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Recovery of three different varieties of tobacco (*Nicotiana tabacum* L.) under waterlogging stress

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Abstract

Tobacco plant is one of the high commercial crops that give the largest foreign exchange sources in Indonesia. It also contributes to providing employment not only for farmers but also industrial sectors. Tobacco plant experiences growth failure due to excessive rain, which causes waterlogging to the plants so that the plants become submerged and show hypoxia. A hypoxia tolerant variety is needed to reduce losses in tobacco cultivation. The research was conducted in a greenhouse and biology laboratory at the University of PGRI Argopuro Jember. This research aimed to study the post-hypoxic stress recovery ability in three previously studied varieties of tobacco plants such as sensitive, moderate, and tolerant varieties. The results showed that the treatment of hypoxic stress on tobacco plants significantly affected the parameters of stomatal activity, transpiration rate, and the amount of reducing sugar. In the sensitive and moderate tobacco varieties, the greater percentage of waterlogging resulted in decreased stomatal activity, transpiration rate, and percentage of sucrose. The tolerant tobacco varieties (Bojonegoro var.) show a tendency to increase stomatal activity (76.203, 79.735, 86.963, and 92.227, respectively, for waterlogging 100%, 120%, 140%, and 160% field capacity), transpiration rate (0.139, 0.124, 0.130, and 0.146, respectively, for waterlogging 100%, 120%, 140%, and 160% field capacity), and percentage of sucrose (3.45, 3.64, 3.73, and 4.31, respectively, for waterlogging 100%, 120%, 140%, and 160% field capacity) with a higher percentage of waterlogging. Three varieties of tobacco with tolerant, moderate, and sensitive categories to hypoxia developed different metabolisms to cope with energy crises caused by waterlogging stress comprised of the stomatal conductance, transpiration rate, and sucrose percentage.

Keywords: Moderate; Reducing Sugar; Sensitive; Stomata Activity; Tolerant; Transpiration; Waterlogging.

Abbreviations: ABA_abscisic acid; ACC_aminocyclopropane carboxylate; ADH_Alcohol Dehydrogenase; ADP_adenosine diphosphate; ANPs_anaerobic proteins; ATP_Adenosine triphosphate; DNS_dinitrosalicylic acid; H_2O_2_hydrogen peroxide; LDH_lactate dehydrogenase; NAD_like nicotinamide adenine dinucleotide; O_2^-_superoxide radicals; SPSS_Statistical Package for the Social Sciences

Introduction

Tobacco plays an important role as the basic ingredient for pharmaceutical, cosmetic, and pesticide industrial products (Prasetyo, 2017). Extreme weather is one of the greatest dangers posed by climate damage. When rainfall increases, there is significant crop loss due to flooding (Costa and Farrant, 2019). Tobacco productivity has been reported to decline due to physical damage caused by waterlogging especially during the rainy season. The high intensity of rain floods the roots of tobacco plants, causing rotting, and leads to death. A waterlogging condition due high rain intensity can decrease 57% of agricultural products around the world (Liu et al., 2020; Raza et al., 2019). Efforts to develop superior varieties that are tolerant to waterlogging are very important in overcoming this problem.

Generally, stress is an alteration of the physiological conditions, triggered by factors that tend to disrupt the stability of the plant (Singhal et al., 2016). The oxygen deprivation condition in plant was the general result from waterlogging. Waterlogging cause a direct effect to the interference of water stability in soil (Tan et al., 2018). The re-vigorous of withered plant leaves at the end of a waterlogging stress showed that the water transport would back to a normal condition in the end of waterlogging condition. The root part of the plant has adapted the metabolism which leads to a better water and nutrient transport and results to a higher accumulation and formation of energy (Kumari et al., 2019; Pahlevi at al., 2019). Waterlogging stress would normally decrease the ATP formation, while the ATP derivation is higher in sensitive plant. It is evidenced that the ability of tolerant plant to bear the waterlogging stress by maintaining the energy supply could be a key factor to survive in this stress condition (Nakamura and Noguchi, 2020; Zahra et al., 2021).

The anatomy of every tobacco plant variety has a different response to adapt to waterlogging stress. This includes the alteration of cortex tissue, stele diameter in root, and formation of aerenchyma tissue under the scaled waterlogging condition (Nurhidayati et al., 2018; Purnobasuki et al., 2018).

Tamala et al. (2019) developed tobacco varieties that are resistant to hypoxic stress and showed three groups such as sensitive, moderate, and tolerant. However, this study did not investigate the effects of some types of tobacco after hypoxia treatment. Sucrose also plays an important role in adaptation to hypoxia (Iqbal et al., 2021). This adaptive process helps plants to relieve stress and recover from the stress.

Plants gradually adjust to overcome stress (Banerjee et al., 2019). The basic knowledge of plant adaptation to the climate change and how plant response to stress would result an effective strategy to alter risks in agriculture sector. In a waterlogging stress condition, the reflection of plant might be different according to waterlogging intensity (Sasidharan et al., 2017). The depletion of oxygen supply is a general condition in the waterlogged plant. The plant growth was inhibited as the water availability and oxygen supply was decreased, causing cell damage. The increased denitrification under anaerobic condition was also an adverse response from waterlogging in plant (Sjøgaard et al., 2018). The extension of waterlogging period and higher temperature would increase the damage of plant because of the increase of transpiration. Some plants have a different critical stage of plant development which is very sensitive to the abiotic stress condition (Considine et al., 2017; Le Gac and Laux, 2019).

Several researches have been conducted to understand the plant response to waterlogging, but less about the reoxygenation after plant has been released from waterlogging stress. The need to study and identify waterlogging tolerant genotypes is urgent (Pérez-Jiménez et al., 2018). Exploration of waterlogging tolerant tobacco varieties was essential to support tobacco cultivation and availability during climate change. The ability to recover from stress increases the plant's resistance to various waterlogging condition in the future which develops superior stress-tolerant plant varieties. Therefore, in this study, we investigated the plants' ability to recover after hypoxic stress in three tobacco varieties with the sensitive, moderate, and tolerant. The ability of plants to recover is observed in three aspects, such as stomatal activity, transpiration rate, and amount of reducing sugar. The results of this study aimed to provide the information needed to develop tobacco varieties that are resistant to hypoxic stress.

Result and discussion

Stomata conductance in tobacco plant after waterlogging stress

In plants, hypoxia (low-oxygen stress) is induced by soil waterlogging or submergence and this major abiotic stress has detrimental effects on plant growth, development, distribution, and productivity (Xie et al., 2021). Plant cells that grow under stress will limit growth to maintain water balance in cells and that cause some inhibition of plant growth (Avivi et al., 2020). One form of physiological response is stomatal closure. Stomata are the modification of leaf epidermal cells in the form of pairs of guard cells and can cause the appearance of gaps that allow the exchange of gas and water vapor between the inside of the stomata and the environment (Anu et al., 2017). Figure 1 shows the appearance of open and closed stomata after the tobacco plant was free from waterlogging. Morphologically, the stomata densities of the three tobacco varieties in this study were different. The moderate and tolerant varieties have small pores and low-density morphological features. The sensitive variety has large pores and high density. However, the three varieties have the same type of stomata, which is the anisocytosis type. The results in Tables 1, 2, and 3 show that higher percentage of waterlogging causes a lower stomatal opening. There was no significant difference in stomata activity between the sensitive and moderate varieties, with values that were lower than those of the resistant variety. This suggests that waterlogging treatment of the three varieties may reduce stomatal conductance and reduce stomatal opening rates. The higher the rate of waterlogging, the more the stomata close. This is the plant's response to waterlogging stress. Research results from Weits et al. (2021) reported the involvement of the availability of molecular oxygen in the process of plant development. Moreover, proliferating and undifferentiated cells from different plant tissues were found under hypoxic conditions.

Tolerance to waterlogging is a complex condition, several physiological mechanisms occur, such as root excretion of sodium, control of sodium transport between roots and shoots, and stomatal conductance (Das et al., 2018). Plants exhibit several restrictions under hypoxic conditions, including stomata limitation, negative impact on gas exchange, lower nutrient uptake, and reduced growth (Pereira et al., 2020). According to Buckley (2019), the stomatal aperture is determined by the displacement of stomatal guard cell walls adjacent to the stomatal pore (the 'ventral' walls). That displacement is caused by the deformation of guard cells due to volume changes, but in most species, it is counteracted to a degree by volume changes in the adjacent epidermal or subsidiary cells. The limited supply of CO_2 can directly affect the electron transfer for the reduction of molecular oxygen to form superoxide radicals (O_2^-) in photosystem I. As explained by Khan et al. (2017). Superoxide dismutation then forms hydrogen peroxide (H_2O_2). H_2O_2 has been shown to affect activation or conditions in cellular processes. The lowest H_2O_2 content can increase plant tolerance to various abiotic and biotic stresses. Stomata closure in plants under hypoxic conditions can also be affected by an increase in the ABA hormone, which plays an important role in signaling pathways that control stomatal closure (Ye et al., 2020).

Duncan's further analysis of the combined treatments of waterlogging treatment and different tobacco varieties found that higher waterlogging rates in the sensitive and moderate tobacco decreased stomatal activity. However, the opposite occurs in resistant tobacco. The higher the waterlogging rate, the greater the activity of the stomata, the more stomata open. In this case, resistant varieties have special recovery or adaptation mechanisms that allow them to survive in hypoxic conditions.

Plants can adapt their growth and development to adverse environmental conditions. This process will be very important for plants to survive in hypoxic conditions. Although plants need molecular oxygen (O_2) to live, they can overcome temporary low O_2 conditions (hypoxia) until they return to a standard 21% O_2 atmosphere (normoxia) (León et al., 2021).

Hypoxia decreases green pigment, gas exchange (stomata conductance and internal CO_2 concentration), and photosynthetic activity in plant leaves (Iqbal et al., 2021).

Selective synthesis of a set of 20 anaerobic stress proteins enables energy-producing metabolic processes in the absence of oxygen under anaerobic conditions. Other types of adaptations that may be present in plants include adventitious root formation, the formation of hypertrophic lenticels, and the development of aerenchyma tissues (Tan & Zwiazek, 2019).

The respiration process in plants slows down and the flow of electrons through the respiratory pathway decreases in hypoxia stress. Therefore, it reduces ATP production and as a result, oxidative chemicals like nicotinamide adenine dinucleotide (NAD), must be produced by an alternative pathway that does not use O_2 as its terminal electron acceptor. When the oxidative phosphorylation of adenosine diphosphate (ADP) is limited, plants change their metabolism from aerobic respiration to anaerobic fermentation (Toro and Pinto, 2015).

Hypoxia tolerant species are generally considered to be able to maintain their energy status through fermentation. In addition to the ability to maintain adequate energy levels, it is also important to maintain cytosolic pH. When hypoxia condition occurs, the cytoplasmic pH decreases due to the production of lactic acid from fermentation. The decrease in pH switches from lactic acid fermentation to ethanol fermentation, inhibiting the lactate dehydrogenase (LDH) and ADH activation. Acidosis or a very high acidity level can lead to cell necrosis, maintaining a pH around 6.8 will maintain cell viability (Loreti and Perata, 2020).

Transpiration rate in tobacco plant after waterlogging stress

ANOVA results in this study showed that variety type and waterlogging stress treatment had a significant effect on the transpiration rate of tobacco plants, but not when it was combined. Tables 4, 5, and 6 showed that higher levels of standing water tend to decrease transpiration rates. The lowest transpiration rates were observed in sensitive, tolerant, and moderate varieties, respectively. This is due to the function of the roots as excess water interferes with the absorption of water and nutrients. Plants compensate for this situation by reducing the rate of transpiration by reducing the number of open stomata. Plant response to waterlogging is premature stomata closure to reduce plant transpiration rate. The function of the stomata itself is the place of entry and exit of water and gas exchange. Stomata play a major role in the transpiration process.

Higher stomatal density and index can increase chlorophyll content and electron transport rate (Pereira et al., 2020). In this study, flood treatment did not significantly affect sensitive and tolerant varieties. In the moderate variety, 20% and 60% waterlogging treatments showed higher transpiration rates than other treatments.

Fig 2. shows that tolerant varieties have the most adventitious roots among other varieties. The presence of adventitious roots indicates the adaptation of tobacco plants to hypoxic stress and replaces the main root that is waterlogged. Adventitious roots improve the rate of diffusion of oxygen at the growth point then reduce water and nutrient deficiencies in waterlogged tobacco plants. From the result of this study, it appears that tolerant plants grow more adventitious roots.

Adventitious root formation occurs when the original root system is damaged and unable to function as a supplier of essential water and minerals. In addition, to improve energy efficiency, a more suitable root system is developed to replace damage to the main root system. Adventitious roots emerge from submerged parts of the plant stem and grow horizontally. This is possible as an adaptive mechanism of newly formed roots to replace the function of the damaged original root system (Xie et al., 2021). Pedersen et al. (2021) stated that the response of plants to waterlogging is to produce new adventitious roots with aerenchyma. Root anatomical phenotypes are dynamic and respond to changes in genotype and environment (Vanhees et al., 2020). In this study, airway tissue began to appear 1 week after hypoxic stress treatment on tobacco plants. Aerenchyma formation can vary at different root positions and with different root lengths and acts as an important index to evaluate the oxygen transportability of roots. (Yamauchi et al., 2019; Chen et al., 2021).

Hypoxia causes changes in metabolic activity in plants. Lack of oxygen facilitates the activation of anaerobic respiration (Jia et al., 2021). Low oxygen levels stimulate the production of the enzyme 1 aminocyclopropane carboxylate (ACC) synthase.

ACC is often used to induce an ethylene response. The hormone ethylene has many effects on plant growth and development. Its direct precursor is 1-aminocyclopropane-1-carboxylic acid (ACC) (Mou et al., 2020).

Amount of reducing sugar in tobacco plant after waterlogging stress

Hypoxic stress causes tobacco plants to go through a series of adaptive processes to survive and escape stressful conditions. One of the substances that change in response to hypoxic stress conditions is sucrose levels. Sugar metabolism is a key component of hypoxia tolerance and acclimatization in plants. The response to increasing waterlogging will be complex and species-dependent (Edge et al., 2020). Figure 3 shows the condition of tobacco plants when waterlogged and after being released from stagnant water which causes hypoxia. Hypoxia caused by waterlogging activates the acclimatization response to stress and limits plant growth. After experiencing hypoxia, plants must restore metabolism dynamically and appropriately to be able to maintain growth (Liu et al., 2021).

Tables 7, 8, and 9 show that plants at 60% waterlogging treatment has the lowest percentage of sucrose among other treatments. The tolerant variety produced the lowest percentage of sucrose among the other varieties, while the sensitive variety produced the highest percentage of sucrose. In the sensitive cultivar, waterlogging treatment tends to increase the amount of sucrose after treatment, whereas the opposite occurs in moderate to tolerant cultivars. The greater waterlogging of moderate and sensitive varieties causes lower the percentage of sucrose.

Hypoxia caused by waterlogging is serious abiotic stress that affects crop productivity.

Hwang et al. (2020) stated that oxygen stress is low. Each species specifically reconfigures energy metabolic pathways including starch sucrose metabolism, glycolysis, fermentation and nitrogen metabolism, tricarboxylic acid flow, and fatty acid degradation via oxidation beta and glyoxylate cycle. Enzymatic sucrose synthesis and degradation are recognized as central to stress-related processes, as they significantly impact stress tolerance. The metabolism of foliar sucrose involves the cleavage of sucrose by invertase and synthesis using ATP catalyzed by hexokinase and sucrose phosphate synthase (Weiszmann et al., 2018).

Table 1. The effect of waterlogging treatment on stomata activity during and after hypoxia stress.

No.	Waterlogging Treatment	Total Number of Stomata Open During Hypoxia Stress	Total Number of Stomata Open After Hypoxia Stress
1.	Waterlogging 100%	76.09^{a}	76.00^{c}
2.	Waterlogging 120%	67.10^{b}	53.83^{ab}
3.	Waterlogging 140%	54.94^{c}	57.00^{b}
4.	Waterlogging 160%	42.81^{d}	50.67^{a}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%

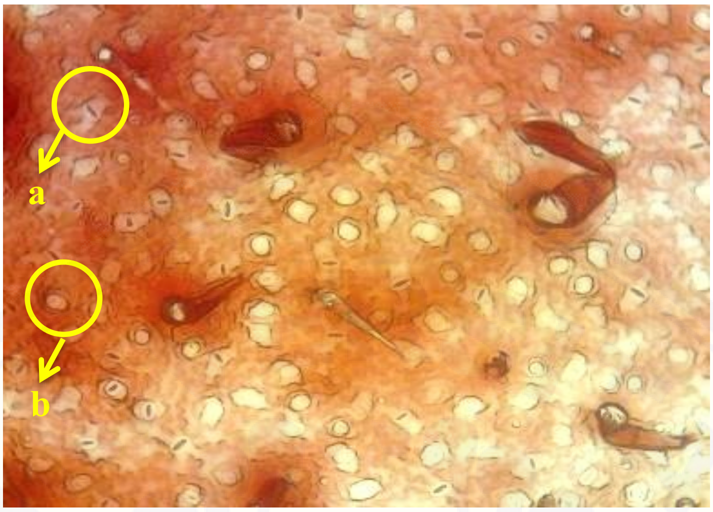

Fig 1. Stomata of tobacco leaf. (a). Stomata open, (b). Stomata closed.

Table 2. The effect of different variety of tobacco plants on stomata activity during and after hypoxia stress.

No.	Variety of Tobacco	Total Number of Stomata Open During Hypoxia Stress	Total Number of Stomata Open After Hypoxia Stress
1.	Sensitive	51.31^{a}	63.38^{b}
2.	Moderate	45.59^{a}	57.00^{a}
3.	Tolerant	83.78^{b}	57.75^{a}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%.

Fig 2. Formation of adventitious roots in tobacco plant after waterlogging. (a) Sensitive tobacco adventitious roots, (b) Moderate tobacco adventitious roots, (c) Tobacco tolerant adventitious roots.

Table 3. The effect of interaction between waterlogging and variety of tobacco plants to stomata activity during and after hypoxia stress.

Variety of Tobacco	Waterlogging 100% Control)	Waterlogging 120%		Waterlogging 140%		Waterlogging 160%	
		During	After	During	After	During	After
Sensitive	76.105^{a}A	59.208^{a}B	54.00^{ab}B	41.765^{b}C	41.50^{a}A	28.165^{a}D	82.00^{c}D
Moderate	75.86^{a}A	62.350^{a}B	36.50^{a}A	36.083^{b}C	61.50^{b}BC	8.045^{b}D	54.00^{b}AB
Tolerant	76.203^{a}A	79.735^{a}A	71.00^{b}C	86.963^{a}A	68.00^{b}B	92.227^{c}A	16.00^{a}A

Note: Numbers followed by the same small letter in the same column and the same capital letter in the same row were not significantly different at DMRT 5%.

Fig 3. The waterlogging treatment condition. (a) Waterlogged conditions, (b) After being released from waterlogging condition.

Table 4. The effect of waterlogging treatment on transpiration rate during and after hypoxia stress. Transpiration rate in tobacco plant after hypoxia stress.

No.	Waterlogging Treatment	Transpiration Rate During Hypoxia Stress	Transpiration Rate After Hypoxia Stress
1.	Waterlogging 100%	0.10225^{b}	0.10708^{a}
2.	Waterlogging 120%	0.08833^{b}	0.10358^{a}
3.	Waterlogging 140%	0.06658^{a}	0.10292^{a}
4.	Waterlogging 160%	0.05325^{a}	0.10358^{a}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%

Table 5. The effect of different variety of tobacco plants on transpiration rate during and after hypoxia stress.

No.	Variety of Tobacco	Transpiration Rate During Hypoxia Stress	Transpiration Rate After Hypoxia Stress
1.	Sensitive	0.04088^{a}	0.05606^{a}
2.	Moderate	0.06156^{b}	0.13438^{c}
3.	Tolerant	0.13038^{c}	0.12244^{b}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%.

Table 6. The effect of interaction between waterlogging and variety of tobacco plants to transpiration rate during and after hypoxia stress.

Variety of Tobacco	Waterlogging 100% Control)	Waterlogging 120%		Waterlogging 140%		Waterlogging 160%	
		During	After	During	After	During	After
Sensitive	0.055^{a}A	0.061bA	0.056^{a}AB	0.029bA	0.056^{a}AB	0.007^{b}B	0.057^{a}B
Moderate	0.128^{b}A	0.081bB	0.137^{c}A	0.042bC	0.137^{b}A	0.007^{b}D	0.135^{c}A
Toleran	0.139^{b}A	0.124aA	0.118^{b}A	0.130aA	0.116^{b}A	0.146^{a}A	0.118^{b}A

Note: Numbers followed by the same small letter in the same column and the same capital letter in the same row were not significantly different at DMRT 5%.

Table 7. The effect of waterlogging treatment to amount of reducing sugar during and after hypoxia stress.

No.	Waterlogging Treatment	Amount of Reducing Sugar During Hypoxia Stress	Amount of Reducing Sugar After Hypoxia Stress
1.	Waterlogging 100%	3.1183^{a}	3.1183^{b}
2.	Waterlogging 120%	4.0400^{c}	3.9833^{d}
3.	Waterlogging 140%	4.1050^{d}	3.6425^{c}
4.	Waterlogging 160%	3.8433^{b}	2.9650^{a}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%.

Table 8. The effect of different variety of tobacco plants to amount of reducing sugar during and after hypoxia stress.

No.	Variety of Tobacco	Amount of Reducing Sugar During Hypoxia Stress	Amount of Reducing Sugar After Hypoxia Stress
1.	Sensitive	3.9325^{b}	4.0562^{c}
2.	Moderate	4.1100^{c}	3.3800^{b}
3.	Tolerant	3.7825^{a}	2.6962^{a}

Note: Numbers followed by the same letter in the same column were not significantly different at DMRT 5%.

Table 9. The effect of interaction between waterlogging and variety of tobacco plants to amount of reducing sugar during and after hypoxia stress.

Variety of Tobacco	Waterlogging 100% (Control)	Waterlogging 120%		Waterlogging 140%		Waterlogging 160%	
		During	After	During	After	During	After
Sensitive	2.95aA	4.35bC	4.35bC	4.31cC	4.31cC	4.12bB	4.12bB
Moderate	3.35cA	4.73cC	4.73cC	4.62bC	4.62bC	3.74aB	3.74aB
Tolerant	3.45cA	3.64aB	3.64aB	3.73aB	3.73aB	4.31cC	4.31cC

Note: Numbers followed by the same small letter in the same column and the same capital letter in the same row were not significantly different at DMRT 5%.

Park et al. (2020) reported that waterlogging tolerant cultivars showed a higher amount of starch and sucrose metabolism compared to intolerant cultivars. Sucrose treatment increased the anthocyanin content. The content of phytohormones showed that the content of jasmonate, salicylic acid, and abscisic acid were increased in response to sucrose treatment (Wingler et al., 2020). In moderate variety, dynamic reconfiguration of energy metabolism occurred at the initial time point of the low oxygen treatment, but energy reconfiguration at the end time point was not as dynamic as at the initial time point. Tolerant variety appears to have a high photosynthetic capacity which can produce more O_2 and have the additional ATP to overcome energy depletion caused by low oxygen stress. Sensitive variety did not show significant changes in the expression of genes involved in anaerobic energy metabolism (Hwang et al., 2020). The results of the study from Iqbal et al. (2021) show that hypoxic conditions reconfiguring transcriptomes with glucose deficiency increase the rate and magnitude of gene induction for core anaerobic proteins (ANPs). Endogenous sugar levels were maintained by exogenous glucose under aerobic conditions and showed a prominent capacity for sucrose re-synthesis which was not detected under hypoxia.

Specific and previously unrecognized roles of sugars in hypoxic responses ranges from the acceleration of the onset of the early adaptive phase by stress to the maintenance and modulation of co-expression relationships by carbohydrate availability.

Materials and methods

Place and plant material

This research was conducted at the Greenhouse of the University of PGRI Argopuro Jember from June to August 2020. The plant material used in this study was three varieties of the tobacco plant which were Sumoris, Merci, and var. Bojoegoro. Materials required for this study were a 50 x 50 cm plastic bag, bucket, scissors, paper, glass slides, microscope (ReichertJung series 150), graph paper, spectrophotometer (Shimafzu UV 1000), 3 varieties of tobacco seeds, nail polish, clear tape, and petrolatum.

Experimental design

This study used a factorial randomized block design with 2 factors, treatment of waterlogging stress and 3 different varieties of the tobacco plant. The waterlogging treatment was investigated at 100%, 120%, 140%, and 160% field capacity. The 3 varieties of tobacco plants were observed, which were selected from previous studies (Tamala et al., 2019), Sumoris var. (sensitive), Merci var. (moderate), and Bojonegoro var. (tolerant).

Treatment of waterlogging stress

Three different varieties of tobacco seed were grown in a pot tray. The 40-day-old tobacco saplings were transferred to polybags. Treatment of hypoxia stress or waterlogging is carried out 60 days after the sampling has been transferred. Four levels of waterlogging stress treatments, which were 100, 120, 140, and 160% of field capacity, have been evaluated.

The field capacity was calculated by weighing 1 kg soil and immersed in 1 L water for 24 hours until saturated. The soil media was allowed to stand until there was no water drips and weighed again. The weight difference was 0.4 kg, and 0.4 kg/L assumed as 100% field capacity. The soil used for growing media was measured for 19 kg per polybag.

waterlogging 100% = 0.4 kg/L * 19 kg soil = 7.6 L water
waterlogging 120% = 120% * 7.6 L = 9.12 L water
waterlogging 140% = 140% * 7.6 L = 10.64 L water
waterlogging 160% = 160% * 7.6 L = 12.16 L water

Waterlogging was conducted by adding water as much as 7.6, 9.12, 10.64, and 12.16 liters corresponding to waterlogging 100%, 120%, 140%, and 160%, respectively. Observations were made 6 hours after waterlogging (for the observation during the waterlogging), then the water was removed. Observations were made again 6 hours after the removed water process (for the observation after the waterlogging).

Data collection and statistical analysis

The stomata conductance, transpiration rate, and the amount of reducing sugar in tobacco plant samples were observed. Samples were taken in 2 times. The first-time data collected was 6 hours after the waterlogging was started, and the second one 6 hours after waterlogging has been removed. The stomata activity is investigated from the number of open pores calculated by a photometer. The transpiration rate is calculated according to the transpiration rate formula, which is the ratio of the photometer scale to the outer surface of the leaf. The amount of reducing sugar was measured by the DNS method (dinitrosalicylic acid method). The data from this study were analyzed by the ANOVA test followed by the Duncan test with a 95% confidence level, using the application of SPSS ver.22.

Conclusion

The results of this study show that hypoxic stress treatment in tobacco plants has a significant effect on the parameters of stomatal activity, transpiration rate, and reducing sugar amount. Higher waterlogging rates in the sensitive and moderate varieties resulted in decreased stomatal activity, transpiration rate, and sucrose rate. Conversely, tolerant tobacco tends to increase stomatal activity, transpiration rate, and sucrose rate with higher waterlogging rates. Three accessions of tobacco with categories tolerant, moderate, and sensitive to hypoxia developed different metabolisms to cope with energy crises caused by hypoxic stress.

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