Novel Split Selectable Markers Allow for Multi-gene Transformation in Plants

Background

- Complex plant traits (e.g., yield) are controlled by multiple genes.
- The ability to stack multiple genes in plants is of great importance in the development of crops with desirable traits but can be challenging due to limited selectable marker options.
- Usually, gene stacking involves using one selectable marker for each gene. The effectiveness of selection may vary depending on the specific selectable markers used and precise optimal conditions.

Approach

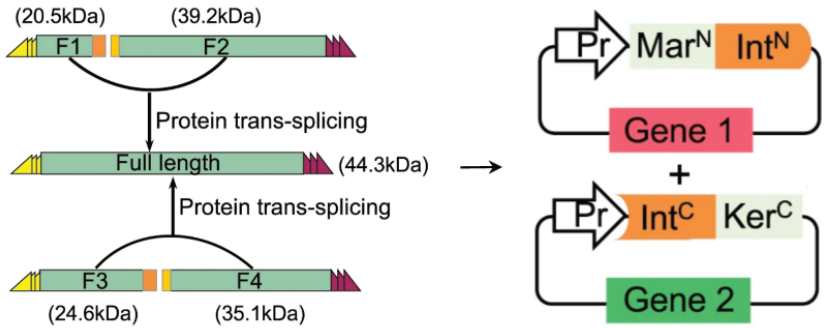
- Each selectable marker gene was divided into two fragments tagged with split inteins (excisable segments).
- For each selectable marker gene, the two partial fragments were cloned into two different plasmid vectors, respectively, which were then co-transformed into plants.
- The partial selectable marker fragments were reassembled into full-length functional proteins via trans-splicing of split inteins.

Results

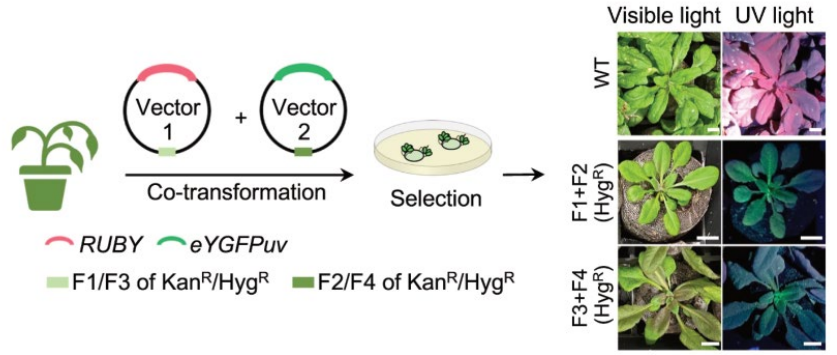
- A visible marker, RUBY, was successfully reconstituted from two non-functional fragments through *Agrobacterium*-mediated leaf infiltration in tobacco.
- Both the split Hyg^R and the split Kan^R selectable marker systems were successfully established in the model plant *Arabidopsis* and the non-model tree *Populus* for *Agrobacterium*-mediated stable transformation, as demonstrated by stacking two reporter genes (eYGFPuv and RUBY).

Significance

- We demonstrate that the systems of split–Kan^R and split–Hyg^R are effective for both *in planta* and plant tissue culture co-transformation in herbaceous and woody plants.
- The split selectable marker systems established in this study provide a valuable tool for gene-stacking in both herbaceous and woody plants. It allows markers to be used multiple times.
- This new technology has great potential for accelerating the improvement of plant traits relevant to bioenergy, climate change mitigation and bioeconomy.



The selectable marker Hyg^R was split into two parts (F1 + F2 or F3 + F4) and cloned into two different plasmid vectors for stacking two genes (Gene 1 and Gene 2).



The split Hyg^R selectable marker system was used to stack two reporter genes (eYGFPuv and RUBY) in Arabidopsis. The RUBY expression shows a purple-leaf phenotype under visible light. The eYGFPuv expression shows green-leaf phenotype under UV light.

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