Abstract

Identification of anonymous proteins from two-dimensional (2-D) gels by peptide mass fingerprinting is one area of proteomics that can greatly benefit from a simple, automated workflow to minimize sample contamination and facilitate high-throughput sample processing. In this investigation we outline a workflow employing robotic automation at each step subsequent to 2-D gel electrophoresis. As proof-of-concept, 96 protein spots from a 2-D gel were analyzed using this approach. Whole protein (1 mg) from mature, dry soybean (*Glycine max* [L.] Merr.) cv. Jefferson seed was resolved by high resolution 2-D gel electrophoresis. Approximately 150 proteins were observed after staining with Coomassie Blue. The rather low number of detected proteins was due to the fact that the dynamic range of protein expression was greater than 100-fold. The most abundant proteins were seed storage proteins which in total represented over 60% of soybean seed protein. Using peptide mass fingerprinting 44 protein spots were identified. Identification of soybean proteins was greatly aided by the use of annotated, contiguous Expressed Sequence Tag (EST) databases which are available for public access (UniGene, http://www.sciencedirect.com/science?_ob=RedirectURL&_method=externObjLink&_locator=url&_cdi=5275&_plusSign=%2B&_targetURL=ftp%253A%252F%252Fftp.ncbi.nih.gov%252Frepository%252FUniGene%252F). Searches were orders of magnitude faster when compared to searches of unannotated EST databases and resulted in a higher frequency of valid, high-scoring matches. Some abundant, non seed storage proteins identified in this investigation include an isoelectric series of sucrose binding proteins, alcohol dehydrogenase and seed maturation proteins. This survey of anonymous seed proteins will serve as the basis for future comparative analysis of seed-filling in soybean as well as comparisons with other soybean varieties.