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PURE SCIENCE

Candidate Genes of molecular regulation of Vascular Cambium as possible targets using modified CRIPSR/Cas9 system using flowering stimulation and precision lighting.

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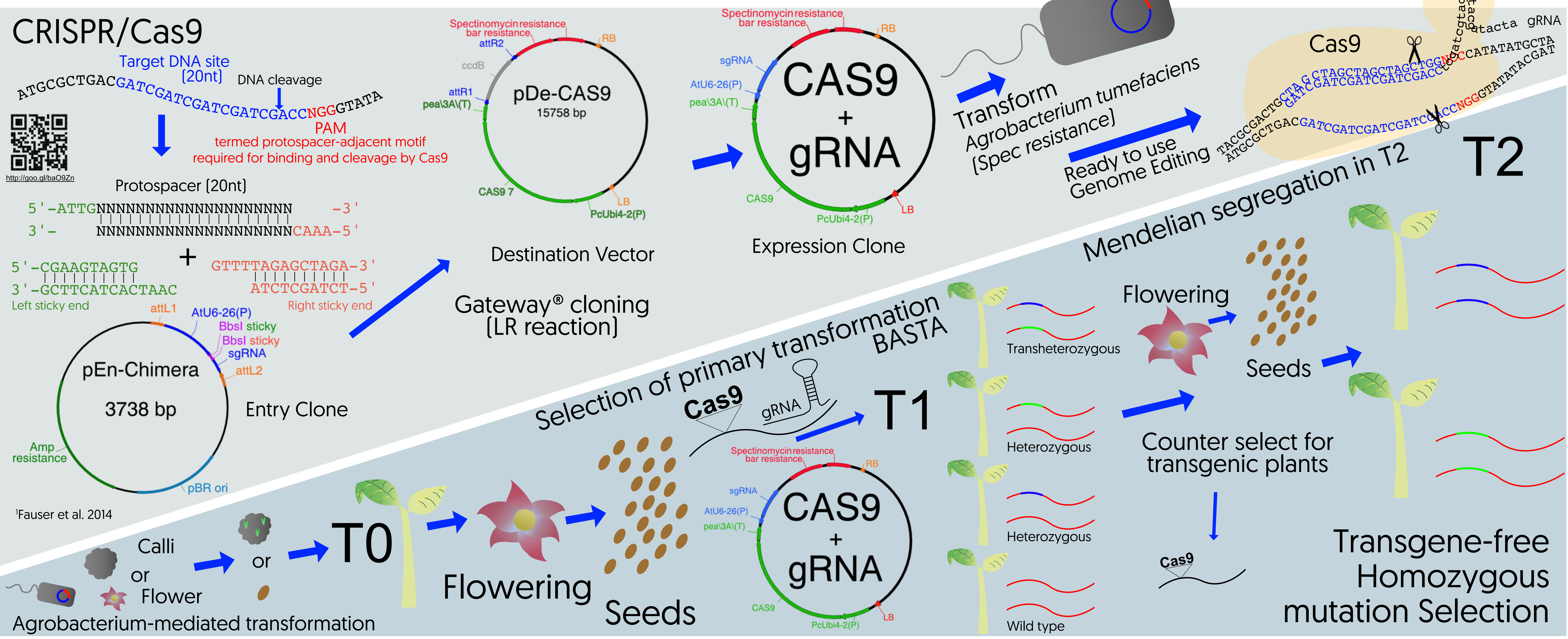
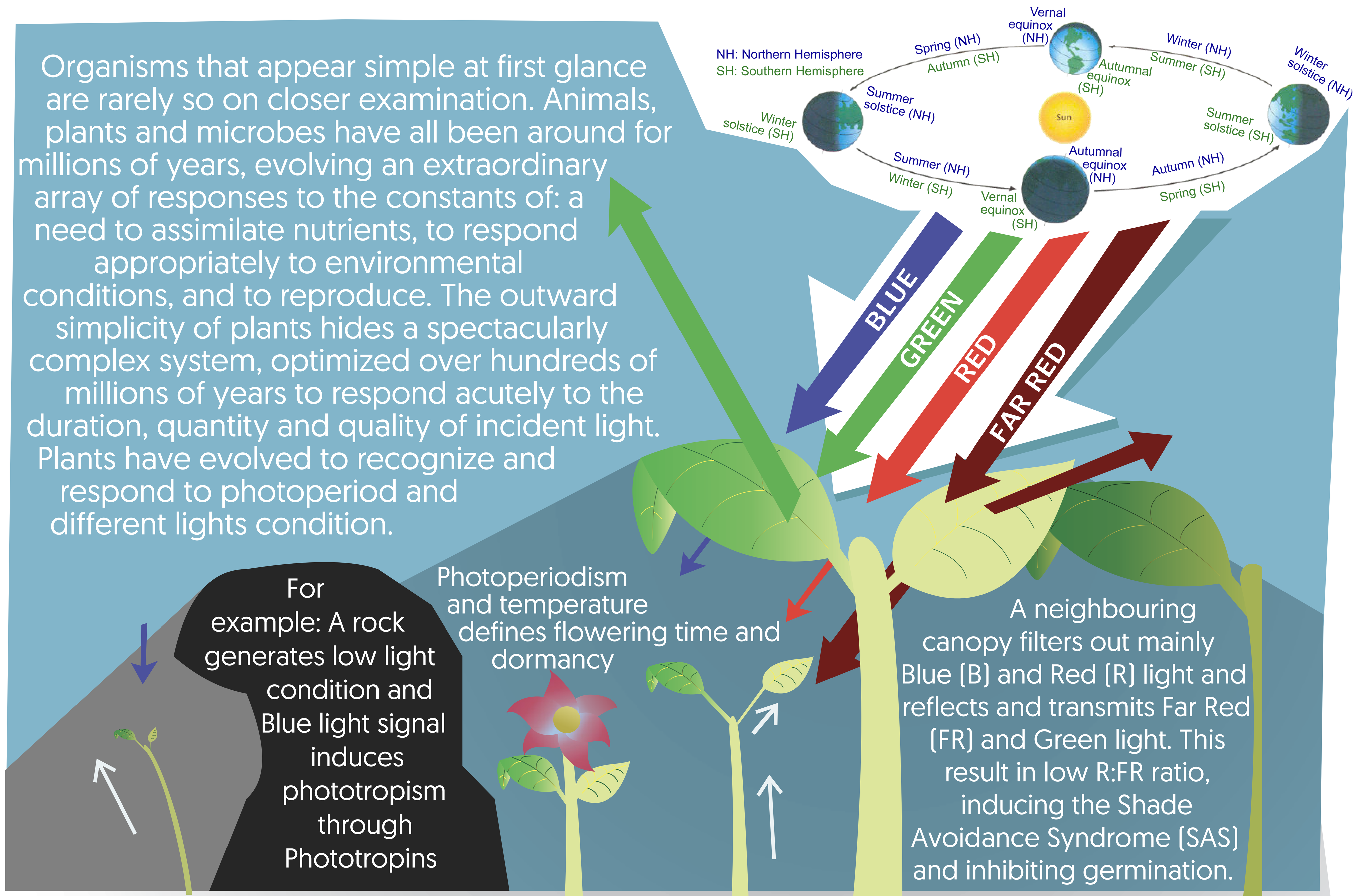
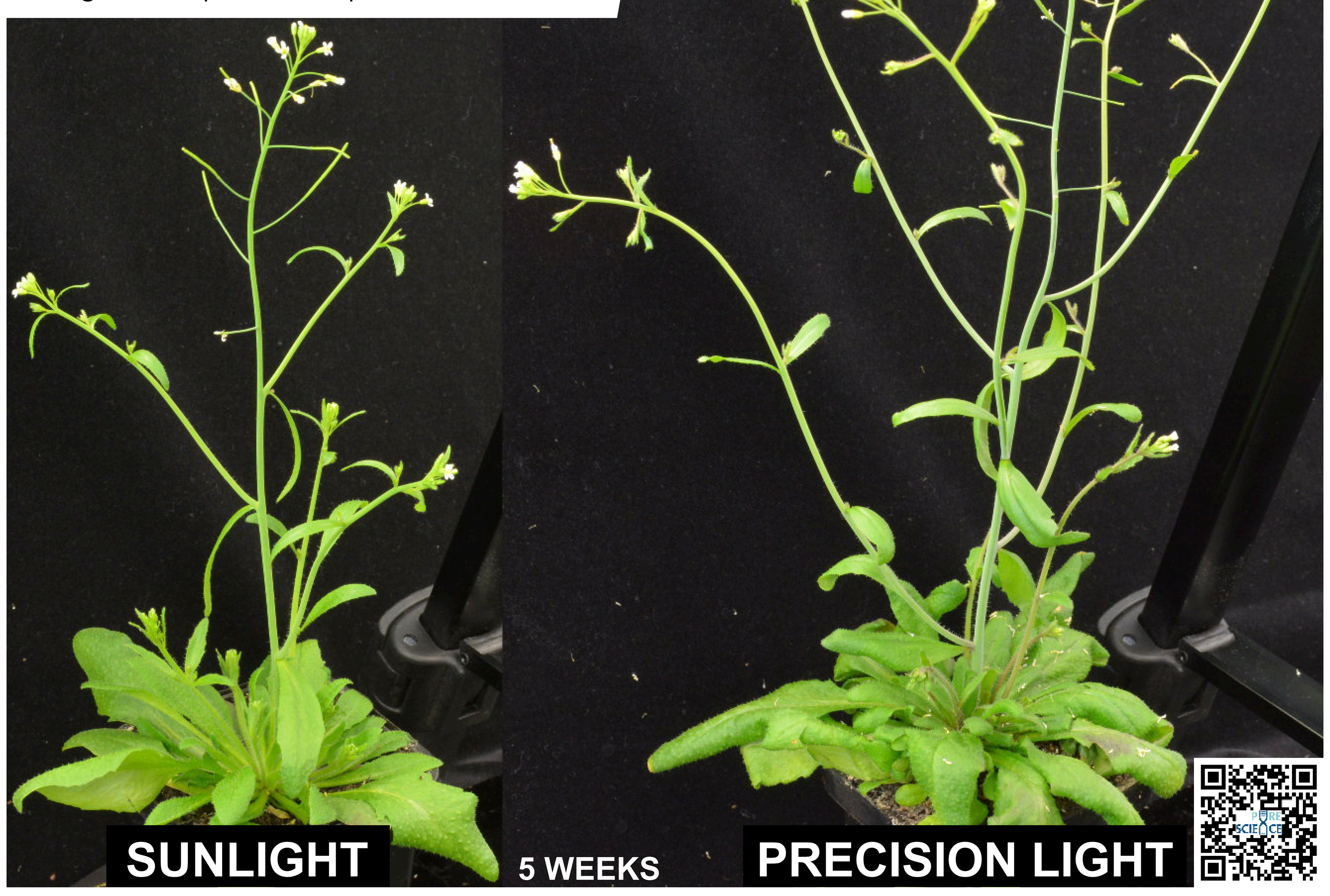
Abstract: Indeterminate growth in plants require a constant supply of new cells. For primary growth of the roots and shoots, the meristems are located in the apical meristems. Woody plants also have secondary indeterminate mitotic regions towards the exterior of roots, stems and branches that produce the cells for continued growth in girth, the vascular cambium. The principle requirements to maintain a functioning meristem in a dynamic system are a balance of cell division and differentiation and the regulation of the planes of cell division and expansion. As a renewable resource, the demand for wood products is expected to continue to increase into the future. Compared to annual crop plants, direct genetic modification of trees species has gained little attention, partially because trees have much longer lifecycles and tight regulations opposing transgenic use in the field. The newly developed gene editing technologies, such CRISPR/Cas9 increase the potential for the modification of species. CRISPR/Cas9 has several major advantages over previous transgenic-based approaches and can work alongside conventional breeding programs by directly improving known yield-related loci or genes. We have a modified CRISPR/Cas9 system that promotes early flowering in plants. Together with precision lighting with different ratios of Blue, Red and Far Red light we have managed to regulate the acceleration of flowering time to get viable flowers. The CRISPR/Cas9 mutated plants flower earlier than the wild type plants, resulting in fast recovery of the second generation (T2) in Arabidopsis. We are now testing this technology in *Arabidopsis thaliana* with the aim to accelerate breeding in arboreus species.





Precision Light

The spectral range from 400 to 700nm has been designated as the photosynthetically active radiation (PAR). The optimum light intensity recommended to grow *Arabidopsis* plants (according to ABRC[2]) is 130-150 umol/m2/s, which mimics 60% shade cloth in summer greenhouses. Using artificial light, this measure is inaccurate, because only a portion of the white light is available. Using the right amount of Blue, Red and Far Red light, including specific photoperiod and night pulses of Red or Far Red, it is possible to modulate and regulate the plant development.



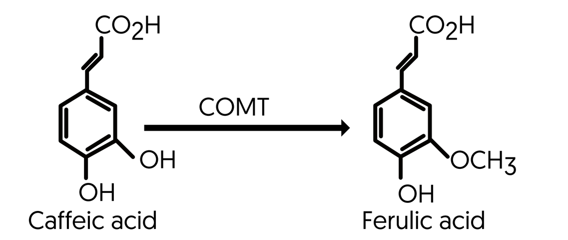
Investigation of the interplay between light and the flowering response are being investigated by application of various light conditions. Once the light variables have been optimized they will be reinvestigated with *Arabidopsis thaliana* transformed with modified CRISPR/Cas9 targeting reporter genes ABH1, SAC9 to resulting in bleached cotyledons or PDS3 resulting in albino plants, obtaining homozygous mutants faster than wild type.

A list of candidate molecular regulators of vascular cambium structure and function has been studied in a previous review paper (3Matte et al. 2010). Lignin pathway, cellulose synthesis or wood formation are also possible targets.

Our current work aims to explore and optimize the variables within this system of CRISPR/Cas9 in conjunction with precision light for future exploitation to deliver positives outcomes for stakeholders in the forestry industry.

Examples:

Protoplasma
DOI 10.1007/s00709-010-0211-z
REVIEW ARTICLE
The vascular cambium: molecular control of cellular structure
Juan Pablo Matte Risopatron · Yuqiang Sun · Brian Joseph Jones



COMT: enzyme involved in methylation in lignin biosynthesis in woody poplar plants

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[1]Fauser, F., Schiml, S. and Puchta, H. (2014), Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*. Plant J, 79: 348–359. doi:10.1111/tpj.12554
[2]Arabidopsis biological resource center
[3]Matte Risopatron J.P. (2010), The vascular cambium: molecular control of cellular structure. Protoplasma 247:145–161. doi: 10.1016/j.tplants.2011.11.004

Aknowledge: CONICYT PAI 82140040 (Concurso nacional apoyo al retorno de Investigadores desde el extranjero. Convocatoria 2014)