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# Response of *Nicotiana tabacum* Plant Under Waterlogging Stress During Vegetative Stage

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**ABSTRACT**

Tobacco is one of the important commodities cultivated in Indonesia. Some varieties have been commonly cultivated such as var. Srumpung, Dixie Bright, dan Somporis. One of the major constraints in tobacco cultivation is high rainfall, which consequently results in waterlogging stress. The later causes decrease in plant growth and productivity. Under waterlogging stress condition, plants develop diverse mechanisms including morphological, physiological and biochemical adaptations. Interestingly, each variety of plants possesses unique response against waterlogging stress. This study aims to investigate the morphological and anatomical responses of some tobacco varieties against waterlogging stress. Treatment with 100%, 150%, 175% and 200% above field capacity represented waterlogging stress and were given to each tobacco variety. Parameters used in this study include plant height, number of tobacco leaves, leaf area, root length, number of adventitious root, number of opening and closing stomata. The study was set up as factorial experiment in completely randomized design. The results showed that waterlogging stress decreased all morphological and anatomical parameters used in this study (number of tobacco leaves, leaf area, root length, number of adventitious root, number of opening and closing stomata). The lowest decline occurred in var. Somporis. In addition, our results also demonstrated that waterlogging stress could trigger adventitious root formation and induce stomatal closing.

***Key word*** *: Nicotiana tabacum*, *Vegetative stage, Waterlogging stress,*

## Introduction

Water excess is one of the common environmental stress conditions that can reduce plant growth and productivity (Taiz, 2010). This situation can be divided into two types: *waterlogging stress*, which occurs when the plant root system is saturated with water and become lack of oxygen supply, the second type is flooding stress, which is demonstrated by the existence of water above the soil surface. The later can be subsequently divided into partial submergence and complete submergence. The first one can happen when few portion of the shoot is submerged. Meanwhile, complete submergence is characterized by submergence of all plant parts (Shimamura, 2002).

*Waterlogging stress* has been reported to cause hypoxia or anoxia stress on the plant (Smith, 2010). This has consequently limited the oxygen availability for plant root system and soil microorganisms. Reduced oxygen supply in plant root system inhibits the water and nutrient transport to the shoot system. This condition results in the stomatal closure, induces lose of cell rigidity which makes plant wilt (Sairam, 2009) and ultimately reduces plant productivity.

Plant adaptation mechanism is the main key for keeping the plant survives under the *waterlogging* *stress* conditions. Plants grown under this situation might be able to survive by regulating their metabolic pathway and doing anatomical, morphological and physiological adaptation (Pourabdal, 2008). According to (Amico, 2001), the initial response in plants grown under *waterlogging stress* is rapid stomatal closing, resulted consequently in plant wilting. Furthermore, stomatal closing causes carbon fixation reduction during photosynthesis (Striker, 2005). Plants also stimulate the formation of adventitious roots that enhances the absorption of oxygen by plant root (Suwarti, 2013). Some plants have been reported to form adventitious roots as a response to oxygen deprivation. This additional root replaces the damaged root system due to oxygen deprivation during *waterlogging stress*. This type of roots is more effective in transferring the oxygen because of the presence of aerenchyma tissues. In addition, the fact that the adventitious root is formed above the soil surface enhances the oxygen absorption efficiency (Smith, 2010). Adventitious roots can reduce the adverse effects of *waterlogging stress* by extending the root system into the air, increasing the aerobic respiration, and oxidizing the rhizosphere (Bacanamwo, 1999).

## Methodology

### Study area

The research was conducted at PT. Sadhana Purwosari Pasuruan East Java and Laboratory of Plant Biosciences and Technology, Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya.

### Procedures

### 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.

### 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).

### 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 : Wed Weight (Gram)
Tk : Dry Weight (Gram)

### 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.

### Parameters of observation

Parameters include the observation of morphological characters (plant height, number of tobacco leaves, leaf area, root length and number of adventitious roots) and anatomical characters (the number of open and closing stomata)

### Data Analysis

The data obtained were analyzed using ANOVA Two Way followed by *Tukey* test.

## Result

Morphological Responses of Tobacco (*Nicotiana* *tabacum*) Varieties During Waterlogging Stress.

Hypoxic condition in the plant root system is one of common effects when plants are exposed to *waterlogging* (Hodson, 2012). This hypoxic condition can negatively affect plant metabolisms, which can decrease the growth and productivity of plants (Taiz, 2010). A plant form adventitious roots as morphological response, which serves to enhance the oxygen supply, and helps the absorption of water and nutrients (Smith, 2010). Other response, such as physiological responses can be also observed. This can be either stomatal closure (Amico, 2001) or reduction in chlorophyll content (Riche, 2004). Each tobacco plant varieties have different responses during exposure to *waterlogging stress*. The morphological response of each variety can be shown in the Table 1.

#### Effect of Waterlogging Stress on the Stomatal Status of Tobacco (*Nicotiana tabacum*) Varieties

Other initial plant morphological response during *waterlogging stress* is an increase of stomatal closure. Like other environmental stresses, *waterlogging stress* also induces accumulation of abscisic acid (ABA). This may lead subsequently to the stomatal closure and further inhibits leaves growth. Opening and closing of stomata can be observed through the anatomical study of the tobacco varieties.

## Discussion

Based on the results shown in both Table 1 and Table 2, *waterlogging stress* treatments were not significantly affect almost all morphological parameters of the tobacco varieties. *Waterlogging stress* was only significantly affecting plant height. During vegetative phase, plants tend to perform growth and cell division to promote the formation of plant organs. Rhizosphere is the main area that can be directly affected by *waterlogging stress*. Subsequently, the function of the plant root system will be perturbed. This means that water and nutrient supply will be inhibited. Furthermore, *waterlogging stress* can block the fixation and distribution of Nitrogen

**Table 1**. Morphological Responses of Tobacco (*Nicotiana Tabacum*) Varieties During *Waterlogging Stress*

| Characters Morphology | Waterlogging stress to Treatment/Tobacco Plant Varieties (*Nicotiana tabacum*) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G1 | | | G2 | | | G3 | | | G4 | | |
| | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 |
| Average High Plant (HP) (cm) | 31.8 | 31.8 | 34.5 | 30.2 | 27.3 | 22 | 21 | 22.5 | 29.33 | 17.83 | 17.17 | 20.4 |
| Average Number Leaf (NL) | 10 | 9.3 | 8.7 | 9.8 | 9 | 8.7 | 9.3 | 8.8 | 8.8 | 8 | 7 | 7 |
| Average Leaf Area (LA) ($cm^2$) | 639.7 | 492.3 | 519 | 555.8 | 443.9 | 488.4 | 414.4 | 379.5 | 368.2 | 387.7 | 240.3 | 284 |
| Average Length Roots (LR) (cm) | 23.8 | 25.3 | 14.2 | 21 | 15.7 | 14.7 | 22.3 | 15 | 14.1 | 16.8 | 14.5 | 11.4 |
| Average number adventitous Root (NAR) | 0 | 0 | 0 | 3.67 | 3.33 | 3 | 6 | 6.67 | 5.33 | 7.67 | 8.67 | 9.33 |

Note: G1 = *Waterlogging Stress* With 100% of Field Capacity (Control); G2 = *Waterlogging Stress* With 150% Field Capacity; G3 = *Waterlogging Stress* With 175% Field Capacity; G4 = *Waterlogging Stress* With 200% Field Capacity. V1= Var. Srumpung; V2= Var. Dixie Bright; V3= Var. Somporis

**Table 2**. Interactions Between Stress *Waterlogging stress* and Varieties of Tobacco Plant Analysis Against Character Plant Morphology

| Characters Morphology | Anova Two WayP Value | Tukey Test |
|---|---|---|
| PH | P value = 0,001 (p<0,05) | Significantly different |
| LN | p value = 0,963 (p>0,05) | - |
| LA | p value = 0,764 (p>0,05) | - |
| RL | p value =0,129 (p>0,05) | - |
| NAR | P value =0,286 (p>0,05) | - |

**Note: PH:** Plant height; **LN**: Leaf Number; **LA**: Leaf Area; **RL**: Root Length; **NAR**: Number of Adventitous Roots

(N) and other minerals to the shoot system are also limited, thereby inhibiting the growth of the top organs including leaves and buds (Dennis, 2000). In this present study, *waterlogging stress* treatments were given at 75 das when the plants were already vegetatively fully formed. This explained the non-significant effect of the treatment to some morphological parameters used, but not plant height, due to the damage in the root system caused by waterlogging stress (Table 1 and 2).

**Fig. 1**. Adventitious Roots (Circled) Of The Tobacco Plant Var. Somporis After Being Treated With *Waterlogging Stress* At 100% Field Capacity

Interestingly, *waterlogging stress* has enhanced the formation of adventitious roots. This result demonstrates the function of adventitious root, which ensure the oxygen supply when the root system is perturbed. When the hypoxic condition (lack of O2) has occurred, the formation of adventitious roots will take place on the unusual location, close to the soil surface, where high oxygen pressure is presence. Adventitious roots grow laterally from the base of the main stem. Adventitious roots can reduce the adverse effects by expanding the new root system to the air, improving aerobic respiration, and transferring oxygen to the rhizosphere area (Bacanamwo, 1999). This additional root might transiently replace the damaged main roots, which importantly function to assure the oxygen, water and nutrients supply during stress (Sairam, 2009).

Furthermore, in this study, we observed that plant height represents the most affected parameters during *waterloggging stress*. In addition, plant height is also representing plant growth (Gardner, 1991). In the period of *waterlogging stress*, water absorption is also inhibited even though an excess of water is omnipresent in plant surrounding. Low level of water availability within the plant reduces the photosynthetic rate. The later lead to a decline of the photosynthate products within the plant organ. Consequently, some vegetative organs, including roots, stems and leaves will compete each other in getting the photosynthate products. Reduced photosynthate allocation has been reported to depress the growth of root, stem and also leaves (Nurhidayati, 2018).

Based on the data (Table 1 and 2), decrease in all tobacco variety height is directly proportional to the increase of *waterlogging stress* status, which is represented by several percentage of field capacity. Tobacco varieties treated with 200% of field capacity

**Table 3.** Effect of *Waterlogging Stress* on the Stomatal Status of Tobacco (*Nicotiana tabacum*) Varieties

| Characters Morphology | Waterlogging stress to Treatment/Tobacco Plant Varieties (Nicotiana tabacum) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G1 | | | G2 | | | G3 | | | G4 | | |
| | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 | V1 | V2 | V3 |
| Average Number of Stomata Closed (ST) | 3.07 | 1.27 | 1.27 | 3 | 1.87 | 3.4 | 8.87 | 2.07 | 5.07 | 5.6 | 6.73 | 8.93 |
| Average Number of Stomata Closed (ST) | 3.07 | 6.73 | 6.67 | 7.67 | 7 | 5.47 | 3 | 14 | 2 | 2.73 | 1 | 1.73 |

Note: G1 = *Waterlogging Stress* With 100% of Field Capacity (Control); G2 = *Waterlogging Stress* With 150% Field Capacity; G3 = *Waterlogging Stress* With 175% Field Capacity; G4 = *Waterlogging Stress* With 200% Field Capacity. V1= Var. Srumpung; V2= Var. Dixie Bright; V3= Var. Somporis

**Table 4.** Statistical Analysis of the Effect of *Waterlogging stress* on the Stomatal Status of Tobacco (*Nicotiana tabacum*) varieties

| Stomatal Status | Anova Two WayP Value | Tukey Test |
|---|---|---|
| ST | p value = 0.03 (p<0,05) | Significantly different |
| SB | p value = 0.04 (p>0,05) | Significantly different |

Note: ST: Stomatal Opening; SB: Stomatal Closurel showed lower plant height compared to varieties, which were treated, with 100% of field capacity. When the plants were treated with 200% of field capacity, var. Srumpung and Dixie Bright demonstrated a non-significant decreased in plant height about 43.98% and 44.39%, respectively. Whereas, var. Somporis showed a significant decrease in plant height compared to var. Srumpung and Dixie Bright. According to (Nurhidayati, 2018), *waterlogging stress* causes hypoxic conditions that may inhibit cellular respiration within the plant root. This condition enforces the plants to switch the respiration pathway, from aerobic to anaerobic-based respiration pathway.

Limited metabolic energy would further inhibit multiple processes including cell division, water and nutrient uptake process and various metabolic processes, which subsequently affect the overall plant growth and development.

The number of closing stomata increases along the increasing status of *waterlogging stress*. This result is in accordance with (Salisbury, 1995), who reported that plants tend to close their stomata during *waterlogging stress* in order to reduce the rate of transpiration.

Furthermore, the stomatal opening and closure might tightly correlate with root damage which perturbes water absorption, resulting in physiological drought stress within the plant cell. Therefore, plants will try to keep the turgidity of the cell by closing their stomata (Damayanti, 2007).

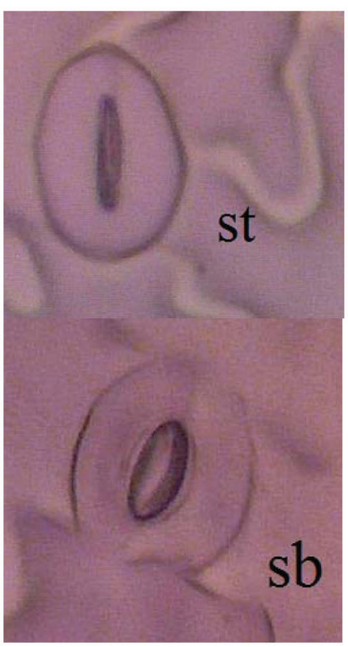


**Fig. 2.** Observation of Stomatal status on Tobacco Somporis Plants by CX 21 Microskop Magnification 400 X (sb:stomatal opening; st: stomata closure)

The overall results demonstrate that the three tobacco varieties used in the study showed different both morphological and anatomical responses during *waterlogging stress*. Decrease in plant height, leaf number, leaf area, root length, number of open stomata as well as increase of closing stomata were observed in the three varieties. However, among those three varieties, var. Somporis showed lower rate response in all parameters used.

Furthermore the formation of adventitious root was observed in all varieties of tobacco plants at each *waterlogging stress* level. Interestingly, highesr number of adventitious root was observed in var. Somporis.

## Conclusion

1. All Tobacco Plant Variety decreased growth (plant height, leaf number, leaf area, root length, number of stomata open)
2. Somporis varieties of tobacco plants have a reduced rate of growth is lower than the other two varieties
3. All varieties of tobacco plants in the treatment of stress puddles forming adventitious roots

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