

ABSTRACT

An efficient protein extraction method is crucial to ensure successful separation by two-dimensional electrophoresis (2-DE) for proteome studies in plant species. Rice variety MR232 was used as an experimental material. The seeds were grown in pots by adopting completely randomized design with three replication. Eight different protein extraction methods were followed in the study to determine the efficiency of protein extraction. Stress was induced artificially by withholding water on 45th day and leaf samples were collected for protein extraction. Among eight extraction methods tested, phenol, TCA-Acetone (4) and direct IEF extraction recorded higher amount of total proteins; Qualitative analysis of SDS also confirmed the efficiency of the three methods. These three extraction methods which gave high intense protein bands with clarity were considered for 2-DE analysis. A clear distinction of protein alteration was observed between control and stress induced leaf tissue in 2-DE gel. Among the three aforementioned extraction methods, phenol extraction method has shown 20 protein spots variation between control and stress, 12 in TCA-acetone (4) method and 13 in direct IEF extraction method. All these altered protein spots were then compared with UniProtKB database based on its MW and pI value to predict the identity of proteins. The investigation has shown that among the three selected methods, phenol extraction method has proved to be effective for further proteome analysis using 2-DE which gave the highest protein yield, a greater spot resolution and a minimal streaking on 2-DE gels for both control and stress induced rice leaf samples. Present results may provide new insight to drought stress proteome analysis in rice and might provide a better understanding of the molecular basis of drought-regulation in plants.