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Protein Expression of Soybean (*Glycine Max* L. Merr) Varieties In Drought Stress

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Abstract

Drought is one of the most severe limitations on the productivity of soybean. There are many genes and proteins involved in drought stress tolerance. Identification of proteins which could be used as the base for the development of molecular study is very important to understand drought tolerance thoroughly. The objective of the research was to investigate protein expression of soybean to drought stress. Changes in protein expression were analyzed using SDS PAGE and two dimensional gel electrophoresis. Image analysis of 2D protein was performed by using the PDQuest 8.0 software program (Bio-Rad). Tolerant variety, Dering-1, was subjected to drought stress using limitation of watering, while Detam-1, a sensitive variety, was used as comparator. The result showed that protein concentration have decreased in drought condition from 3,22 mg/ml to 0,77 mg/ml. The new protein band with the 24,95 kDa have been found in drought condition. This protein is osmotin like protein with the accession number NP915414 which may play a role in the mechanism of drought resistance. The identification of the protein based on sequence amino acid literature review.

1. INTRODUCTION

Wetland, characterized by two dry season (I and II), is commonly used as the main agro-ecosystem soybean production in Indonesia. As a result of this condition, soybean cultivation often have the risk of crop failure due to the drought. Global warming causes the increase in the intensity of extreme drought and the risk of such failures. Recently, soybean varieties which are specifically released for the purpose of drought tolerant are not available yet. Varieties Dering-1 (strain DV / 2984-330) is a soybean variety that is assembled to solve the problem.

The research on Dering-1 has been performed over six years to solve the risk of

drought on soybean production in the dry season. As a result, strain DV / 2984-330/ Dering-1 variety provides the highest grain yield (1.95 t / ha), 14% higher than Tidar (1.71 t / ha) and 16% higher than Wilis variety (1.68 t / ha). Tidar and Willis have been used as comparator varieties. Lines DV / 2984-330/ Dering-1 cultivar, able to grow well even in drought conditions. Results of multi-location experiment at 16 dry locations in Probolinggo, Jombang and Mojokerto prove promising lines which have the potential to achieve the highest yield of 2.8 tons per hectare, with an average yield of 2 tons per hectare (Suhartina, 2014).

Arumingtyas & Savitri (2014), used two varieties of soybean Tidar tolerant varieties sensitive variety Detam-1 were subjected to drought stress using limitation of watering in greenhouse. Two new protein bands, 13 kDa and 52 kDa, were found in Tidar variety in drought condition. Proteins identified in previous experiment were separated using 2D-PAGE. The result shows that the 13 kDa protein band in tolerant variety was thicker when the plant is 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* and have role in the drought tolerance mechanism.

Expressed protein plants during drought can vary regarding the condition of time and different growing season, so need to confirm repeatedly with different conditions. In this study the varieties used are local superior varieties which tolerant to drought and planted in the field.

Identification of drought-resistant protein on drought-resistant varieties is important to know the protein expressed by the gene in drought condition. The result of this study can be used as molecular markers to study drought resistance in soybean and further can be used to identify several genes responsible for drought resistance and their mechanisms. The aim of the study to get profile protein expression of soybean varieties in drought stress condition.

2. MATERIALS AND METHODS

Growing plants, the Glycine max L. Merrill cultivars Dering-1 and Detam-1 have contrasting responses to water deficit; high tolerance to drought, and very sensitive to drought stress (Research Institute of Legume and Tuber Crops, 2012). Plant were grown in the field under severe drought stress by applying soil water content of 25% field capacity at the early growth phase until harvest time, and compared to plant grown in normal condition (100% field capacity) as normal condition.

The materials used for the isolation of protein and SDS- PAGE were: 1 M Tris-HCl pH 8.3, 5 M NaCl, 1 M DTT, 0.1 M EDTA, aquabidest, 40 mM PMSF, 10% SDS, Tris-HCl pH 8, ammonium persulfate (APS), TEMED (Sigma), 12.5% separating gel, 3% stacking gel, RSB (Reducing Sample Buffer) for the plant (0.1 M Tris-CI, 4% SDS, 0%, 2% Bromophenol blue, 200 mM DTT), the staining solution (Coomasie Blue R-250, methanol, distilled water, glacial acetic acid), destaining solution (methanol, glacial acetic acid, distilled water).

Protein isolation was determined by following the procedures of Stacy and Aalen (2003). A total of 0.1 g soybean leaves was homogenized in 500 µL cold extraction buffer. The homogenate was then moved into eppendorf tube which has been filled with 250 µL extraction buffer, mixed well, added with 40 mM PMSF 25 µL, incubated for 1 h in a refrigerator and vortexed every 15 min. The mixture was then centrifuged at 13,000 rpm and temperature 4°C for 5 min. The supernatant was moved into Eppendorf tube. Protein concentration was measured using Nano- drop apparatus.

Precipation with TCA. One volume of 20% TCA was mixed with one volume of protein sample, and the mixture was vortexed. After 1 h of incubation at 20 the sample was

sentrifuge at 15 000 g for 15 min at 4 °C and the supernatant was removed. Then, 0.5 mL of ice-cold acetone containing 20 mM DTT was added, and the mixture was centrifuged at 13 000 g for 15 min at 4 °C C. The supernatant was discarded, and the pellet was air dried (Fic *et al*., 2010).

Electrophoresis. The protein resulted from previous step was added with sampel buffer (RSB) with the ratio of 1:1 (10 µl:10 µl), then run in SDS-PAGE. The gel use in this experiment was 12% separating gel and 3% stacking gel. Electrophoresis was done using a constant current 20 mA for 4 hours. The gel then stained using Coomassie Brilliant Blue (CBB) and destained. The molecular weight was determined using standards protein.

Isoelectrofocusing. Two-dimensional protein analysis was performed in two stages (Natarajan, 2005); Isoelectro focusing as the first dimension and SDS-PAGE as the second dimension. Protein samples isolated from the leaves were firstly extracted by using acetone/trichloroacetic acid (TCA). The sample was poured into a column tray, covered with IPG strip (pH 4-7), incubated for 1 h at room temperature, added by 2 ml of mineral oil on a tray and incubated overnight. Attach strips focusing on the tray, add 2 ml of mineral oil and then leveled. Running in the IEF until it reaches 4 cycles IEF. After the strip is taken and drained, put on a clean tray plus 2.5 ml equilibration buffer I, shaker for 10 min and then drained. Added 2.5 ml equilibration buffer II shaker for 10 min, drained, dipped three times in a solution of 1 x TGS buffer. The strip is inserted into the plate to stick on separating gel, agarose overlay was then added 1 ml to condense. The electrophoresis was run for 45 min at a voltage of 200 V 85 mA. Visualization was performed by using coomasie blue staining, and shooting was performed by using Gel Doc.

Image analysis was performed with the PDQuest 8.0 software program (Bio-Rad). One gel image was selected as a reference followed by automated spot matching among the gels. The unmatched spots of the member gels were added to the reference gel. The amount of protein spot was expressed as the volume of that spot which was defined as the sum of the intensities of all the pixels that made up that spot. To correct the variability due to CBB staining, and to reflect the quantitative variations in intensity of protein spots, the spot volumes were normalized as the percentage of the total volume in all of the spots present in the gel (Xiong *et al*., 2010).

3. RESULTS

The results of protein isolation showed that protein precipitation with TCA treatment showed the higher concentrations than that of without precipitation in all varieties of soybean (Table 1). Whereas the general condition of drought stress causes a decrease in protein concentration. In normal condition, protein concentration of variety Dering-1 with and without TCA precipitation was 4.79 and 3.22 mg/ml, respectively. The concentration of protein in the stress condition decreased which ranges from 3.22 to 0.77 mg / ml

The result of SDS-PAGE analysis revealed that the protein bands with TCA precipitation were more appeared than without precipitation (Figure 1). The profile of variety Dering-1 protein also indicated that there were 10 bands with TCA precipitation and only 7 bands without precipitation. However, on the protein without precipitation indicates the induced a new protein in drought stress conditions.

Variety Dering-1 showed the different protein band profiles between normal and drought stress conditions. In normal conditions, 7 protein bands were observed and 10 bands in the drought condition. In the drought-sensitive varieties showed differences in the protein profiles of drought tolerance varieties. In the drought-sensitive there was proteins that decrease the quality of thickness is in the BM 24,5 kDa. Figure 1 showed changed of sample B1 and B2 to D1 and D2.

Table 1. Protein concentration

NO	Sample	Concentration (mg/ml)
1	A1	4,79
2	A2	3,22
3	B1	6,21
4	B2	5,34
5	C1	4,86
6	C2	0,77
7	D1	4,74
8	D2	0,79

A1 : variety Dering-1 precipitation with TCA normal condition
A2 : variety Dering-1 non precipation normal condition
B1 : variety Detam-1 precipitation with TCA normal condition
B2 : variety Detam-1 non precipation normal condition
C1 : variety Dering-1 precipitation with TCA drought stress
C2 : variety Dering-1 non precipation drought stress
D1 : variety Detam-1 precipation with TCA drought stress
D2 : variety Detam-1 non precipation drought stress

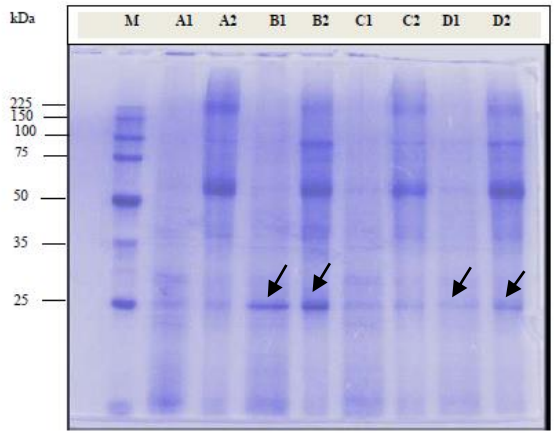

Figure 1. Analysis SDS-PAGE used separating gel 12,% and stacking gel 3% coloured with *Commassie Briliant Blue (CBB)*

M : Marker
A1 : variety Dering-1 precipitation with TCA normal condition
A2 : variety Dering-1 non precipation normal condition
B1: variety Detam-1 precipitation with TCA normal condition
B2: variety Detam-1 non precipation normal condition
C1: variety Dering-1 precipitation with TCA drought stress
C2: variety Dering-1 non precipation drought stress
D1: variety Detam-1 precipation with TCA drought stress
D2: variety Detam-1 non precipation drought stress

2D electrophoresis showed Dering-1 in drought condition (a) and normal condition (b). (Figure 2). In the stress condition showed that the induction of new proteins in BM 24.95 kDa; pI 6, there are several proteins that increase the quality of its thickness is in the BM 90.26, pI 7 and 45.17 kDa, pI 4.

4. Discussion

In general, the increase in the concentration of total protein occurred in the treatment of precipitation with TCA. Mechanism TCA precipitation 10% as the agent of the negative ions of the TCA will join the proteins that are in a state as cations (pH in acidic conditions to the protein isoelectric pH) to form salts protein. Some of the resulting salt does not dissolve so this method can be used to separate proteins from solution. General agent salt precipitation will dissolve while the protein will be decomposed by the addition of base (to form a negatively charged protein or anionic protein).

Drought stress caused reducing the concentration of the total protein, because the response to drought occur at the molecular

level, the cellular, biochemical and physiological. The response is generally decline in cellular processes that will be continued to physiological processes and the production of total proteins in drought stress. Under conditions of drought stress although the total protein concentration decreased but it could have happened the emergence of new protein bands and possibility the loss of certain protein bands. In this condition the SDS PAGE electrophoresis results could indicate an increase in the quality of the band become thicker or otherwise visualization becomes thinner.

The tolerant varieties showed induction of new protein drought stress, namely 90.26; 45.17; and 24.95 kDa. There was induction of new protein different with susceptible varieties (Detam-1) on the molecular weight 174.01 and 24.95 kDa. Further identification focused on protein 24.95 kD, it was believed to osmotin like protein (Hajheidari et al., 2005). Osmotin like proteins play a role in the mechanism of drought resistance. Osmotins (osmotin-like proteins or OLPs) are members of the Pathogenesis-related protein 5 (PR-5) family (Van Loon and Van Strien 1999), which are produced in plants under different abiotic and biotic stresses (Singh et al., 1987; Zhu et al.,1995; Gimeno et al.,2009; Zhang and Shih 2006). Among the several stress-related proteins, osmotin is one of the unique proteins, which is induced in response to both abiotic and biotic stresses in plants (Parkhi et al., 2009).

Transgenic research osmotin like protein coding genes showed that transgenic soybean able to manage the leaf water potential, CO_2 assimilation rate is higher, stomatal conductance (Grossi-de-Sa and Zanettini, 2014). Barthakur et al., (2001), the transgenics tobacco maintained higher leaf relative water content (RWC), leaf photosynthesis and free proline content than the wild type plants during water stress and after recovery from stress. These results suggest the involvement of the osmotin-induced increase in proline in imparting tolerance to salinity and drought stress in transgenic plants over-expressing the osmotin gene and tomato (Goel et al., 2010).

The proteins produced by plants in drought stress conditions were analyzed and characterized. This approach allowsto know several proteins responsible for stress conditions, plant growth and development. Attempts to improve the properties of plant resistance to drought stress within understanding the function of proteins involved in the defense mechanism against stress.

These proteins are produced from gene expression induced by environmental stress. Some proteins have a function as an introduction to signals from the cell surface to the cell plant, enzymes involved in the biosynthesis of molecules that influence the defense mechanisms (such as proline, some types of carbohydrates and polyamine), or a transcription factor that activates the expression of genes play a role in plant defense mechanisms against the stress.

Proteins on the molecular weight 24,6 kDa were expected induced by the genes responsive to drought stress conditions. Shinozaki and Shinozaki (1997), the expression of certain genes is influenced by a number of reaction of a number of genes that can be active (on) or inactive (off) because the time and the environment.

5. CONCLUSION

The result showed that protein concentration have decreased in drought condition from 3,22 mg/ml to 0,77 mg/ml. The new protein band with the 24,95 kDa have been found in drought condition. This protein is osmotin like protein with the accession number NP915414 which may play a role in the mechanism of drought resistance. The identification of the protein based on sequence amino acid literature review.

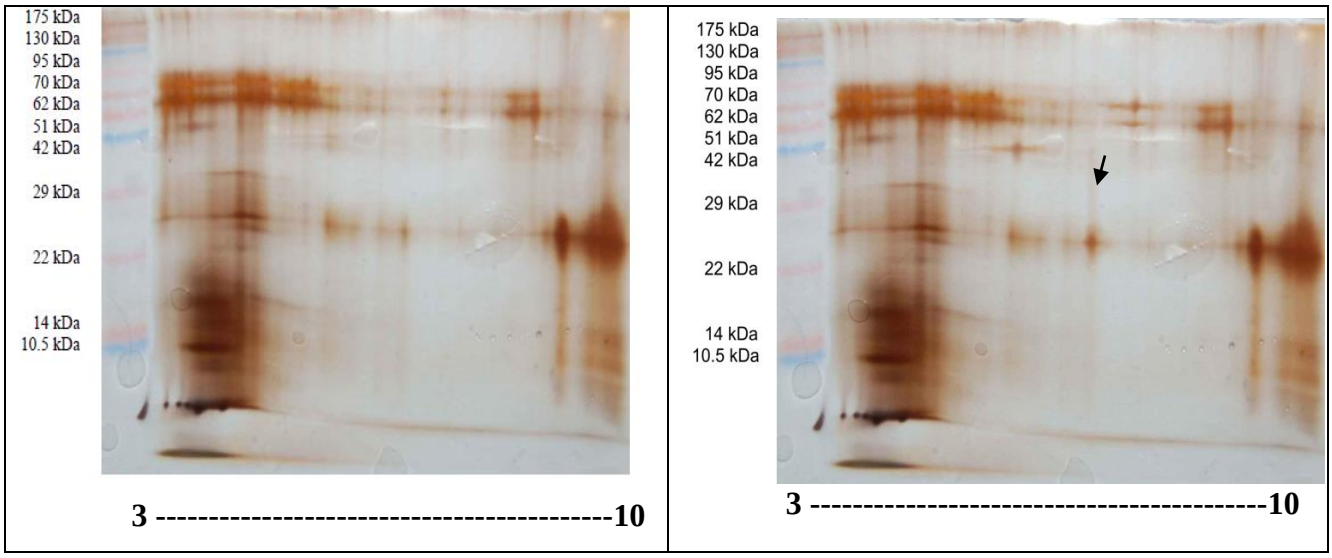

Figure 2. 2D-PAGE electrophoresis protein profile of leaves of soybean varietiy (Dering-1), in in normal condition (left) drought condition (right). (Note: The arrows indicate the specific protein with a molecular weight of 24,5 kDa)

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