

## Overexpression of *Ricinus communis* Malate Synthase Gene in *Arabidopsis thaliana* Enhances Seed-Thermotolerance During Germination at High Temperatures

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Introduction: Ricinus communis seeds germinate to a high percentage and faster at 35°C than at lower temperatures, but with compromised seedling establishment. High temperature during seed germination leads to down-regulation of important metabolic processes which are crucial for successful seedling establishment. Overexpression of malate synthase (MLS) resulted in higher starch levels in Nicotiana benthamiana leaves, which highlights the importance of this gene in energygenerating pathways for seedling establishment. **Objectives:** The objectives were to overexpress Ricinus communis malate synthase (RcMLS) gene in Arabidopsis thaliana and to evaluate the responses of the transformed seeds to high temperatures during germination. Material and Methods: RcMLS was cloned into Agrobacterium tumefaciens using the Gateway cloning-system. Subsequently, the transformed bacteria were used to (stably) transform Arabidopsis thaliana by a floral dip procedure. Seeds of A. thaliana overexpression lines and A. thaliana wild type (Col-0) were used to phenotype seed germination at different temperatures (22°C. 31°C, 33°C, 34°C, and 36°C). Results and Discussion: Col-0 seeds germinated nearly 100% at 22°C, 31°C and 33°C. At 34°C total germination lowered to 92%, whereas at 35°C total germination lowered to 36%. At 36°C or above none of the seeds germinated, not even after relocation to 22°C for a week. Therefore, 35°C was selected as the threshold temperature for further germination experiments. Total germination of Arabidopsis seeds overexpressing RcMLS was 65%, when seeds were germinated at 35°C. This is significantly higher than the total germination of the Col-0 seeds at the same temperature. Conclusions: Overexpression of RcMLS enhanced thermotolerance in Arabidopsis seeds during germination at 35°C. RcMLS might be a key responsive gene in lipid mobilization and gluconeogenesis to sustain successful seedling growth at high temperatures.

**Key words:** functional characterization, high-temperature stress, temperature-responsive genes

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