

Genotyping *myb36* and *sng3* *Arabidopsis* mutants to be used as controls for suberin and lignin measurements in *Linanthus parryae*

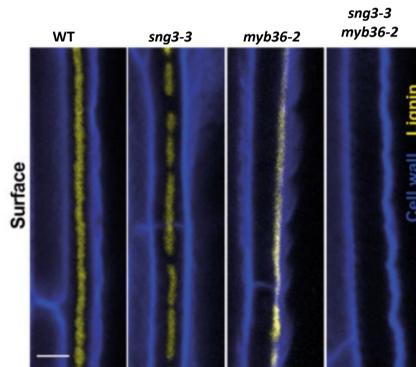


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Introduction

Linanthus parryae (*L. parryae*) is a small annual plant of the Mojave desert with blue and white flowers. Plants are obligate outcrossing and interfertile; however, they show constant spatial segregation often observed along the sides of shallow desert ravines (Schemske and Bierzychudek, 2001). Moreover, blue morphs are favored in dry years, while white morphs are favored in wet years. The mechanisms that control the spatial segregation of flower morphs and increased tolerance to high or low humidity are not known and while a lot of attention has been given to flower color, it is not known if this spatial segregation is instead linked to a root phenotype.

Objective



The objective of this study is to investigate whether there is a pleiotropic connection between flower color morphs and root phenotypes linked to the deposition of suberin and lignin in the root endodermis. Previously it has been shown that *Arabidopsis thaliana* (*Arabidopsis*) mutants *schengen3* (*sng3*) and *myb36*, which show differential deposition of suberin and lignin in the root endodermis (Figure 1), have altered tolerance to high or low humidity (Kamiya et al., 2015; Rey et al., 2021). To assess whether *L. parryae* shows altered deposition of lignin and suberin content in the root endodermis, *sng3* and *myb36* will be utilized as a control. Therefore, this study was done to identify homozygous lines of *sng3* and *myb36* to be utilized in future experiments.

Figure 2: Lignin deposition in the root endodermis of Col-0, single mutant *sng3-3*, *myb36-2*, and double mutant *sng3-3myb36-2*. Cellulose was stained with Calcofluor (blue) and the roots were stained with basic fuchsin (yellow) to view lignin. Photos were taken under a fluorescent microscope (Rey et al., 2021).

Methods

- Seeds of Col-0, *sng3* (At4g20140) and *myb36* (At5g57560) were purchased from the ABRC stock center, which classified them as a mixture of heterozygous and homozygous
- Seeds were stratified in tap water at 5°C in the dark for 5 days to synchronize seed germination
- After stratification, the seeds were sown on common soil and the plants were grown in a semi-controlled environment (photoperiod: light/dark, 12/12h) and watered with Hoagland's solution (strength 0.25x; pH of 6.2) twice a week. Seedling were thinned to have only one plant per pot (Figure 2)
- One leaf from a young rosette was snap-shot in liquid nitrogen and reduced to a fine powder with a pestle
- Genomic DNA (gDNA) was extracted from 12 plants per genotype with the CTAB buffer
- Amplifications of target regions in the gDNA were performed via PCR using the touch-down method
- Two PCR reactions were performed for each mutant: one to assess amplification of intact gDNA, and one to detect amplification of T-DNA. Specific combinations of oligonucleotides were utilized for specific reaction (Table 1)
- Presence and size of PCR amplification results were visualized after gel electrophoresis

Table 1: Sequence, melting and annealing temperature and target templates (genomic or T-DNA) of the primers utilized to genotype *sng3* and *myb36*.

Gene	Primer Name	Primer Sequence	Melting Temperature (Tm)	Annealing Temperature (Ta)	PCR
<i>sng3</i>	75_F (LP)	ATTCTACGAGCCTTCCCATTC	59	70	genomic PCR
	76_R (RP)	TCTCCGGTGAGACTGTTGTTC	60		
<i>sng3</i>	76_R (RP)	TCTCCGGTGAGACTGTTGTTC	60	57	T-DNA PCR
	59_F (LBb1.3)	ATTTTGCCGATTTCGGAAC	52		
Gene	Primer Name	Primer Sequence	Melting Temperature (Tm)	Annealing Temperature (Ta)	PCR
<i>myb36</i>	60_F (LP)	CGAATTGCTCAGATTCTGAGG	60	70	genomic PCR
	77_F (RP)	ATGCAGAAAAAGCAAAAACCC	60		
<i>myb36</i>	77_F (RP)	ATGCAGAAAAAGCAAAAACCC	60	77	T-DNA PCR
	62_F (LB)	ATAATAACGCTGGGACATCTACATTTT	69		

References

1. Schemske D W., Bierzychudek P (2001). Perspective: Evolution of Flower Color in the Desert Annual *Linanthus Parryae*: Wright Revisited. *Evolution*, 55: 1269-1282.
2. Kamiya T, Borghi M, Wang P, Danku J M.C., Kalmbach L, Hosmani P, Naseer S, Fujiwara T, Geldner N, Salt D E. (2015). The MYB36 Transcription Factor Orchestrates Casparian Strip Formation. *Proceedings of the National Academy of Sciences*, 112: 10533–10538.
3. Rey G, Ramakrishna P, Salas-González I, Fujita S, Love A, Tiemessen D, Lapiere C, Morreel K, Calvo-Polanco M, Flis P, Geldner N, Boursiac Y, Boerjan W, George M W., Castrillo G, Salt D E (2021). Two Chemically Distinct Root Lignin Barriers Control Solute and Water Balance. *Nature Communications*, 12: 1–15.



Figure 2: Images of *Arabidopsis thaliana* plants. A) *myb36*; B) *sng3*; C) Col-0 wild type; D) all the plants growing in the tray.

Results

All samples of both *myb36* and *sng3* had amplification of T-DNA during T-DNA PCR with a band size of approximately 600 bp for *myb36* and approximately 400 bp for *sng3*. Samples 1-9 had amplification of genomic DNA in both *sng3* and *myb36* during genomic DNA PCR with a band size of approximately 1200 bp for both. Samples 11-12 of both *myb36* and *sng3* demonstrated no amplification of genomic DNA. These results show that individuals 1-9 of both *myb36* and *sng3* are heterozygous because they showed amplification of both T-DNA and genomic DNA. Individuals 11-12 of both *sng3* and *myb36* are homozygous lines because they had amplification of T-DNA but not of genomic DNA.

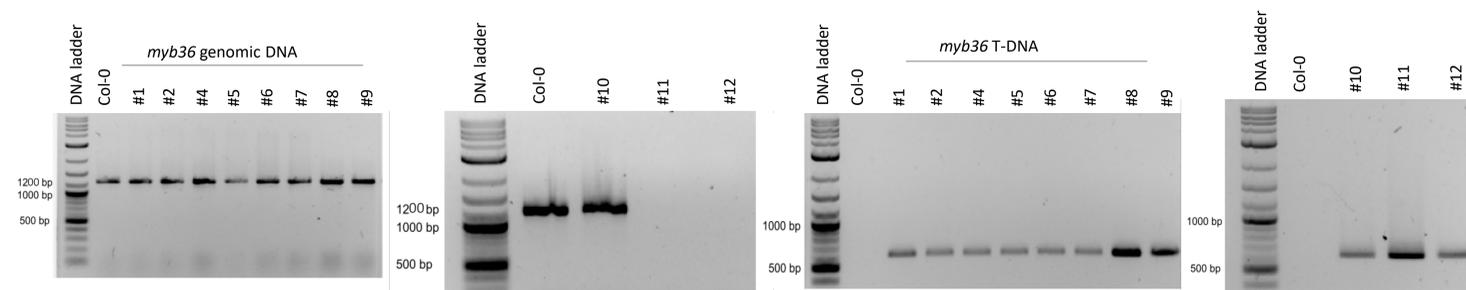


Figure 3: Gel electrophoresis images of the PCR results obtained with the primer pairs 60_F and 77_R to amplify the genomic sequence of gene At5g57560 and pairs 77_F and 62_F to amplify the T-DNA insertion.

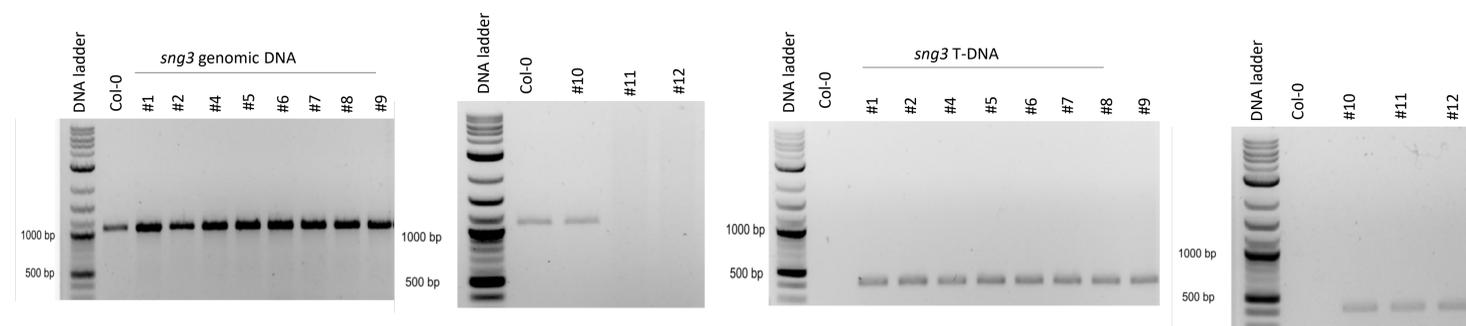


Figure 4: Gel electrophoresis images of the PCR results obtained with the primer pairs 75_F and 76_R to amplify the genomic sequence of the gene At4g20140 and pairs 76_R and 59_F to amplify the T-DNA insertion in *sng3*.

Future Directions

The homozygous plants from both mutants will be used as controls to investigate the lignin and suberin deposition in the root of desert plant *L. parryae*. Further experiments will be conducted to investigate the chemical nature of the floral pigments and how they relate to the root chemical phenotype.