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Low-frequency Transgene Leakage from a Natural Confinement System

Transcriptional profiling of a Petunia hybrida mutant line with increased transmission of plastid encoded transgenes through pollen donor

Reference: Transgene Leakage

Header image provided by the university

Seeking

Development partner

About **LMU Munich**

Ludwig-Maximilians-Universität München is the University in the heart of Munich. LMU is recognized as one of Europe's premier academic and research institutions. The LMU Munich community is engaged in generating new knowledge for the benefit of society at large.

Background

Chloroplasts are integrated into plant cells as organelles and can be traced back to an endosymbiotic event, when a cyanobacterium was engulfed by a eukaryotic cell. Beside chloroplasts, plants contain other types of plastids, e.g. leucoplasts, which do not carry out photosynthesis. All types of plastids have in common that they contain their own genome, which is thought to be derived from the ancestrial cyanobacterial genome. Although most of the former genes are now located in the nuclear plant genome, about 100 genes are still present on the plastid genome. In most angiosperms, plastid genomes are mainly transmitted through the egg cell of the female gametophyte, and not through sperm cells of the pollen grain, leading to maternal inheritance of the respective genes. Integrating transgenes into the plastid genome therefore prevents outcrossing of transgene-encoded traits via pollen distribution and thus can be regarded as a natural confinement system. However, a low-frequency leakage of plastid genomes via pollen seems to be universal in plants. In a study employing the model plant *Arabidopsis thaliana* and the crop plant *Brassica napus*, those frequencies were determined [1]. So far, little is known about the molecular mechanisms influencing plastid genome distribution during the reproductive phase of plants.

Tech Overview

In a search for angiosperm species, which are suitable to serve as subjects in elucidating the molecular mechanisms, LMU researchers selected *Petunia hybrida* as a model plant. *Petunia hybrida* is accessible to genetic manipulation of the plastid genome, endogenous transposons enable nuclear genome mutagenesis, and frequency of paternal leakage offered a good starting point.

The researchers observed that paternal transmission of a transgenes occurred in approximately 1 in 2000 cases in greenhouse conditions and 1 in 8000 cases in field conditions [2], and is thus increased severalfold in comparison to *Arabidopsis thaliana* (1 in 50.000 cases). With the help of endogenous transposable elements, a collection of nuclear mutant lines were established, in the assumption that respective molecular mechanisms are under nuclear control. The endogenous transposable elements of strain W138 were introgressed into the transplastomic strain T16 and 450 lines were generated. Subsequently, transposons were stabilized and reciprocal test-crosses were performed. To this end the team identified one mutant line which indicated a 10-fold increased paternal transmission of the transgene (1 in 200 cases). It turned out that the number of transposable elements in the nuclear genome of the mutant line is very high and complicates the recognition of the responsible gene locus.

The aim of this project is to apply a further method which allows simultaneous examination of respective pollen transcripts in the mutant line versus control line.

Further Details

[1] Schneider, A., Stelljes, C., Adams, C., Kirchner, S., Burkhard, G., Jarzombski, S., Broer, I., Horn, P., Elsayed, A., Hagl, P., et al. (2015). Low frequency paternal transmission of plastid genes in Brassicaceae. Transgenic Res 24, 267-277. DOI: 10.1007/s11248-014-9842-8

[2] Horn, P., Nausch, H., Baars, S., Schmidtke, J., Schmidt, K., Schneider, A., Leister, D., and Broer, I. (2017). Paternal inheritance of plastid-encoded transgenes in Petunia hybrida in the greenhouse and under field conditions. Biotechnol Rep (Amst) 16, 26-31. DOI: 10.1016/j.btre.2017.11.001

Stage of Development

Proof of concept for estimating paternal transmission frequencies of plastid transgenes has been achieved. A study subject towards the molecular analysis has been established. Currently a *Petunia hybrida* mutant line is under investigation.

Benefits

The identification of transcripts/proteins will help to understand the molecular mechanisms preferring maternal plastid gene inheritance in Angiosperms.

Applications

Genetic modified plants are under debate, including concerns about potential introgression of transgenic traits into wild relatives. Integrating transgenes into the plastid genome decreases this risk enormously and transgene escape can be further reduced once the molecular mechanisms are elucidated.

Opportunity

Currently seeking an experienced partner to perform transcriptional profiling on pollen RNA of *Petunia hybrida* mutant lines and control lines. The partner should optimally also support bioinformatic analysis.

For further information, please contact us.

Ludwig-Maximilians-Universität München (LMU Munich)

Office for Research and Technology Transfer

- Corporate Partnerships -

Dr. Barbara Blaurock

+49(0)89 2180-722 13

Dr. Laura Gerwin

+49(0)89 2180-722 12

corporatepartnerships@lmu.de

www.lmu.de/researchservices/corporatepartnerships/