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Identification and Characterization of Drought Stress Protein on Soybean (*Glycine max* L. Merr)

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ABSTRACT

This research aims to identify the changing of protein of soybean (*Glycine max* L. Merr) in response to drought stress. Four tolerant varieties of soybean Tanggamus, Nanti, Seulawah, Tidar, two moderately tolerant varieties Wilis and Burangrang and one sensitive variety Detam-1 were subjected to drought stress using limitation of watering. Proteins were isolated from leaf using presipitation method with TCA, and then run on SDS-PAGE. The suspected protein then sequenced at Proteomics International, Western Australia and analyzed using *Malditoff Mass Spektrometer Proteomics Analyzer*. Spectrum analysis based on hint peptide was identified using *Mascot Sequence Matching Software and compare to the* protein database and aligned using *Clustal X software* Bioedit and BLAST (*Basic Local Alignment Search Tool)* at NCBI. Two new protein band, 13 kDa and 52 kDa, identified in previous experiment were separated using 2D-PAGE. The result shows that the 13 kDa protein band in tolerant varieties were thicker when the plant subjected to drought stress than in normal condition. This protein show high (96%) homology to *auxin binding protein* and 88% homology to *germin like protein*, which has enzymatic activity as detoxification enzyme *oxalate oxidase* and *superoxide dismutase* which has a role in the drought tolerance mechanism.

Key words: Protein, drought stress, soybean, 2D-PAGE

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INTRODUCTION

The insufficiency of soybean production to meet domestic demand was happened due to the low and instability of the productivity (0.5 to 1.6 tons/ha). One of the causes of the low productivity is drought stress which causes insufficient soil water availability for soybean to grow. Drought has been known as one among the most limiting environmental stresses on plant growth and productivity. Drought stress causes biochemical changes such as osmolite and specific protein accumulation caused by changes in the cellular and molecular level [1]. Plant responses to drought as a result of some physiological and biochemical mechanisms are integrated events ranging from signal perception, transduction and regulation of gene expression that leads to adaptive changes in plant growth such as: changes in growth rate, stomatal conductance, osmotic potential network, and antioxidant defences [2-5]. Changes in protein expression, accumulation and synthesis of protein has been observed in several plant species under conditions of drought stress during the growing [6, 7]. Protein changes that occur both qualitatively and quantitatively detected during drought stress [8], drought stress increased the expression of a number of 50 proteins, decrease the expression of 23 proteins and induction of 10 proteins were detected by 2-dimensional gel electrophoresis.

In addition to the decrease in protein synthesis, new proteins also occur under drought stress conditions. There are hundreds of proteins induced by environmental stresses as plant defence responses to stress, but the mechanisms of plant resistance that caused by the synthesis of new protein is not yet [9, 10]. The concentration of a particular protein (10-70 kD protein) increases in drought conditions, in addition to the decline in the concentration of several other proteins as well as the synthesis of new proteins [11-13]. Late embryogenesis abundant (LEA) protein especially with a molecular weight of 10-30 kDa involved in the protection of higher plants from damage caused by environmental stresses, especially drought [14-16].

Drought stress causes changes in physiological processes, metabolism and the expression of several genes that are thought to play an important role on adaptive response of plants to water stress. Some of the genes responsive to drought stress , high salinity and cold temperatures at the level of transcription (mRNA) has been widely reported [17, 18] . The amount of mRNA of genes responsive to drought stress decreased when the stress condition ceased. This suggests that the genes expression is induced by water deficit in environment. The functions of several gene products based on predicted amino acid sequence is protecting plant cells during dehydration [19]. So, in this research protein expressed during drought stress were identified and sequenced in order to develop candidate marker for drought tolerance varieties.

MATERIAL AND METHODS

Plant Material and Protein Isolation

The plant materials used for the isolation of protein is the leaf of seven soybean varieties including: four drought tolerant varieties Tanggamus , Nanti , Seulawah , and Tidar,

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two moderately tolerant varieties Wilis and Burangrang and one sensitive variety Detam - 1. Two-dimensional protein analysis is done in two stages, i.e. isoelectrofocusing as the first dimension and SDS - PAGE as the second dimension. Protein was isolated from leaves using acetone/trichloroacetic acid (TCA) method [20].

Amino Acid Sequencing

2D - PAGE protein profile that indicates the position of the target protein in a specific molecular weight and specific isoelectric point (pI), was determined to decide the transfer buffer solution to be used. Sample preparation for amino acid sequencing includes two phases: the first phase was SDS - PAGE electrophoresis protein, the second stage is staining and destaining. Gel containing the target protein band was cut and sends for amino acid sequencing using the N terminal acid method to Proteomics International, Western Australia.

RESULT AND DISCUSSION

Identification of Drought Stress Protein

Protein analysis by 2D-PAGE technique (two-dimensional polyacrylamide gel electrophoresis) was conducted to determine the protein profile differences of drought tolerant soybean varieties under drought conditions compared to the control which grow under normal condition based on differences isolektric point (pI) and molecular weight (kDa). In normal conditions the plant showed the presence of several proteins of a molecular weight of 14 kDa - 97 kDa, with a pI range 4-7 and there are about 90-100 points (Figure 1).Electrophoresis results showed that there were differences between the control condition with stress, soybean varieties tolerant to the presence of specific proteins appear different when compared to the control condition with a molecular weight of about 13 kDa and an isoelectric point of 5.3.

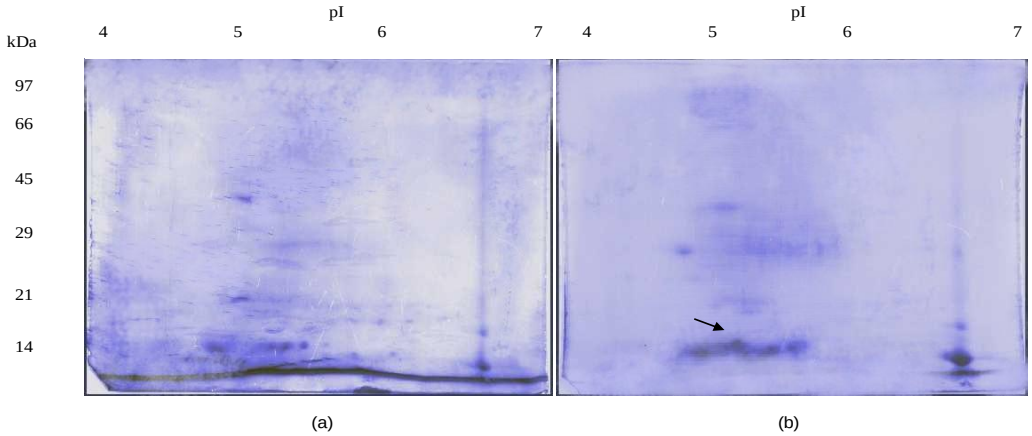

Figure 1: 2D-PAGE protein profile of leaves of soybean varieties tolerant (Tanggamus), (a) in normal condition, (b) in drought stress condition. (Note: The arrows indicate the specific protein with a molecular weight of 13 kDa)

Amino acid sequencing

Amino acid sequencing of the 13 kDa protein yielded a sequence of 19 peptides. This peptide sequence is then compared to the existing database at NCBI using the BLAST program (Basic Local Alignment Search Tool). The analysis showed that the 13 kDa protein fragment has 96% homology with *Glycine max* ABP19a-like auxin-binding protein, 88% the protein germin-like protein subfamily 3 member 4 precursor *Arachis hypogaea*, 44% GLP *Pisum sativum*, 83% GLP *Capsicum chinense*, 83% GLP *Nicotiana tabacum*, 83% GLP *Chimonanthus praecox*, 79% GLP *Gossypium barbadense*, 79% GLP *Hordeum vulgare* subsp. Vulgare, 79% GLP *Oryza sativa*, *Arabidopsis thaliana* 64% GLP.

It is known that the resistance of plants to face stress involve a change in gene expression, in addition to changes in the genes themselves. Changes in proteins is an important part of plant response to environmental stress conditions and for adaptation to specific environmental conditions [21, 22]. Under drought conditions some process changes, including changing in protein levels in plants. Some researchers showed that the protein content decreased in drought stress conditions [23, 24], another study showed increased levels of protein in drought stress conditions [25].

Changes in protein expression, accumulation and synthesis of new protein has been observed in several species of plants in drought stress conditions during growth [6, 7]. Drought stress has been reported to increase the expression of 50 proteins, formation of 23 proteins and a decrease in the number of 10 proteins [8].

In the previous research, characterization of proteins using SDS-PAGE electrophoresis results showed the formation of new proteins with a molecular weight of 13 and 52 kDa in drought-tolerant varieties Tanggamus, Nanti, Seulawah and Tidar [26]. This protein is not found in the moderate tolerant varieties Wilis and Burangrang and drought sensitive variety, Detam-1. Induction of new proteins in drought tolerant varieties showed the mechanism of these varieties to coupe with drought stress conditions. Tolerance in these varieties is indicated by the appearance of new proteins in stress conditions which thought to play a role in the mechanism of drought resistance.

Confirmation using 2D-PAGE electrophoresis in this research showed that the drought tolerant varieties induced new proteins which different in the thickness compared to the control condition. In drought tolerant soybean varieties showed a thicker protein than the control. This protein has a molecular weight of about 13 kDa and an isoelectric point of 5.3. On the other hand the moderate and sensitive varieties was not any induction of new proteins in drought stress conditions.

The change of protein type or the emergence of new protein is also found in other plants. Research on palm oil showed the formation of a new proteins in the molecular weight of approximately 60 kDa in drought stress conditions [27], whereas severe drought stress conditions reported to cause the induction of 40 kDa new protein on wheat [12]. Sugarcane

which experiencing drought stress accumulated a specific protein with a molecular weight of 22 kDa and an isoelectric point of 6.0 [28]. Generally, accumulation of low molecular weight proteins increase when plants experience drought stress [29, 30], but the total protein decreased.

The 13 kDa protein identified in this research showed 96% homology with auxin - binding protein - like ABP19a of *Glycine max*, 88 % with germin - like protein (GLP) subfamily 3 member 4 precursor *Arachis hypogaea*. Homology between auxin - binding protein with GLP are also found on peach. Cloned of a gene that encodes auxin - binding protein (ABP19/20) showed high homology with proteins in the germin family [31]. Auxin binding protein has enzymatic activity as glutathione S - transferase [32], and manganese superoxide dismutase [33]. Glutathione is one of the endogenous antioxidants in plants [34] which has an important role in defence mechanisms.

Some important catalytic enzymes that use glutathione in the mechanism of resistance in stress conditions such as cold temperatures and drought is the glutathione S - transferase (GST) and glutathione reductase (GR) [35] . Increased activity of GST on suspected tolerant varieties have an important role in protecting plants again drought stress [36], by the mechanism of antioxidant defence as also showed by catalase (CAT), superoxide dismutase (SOD) , ascorbate peroxidase (APX) , and glutathione reductase (GR) [37].

GLP subfamily are classified into two groups based on the activity of oxalate oxidase (OXO) and superoxide dismutase (SOD). Germin has been identified as oxalate oxidase [38, 39]. Oxalate oxidase and Super Oxide Dismutase (SOD) produces hydrogen peroxide (H_2O_2) which catalyzes the oxidative breakdown of oxalate ($C_2H_2O_4$) to 2 CO_2 and H_2O_2. Germin was suspected to contribute in the preparation of cell wall [39] by producing H_2O_2 required by peroxidase to catalyze the crosslinking of several components of the cell wall [40]. Germin and germin - like proteins play a role in strengthening the cell walls of plants for resistance to biotic and abiotic stress [41, 42, 43].

The expression of gene encoding germin-like proteins (GLP) observed in experimental trials of plant subjected to drought , high salt stress and high temperature stress [44, 45, 46, 47]. GLP gene function are not clearly understood, but is thought to be involved in the regulation of cell wall expansion. Germin protein first identified in wheat as genes expressed during germination. In Mesembryantemum crystallinum plants, germin - like protein (McGLP) expressed in roots and decreased in response to salt stress [48].

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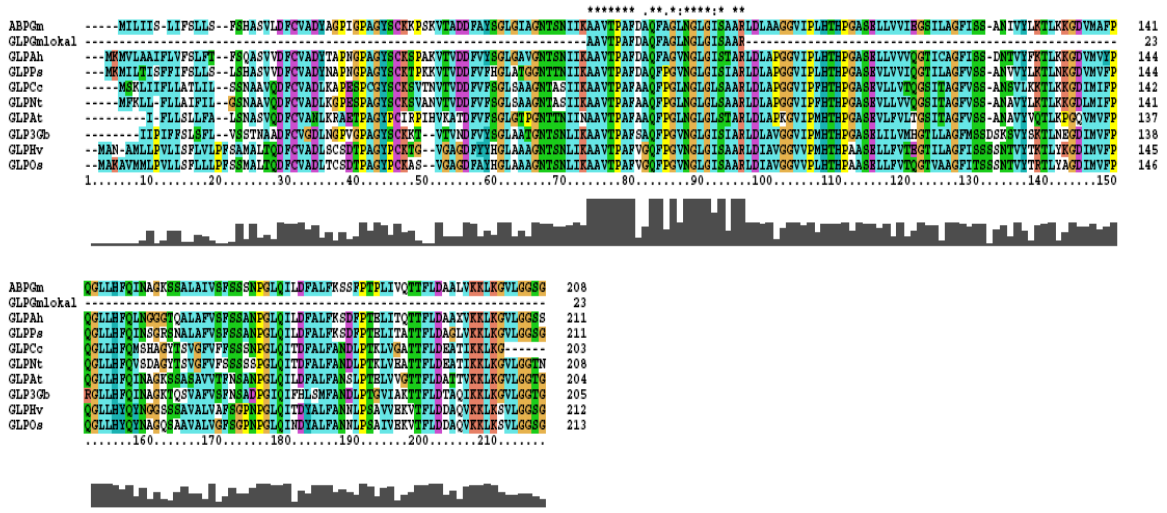

Figure 2: Alignment of amino acid sequences of soy protein 13 kDa locally with existing sequences in the NCBI database. ABPGm (*Glycine max*), GLPGm (*Glycine max)* local, GLPAh (*Arachis hypogaea*), GLPPs (*Pisum sativum*), GLPCc (*Capsicum chinense*), GLPNt (*Nicotiana tabacum*), GLPAt (*Arabidopsis thaliana*), GLPGb (*Gossypium barbadense*), GLPHv (*Hordeum vulgare*), GLPOs (*Oryza sativa*). Identical amino acids are marked symbols which conserved marked box and star symbol.

CONCLUSION

A new protein of the molecular weight of 13 kDa was produced by drought tolerant soybean varieties in response to drought stress. Based on the 2D-PAGE electrophoresis results, the production of this protein was higher under drought stress than that in normal condition. The 13 kDa protein was identified as a protein which homologous to the detoxification enzyme oxalate oxidase and superoxide dismutase which plays a role in the mechanism of drought resistance

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