Molecular-Based Genotypic Selection for Anthocyanin in Lactuca sativa, Year Three

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The overall goal is to identify a non-destructive real-time PCR-based method allowing for rapid genotypic selection in Lactuca sativa (lettuce) seedlings at an early stage of development. The obligate self-pollination aspect in lettuce complicates the propagation of traits of commercial interest. A novel nested microplate was developed to facilitate the sampling of root cap cells as a source of DNA for genotyping. The model gene was LsANS (L. sativa anthocyanidin synthase), which is functional in red-leaf lettuce and non-functional in green-leaf lettuce. Analysis of the LsANS transcript by RT-qPCR found that red-leaf cultivars have a higher level than green-leaf cultivars. Cultivars were crossbred to hybridize for a functional LsANS gene. Only 1% of crossbred cultivars produced seeds, demonstrating the need for a scalable and cost-effective selection tool. Next, the nested microplate was improved by germinating the seeds under an LED light for a 16-hour photoperiod to yield 91.6% germination within six days. The genotyping workflow using real-time PCR followed by high-resolution melt analysis (HRM) was refined by confirming that plant PCR inhibitors in the receiving medium do not affect PCR melting temperatures (Tm). Only 43.9% of samples with known genotypes were correctly identified using PCR (<40 cycles) and HRM (Tm difference > 0.4°C) parameters. Fluorescence microscopy of the receiving medium confirmed insufficient shedding of root cap cells. Current research focuses on developing techniques to increase the amount of accessible DNA in the lower plate. These studies show that the traditional breeding process can be considerably streamlined on a high-throughput scale.

Awards Won:

Fourth Award of $500