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Editorial Office

Faculty of Science and Technology,
Universitas Islam Negeri Maulana Malik Ibrahim Malang
Jalan Gajayana 50 Malang, Jawa Timur, Indonesia 65144
Phone (+62) 81336397956, Faximile [\(+62\) 341 558933](tel:+62341558933)
e-mail: greentech@uin-malang.ac.id



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Profile of Protein Levels Some Tobacco Varieties (*Nicotiana tabacum* L.) On Waterlogging Stress

Tutik Nurhidayati*, Novitasari**, Herry Purnobasuki*, Sucipto Hariyanto*, Nurul Jadid**

*Department of Biology, Faculty of Mathematics and Natural Sciences Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia

**Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

*Corresponding author: tutikhd72@gmail.com

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ABSTRACT

Tobacco is a high-value crops that are sensitive to waterlogging stress. Some tobacco varieties have been widely cultivated in Indonesia, including Jepon Mawar, Jepon Banyak and Rejeb. Some species have different abilities to withstand the conditions of waterlogging stress by morphological adaptation, anatomy, physiology, and metabolic pathway changes. Changes in protein profiles is one form of plant defense response to waterlogging stress. Profile proteins experienced upregulation in hypoxia and anoxia conditions when gripped waterlogging stress known as anaerobic polypeptides. In addition to the waterlogging stress will cause the decrease in chlorophyll levels as a result of chlorosis during the stress. The purpose of this research is to know protein profile and chlorophyll content of several varieties of tobacco (*Nicotiana tabacum* L.): Rejeb, Jepon Mawar and Jepon Many against stagnant waterlogging stress. The protein profile was analyzed by SDS-PAGE method. While the chlorophyll content was analyzed by spectrophotometric method. The protein profiles expressed from the three test varieties were present in the molecular weight range 85.38-153.33 kDa. Proteins with a molecular weight of 85.38 kDa are thought to have similarities to the Peroxidase group (BM = 85 kDa) that play a role in the ROS detoxification process. While the chlorophyll content in the three varieties decreased with the increasing of waterlogging stress except on Rejeb varieties treated by 175% and Jepon Mawar varieties on 200% waterlogging stress treatment.

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Corresponding Author:

Tutik Nurhidayati,
Department of Biology,
Faculty of Mathematics and Natural Sciences
Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
Email: tutikhd72@gmail.com

1. INTRODUCTION

Waterlogging of an abiotic stresses which may affect growth and yields. One of the causes of waterlogging is the high rain insensity. The main cause of damage to the plant during the waterlogging is the low oxygen, which indicated the existence of wilting symptoms due to the absorption of nutrients and water that hampered [1]. Waterlogging can also cause the condition of hypoxia and anoxia in the soil. Stress reduces the pool of gas exchange between the plant tissue and air as gas diffusion layer in water 10,000 times slower than in air [4]. Despite the low oxygen availability is an important factor affecting plant growth, soil chemical element content, such as pH and redox potential, is also changing under stress conditions inundation and can also affect the survival and growth of plants [5,6

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as

degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10].

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10]. Reported by Ahzan (2007), found a total of 35 proteins in the roots of tomato plants (*Lycopersicon esculentum*) in response to the stress puddle. 16 proteins had increased expression and 13 proteins had decreased expression. In maize (*Zea mays*), the treatment of waterlogging stress changes the pattern of protein synthesis in the primary root [12].

Response of the other plant in response to the stress puddle is a decrease in the conductance of stomata (Folzer et al., 2006), changes in hormone balance (Else et al., 2001), decreased transpiration and inhibition of photosynthesis rate due to a decrease in leaf chlorophyll content (Cao and Conner, 1999). These responses occur within hours or days, depending on the level of tolerance of plant species (Striker et al., 2005). The decline of chlorophyll and chlorophyll degradation massively occur during leaf senescence, fruit ripening, and also in response to environmental stress (Hortensteiner and Bernhard, 2010). The decline in the chlorophyll content due to intake of nutrients, especially of N low due to damage to the root system due to flooding.

Determination of protein profiles and chlorophyll content of some varieties of tobacco in response to stress puddle provide a better understanding of its function in adaptation to stress.

2. RESEARCH METHOD

2.1 Preparation of tobacco seeds

Tobacco varieties seed in this study include var. Srumpung, Dixie Bright and Somporis. The seeds were germinated in germination media containing compost and chaff (2:1). Germination was carried out for 15 days after seeding (15 das). Germinated seeds were then pricked (until seedlings aged 54 das) and transferred *pottray*.

2.2. Planting tobacco seeds in polybag

54 das tobacco seedlings were transferred to non-perforated polybags. Planting medium used in this procedure was compost and chaff with a ratio of 4:1. Each polybag contains 1 kg of planting medium. 5 gr NPK fertilizer was applied for each plant. Pest and disease control was done using insecticides organtrin 1.5 ml / L. The control carried out at the time of old tobacco plants (15 HST). Tobacco plants were grown for 3 weeks (21 days after planting (dap)).

2.3. Field capacity measurement

Measurements of field capacity were conducted to determine the volume of the water in the treatment of *waterlogging stress*. Planting media that were placed in polybag *were watered* until the water passed through the media. The media was subsequently *left to stand for 3 days* until there were no *the dripping water occurred*. Furthermore, the media were directly weighted. Meanwhile, dry weight media were measured by placing the

planting medium in the oven at 100° C for 24 hours until reaching a constant weight. Field capacity is calculated using the formula:

$$W = \frac{Tb - Tk}{Tk} \times 100 \%$$

Information:

W : Field Capacity

Tb : Wet Weight (Gram)

Tk : Dry Weight (Gram)

2.4. Stress Waterlogging Treatment

Waterlogging stress treatments were carried out using 21 dap tobacco plants. *Waterlogging stress* treatment used in this study were 100%, 150%, 175% and 200%. These treatments were applied during 10 days. The volume of water was maintained for 10 days of treatment.

2.5 Sample Preparation (Protein Extraction)

Protein profile analysis using protein electrophoresis method. Plants that have been taken are washed with distilled water. A total of 0.250 grams of leaf organs were washed with phosphate buffered saline (PBS) pH 7.4. The organs were homogenized with cold mortar and added 500 µl of protein extract buffer. Homogenate is inserted in a 1.5 ml tube and centrifuged at 10000 rpm at 4 ° C for 10 minutes. The pellet is removed and the supernatant is inserted a new 1.5 ml tube then stored at -20 °C. For electrophoresis used 12.5% separating gel and 5% stacking gel.

2.7 Parameters of observation

Parameters include the observation of profile protein.

2.8 Data Analysis

The data chlorophyll content obtained were analyzed using ANOVA Two Way followed by *Tukey* test and descriptive to analysis protein.

3. RESULTS AND ANALYSIS

3.1 Protein Profile Japon Variety

Based on the results of SDS-PAGE analysis Japon Many varieties are shown in Figure 1 shows that a protein with a molecular weight of 153.33 kDa decreased expression represented by the thickness of the protein band with increasing level of flooding. Protein band with a molecular weight of 119.51 and 103.98 kDa expressed only in the treatment of 100% waterlogging stress and degradation as indicated by the loss of protein bands on the treatment of 150%, 175% and 200%. The protein band with a molecular weight of 90.48 kDa is expressed only in the concentration of 175% and 200% waterlogging. While the protein with a molecular weight of 85.38 kDa was uniformly expressed on all the treatment of waterlogging stress. The results show that there are some proteins that degrade or decrease expression when the waterlogging stress. In addition there are also proteins that are only expressed at high waterlogging rates such as proteins with molecular weight of 90.48 kDa. The weight of expressed protein molecules differs across all treatments ranging from 85.38-153.33 kDa.

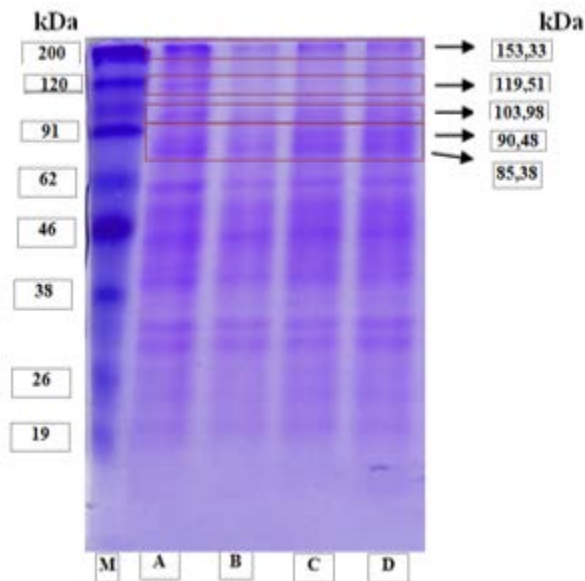


Figure 1. Results of Analysis of SDS-PAGE Profiles of Many Jepon Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

Research on tomato plants (*Solanum lycopersicum*) treated with waterlogging for 14 days showed that expressed proteins ranged in molecular weight 50-110 kDa [21]. The 50-55 kDa molecular weight protein is a member of the Rubisco Large-Subunit (RLS). The 60-61 kDa molecular weight protein is Rubisco Binding Protein (RBP), a molecular weight protein of 93-95 kDa which is an ATP-dependent protease belonging to serine protease (Clp-P), and a protein with a molecular weight of 110 kDa is a protein Rubisco Activase (RA). Based on the results of identification, these proteins play a role in the process of photosynthesis. Rubisco's expression decreased in total dissolved protein during response to the inundation stress. Rubisco has two functions that act as carboxylases that mediate CO₂ assimilation and as oxygenase in catalyzing the early photorespiration stage [22]. In addition, the presence of inundation can also induce the formation of ROS (Reactive Oxygen Species) which may lead to the degradation of Rubisco subunit and Rubisco Activase.

3.2 Protein Profile Rejeb Variety

In Rejeb varieties, occurring protein with molecular weight of 153.33 kDa increased expression in the treatment of waterlogging stress 175% as indicated by the band thicker than other treatments. Protein with a molecular weight of 119.21 and 103.98 kDa was also expressed on the previous varieties. These proteins are expressed in the treatment of 100% and have been degraded in the treatment of 150%, 175%, 200%. In addition, there is an increased expression of the protein bands with molecular weight of 85.38 kDa when the waterlogging stress treatment 175%, it is seen from the thickness of the protein bands. Band thickness decreases as 200% inundation concentration as shown in Figure 2.

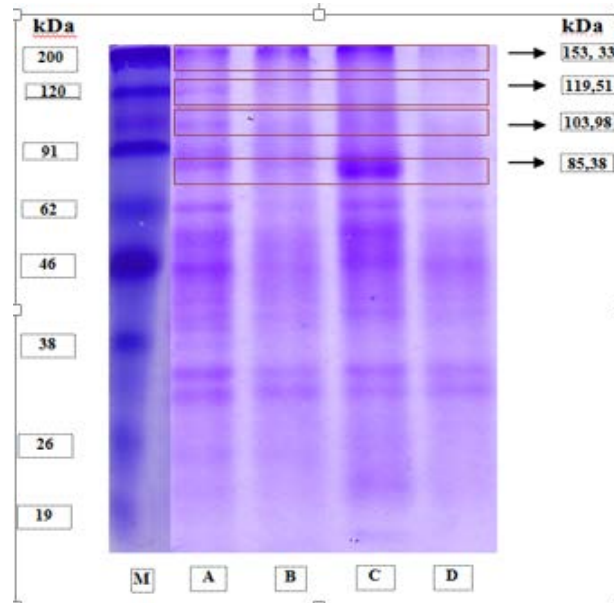


Figure 2. Results of Analysis of SDS-PAGE Profiles of Many Jepon Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

According to [23], maize (*Zea mays*) were flooded for 52 hours showed increased expression of a protein with a molecular weight of 85 kDa and 133 kDa. Protein with a molecular weight of 133 kDa initially decreased expression during the initial 4 hours of waterlogging stress, and increased after 28 to 52 hours of waterlogging stress. While the protein with a molecular weight of 85 kDa significantly increased its expression during 52 hours of waterlogging stress. Based on the results of identification, both proteins are included in the peroxidase group that play a role in the physiological functions of plants including plant development, oxidative stress and processes associated with plant cell walls. Peroxidase also plays a role in the production and detoxification of ROS (Reactive Oxygen species) caused by stress.

Oxidative stress occurs when unbalanced production and neutralization of ROS within cells. High light, heat, pathogen attack, low oxygen levels, and re-aeration after hypoxia phase can increase ROS production [25]. ROS is produced from molecular oxygen through several reduction steps. Anion superoxide (O_2^-), hydroxyl radical (OH), singlet oxygen (1O_2) results from the reduction of one or three electrons from oxygen by the reduction energy provided by the electron carriers in mitochondria and chloroplasts [26]. ROS is highly reactive and can cause damage to lipid membranes and proteins [23]. Plants have a protective system to protect mitochondria from overproduction of ROS such as antioxidants (glutathione, ascorbic acid, tocopherol, tannins, ubiquinol, and phenolic acid), ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX). So it can be predicted that an increase in protein expression with a molecular weight of 85.38 kDa in the Rejeb variety (175% stroke) is one form of protection mechanism of oxidative stress that can damage the lipid membrane and other proteins. It is also supported with Rejeb Reef morphology data of 175% treatment which showed better growth compared to 100% treatment.

3.3 Protein Profile Jepon Mawar Variety

In Jepon varieties of roses, also express proteins that appeared in previous varieties as shown in Figure 3, which is a protein with a molecular weight of 119.51 and 103.98 kDa that appears only in the treatment of stress puddle 100%. Protein with a molecular weight of 153.33 kDa stress treatment increased expression as a pool of 200%. Increased expression also occurs in Rejeb varieties with 175% stress treatment. While the protein with a molecular weight of 85.38 kDa expressed only in the treatment of a pool of 100% stress and degradation or decreased expression with increasing level of flooding. So it can be said that the majority of proteins that appear on the treatment of 100% have been degraded in the other treatment.

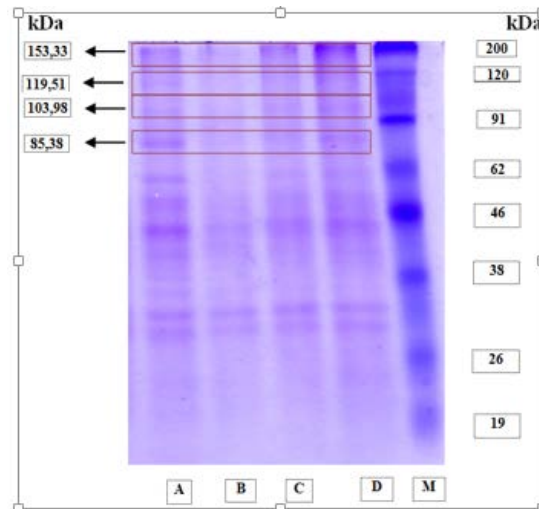


Figure 3. Results of Analysis of SDS-PAGE Profiles of Many Jepon Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

4. CONCLUSION

Stress can alter protein expression inundation of some varieties of tobacco. SDS-PAGE analysis of tobacco leaf protein indicates that there are 5 different proteins expressed in each variety and treatment of waterlogging stress. proteins are expressed differently in all three test varieties contained in the molecular weight range from 85.38 to 153.33 kDa. Varieties Rejeb increase protein expression at a molecular weight of 85.38 kDa in the treatment of 175%, as indicated by the thickness of the protein bands. The protein is degraded in the varieties of roses Jepon treatment 150%, 175% and 200% and expressed the same in all treatment Jepon Many varieties. Protein with a molecular weight of 85.38 kDa allegedly has in common with Peroxidase group (molecular weight 85 kDa), which plays a role in detoxification of ROS.

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