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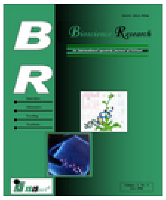

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Solubilization of inorganic phosphate by phosphate solubilizing fungi isolated from oil palm empty fruit bunches of Central Kalimantan

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Growth responses of tobacco (*Nicotiana tabacum* L.) varieties to water logging stress

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Having high economic value makes tobacco becomes favorite industrial crop cultivated in Indonesia. However, several negative environmental conditions, including heavy rainfall season, affect the successfulness of tobacco cultivation. Heavy rainfall season is the major cause of waterlogging stress, which affect plant productivity. This study aims to determine the growth responses of some tobacco varieties under *waterlogging stress* and to evaluate its tolerance against *waterlogging stress*. Five tobacco varieties were used in this study, including var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95. The results were analyzed using two way Anova test and followed by Tukey test. The results showed that *waterlogging stress* decreased plant growth, induced the formation of aerenchyma tissues and adventitious root. In addition, this environmental stress also induced stomatal closure. In this present study, we observed that var. Bligo 1 and Grompol Jatim 1 are medium tolerant against waterlogging stress. Meanwhile, var. Bojonegoro 1 is considered as tolerant variety. Furthermore, the rest of tobacco varieties are sensitive to *waterlogging stress.* The overall results demonstrated *waterlogging stress* affect plant growth; induce stomatal closure, adventitious root and aerenchyma root formation. Moreover, each tobacco variety showed different level of tolerance against waterlogging stress

Keywords: *Nicotiana tabacum,* Plant growth, Sensitivity Index, Tolerant, Varieties, Water logging Stress

INTRODUCTION

Tobacco is considered as industrial crops possessing high economic value (Simpson and Ogorzaly, 2001). Not only being used as the main material in cigarette industry, tobacco has been used in medicinal and agricultural-based products, including pesticides (Simpson and Ogorzaly, 2014). However, the cultivation of this promising plant face major environmental constraint, including high rainfall season, which is typically occurred in tropical region. This uncertain season might cause flooding or waterlogging stress.

One of the major effects of waterlogging stress is the emergence of hypoxic or anoxic condition in the plant root system (Smith et al., 2010). In addition, the waterlogging stress causes increased soil density, plant phytotoxicity due to the formation of toxic compounds, decreased in soil pH and oxygen supply (Parent et al., 2008). Previous research has reported that decreased in soil pH causes plant cell damage and often leads to acceleration of cell death in sensitive to waterlogging stress plants (Hodson and Bryant, 2012).

However, tolerant plants may survive under waterlogging stress by developing adaptation mechanisms, which includes anatomical and morphological adaptation as well as metabolic regulation mechanisms (Susilawati et al., 2012). An example of anatomical adaptation is the formation of aerechyma tissues in the root system. In maize, hypoxic condition stimulates the ethylene biosynthesis. This may induces subsequently to cell death in the root cortex, giving rise further to the formation of aerenchyma at the root (Rajhi et al., 2010).

In addition, plants also develop adventitious root when they are suffering from the waterlogging stress. The presence of adventitious root might help plants to easily absorb the oxygen (Dawood et al., 2014). Research on tomatoes (*Solanum lycopersicum*) indicates that one week treatment of waterlogging stress increases the formation of adventitious roots (Vidoz et al., 2010). This study aims to determine the growth responses of some tobacco varieties (var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95) under waterlogging stress and evaluate its tolerance againts waterlogging stress.

MATERIALS AND METHODS

Tobacco varieties used in this study include var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95, which were obtained from the Indonesian Sweetener and Fiber Crops Research Institute (ISFRI). The study was conducted at the green house of the Laboratory of Plant Biosciences and Technology, Department of Biology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia.

Preparation of growing media

:Growing media used in this study is composed of mixture of soil and compost with a ratio of 1: 1. The total weight of the planting medium was 1 kg, where 1 g additional NPK fertilizer was added to each polybags.

Preparation and Planting of Tobacco Seedlings:

Tobacco seeds were germinated in potting trays containing growing media (soil and compost mixture in the ratio 1: 1). Germination is done for 4 weeks. Furthermore, young juvenile tobacco plants are transferred to polybags that have contained planting media (Hurng et al., 1994).

Waterlogging stress treatment:

Tobacco plants aged 40 days after planting (dap) were treated with waterlogging stress (Hurng et al., 1994).Each varieties were subjected to waterlogging stresses of 150%, 175%, and 200% of the field capacity. Meanwhile, the control plants were treated with 100% of the field capacity. Waterlogging stress were conducted for 10 days. The volume of water in each treatment is regularly adjusted and sustained for 10 days of treatment.

Plant growth parameters used in this study include plant height, number of adventitious roots, canopy-root ratio, the total number of aerenchyma formation. The sensitivity index was measured against the total dryness observation parameter by the equation:

$$S = \frac{\left(1 - \frac{Yp}{Y}\right)}{\left(1 - \frac{Xp}{X}\right)}$$

Information:

S = Sensitivity Index of waterlogging stress
Yp = the average values of a treated variety
Y = the average values of a non-treated variety
Xp = the average of total treated varieties
X = the average of total non-treated varieties

The criteria for tolerance level of *waterlogging stress* are: **tolerant variety** (when S < 0,5), **medium tolerant variety** (0.5 < S < 1.0), and **sensitive variety** (S > 1.0).

The data obtained were analyzed using ANOVA Two Way and followed by Tukey test. Aerenchima tissue observation was analyzed descriptively.

RESULTS

Tobacco Plant Morphology Response At Waterlogging Stress

Our statistical analysis (Table 1) showed that waterlogging stress significantly influences the plant height, canopy-root ratio, root length and adventitious root quantity. Based on Table 1 and Figure 1, plant height growth tends to decrease when the plants were treated under waterlogging condition. Maximum treatment at 150% of field capacity, the crops showed a significant decrease in growth (plant height), and further decrease with increasing waterlogging stress. Similarly, the root length begins to decrease significantly at 150% of field capacity (Figure 2). Decrease in plant growth further affects the root and canopy ratios. Based on Figure 3, it is known that the ratio of root canopy increases following an increment of field capacity (waterlogging stress). Finally, plants adapt by inducing and accelerating the formation of adventitious roots (Figure 4).

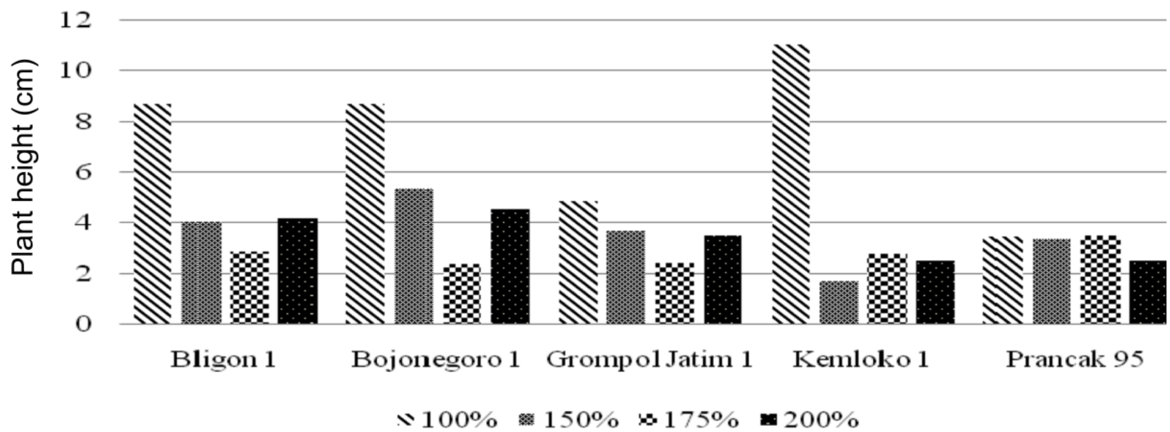

Figure 1. Plant height response of tobacco varieties after waterlogging stress treatment

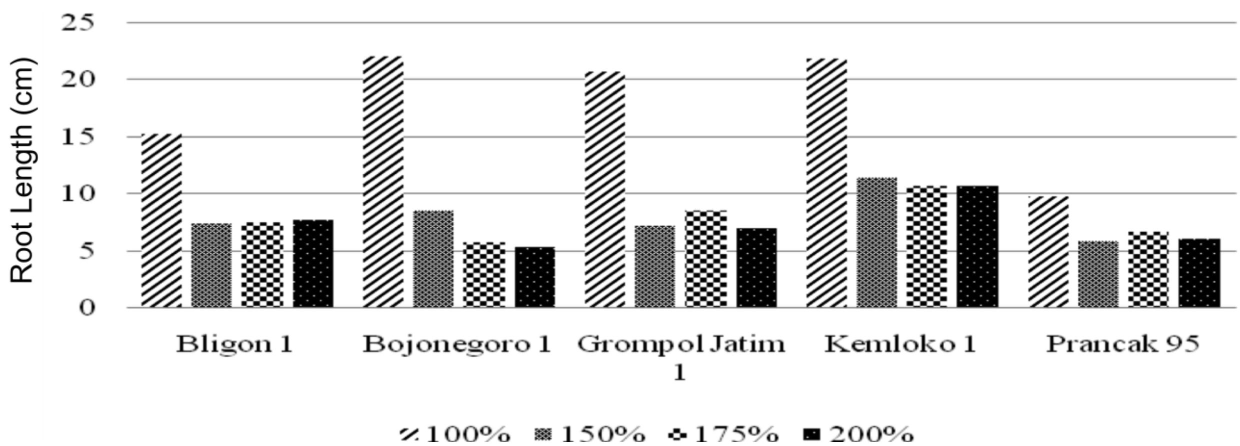

Figure 2. Growth Response in root length of tobacco varieties after *waterlogging stress* treatment

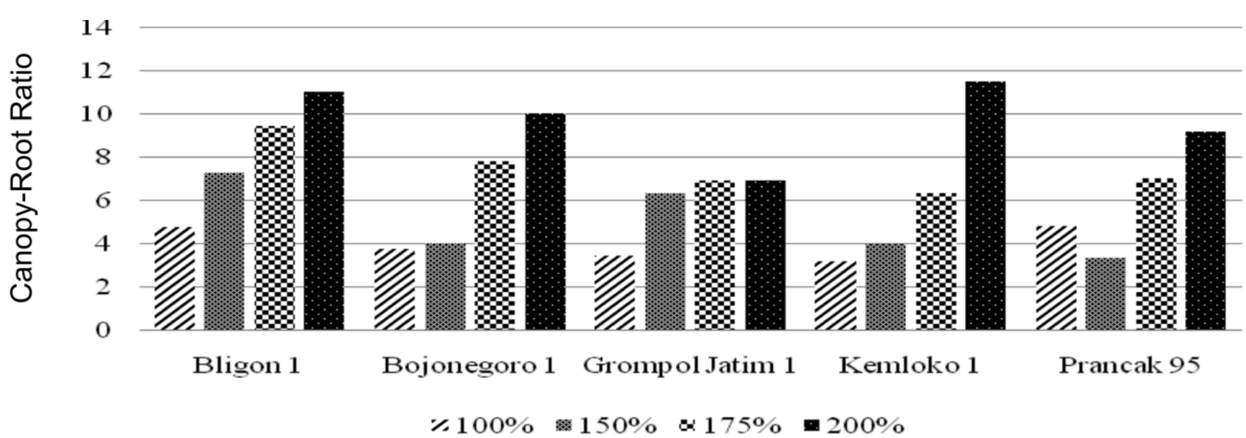

Figure 3. Growth Response in canopy-root ratio of tobacco varieties after waterlogging stress treatment

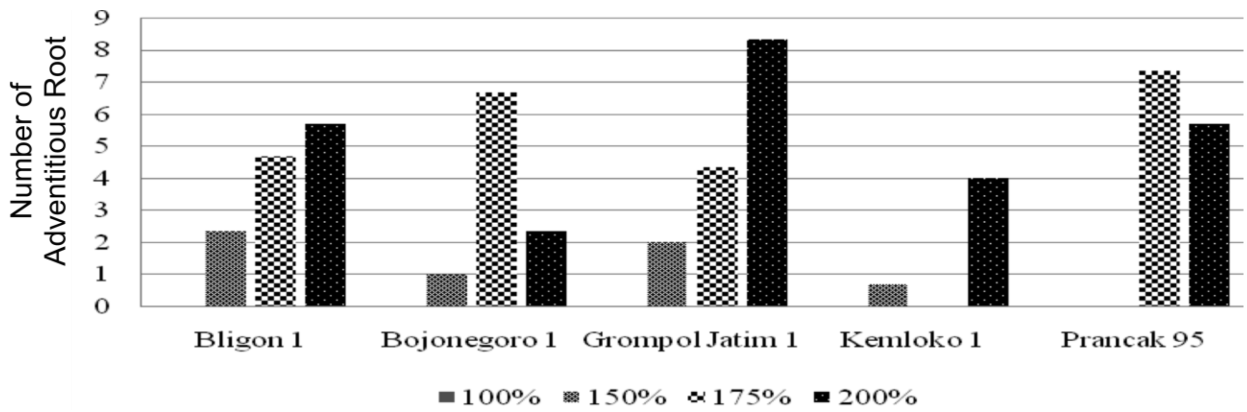

Figure 4. Number of adventitious roots of tobacco varieties after waterlogging stress treatment.

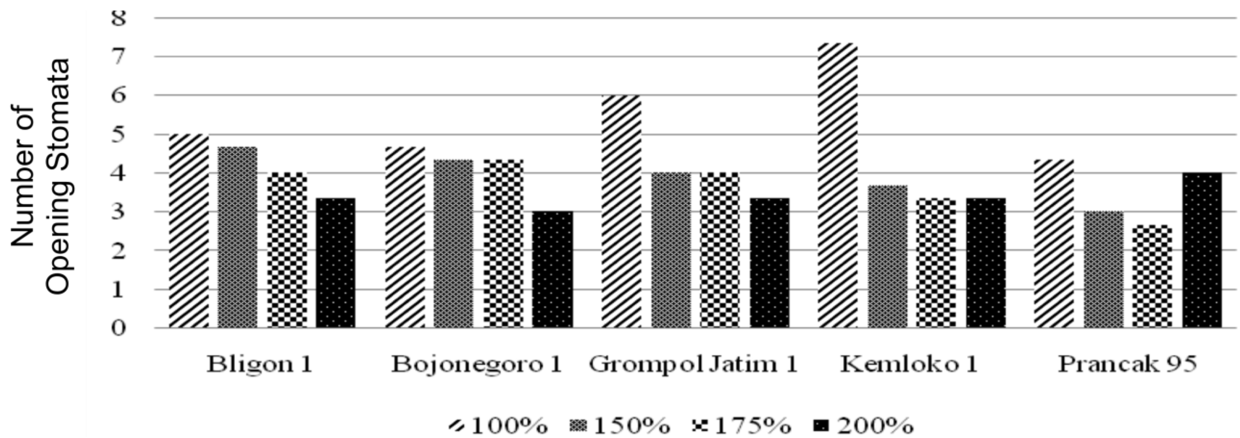

Figure 5. Number of opening stomata in tobacco varieties after waterlogging stress treatment.

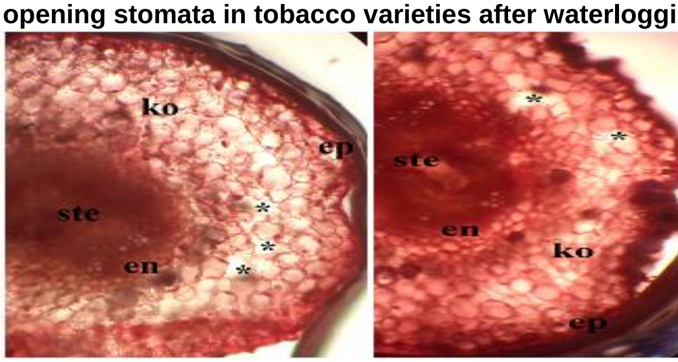

Figure 6. Aerenchymal observation of tobacco Bojonegoro 1 varieties treated with *waterlogging stress*: (A) Plant tobacco control; (B) Plant tobacco Treatment (ep: epiderm; co: cortex; en: endoderm; ste: stele; asterisk (*) aerenchy

Tobacco Plant Anatomy Response At Waterlogging Stress

Anatomical observation in response to waterlogging stress includes morphological observation of the stomata and plant aerenchyma tissue observation. Our data (Table 1) showed that waterlogging stress had significant effect to total number of open stomata. Based on Table 1 and Figure 5, it is known that the total number of opening stomata decreases with an increase of waterlogging stress level. Root anatomical observation showed the formation of aerenchyma (Figure 6).

Table 1. ANOVA for Plant Height, Root Length, Canopy/Root Ratio, Number of Adventitious Roots and Number of Stomata Using Adjusted SS for Tests

Source of variations	Tukey Test				
	Plant height (cm)	Root length (cm)	Canopy / Root ratio	Number of Adventitious Roots	Number of Opening Stomata
Varieties	0,308	0,000*	0,108	0,095	0,211
Waterlogging stress	0,000*	0,000*	0,000*	0,000*	0,000*
Varieties* Waterlogging stress	0,241	0,092	0,300	0,302	0,074

Note: The symbol "*" indicates high significant difference

Table 2. Sensitivity Index of Tobacco Varieties in Waterlogging Stress

Varieties	150%			175%			200%		
	S	MT	T	S	MT	T	S	MT	T
Bligon 1	√			√				√	
Bojonegoro 1			√			√			√
Grompol Jatim 1		√			√			√	
Kemloko 1		√		√			√		
Prancak 95	√			√			√		

Description: S = sensitive, MT = medium tolerant, T = tolerant

Sensitivity Index Tobacco Plants In Waterlogging Stress

Determination of the sensitivity index is based on the plant dry weight parameters. The tobacco plant sensitivity index at 150%, 175% and 200% stresses is presented in Table 2. Varieties Bojonegoro 1 seems to be more tolerant compared to others. Meanwhile var. Bligon 1 and Grompol Jatim 1 is considered as medium tolerant

DISCUSSION

Plants develop unique strategy to face adverse environmental condition. The later includes abiotic stress which seems to be more pronoounced in tropical region. Drought and flooding stress are two major causes of low yield of industrial crops. To better adapt and response this environmental condition, plants perform a complex combination of anatomical, physiological and molecular responses (Kumutha et al., 2009, Jadid et al., 2017). In addition, excessive production of reactive oxygen species (ROS) during the environmental stress could also severely affect plant growth. Therefore, environmental stress could also induce the formation of antioxidant compounds. The later is not only useful for human health (Jadid et al., 2016) but also vital for plant growth and development, especially when plants are subjected to severe environmental stress. Additionally, some reports also demonstrate that abiotic stress could enhance chlorophyll breakdown, protein degradation, decrease in membrane permeability and stability (Jadid et al., 2017), slower leaf expansion, stomatal closure and petiol epinasty (Lin et al., 2006). Based on Figure 1 - 2, the plant height and root length of tobacco varieties tend to decrease following the level of waterlogging treatment. At the 150% of field capacity level, the plants begin to decrease its plant growth and it was subsequently decline following the increase of waterlogging stress levels. This might be caused by the nutrient and water competition within the vegetative organs such as roots, leaves and stems occured during the treatment. This competition could inhibit the plant height but in contrast, it could also increase the leaf area of tobacco varieties.Lin et al., (2006) reported also that waterlogging stress caused hypoxic to anoxia conditions. This might slow the cellular respiration within the root tissues. Thereby, plants tend to temporary change cellular respiration pathway from aerobic to anaerobic respiration / fermentation. However, Fermentation is less efficient in converting ADP to ATP, compared to aerobic respiration. Limited availability of metabolic energy would inhibit multiple biological processes in the plant including cell division, water and nutrient uptake and other metabolic processes.

The root-canopy ratio is the ratio between the canopy's dry weight and root dry weight. According to Firdaus et al. (2013), high canopy-root ratio indicates good upper vegetative growth. In this study, we observed an increase of canopy-root ratio of treated varieties following an increase of waterlogging stress levels (Figure 3). This suggests that root growth is more inhibited than shoot growth during waterlogging stress. In addition, this also suggests that there were unbalanced accumulation of photosynthate products between shoot and root. The canopy-root ratio begins to increase significantly at 175% of waterlogging stress. This result is in line with Florentine and Fox (2002) which states that in Eucalyptus victrix, E. terminalis, and E. leucophloia, the ratio of canopy-root in the plant is higher than the unheated control plants

.Waterlogging stress further suppresses root growth compared to shoot growth (Lin et al., 2006). The change of cellular respiration pathway from aerobic to anaerobic might play role in this process, which subsequently result in cell damage and often leads to cell death in sensitive plants (Hodson and Bryant, 2012). The cell death phenomenon begins probably at root tissue since it is the first plant organ affected by waterlogging stress.

Adventitious roots were observably found in tobacco varieties treated with waterlogging stress.

This type of root is actually formed from organ other than root tissues. Adventitious roots arised after plants were treated with waterlogging stress (Figure 4). This formation is common plant adaptation against waterlogging stress. Based on the reference (Cronk and Fennessy, 2001), within a few days of flooded condition, some plants will initiate the formation of adventitious roots that grow laterally from the main stem. Adventitious root formation occurs due to the interaction of several plant hormones, auxin and ethylene (Akhtar and Nazir, 2013). In the absence of oxygen, the Krebs cycle cannot be run due to lack of a terminal electron acceptor for the oxidation of NADH. The next ATP can only be produced by fermentation, in which the first pyruvate is converted into lactate. But this has not happened in a long time, as well as a decrease in pH in the cytosol causes inhibition of lactate dehydrogenase activity and turned into ethanol fermentation. Under anaerobic conditions, the proton released from the vacuole to the cytoplasm will increase the acidity caused by lactate dehydrogenase

.Decreased pH and occurrence of cytosolic acidosis are the main causes of cell damage, and often lead to cell death in sensitive plants (Hodson and Bryant, 2012). This further causes root damage and inhibition of auxin transport to the root. Based on reference (Cronk and Fennessy, 2001), auxin is involved in the formation of adventitious root. Diffusion of auxin to the root of was inhibited in less-oxygen root. This might cause the accumulation of auxin in a zone between the shoot and root in which adventitious roots are formed. Adventitious roots help the absorption of water and nutrients in plants that are tolerant to waterlogging stress. Adventitious roots also facilitate the diffusion of end-products alcohol fermentation within the plant to avoid alcohol accumulation.

Stomata consist of a pair of guard cells and some additional cells. Stomata serve to regulate the assimilation of carbon dioxide (CO_2) and the transpiration process through changes in guard cell turgidity. This specific feature is critical for the global water-carbon cycle and the ability of plants to respond to environmental changes (Gan et al., 2010). Based on Table 1 and Figure 5, waterlogging causes decrease of opening stomata. This indicates that there has been a root damage that causes an inhibition of water absorption. The plants further respond with stomatal closure to reduce the transpiration rate. At physiological level, stomatal closure reduces transpiration but inhibits photosynthetic process. This response can occur within a few hours or days, depending on the degree of tolerance of each plant to the waterlogging (Striker, 2012). Excessive ground water inhibits respiration of roots and water and nutrient absorption from soil, induces stomatal closure and finally dramatically.

Reduces plant growth.

Determination of the sensitivity index is based on the total dryness (total dry weight) observation, since the dry weight indicates the positive or negative effect of plant growth. Based on the observation, the most tolerant plants tolerant to the waterlogging stress (highest level of waterlogging stress, 200%) is var. Bojonegoro 1, while Bligon 1 and Grompol Jatim 1 is considered as medium tolerant (Table 2). These results might reflect their native habitat where these three varieties are commonly cultivated in the area with an excess of water. Var. Bligon 1, Bojonegoro 1, and Grompol Jatim 1 are more suitable for being cultivated in ex paddy fields. Var. Prancak 95 is sensitive to all level of waterlogging stress. This is in line with the native condition where var. Prancak 95 is cultivated. This variety is commonly grown in dry climate area. Based on the reference (Anonymous, 2012), varieties Prancak 95 are selected from local varieties derived from Prancak, District Pasongsongan, Sumenep, Indonesia. This variety is more suitable to be grown in dry land.

CONCLUSION

The overall results demonstrated waterlogging stress affect plant growth, induce stomatal closure, adventitious root and aerenchyma root formation. In addition, each tobacco variety showed different level of tolerance against waterlogging stress. Bojonegoro variety is a tolerant varieties in 200% of waterlogging Stress level.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TN and NJ designed the experiments and analyze the data. HC performed the experiments. TN, HP, SH and NJ supervised all the biological experiments. TN and NJ wrote the manuscript. All authors read and approved the final version

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