# Molecular-Based Genotyping of Lactuca sativa for Accelerated Genotypic Selection

Swartz, Sophia

The obligate self-pollination aspect in mature Lactuca sativa (lettuce) plants impedes the propagation of traits of commercial interest and makes crossbreeding difficult and tedious. The purpose of this experiment was to identify a minimally destructive, qPCR-based method allowing for rapid genotypic selection in lettuce seedlings. Reproductive physiology studies characterized the obligate self-pollination aspect of lettuce. The overall objective was to target a plant structure for DNA extraction that was accessible at an early stage of seedling development and easy to isolate without inhibiting continued seedling growth. Several possibilities were tested, beginning with targeting the endosperm of the dormant seed, sampling detached root cap cells, and ultimately excising a part of the seedling root. In the process of growing the lettuce seedlings, a novel nested microplate model with the potential for high-throughput application was developed to facilitate the sampling of root tissue. Four simplified DNA isolation protocols were developed and tested to accelerate the isolation of genomic DNA and minimize potential for error. DNA isolated with simplified protocols produced successful qPCR products utilizing primers targeting genomic and plastid genes. A robust qPCR protocol using a 96-well microplate set-up resulted in successful DNA amplification, thus demonstrating qualitative proof of concept. The results show that the genotypic selection process can be considerably accelerated and simplified on a potentially high-throughput scale.

**Awards Won:**
Second Award of $2,000