Letter to the Editor

Reporter genes confer new-to-nature ornamental traits in plants

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Dear Editor,

Ornamental plants (trees, shrubs, and herbs) beautify our urban and rural environments, enrich the quality of human life, and represent a vital component of the horticultural industry. The introduction of novel plant varieties and cultivars is critical to the ornamental horticultural industry [1]. To develop ornamental plants with desirable traits, different approaches, such as ploidy manipulation, interspecific hybridization, and physical/chemical mutagenesis, have been used for decades [2, 3]. However, the breeding of new ornamental varieties of trees and shrubs is a time- and labor-consuming process, because these plants may have a long juvenile growth period, large physical size, or altered floral structures, and consequently require long-term observations, large areas for progeny testing, and/or special equipment for pollination and/or seed collection [4].

The utilization of genetic transformation to transfer genes cannot only expand the available toolkits for plant breeders, but also save considerable time in the breeding process [5]. For instance, Chin et al., generated brilliant green fluorescent Petunia plants by using a potent fluorescent protein eYGFPuv [6]. Furthermore, we recently successfully demonstrated the use of this eYGFPuv into additional herbaceous and woody plants, including tobacco (Nicotiana benthamiana and Nicotiana tabacum), Arabidopsis thaliana, poplar clone “717” (Populus tremula x alba INRA “717-1B4”), and citrus rootstock (Citrus sinensis “Washington” sweet orange x Poncirus trifoliata; Carrizo citrange) [7]. Similarly, the heterologous engineering of betalain pigments in plants has been achieved in a variety of plants. For example, the heterologous production of betalain pigments was reported in food crops tomato (Solanum lycopersicum), potato (Solanum tuberosum), eggplant (Solanum melongena), and ornamental petunia (Petunia sp.) by coexpressing three genes of the elucidated betalain biosynthetic pathway [8]. Along these same lines, a new reporter called RUBY was recently created. This reporter converts tyrosine to vividly red betalain, and was used as an effective selection marker for transformation events in both rice (Oryza sativa) and A. thaliana [9]. Considering that plant color is one of the most important ornamental traits, heterologous betalain production may offer exciting opportunities for creating new horticultural value for consumers, producers, and suppliers of ornamental plants [8]. However, the heterologous engineering of betalain pigments remains to be elucidated in woody plants. We, therefore, applied the reporter RUBY in the woody plant poplar in this study.

Using gblocks from Integrated DNA Technologies (IDT), we created the RUBY vector pAXY0003 containing 35S:RUBY using Gibson cloning [9]. After Agrobacterium-mediated tobacco leaf infiltration, we observed strong red pigment in the leaf of N. benthamiana, indicating the 35S:RUBY worked efficiently in transient expression. Since we already demonstrated the efficacy of eYGFPuv in tobacco leaves, we then combined the features of both eYGFPuv and RUBY reporters in order to enable color change under different lighting conditions. As such, we created the first dual reporter system pAXY0004 containing 35S:RUBY and 35S:eYGFPuv. Through tobacco leaf infiltration, we were able to observe brilliant green fluorescence and red pigment simultaneously in the infiltration area, suggesting that this dual reporter system worked efficiently in transient expression.

We then examined the efficacy of 35S:RUBY in a woody plant through the use of stable transformation in poplar clone “717” [7]. We were able to see red callus in the RUBY transformation. However, we were unable to recover
any shoots from these red calluses in shoot induction medium. One possibility is that the high expression level of 35S:RUBY inhibited the shoot induction during RUBY transformation. In parallel, we created another dual reporter construct pAXY0005 in which we used a constitutive 35S promoter to drive the expression of
After competent cells during early organogenesis in poplar. Selected under UV light in rooting medium (Fig. 1B). We chose the promoter of Potri.008G065400 to create a tissue-specific construct that allows for RUBY expression in regeneration-competent cells during early organogenesis in poplar. After Agrobacterium-mediated stable transformation in poplar clone “717”, multiple independent transgenic events exhibiting green fluorescence were successfully expressed in regeneration-competent cells during early organogenesis in poplar. Arabidopsis root formation in regeneration-competent cells during early organogenesis in poplar. Arabidopsis [10]. We chose the promoter of Potri.008G065400 to create a tissue-specific construct that allows for RUBY expression in regeneration-competent cells during early organogenesis in poplar. After Agrobacterium-mediated stable transformation in poplar clone “717”, multiple independent transgenic events exhibiting green fluorescence were successfully selected under UV light in rooting medium (Fig. 1B). After multiple rounds of propagation in rooting medium, we finally produced more than 26 ramets from this initial event. We were able to continually observe homogeneous and uniform traits of RUBY and eYGFPuv in all ramets, indicating the stability of these novel ornamental traits during propagation. Next, we transferred multiple ramets into soil for greenhouse observation. After three weeks in soil, we observed typical phenotypes of RUBY and eYGFPuv under visible light and UV light, respectively, indicating that the traits of RUBY and eYGFPuv are robust and stable (Fig. 1C). As the plants grow, the traits of RUBY became more apparent in the larger leaves and longer stems due to the accumulation of red pigment (Fig. 1D and E). Analysis of photosynthesis in leaves from three ramets of transgenic line PAXY0005 showed that the maximal rates of net photosynthesis (Amax), electron transport (Jmax) and Rubisco carboxylation (Vcmax) were lower in the transgenic plants than the wild type plants (Fig. 1F). There is no noticeable difference in the growth rate of the transgenic plants compared with the wild type plants by week 16 (Fig. 1G). Notably, we observed both phenotypes of RUBY and eYGFPuv in multiple ramets cultured in greenhouse. These results support that the traits of RUBY and eYGFPuv can be easily maintained and stably spread in both tissue culture and greenhouse conditions.

In summary, we created an ornamental poplar genotype harboring novel decorative traits using reporter-gene-based transformation for the first time in woody plants. The pattern of RUBY phenotype of the transgenic line PAXY0005 could be resulted from the specificity of the promoter of Potri.008G065400 or of the insertion of T-DNA to a special location of the poplar genome, or both. It is possibly, though, the location of the insertion played a main or a larger role, as only one of the four recovered PAXY0005 events with strong eYGFPuv expression showed the RUBY phenotype (Data not shown). Nonetheless, our results demonstrated the potential of using RUBY and eYGFPuv to create novel decorative traits. Besides, the lower photosynthesis rate of transgenic line PAXY0005 could be a desired feature for indoor ornamental plants. Given that RUBY and eYGFPuv have been successfully applied in various plant species, we propose that this dual reporter system can be directly applied in other herbaceous and woody plant species in order to develop novel ornamental plants. In comparison with the conventional ornamental breeding tools, firstly, this approach dramatically decreased the cultivar creation period with easily selectable reporters. Secondly, the ornamental traits are predictable, desirable and manipulable. Technically, the desired traits can be either expressed at the whole-plant level using a constitutive promoter (like 35S or ubiquitin promoter) or in the specific plant tissue or organ (e.g. flower, leaf, and root) using a tissue-specific promoter, which is usually unachievable using conventional ornamental breeding techniques. Thirdly, the application of this approach is flexible and adjustable. In addition to RUBY and eYGFPuv, many other colorful reporters, such as chromoproteins, can be potentially incorporated into this system. For example, many eukaryotic chromoproteins, such as, mRF1, amilCP and amilGFPHavebeenusedforbacterial synthetic biology, because these chromoproteins can be directly detected by the naked eye without requiring pretreatment [11]. Notably, the application of these chromoproteins in plants remains to be studied. Hence, with the exploration of new colorful reporters, the desired ornamental traits can be expanded through gene stacking. Finally, these colorful trees might also be a good vehicle to gain public acceptance of genetically modified woody plants.

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**Author contributions**

G.Y., H.L and X.Y conceived the research. H.L. conceived the idea of WOX-RUBY construct. G.Y. and H.L. conducted the experiments. D.W and S.J carried out the photosynthesis analysis. G.Y. wrote the first draft of the manuscript with the input from H.L. and other authors. G.T. and X.Y. supervised the research. All authors contributed to manuscript revision, read, and approved the submitted version.

**Data availability**

The plasmids will be available at Addgene (http://www.addgene.org). The construct data were deposited in Dryad (doi:10.5061/dryad.gf1vhhmrr).
Competing interests
The authors declare no conflict of interests.

Supplementary data
Supplementary data is available at Horticulture Research online.

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