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Glutathione S-transferase as a potential marker for mutation breeding in poinsettia (*Euphorbia pulcherrima* willd. Ex klotsch).

Introduction and Methods

Poinsettia is a popular ornamental crop due to its range of bract colourations, which is obtained either through classical (crossing) or **mutagenic breeding (radiation)** (Fig. 1). The success of radiation breeding is highly genotype-dependent, and molecular markers have not yet been described for pre-selection of promising genotypes heterozygous at the GST locus.

We developed a PCR-based marker for an anthocyanin-related ***Glutathione S-transferase gene* (*GST*)** containing a **highly mutable SSR locus**. The CDS of the gene was sequenced from red and white varieties. Moreover, the varieties were genotyped using an approach based on the fluorescent labelling of PCR fragments.

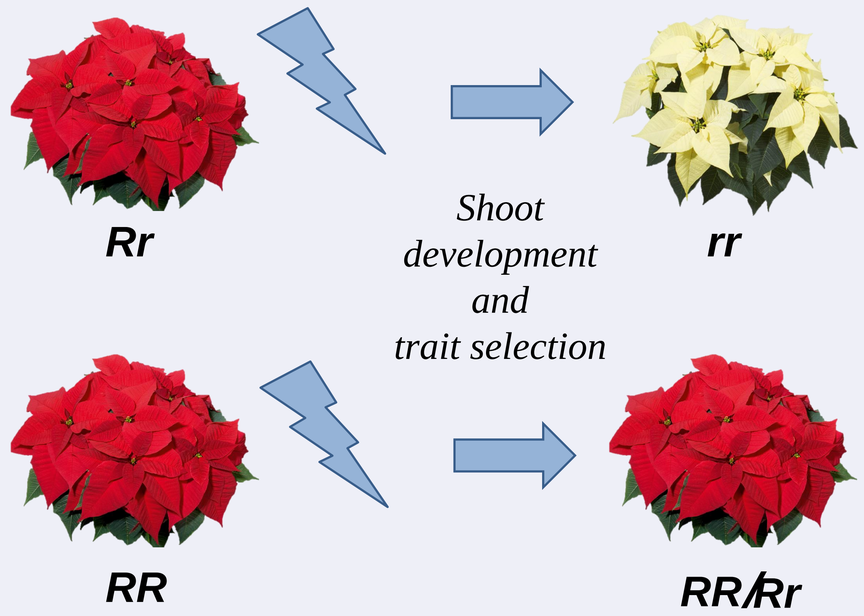

Figure 1. Schematic representation of mutagenic breeding in poinsettia. Upon irradiation, heterozygous red varieties for the *GST* locus (*Rr*) are capable of generating homozygous white sports (*rr*), unlike homozygous red varieties (*RR*).

Results and Discussion

The CDS sequencing showed a **4 bp *indel*** in all white varieties (*rr*), but only for a few of the heterozygous red (*Rr*) and in none of the homozygous red (*RR*) (Fig. 2). A region surrounding the 4 bp *indel* was PCR-amplified and resolved in an acrylamide gel in order to check for allelic configurations.

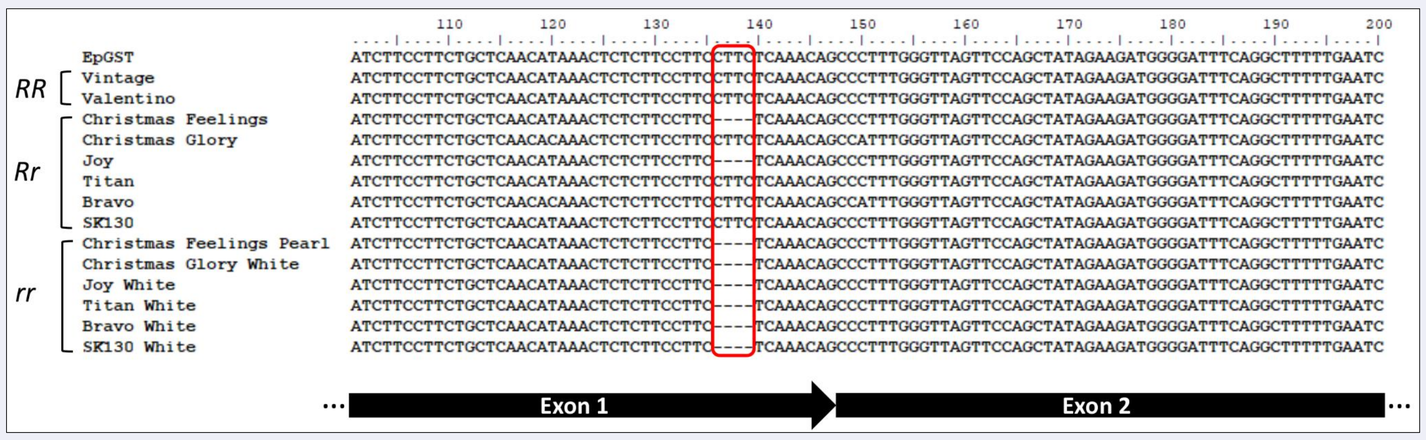

Fig. 2. Nucleotide alignment of the *GST* CDS for red and white poinsettia genotypes.

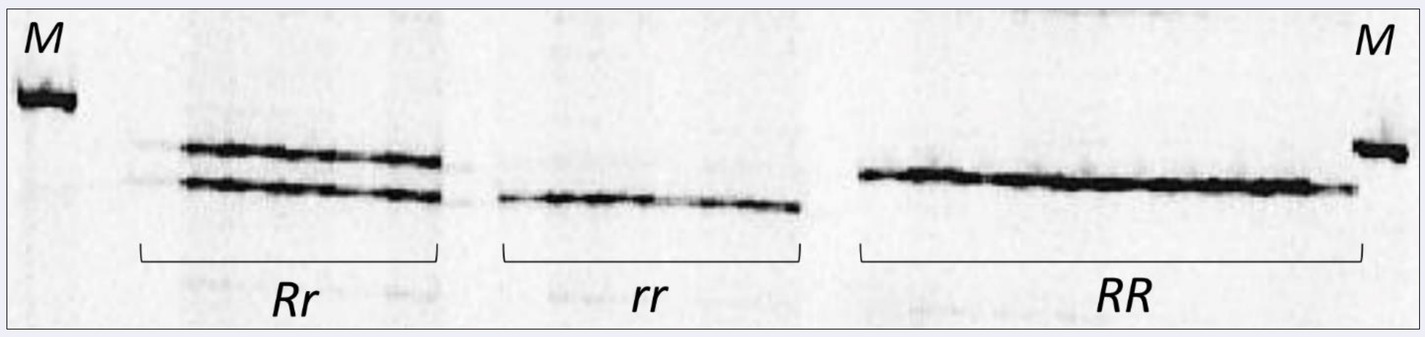

Figure 3. PCR amplification of the trinucleotide motif SSR locus ($CTTC_3$) in the *GST* for red and white poinsettia varieties. M = Marker (210bp)

All white varieties showed a homozygosity status for the allele containing the 4 bp *indel*. In contrast, heterozygous red varieties showed both copies of the allele, and homozygous red varieties showed a homozygosity status for the allele without the *indel* (Fig. 3).

The *indel* is located in a SSR locus with a tetranucleotide motif ($CTTC_3$) composition. Deletion of one repeat results in a putative early stop codon on the amino acid sequence of the GST protein. *GST* genes play an important role in anthocyanin transportation, since *GST* mutants show phenotypes with a visible lack of pigmentation, such as *bz2* from maize[1], *an9* from petunia[2], *tt19* from Arabidopsis[3], *fl3* from carnation[4] and *rap* from strawberry[5].

Conclusions

The SSR locus in the *GST* gene is a potential marker to aid the identification of heterozygote genotypes for irradiation mutagenesis in poinsettia breeding. Moreover, the presence of the 4 bp *indel* in white varieties in a recessive homozygosity state might explain the phenotype, which may be due to the lack of a functional GST protein.

References

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