STRUCTURAL and FUNCTIONAL GENOMICS APPLIED to an ANTHOCYANIN-FREE EGGPLANT GENOTYPE for the ANALYSIS of COLOUR REGULATION in PEEL

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RATIONALE

- In **eggplant (*Solanum melongena* L.)** purple fruit varieties, **anthocyanins** are synthesized in the **fruit peel**.
- In a previous study(1) a green-fruited eggplant plant **'Green Beauty' (GB)** originated by spontaneous mutations from the violet-fruited **'Black Beauty' (BB)** was identified (**Fig. 1**).
- With the goal to elucidate the molecular mechanisms responsible for the berry green phenotype, **peel RNAseq** and **genome re-sequencing** analyses were performed in both the **BB** and **GB**.

DIFFERENTIAL EXPRESSION ANALYSIS

- RNA sequencing data were used to obtain the annotated de-novo transcriptome of BB (**Fig. 2.1, 2.2**).
- GB and BB clean reads were then aligned on the annotated transcriptome and the differential expression analysis (DEA) were performed using **GFOLD** (**Fig. 2.3, 2.4**).
- The DEA showed a set of 7,458 differentially expressed genes (DEGs, ±1 fold) in the peel of GB in respect to BB, of which 68.76% were up- and 31.24% down-regulated (**Fig. 3**).

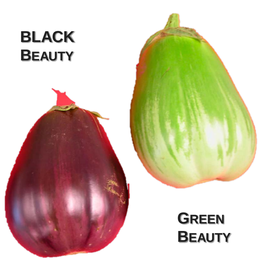

Fig. 1. *'Black Beauty' (BB, left) and 'Green Beauty' (GB, right) fruit.*

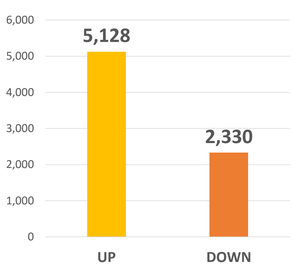

Fig. 3. *Differentially expressed genes (DEGs) between GB and BB*

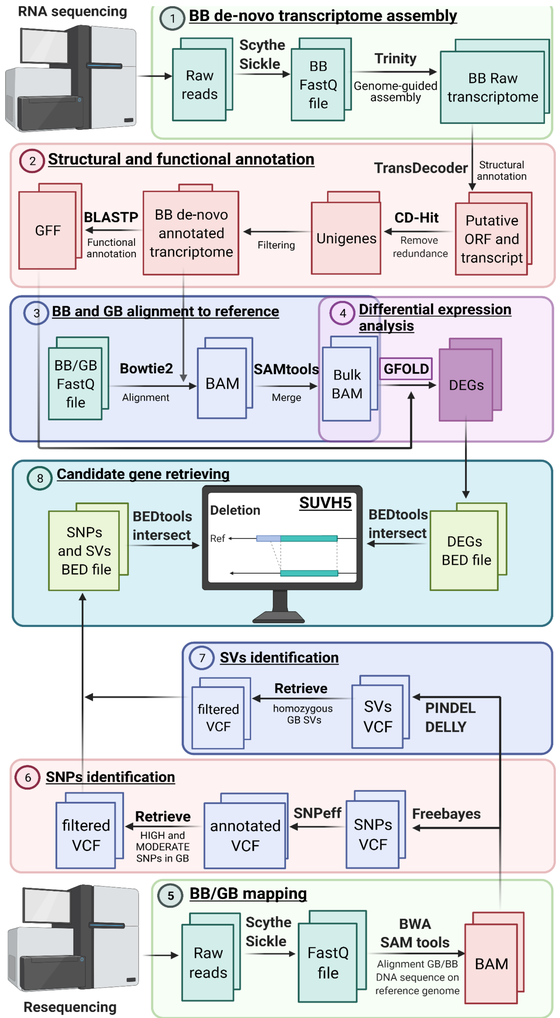

Fig. 2. **Bioinformatic pipeline**

Literature

1 - Gisbert et al., 2016, Hortscience 51(7):793–798
2 - Fan et al., 2018, Plant J., 96(6):1121-1136,
3 - Zhang et al., 2002. Cell,111(1):117-27

SNPs/SVs IDENTIFICATION

- Resequencing data (**Fig. 2.5**) were used for the **Freebayes** analysis (**Fig. 2.6**) which highlighted a set of 842 moderate impact SNPs/indels (59 in DEGs) and 97 deleterious SNPs/indels (4 in DEGs) (**Fig. 4a**).
- Structural variation (SV) analyses (**Fig. 2.7**), performed with **Pindel** and **Delly,** revealed 20,528 SV (2,149 in DEGs) and 268 SV (77 in DEGs) respectively (**Fig. 4b**).

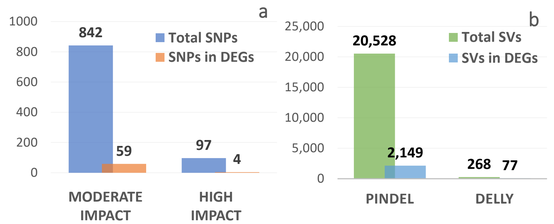

Fig. 4. **Polymorphisms** in **DEGs**. 4.a) Moderate and high impact SNPs identified in DEGs. 4.b) Structural variation (SVs) identified in DEGs.

CANDIDATE GENES AND SEQUENCE VALIDATION

- Joining SNPs/SVs and DEGs, and selecting only homozygous mutations, 5 SNPs and 1 SV were identified.
- Due to evidences previously reported in literature(2), the histone H3 lysine 9 methyltransferase SUVH5 was selected as candidate gene (**Fig. 2.8**).
- In **GB**, the SUVH5 gene showed a deleterious deletion of 591 bp located downstream the gene (**Fig. 5**).

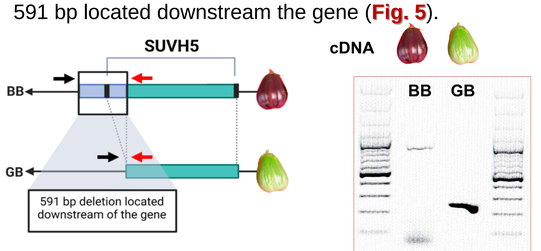

Fig. 5. **SUVH5 deletion** in **GB** and its **validation** by **PCR**.

SUVH5

- The SUVH5 protein contributes to the maintenance of histone H3 methylation at the level of lysine 9 (H3K9me1/me2) and to the CMT3-mediated methylation of DNA of non-CG sites (**Fig. 6**) .

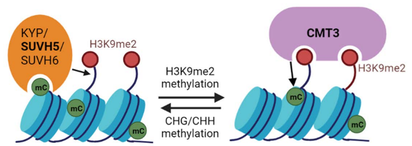

Fig. 6. **Epigenetic mechanism regulation** of **SUVH5 protein**.

- The SUVH5 deletion (591 bp) causes the loss of the stop codon and the ablation of one of the four key cysteines at the C-terminal level (**Fig. 7**).
- This substitution with a serine residue can abolish the methyltransferase activity of the protein(3), resulting in the loss of H3K9me2 methylations and DNA methylations at other specific target regulatory genes (e.g., MYB-like).
- The level of H3K9me2 methylation/de-methylation in specific target loci has been recently highlighted as involved in the regulation of the anthocyanin pathway(2).

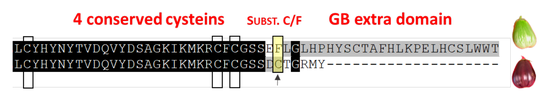

Fig 7. **C-term alignment** of **GB** and **BB SUVH5 protein**.

CONCLUSIONS

- SUVH5 could contribute to the peel colour regulation in the GB variety through an epigenetic mechanism as proposed in the model (**Fig. 6**). Further investigation through the knock out of the gene (CRISPR-Cas9) and quantitative gene expression analyses will be performed to better clarify the role of SUVH5 in the regulation of the anthocyanin pathway.