



# Genetic analysis of bract colour formation in Poinsettia (*Euphorbia pulcherrima* Willd. ex. Klotzsch)

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## *Euphorbia pulcherrima* Willd. ex Klotzsch

- Euphorbiaceae family
- Cassava (*Manihot esculenta*) and the castor oil plant (*Ricinus communis*)
- **Mexico** as center of origin
- Genus *Euphorbia* contains ~2000 species

picture  
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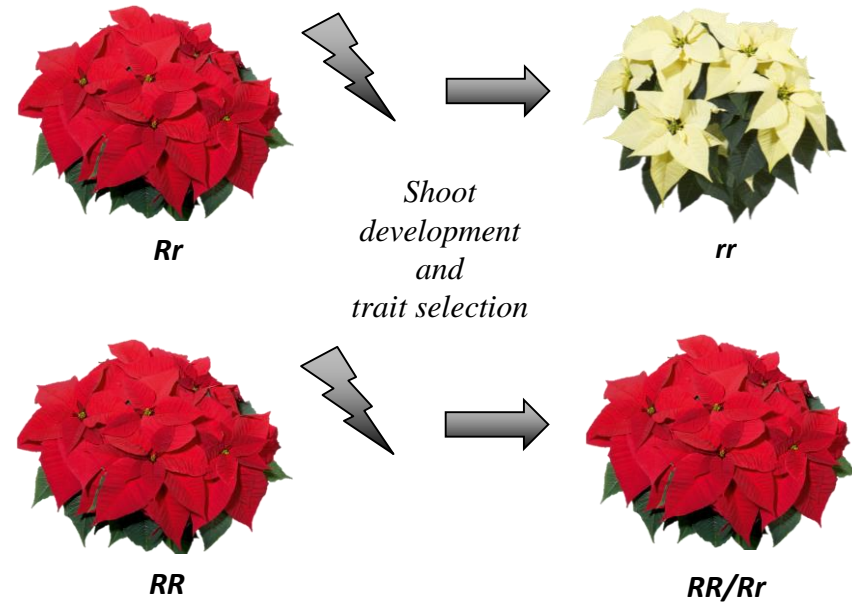
<https://bsapubs.onlinelibrary.wiley.com/doi/full/10.3732/ajb.1200072>

(TREJO ET AL. 2012)



## *Euphorbia pulcherrima* Willd. ex Klotzsch

- Euphorbiaceae family
- Cassava (*Manihot esculenta*) and the castor oil plant (*Ricinus communis*)
- **Mexico** as center of origin
- Genus *Euphorbia* contains ~2000 species
- **Breeding** → Mutagenesis (X-ray irradiation)
- **'White paradox'** →



Expression of genes and enzyme activities involved in the formation of anthocyanin

Vilperte et al. *BMC Genomics* (2019) 20:900  
https://doi.org/10.1186/s12864-019-6247-3

BMC Genomics

RESEARCH ARTICLE

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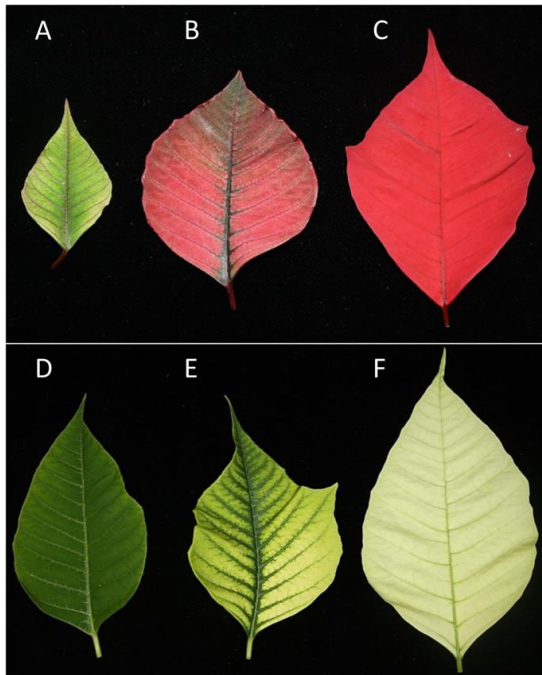


## Hybrid de novo transcriptome assembly of poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotsch) bracts

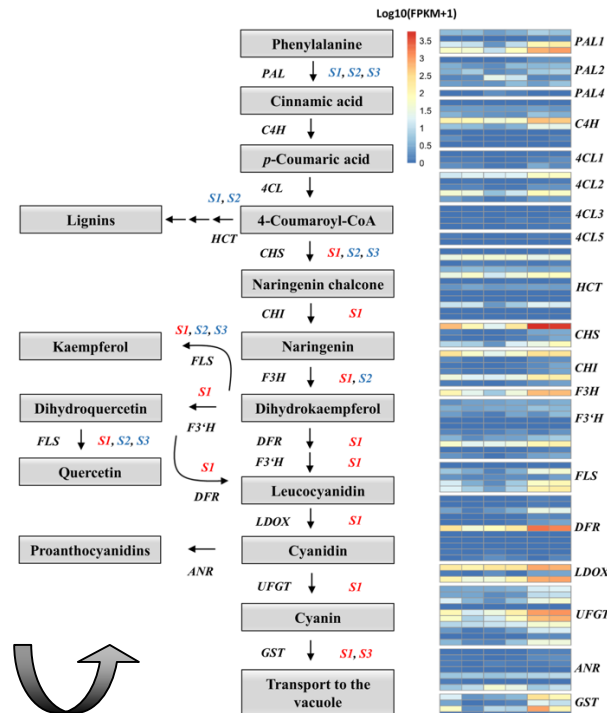
Vinicius Vilperte<sup>1,2†</sup>, Calin Rares Lucaci<sup>3†</sup>, Heidi Halbwirth<sup>4</sup>, Robert Boehm<sup>2</sup>, Thomas Rattei<sup>3\*</sup> and Thomas Debener<sup>1\*</sup>

First poinsettia transcriptome

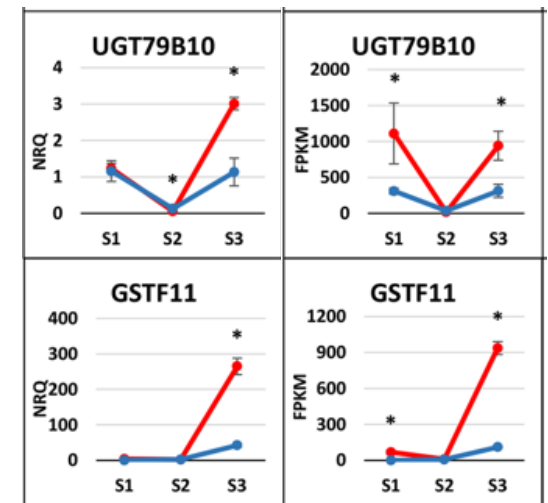
Use in genetics and plant breeding



**Fig. 1:** Bracts of red and white poinsettia varieties for three color developmental stages.



**Fig. 2:** Anthocyanin biosynthetic pathway and expression of related genes during bract development.



**Fig. 3:** RNA-Seq and RT-qPCR expression profiles during bract development.

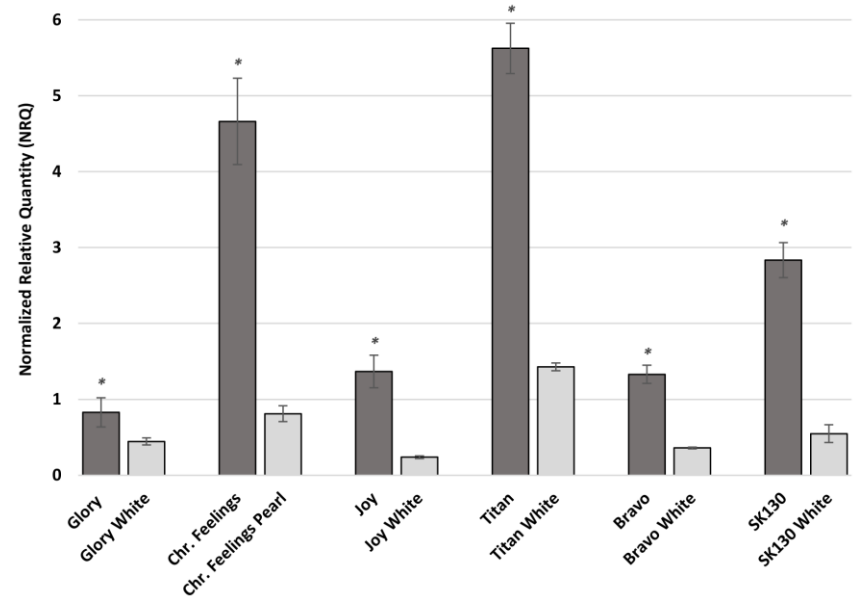


## Major detoxifying enzymes

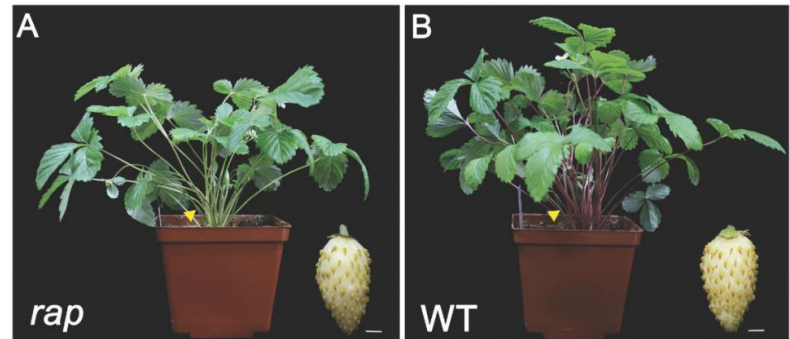
- Abiotic and biotic stress modulation pathways (ISLAM ET AL. 2017)
- **Anthocyanin-related GSTs**
  - *Z. mays* (*Bz2*) (MARRS ET AL. 1995)
  - *A. thaliana* (*TT19*) (KITAMURA ET AL. 2004)
  - *P. hybrida* (*PhAN9*) (ALFINETO ET AL. 1998)
  - *D. caryophyllus* (*fl3*) (LARSEN ET AL. 2003)
- **Mutations**



(CHENG ET AL. 2015)



**Fig. 4:** RT-qPCR of *EpGST* for 12 red and white poinsettia varieties.



(LUO ET AL. 2018)

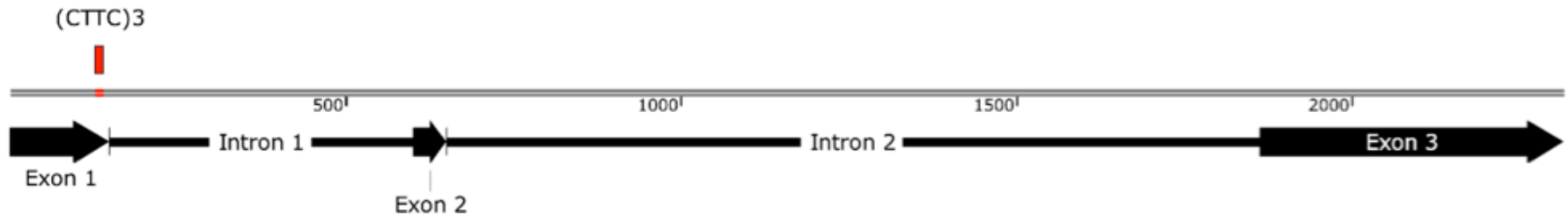
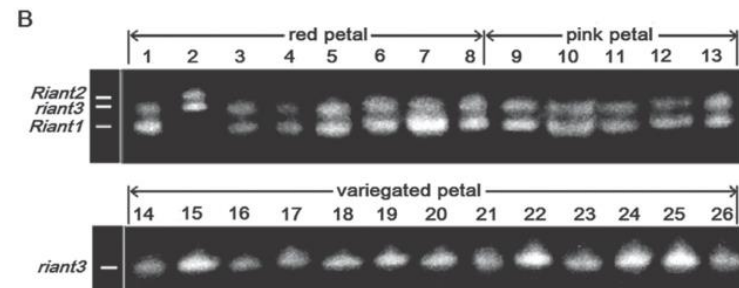
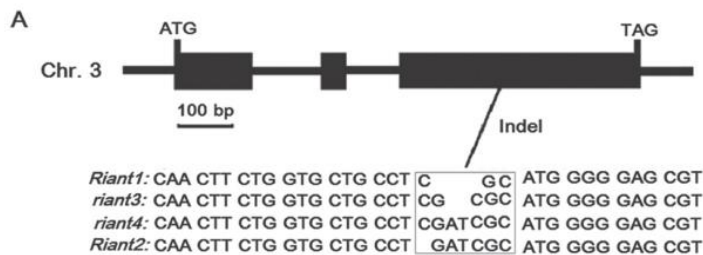
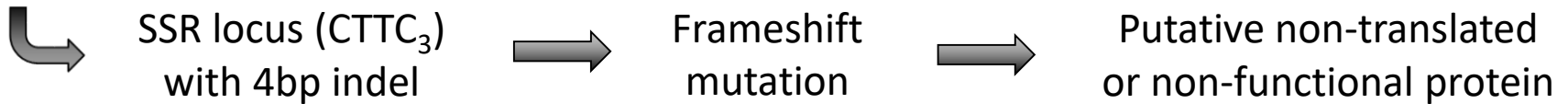


Fig. 5: Schematic representation of the full-length sequence (2314 bp) of the *Euphorbia pulcherrima* GST gene (*EpGST*).

- 2 introns and 3 exons → CDS of 645 bp
- Similar structure to *AtTT19* and *PhAN9*



(CHENG ET AL. 2015)



# GST genotyping

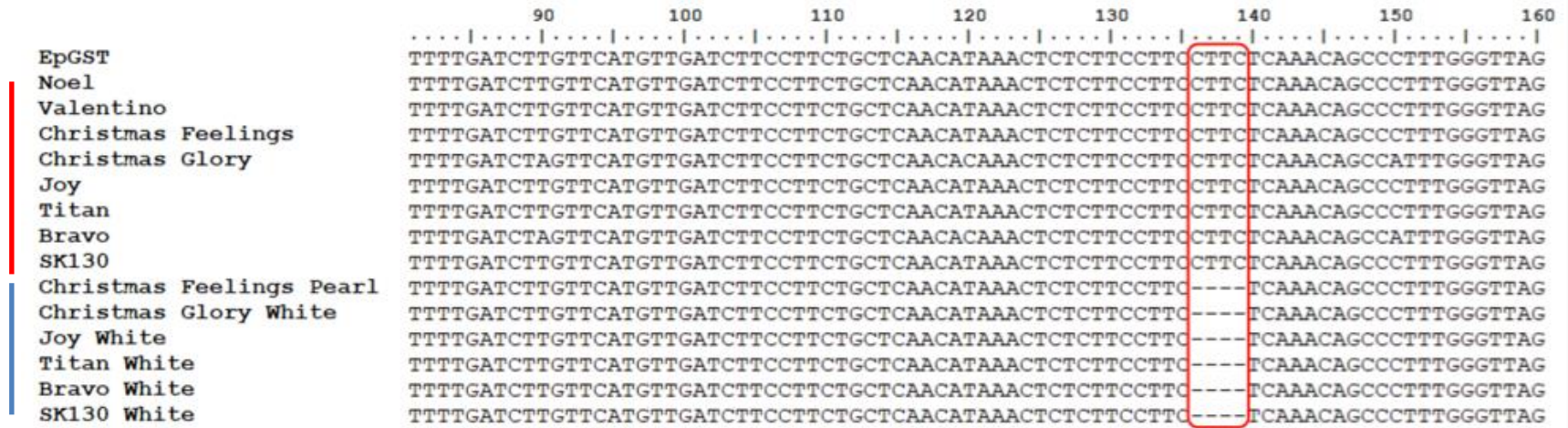
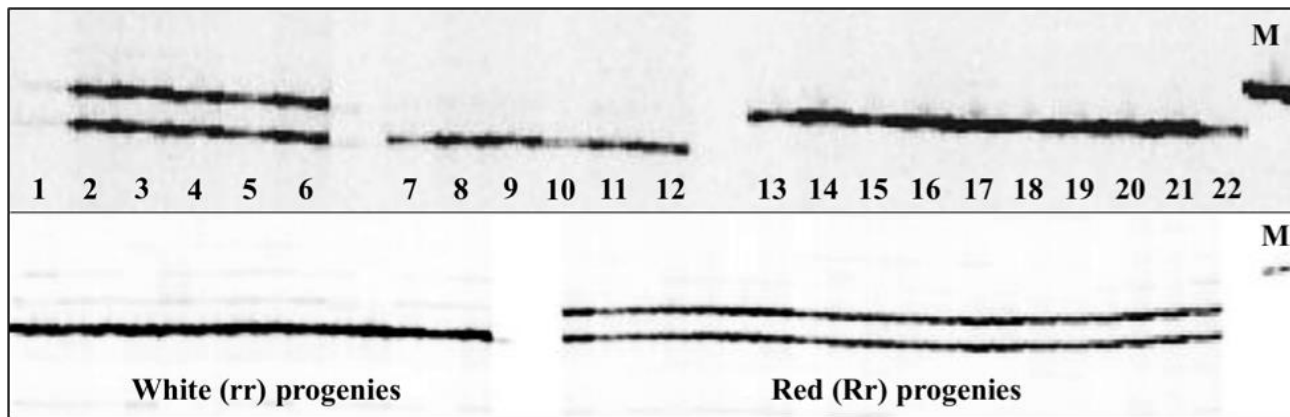


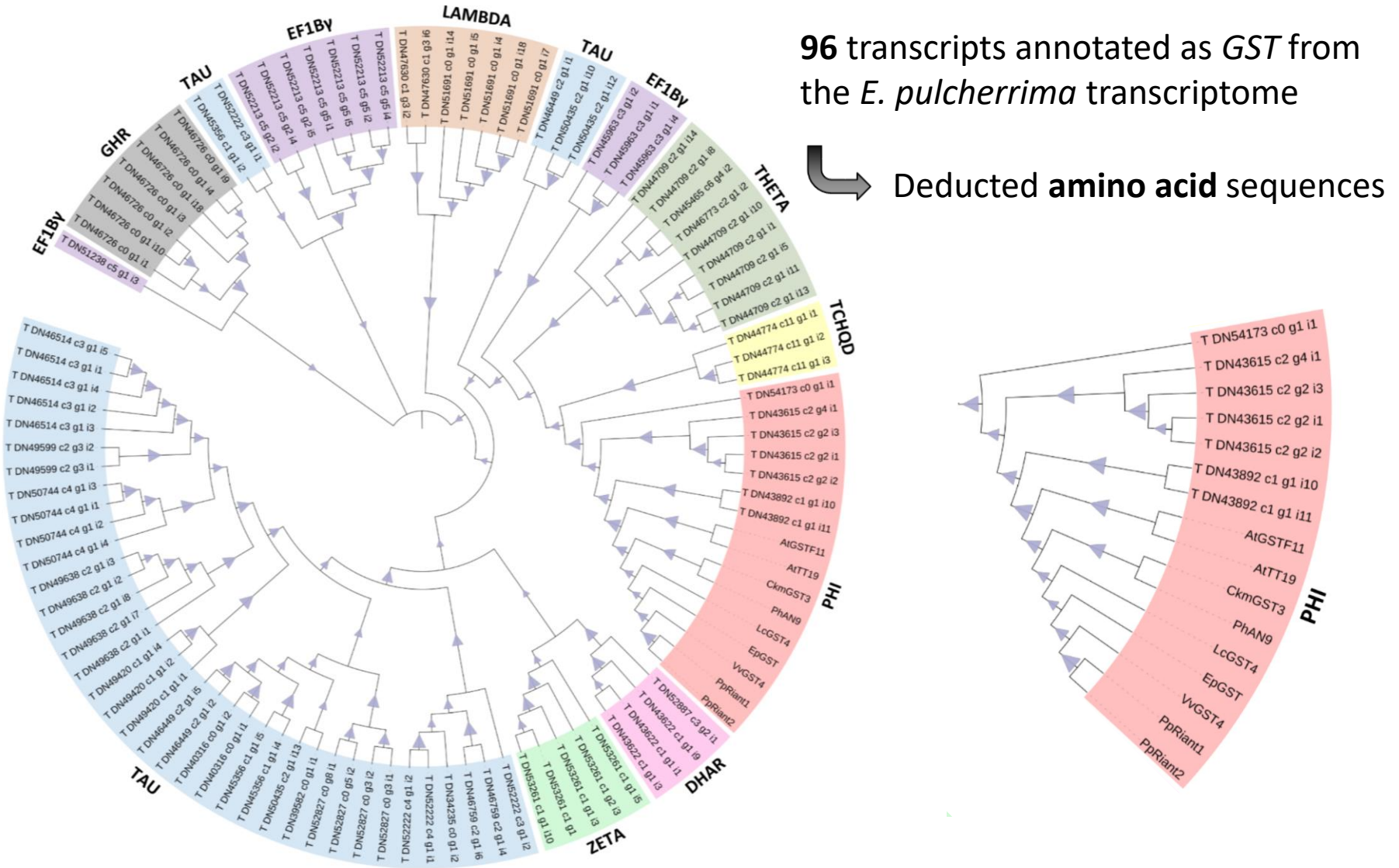
Fig. 6: Alignment of the *EpGST* CDS for 12 red- and white-bracted poinsettia genotypes.



Joy (Rr) x Joy white (rr)



Fig. 7: Genotyping of the 4 bp *indel* in the *EpGST* gene for red and white poinsettia genotypes.



**Fig. 8:** Neighbour-joining tree of poinsettia *GST* genes and known anthocyanin-related genes. Maximum Likelihood as statistical method and 1000 bootstraps.





# Functional complementation – *tt19*

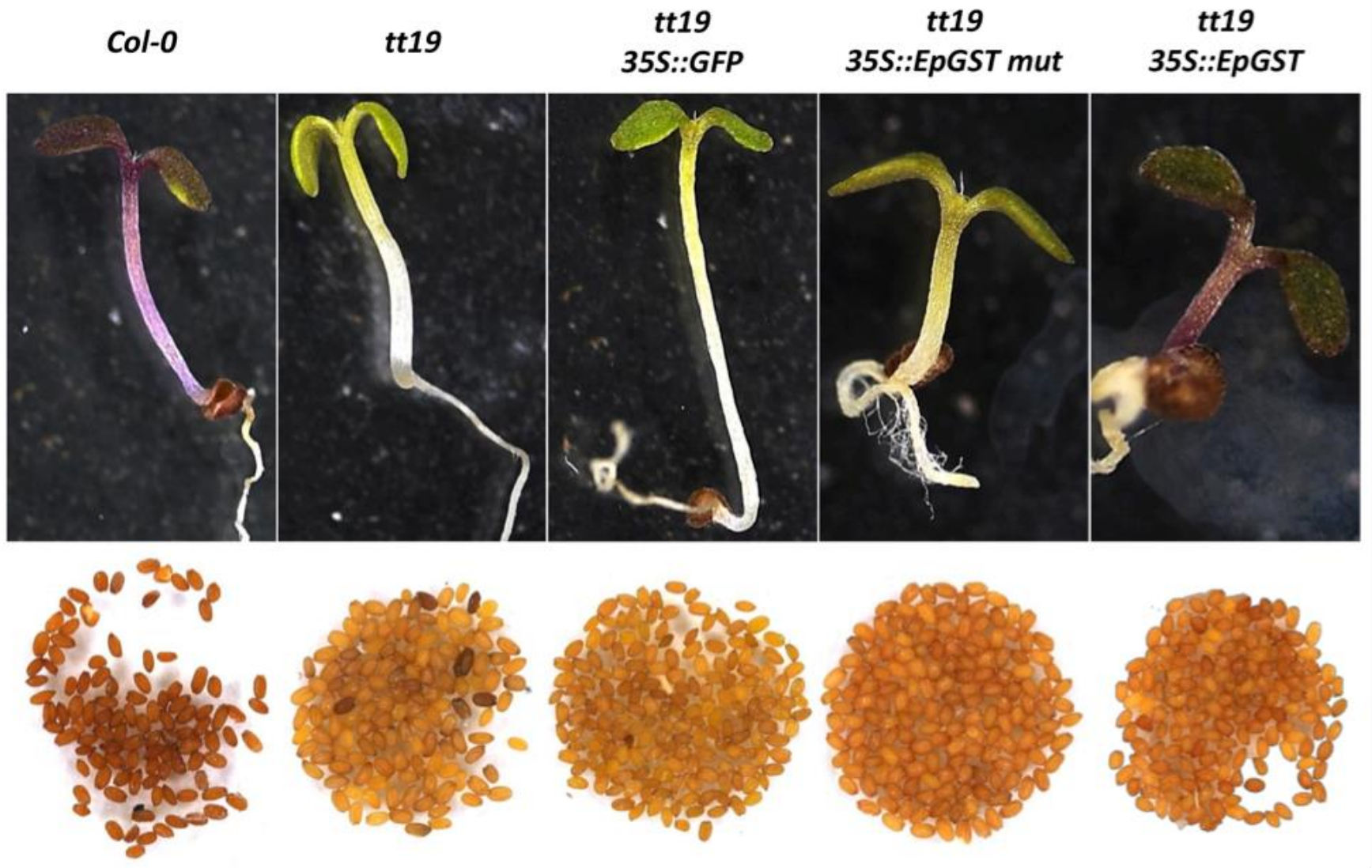


Fig. 9: Functional complementation of Arabidopsis *tt19* mutant with the *EpGST* gene



## KASP Assay

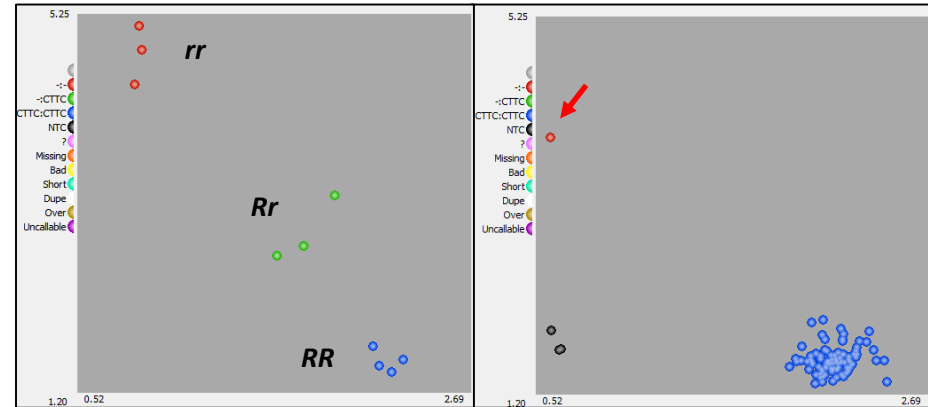
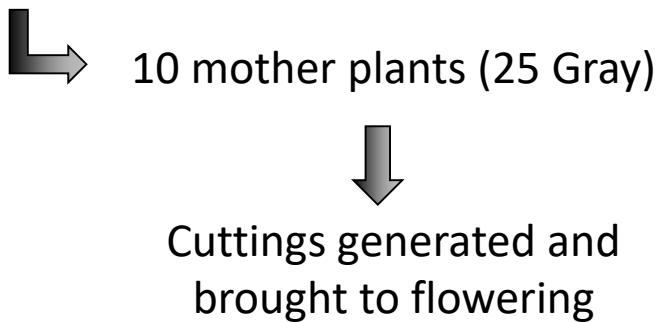
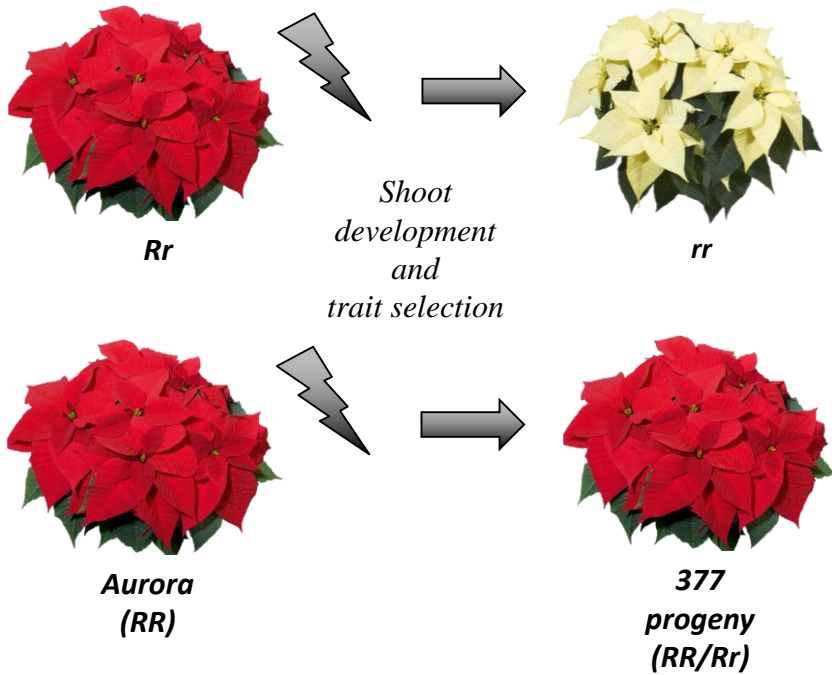


Fig. 10: KASP assay for the 4 bp indel in the *EpGST* gene.

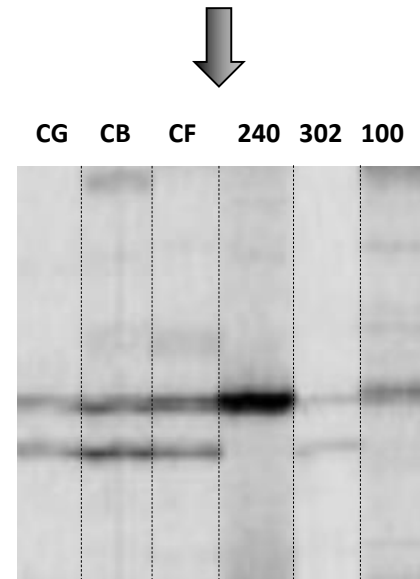


Fig. 11: Genotyping of the 4 bp indel in Aurora progeny.



## Amplicon-Seq

Variety	Total coverage (#reads)	Zygoty
Chr. Feelings	468,931	Rr
Chr. Feelings Pearl	392,563	rr
Valentino	405,905	RR
Vintage	325,598	RR
Aurora	513,140	RR
Aurora P1	448,446	RR
Aurora P2	465,673	RR
Aurora P3	488,369	Rr
35S:: <i>EpGST mut</i>	373,687	rr
35S:: <i>EpGST</i>	538,723	RR

Table 1: Summary of amplicon sequencing.

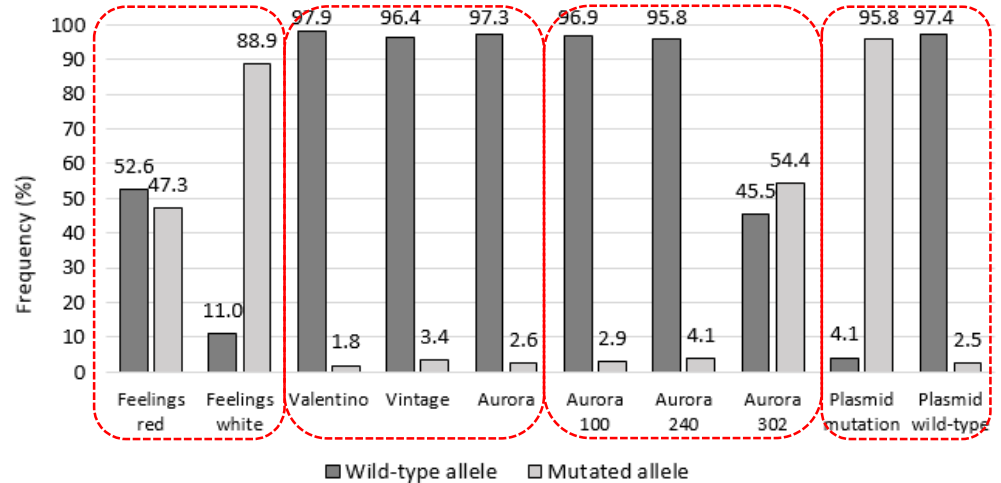


Fig. 12: Distribution of wild-type and mutated alleles from the *EpGST* for different poinsettia genotypes.

## Fragment Length Analysis

SK159 Dark Pink (RR) with 525 progeny

**3 heterozygous progeny out of 190**

Aurora Jingle (RR) with 750 progeny

**3 heterozygous progeny out of 188**

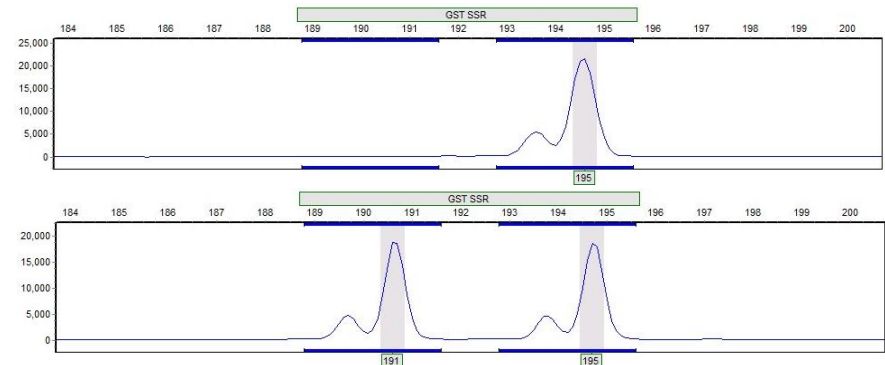


Fig. 13: Example of fragment length analysis for the progeny of irradiated homozygous varieties.

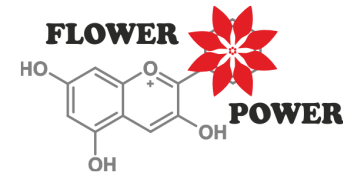


- 1) Identified and characterized the **anthocyanin-related GST** in poinsettia
- 2) Identified a **4bp indel** highly related to the white phenotype
- 3) Developed a **molecular marker** to select promising genotypes for poinsettia breeding
- 4) High **similarity** with other anthocyanin-related GSTs
- 5) Functional analysis in **Arabidopsis tt19** mutant
- 6) Identified the mutation arising from **homozygous red varieties**



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# Thank you for the attention.