

BRE1409: Virus Induced Gene Silencing (VIGS) to test gene function in pulse crops

With advances in DNA sequencing technology the pace of genome sequence data becoming available is rapidly increasing. For agriculture, this means that the DNA sequence of important crop species can be used to identify genes that may contribute to beneficial traits, however, testing the function of these genes remains problematic. The goal of this project was to develop a laboratory technique to transiently silence specific genes in a number of legume species for use in testing gene function. We tested a simplified virus induced gene silencing (VIGS) protocol using Apple Latent Spherical Virus (ALSV) in a number of crop species. The goal was to develop a simple technique that could be applied broadly in a typical molecular biology lab that did not require specialized equipment. Direct inoculation of ALSV of ALSV virus, or inoculation using a bacterial carrier was not effective on soybeans, common beans, or mung beans, however we developed a simple protocol that was effective in silencing the target gene in pea plants. We also developed a novel technique based on root regeneration to introduce ALSV vector into root tissue that showed 100% effectiveness in peas. Using this technique we were also able to demonstrate gene silencing in lentils with ALSV. Using this approach we were able to introduce a Bean Pod Mottle Virus into germinated soybean and common bean seedlings, but not ALSV, suggesting that specific interactions between ALSV and members of the bean family were responsible for the lack of gene silencing observed with other inoculation methods.