
Thesis Advisors: Assoc. Prof. Dr. Sompong Thammasirirak, Assoc. Prof. Dr. Prasit Jaisil

ABSTRACT

Drought is a major abiotic stress that causes severe limitations on the productivity of sugarcane. Biochemical marker and physiological traits associated with drought tolerance are suitable indicators for selection of drought tolerance plants to decrease the impact of drought stress on crop yield in breeding program. Therefore, the sugarcane leaf proteins associated with drought tolerance sugarcane cultivar were analyzed using drought tolerance (K86-161) and drought susceptible (Khon Kaen 1) cultivars. The protein analysis was started by separation of total protein which extracted from sugarcane leaves by 2D-PAGE. The silver stained protein pattern from both cultivars were analyzed and compared. From the results, the proteins that associated with drought tolerance cultivar were classified into five groups according to their molecular weights, namely, 14-19 kDa, 20-22 kDa, 24-28 kDa, 38-44 kDa and 48-50 kDa. These five groups of proteins were extracted from K86-161 sugarcane leaves, separated by SDS-PAGE, and used as antigen for antibody production in mice. Mice were bled and the titers of antisera were assayed by Western blotting technique. The polyclonal antibody elicited to 14-19 kDa protein group indicated high specific antibody to 18 kDa protein and the titer of antigen-antibody interaction at around 1:100 was obtained. Whereas, the titer of other protein groups was very low. So, we focused on 18 kDa protein. To study the 18 kDa protein to drought stress, the representative sugarcane cultivars; K86-161 and Khon Kaen 1 were submitted to progressive water stress for 20 days. The effects of drought stress on protein in the sugarcane leaves were also analyzed using 2D-PAGE. The patterns of the electrophoresis were compared to those of control plants grown normally. Among the modifications induced by drought, the accumulation of a high intense 18 kDa protein with a pI value of 7 was particularly noticeable. As a result, the 18 kDa protein was chosen as drought tolerance associated marker. Then anti-18 kDa protein antibody was used to compare the expression level of this protein in K86-161 and Khon Kaen 1 sugarcane leaves by Western blotting technique. The result indicated that 18 kDa protein band from K86-161 expressed higher intensity than that of Khon Kaen 1 in equivalent total protein amount. The specificity of this antibody was also tested using other two drought tolerance sugarcane cultivars; K88-92 and Uthong 1 and other two drought susceptible sugarcane cultivars; Phill.66-07 and K84-200 by ELISA and Western blotting technique. The results from both techniques demonstrated that 18 kDa proteins were expressed much more intense in drought tolerance sugarcane cultivars with a high specificity. Moreover, those sugarcane plants were further analyzed for drought tolerance by detection of two physiological indexes. The results indicated that the values of chlorophyll contents and SOD activity of drought tolerance plants were higher than those of drought susceptible plants. After the association between 18 kDa protein and drought tolerance was confirmed, the highly sensitive ELISA method was established for detection of the additional 52 sugarcane cultivars for their drought tolerance potential on the expression of 18 kDa protein. This result suggested that six cultivars which contained relative absorbance at 405 nm more than 60% could be used as the parental drought tolerance sugarcane cultivars for further breeding program. These studies indicated that the expression of 18 kDa protein in sugarcane leaves show correlation with drought tolerance and may play important role to preserve water in plant. In addition, this protein is probably able to be developed as a marker in any screening techniques for drought tolerant sugarcane cultivars.