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Particular variety of tobacco Nicotiana tabacum) exhibits distinct morphological and physiological responses against periodic waterlogging stress

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Abstract. Waterlogging Stress influences crop productivity, especially high commercial crop like tobacco, which is very sensitive to an excess of water. This type of environmental stress might happen because of unpredictable season occurred in tropical region, including Indonesia. Global climate change as a consequence of rapid growing industries all over the world might responsible for this. This study aims to investigate the response of some *Nicotiana tabacum* varieties which are treated with periodic waterlogging stress. Some morphological aspects including plant height, stem diameter, leaves width and the emergence of adventitious roots were investigated. In addition, total chlorophyll content was also measured as physiological parameter. Three different varieties are used such as var. Jepon Pelakean, Jinten, Manilo and Morakot. Data were analyzed using One-Way Annova followed by Tukey *post-hoc* analysis. In all morphological aspects var. Jepon Pelakean demonstrated best responses compared to other varieties. Meanwhile, we observed a sharp decline of total chlorophyll content in var. Manilo under this periodic waterlogging stress. The overall results suggest that each variety of tobacco possess a specific mechanism against periodic waterlogging stress.

1. Introduction

Tobacco plant is one of important trading commodity in the world, including Indonesia and therefore play a significant role in the national economy [1]. Global climate change, especially on agriculture sector in Indonesia has a significant effect on tobacco plant productivity. Tobacco productivity has been reported to decline due to biological damage caused by waterlogging [2]. This is particularly problematic since the frecuency of flood disaster has increased in the last [3]. The term of waterlogging or flood often used to describe the different situations in which excess of water can range from saturated soil water conditions (waterlogging) until the formation of the water column causing a thorough immersion in plants (complete submergence) [4]. Saturated soil results in in a drastic reduction of gas exchange rate [5]. This condition might provoke serial perturbation on the biological system of the plant, since oxygen is required to maintain aerobic respiration in submerged tissue [6]. Therefore, the survival of plants under these conditions depends on physiological, morphological and metabolic adaptations [7].

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Morphological responses might obviously occurred when plant face the waterlogging stress. This includes the formation of adventitious roots and an increase in plant height. These will Additionally, plant also develops aerenchyma tissues for facilitating the oxygen distribution throughout the plant body [9]. An excess of water that drowns the aerial part of the plant would reduce external carbondioxide levels. The later will cause further decline of photosynthetic rate [10]. This condition is exacerbated by the changes in stomatal conductance [4] and a decrease in chlorophyll content [11]. This study will investigate the morphological and physiological responses of some tobacco varieties against periodic waterlogging stress.consequently result in an increased of aerial organs biomass [8].

2. Experimental Details

Seeding is done in mixed media tray containing vermicompos and coco peat (1: 1). Furthermore, the growth medium was added NPK (5 g / l medium) and Ridomil solution (0.5 grams / liter of water). Pricking was done using seedlings with 3-4 leaves. Plants that have reached the age of 60-70 das (possessing 4-5 leaves), were then placed into plastic container (40 cm x 30 cm x 20 cm). Each container is filled with 3 polybags of tobacco from the same variety. Stress treatment was firstly done by filling the container with water until reached 13 cm. This condition made the root of the tobacco plants saturated with water (soil waterlogging). This step was maintened for 5 days. The water level was continually maintained at 13 cm height (figure 1). Second step of treatment was done by adding water into the container until part of the stems and the first-second leaves drowned (partial submergence). This second step as also carried out for 5 days, and therefore, the total treatment was maintained. The morphological data includes plant height, stem diameter, leaf width and the number of adventitious roots. Physiological data collection includes the total chlorophyll content. Briefly, leaves were extracted using acetone. The extracts were then analyzed using a spectrophotometer at wavelengths 663 and 645 nm. The experiment was designed using Completely Randomized Design (RAL) using 4 varieties namely Jepon Pelakean, Jinten, Manilo dan Marakot. All data was replicated three times. Data were analyzed using ANNOVA One Way followed by Tukey *post-hoc* test.

Figure 1. Waterlogging Stress treatment at First Stage

Figure 2. Waterlogging Stress treatment at Second Phase (*Partial submergence*)

3. Results and Discussion

Morphological responses generally carried out by the plant, including the formation of adventitious roots and an increase in plant height. This resulted in increased biomass aerial organs, namely the trunk organs. The morphological response in plants to facilitate the distribution of oxygen to the tissues were soaked through aerenchyma network. The observed morphological parameters plant height, stem diameter, leaf width and the formation of adventitious roots are presented in Table 1.

Table 1. Morphological Characteristic of Tobacco Plant Varieties In Condition of Periodic Waterlogging Stress

Morphological Character	Tobacco Plant varieties							
	Jepon Palakean		Jinten		Manillo		Marakot	
	1	2	1	2	1	2	1	2
Plant Height (Cm)	9.63	10.5	11.53	10.5	14.2	10.27	14	9.03
Stem Of Diameter (Cm2)	8.23	9.13	6.77	8.87	8.43	8.03	7.83	8.77
Leaf Widht (Cm2)	11.07	12.1	8.47	10.5	12.23	9.93	11.8	11.1
Number of Adventitious Roots	0	11.33	0	5.67	0	3	0	10.33

Description: (1) Control Plants and (2) Treatment Plants of Periodic Waterlogging Stress

Plant height is a common parameter that should be measured in response to waterlogging stress. This can occur as a result of the interaction of plant hormones, such as abscisic acid (ABA), Gibberellins (GA) and Ethylene (Jackson, 2008). Based on our observation, var. Jepon Pelakean showed an increased of plant height compared to other varieties (Table 1). Meanwhile, three other test varieties showed a decreased of plant height compared to control. Basically, each variety has a different response based on internal interactions within the plant hormone. This is in accordance with previous study, conducted by [15], which showed that under waterlogging stress, two different rice varieties also demonstrated distingush responses. This might be due to hormonal factors involved, such as ethylene, ABA and GA. Var. Jepon Pelakean exhibited common morphological response under waterlogging stress, ie stem hypertrophy. This type of adaptive response is characterized by the production of GA that in some extent it depends on the availability of ABA. Basically, the accumulation of ethylene is also increased under hypoxic conditions. This will lead to a decrease of ABA accumulation through the inhibition of the expression of 9-cis-epoxycarotenoid dioxygenase and via activation of ABA into phaseic acid (PA). This certainly causes a decrease of endogenous ABA that is also required to enhance the expression of GA3 oxidase (an enzyme which catalyzes the conversion of PA into an active gibberellins / GA1). In addition, an increase of ethylene will also result in a lower pH in the apoplast which helps to stimulate the cell wall loosening, a critical step in cell elongation. Stem hypertrophy as a consequence of either hypoxic or anoxic conditions, possibly occurred because of the development of white spongy tissues. This type of tissue is further called as aerenchyma, which is formed externally from felogen and is a homologue of cork tissue. The aerenchyma tissue functions to increase the gas movement between submerged and non-submerged tissues. Some reports have demonstrated the formation of aerenchyma tissues as well as stem hypertrophy in some species such as *Lythrum salicaria*, *Lotus uliginosus*, *L. Tenuis*, *Glycine max*. In contrast to the result obtained in the var. Jepon Pelakean, the other varieties, var.Morakot, Manilo and jinten showed the opposite responses. These varieties showed reduced plant height, compared to control. Dubois et al. (2011) has demonstrated that lowland-rice varieties showed different responses compared to deepwater-rice varieties that undergo stem hypertrophy. Lowland-rice varieties exhibited an increased of gene expression that functions to suppress the formation of GA SUB1A through an increased of expression of SLR1 and SLRL1 repressor. Thus, it can be assumed that var. Marakot, Manilo, and Jinten might exhibit an overexpression of the SUB 1A-like gene that plays a role in GA biosynthesis. Then SUB 1A-like gene would regulate the SLR1 and SLRL1 which suppresses the synthesis of GA. The later would end up with an inhibition of stem elongation.

An increased of stem diameter correlate with the formation of aerenchyma tissue when the plants are facing the waterlogging stress. The formation of this type of tissues might increase the stem diameter. Plant physiological response to waterlogging stress depend on the tolerance level of the plant species. These physiological responses can be stomatal closure, reduction in transpiration and photosynthesis inhibition. Plants also respond to this environmental stress by regulating protein synthesis specifically for anaerobic conditions (anaerobic polypeptide / ANPS). In addition, reduce chlorophyll content could be also considered as physiological response of plants under waterlogging stress [4]. Simultaneous decline of total chlorophyll content in all tested varieties was obviously observed in this study. The highest chlorophyll content can be observed in var.Morakot under this type of stress, which only decreased by 2.8 %, followed by var.Jepon Pelakean in which its chlorophyll has decreased by 7.6 % compared to controls. Meanwhile, biggest decline of chlorophyll content occurred in var.Manilo that is equal to 41.5 %, followed by var.Jinten (33.8 %), compared to controls. Thus, it can be suggested that var.Morakot and Jepon Pelakean possess better responses in term of its total chlorophyll content compared to others.

4. Conclusion

Tobacco var. Jepon Pelakean demonstrated best responses compared to other varieties in all morphological parameters. Meanwhile, we observed a sharp decline of total chlorophyll content in var. Manilo under this periodic waterlogging stress. The overall results suggest that each variety of tobacco possess a specific mechanism against periodic waterlogging stress.

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