Cory Weimer

Major: Biology
University: University of Missouri-Columbia
Faculty Mentor: Dr. Jay Thelen
Mentor Department: Biochemistry
Funded by: MU Monsanto Undergraduate Research Fellowship

Selective precipation of seed storage proteins in soybeans: Reducing the dynamic range of protein expression for in-depth proteome studies

Seed storage proteins (SSPs) are a class of proteins which accumulate at high levels (up to 60% total protein) during plant seed development as a nitrogen reserve for germination. Due to the preponderance of SSPs, the dynamic range of protein expression in seeds can easily exceed 1000-fold. A consequence of this broad dynamic range is that SSPs "mask" lower-abundance proteins in proteomic analyses. To study these concealed proteins I experimented with and developed a procedure by which the major groups of SSPs were selectively removed from a total protein extract prior to electrophoresis. Two different approaches, ethanol and isoelectric precipitation, were compared for their effectiveness at precipitating SSPs from total soybean seed extracts. For both procedures, mature soybeans were homogenized with a mortar and pestle, resuspended in extraction media and filtered through miracloth. From there samples were adjusted upward to a range of 5-50% ethanol for the alcohol precipitation, and downward for a pH range of eight to two with isoelectric precipitation. All samples were centrifuged at 14 k g for 30 minutes after which supernatants and pellets were separated. The pellets were resuspended in the same extraction media used for the initial suspension. All samples were then analyzed by SDS-PAGE and Coomassie staining to determine if the SSPs were precipitated. Results showed that ethanol effectively precipitated soybean proteins, but was not selective for SSPs. Titration analysis showed that at 5% ethanol few proteins were precipitated and by 50% nearly all protein was precipitated. In contrast, adjusting the pH of the soybean protein extract to coincide with the isoelectric point of many SSPs (i.e. isoelectric precipitation) was qualitatively more selective at precipitating SSPs. In a pH range between 3.0 and 5.0 nearly all SSPs were removed from the soluble phase. Isoelectric precipitation was performed in 0.25 pH increments from 3.00 to 4.50 to further determine the optimal pH for isoelectric precipitation. This revealed that the optimal level to remove the glycinin and β =-conglycinin seed storage proteins is in a narrow range between pH 4.00 and 4.50. Future work will include basic pH adjustments to precipitate those SSPs with isoelectric points greater than pH eight. Also, developing stages of soybeans (2, 3, 4, 5, and 6 weeks after flowering) will be tested with isoelectric precipitation for future two-dimensional electrophoretic proteome studies. Other oilseed crops such as *Brassica napus* and castor will be tested with isoelectric precipitation to determine if this approach has broad practical use in reducing sample complexity for proteomics.