M-167

# Analysis of the RLK LRR domain and its synthetic mutants and preparatory binding studies to some of its peptide ligands

*Azam Baharlouei**, Southern Illinois University, Illinois, USA
*David Lightfoot*, Department of Plant Biology, Southern Illinois University, Illinois, USA

The Rfs2 region on chromosome 18 of soybean encodes, among other genes, a receptor like kinase (RLK) protein known to mediate resistance to soybean cyst nematode (SCN) *Heterodera glycines* (I.) and *Fusarium virguliforme* (Aoki) causal agent of sudden death syndrome (SDS). In this present study, the extracellular leucine rich repeat (LRR) domain of RLK and its mutants were cloned in *Escherichia coli* and its expression optimized. The mutants of the RLK-LRR domain (H143D,N,Y) was generated using site-directed mutagenesis. Previously, the amino acid residues at that site was shown to be involved in mediating crucial interactions for the stability of the RLK-LRR homodimer and thus its function. One of our long term goal is to correlate the effect of the synthetic mutations to the binding of LRR domain to its cognate CLE (CLV3/ESR) peptide ligands found in soybean plant secretome and SCN. CLE peptides mediate signaling in developmental and disease resistance pathways in soybean plants. Fluorescein isothiocyanate (FITC) labeling of several of the peptides were optimized to be used in fluorescence polarization (FP) based binding studies. Immunohistochemistry assays were performed to show the localization of the RLK protein in root tissues of soybean plants. Although RLK-LRR expression was optimized, almost 100% of the protein formed inclusion bodies and attempts to refold the protein to native functional state was unsuccessful thereby limiting its use in functional binding assay. The work presented here motivates future studies of LRR domain interaction with its binding partners and relate it to its function.