B-107

A quantitative HPLC method to determine trypsin inhibitor concentration in soybean seeds

*Luciana Rosso**, Crop and Soil Environmental Sciences, Virginia Tech, Virginia, USA
Chao Shang, Crop and Soil Environmental Sciences, Virginia Tech, Virginia, USA
Edgar Correa, Crop and Soil Environmental Sciences, Virginia Tech, Virginia, USA
Bo Zhang, Crop and Soil Environmental Sciences, Virginia Tech, Virginia, USA

Trypsin inhibitor (TI) is an important anti-nutritional factor present in soybean seeds that prevent animal's protein digestibility. Determining seed trypsin inhibitor concentration is essential for screening and selecting soybean germplasm and breeding lines with low TI. Currently, a colorimetric bioassay that measures TI activity (TIA) is widely used to measure TI in soybeans. This bioassay is time consuming, expensive and has repeatability issues. This study developed a high performance liquid chromatography (HPLC) method, based on quantifying Kunitz trypsin inhibitor (KTI), as a high throughput, less expensive and more reliable assay to quantify TI in soybean seeds. The HPLC method was evaluated on 100 lines with various TI concentration and compared to the modified colorimetric bioassay. For the bioassay, TI was extracted by mixing soybean seed powder in 9 mM HCl and TIA was determined based on Kakade et al.,1974. For comparison, the TIA was converted into weight-based unit assuming that KTI is a dominant TI contributor. For the HPLC method, KTI was extracted by using 0.1M NaOAc, separated on a Poros R2/H perfusion column and detected at 254 nm. Each method was performed by duplicate. KTI from the bioassay ranged from 3.69 to 8.43 mg/g with an average of 5.21 mg/g, and the KTI from HPLC method ranged from 0.61 to 11.21 mg/g with an average of 5.25 mg/g. Data from both methods were strongly correlated ($r = 0.78$, $p \leq 0.0001$). The coefficient of variation (%) of KTI data (66% HPLC and 19% bioassay) suggests that the HPLC method reveals a wider range of KTI data that may have a better sensitivity to detect KTI than the bioassay. In addition, the colorimetric bioassay is experimentally tedious and labor intensive. Therefore, the HPLC method is highly preferred to provide a simpler, faster, more sensitive and reliable quantification of TI in soybean seeds.