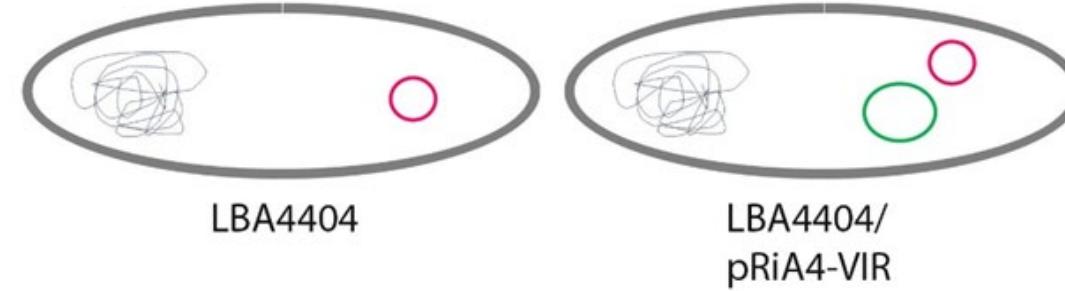
C



A



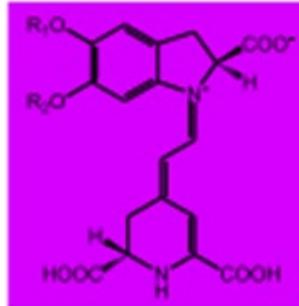
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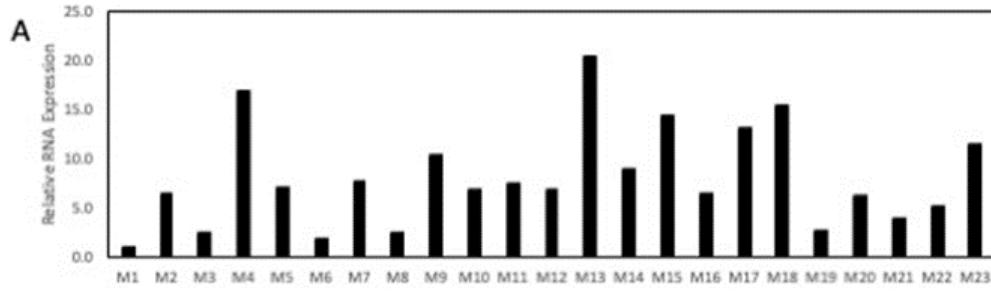
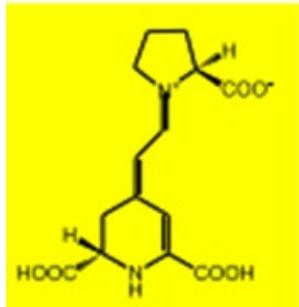
# Expression vs Concentration



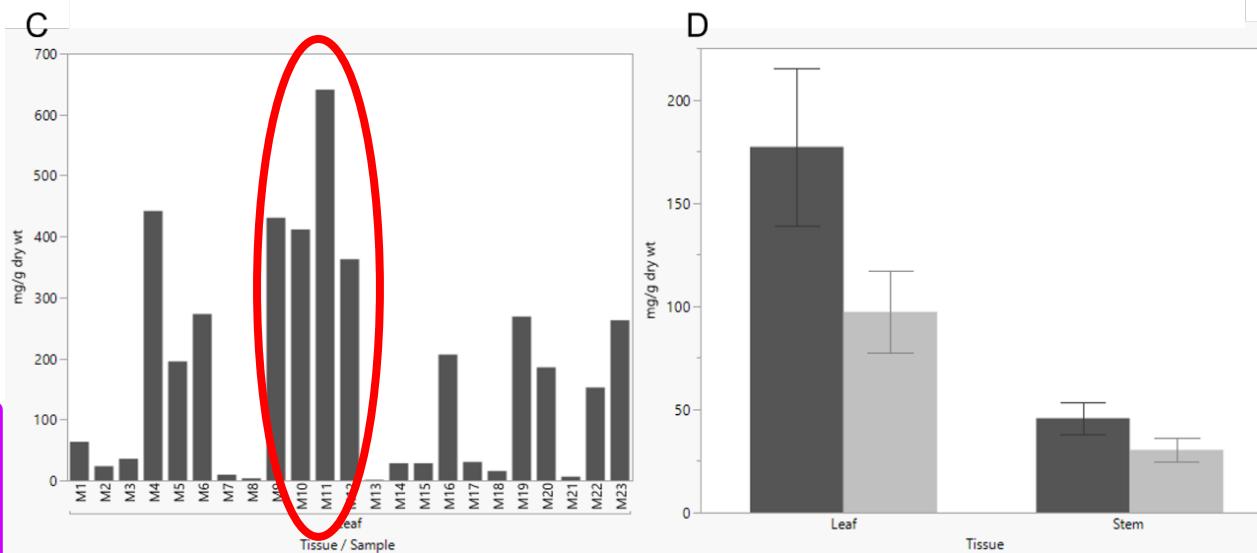
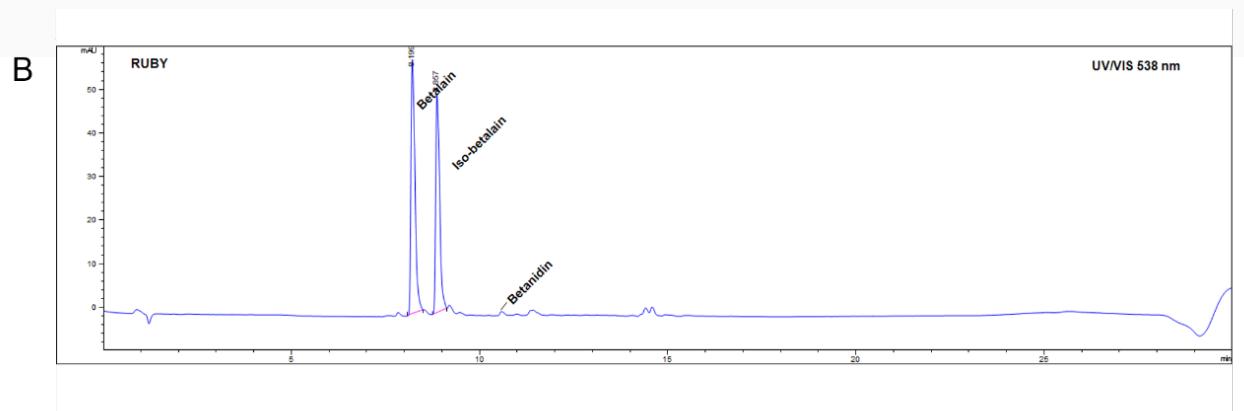
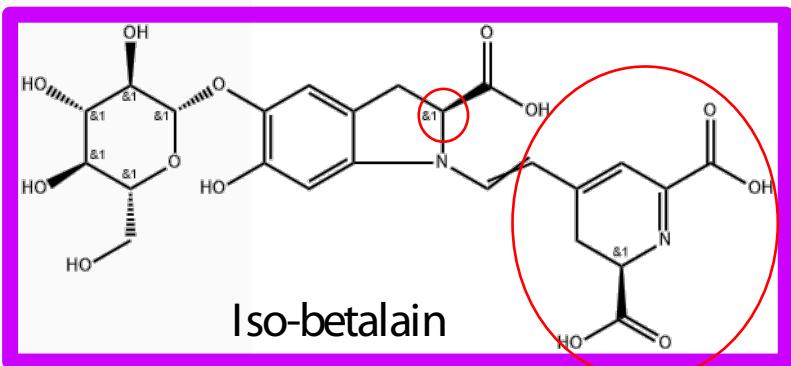
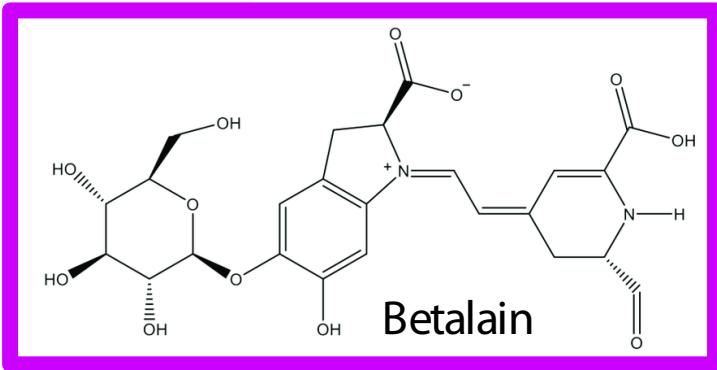
Betacyanins



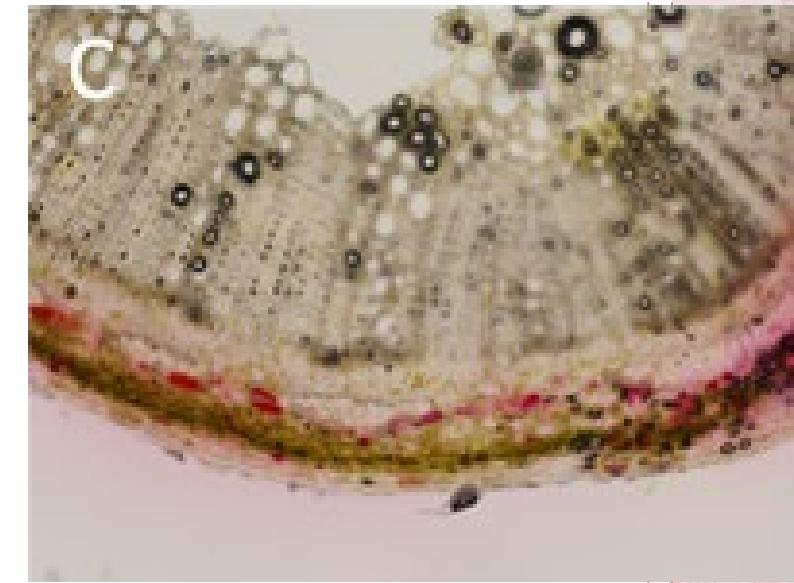
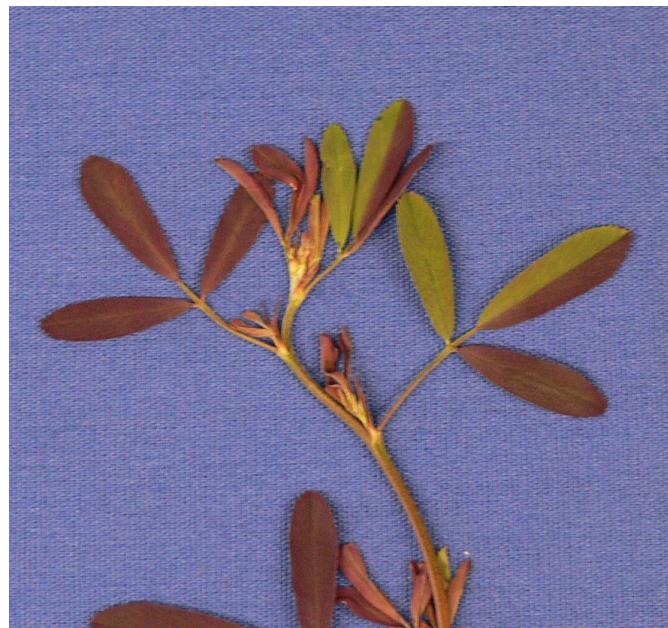
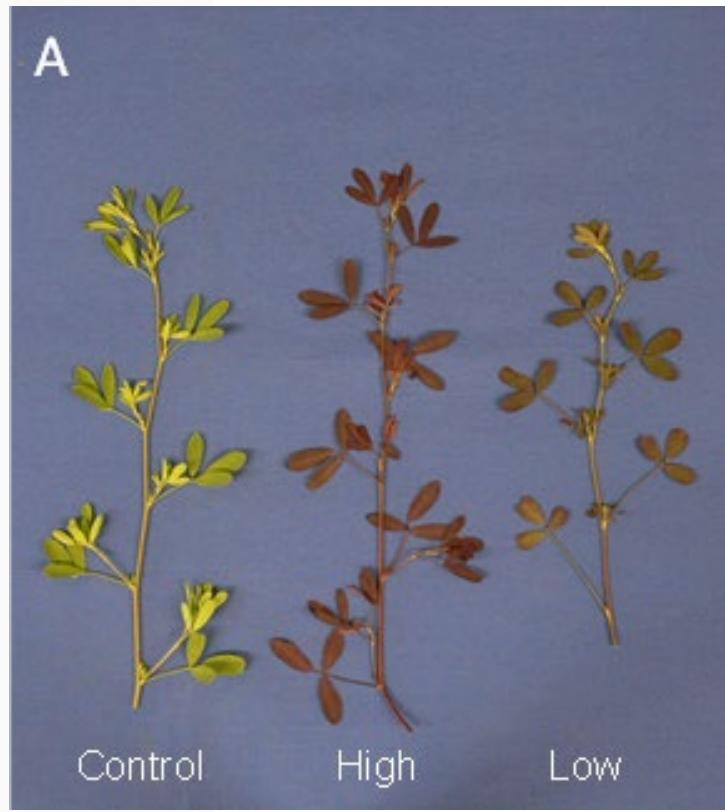
Betaxanthins



# Betacyanin



# Alfalfa's Gem



# Alfalfa's Gem



# Potential Uses

## Scientific

- Marker
  - Transgenic
  - Field



## Commercial

- Native source of Betalain
  - Dye
  - Bio-Available Antioxidant
  - Interaction with saponins
- Stress tolerance
  - Salt
  - Drought
- Reduction in enteric methane

# Thank You

- Mindy Dornbusch (Samac Lab)
- Curtin Lab

