Root architecture of tobacco (*Nicotiana tabacum* L.) under periodic waterlogging stress

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Abstract. Tobacco is a plant that has high economic value and is sensitive under waterlogging stress. Waterlogging stress has a major impact on root organs, so it can change root architecture. The aim of this study was to determine the root architecture of tobacco plants against periodic waterlogging stress through morphological parameters. The first factor is the tobacco plant varieties and the second is periodic waterlogging stress (waterlogging stress for 12 days followed by flooding stress for 12 days), each treatment has 4 replications. Morphological parameters measured were root length, number of roots, root diameter, number of adventitious roots, root fraction (horizontal and vertical), shoot-root ratio, IJA and ICA. The results of the study were modeled using AutoCAD and then illustrated by description. Morphological parameter results showed that Prancak 95 variety had the highest decrease (15%–78%) in parameters number of roots, root diameter, horizontal root fraction, IJA and ICA. Jinten variety had the highest decrease (15%) in root length. Jepon Emas variety had the highest decrease (35%) in vertical root fraction and the highest increase (27%–35%) in adventitious roots and shoot-root ratio. These findings indicate that each tobacco variety exhibits different morphological adaptive strategies to periodic waterlogging stress, with Jepon Emas showing the strongest adaptive response through increased adventitious roots and shoot-root ratio.

1 Introduction

Tobacco (*Nicotiana tabacum* L.) plays an important role in improving the national economy through excise taxes and trade. In East Java Province, tobacco is a leading commodity with a higher economic value compared to other crops. Its contribution to the national income reaches 6.58%, equivalent to approximately Rp 38.5 trillion, and continues to increase up to Rp 43.8 trillion [1]. However, tobacco is highly sensitive to excess water, which can reduce both the quality and quantity of its production [2]. Global climate change, characterized by increased rainfall, has caused periodic flooding in several regions [3], leading to waterlogging stress in plants. This stress is a complex abiotic factor that significantly affects plant growth and productivity [4].

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The response of plants to flooding varies depending on the variety. This study used Jinten, Jepon Emas, and Prancak 95 varieties, which have different levels of sensitivity to waterlogging. Jinten and Jepon Emas are classified as moderately tolerant, while Prancak 95 is considered sensitive [5]. During waterlogging, the root system becomes the primary organ affected due to anaerobic conditions caused by oxygen deficiency. Such conditions can inhibit growth and lead to root damage [6]. To adapt, tobacco plants develop various morphological, anatomical, and metabolic mechanisms [7]. These adaptations are reflected in changes in root length, the number of adventitious roots, root and shoot dry weight, and the root-to-shoot ratio [8].

Root architecture is the result of the activity of apical meristems at the root tips, forming a complex morphological structure [1]. Parameters such as root length, number of roots, root diameter, number of adventitious roots, root fraction, shoot-to-root ratio, Root Anchorage Index (IJA), and Root Grip Index (ICA) can be used to describe this architecture. Waterlogging stress that induces morphological changes in roots will indirectly affect the overall root architecture. Based on this background, it is necessary to conduct research on the root architecture of *N. tabacum* under periodic flooding conditions, based on its morphological components, to understand the plant's adaptive mechanisms to waterlogging stress.

2 Methods and Material

The preparation of *Nicotiana tabacum* seeds involved three varieties Jinten, Jepon Emas, and Prancak 95 which were soaked in water for 24 hours to accelerate germination, then drained and air-dried for two days before sowing in a germination medium composed of soil, vermicompost, and cocopeat (2:1:1) supplemented with Ridomil antifungal solution (0.5 g/L) and NPK fertilizer (5 g/L). The medium was moistened through bottom-watering, and seeds mixed with finely sieved compost soil were evenly spread, covered, and monitored daily until germination, after which trays were uncovered, placed on racks, and misted regularly until seedlings developed 3–4 leaves at 20 DAS. Pricking was performed using sterilized tweezers into 72-hole trays filled with the same medium, followed by a three-day indoor acclimation, transfer to shaded racks, regular misting, and clipping to strengthen stems and standardize growth until seedlings reached 25 days.

Waterlogging stress was applied to 60–70 DAS seedlings with 4–5 leaves, with control plants watered 50 mL/day and treated plants placed in containers holding six polybags per variety; Stage 1 involved maintaining a 13 cm water level for 12 days to saturate the root zone, followed by Stage 2 flooding for 12 additional days to partially submerge the stem and the first 1–2 leaves, totaling 24 days of waterlogging. Root morphology was assessed through direct observations by measuring root length using a ruler, counting main and lateral roots manually, determining root diameter with a caliper for main roots and a micrometer under a microscope for lateral roots, recording the number of adventitious roots emerging near the stem base above the waterlogged zone, and calculating the root fraction as the ratio of horizontal or vertical roots to the total root count.

3 Result and Discussion

Roots are one of the plant organs that play an important role in plant growth and development [3,8]. Roots are organized into a system known as the root architecture. Root architecture refers to the morphological visualization of roots as a result of growth driven by the apical meristem located at the root tip [2]. Root architecture plays a crucial role in providing structural anchorage or root grip strength to support the plant, absorbing water and nutrients available in the soil, and distributing them throughout the entire plant body [7]. Root architecture itself consists of several aspects; however, this study focuses mainly on the morphological aspects.

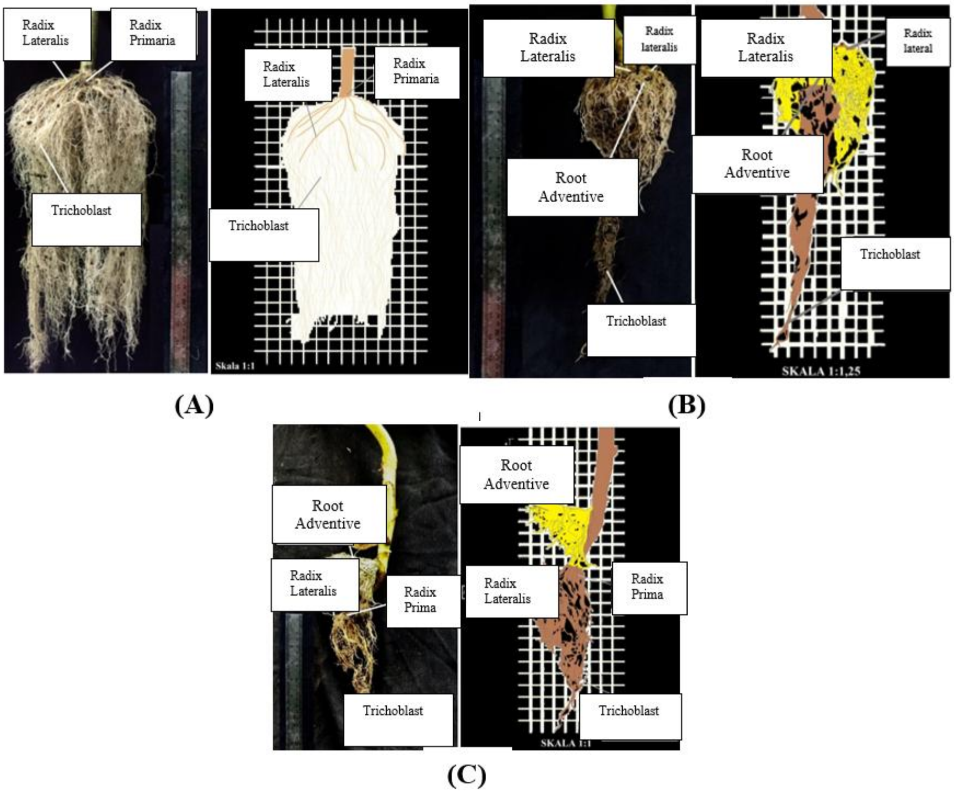

Fig. 1. Root Architecture Model of *Nicotiana tabacum* var. Jepon Emas (A) (Control), (B) (Waterlogging), and (C) (Flooding).

From the root architecture model shown above, it can be observed that the morphological aspects are determined by several parameters characterized by changes in the number, formation (shape), elongation, and growth angle of the roots. These parameters may alter the overall root architecture under environmental stress conditions, particularly flooding stress [3]. The changes in root architecture represent the development of plant adaptive mechanisms during submergence caused by flooding stress. These modifications aim to reduce oxidative damage and enable the plant to adapt, thereby supporting its survival [2].

3.1 Root Length

Root length increases due to the activity of primary meristematic tissue, or the apical meristem, located at the root tip as the main elongation zone [4]. Root length, defined as the extension from the root collar to the root tip, is strongly influenced by water availability, with plants generally forming longer roots under sufficient moisture and modifying root elongation or shortening as adaptive responses to water deficit or excess [5]. Based on **Fig. 2**, root length was affected by both waterlogging stress and varietal differences. Under control conditions, Jepon Emas had the longest roots (11.6 cm), followed by Prancak 95 (10.5 cm) and Jinten (10.1 cm). Under waterlogging stress, all varieties showed reduced root length, with Jepon Emas exhibiting the highest reduction (49%, 5.9 cm), followed by Prancak 95

(48%, 5.4 cm) and Jinten (37%, 5.9 cm), demonstrating variability in tolerance among varieties.

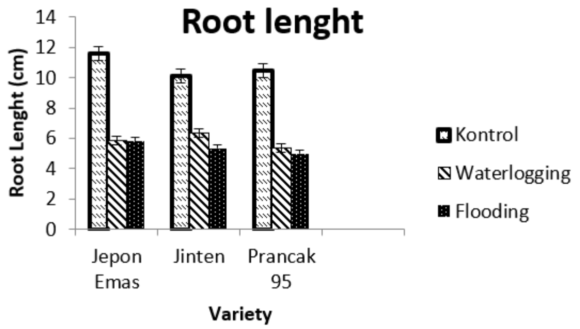

Fig. 2. Root Length of *Nicotiana tabacum* L.

This finding aligns with a previous study [4] showing that flooding stress reduces root length in *Glycine max* L. and supports the observation that Jepon Emas experienced a greater reduction in root length under waterlogging stress than the other varieties, yet showed a milder reduction under flooding conditions. This pattern is consistent with the explanation by [8], who reported that increased formation of adventitious roots under more complex flooding stress helps reduce damage in the root zone, allowing roots to maintain their length. It also agrees with [3], who stated that waterlogging-intolerant plants experience drastic root length reduction under flooding, while tolerant plants can still develop roots in submerged conditions. Because roots require oxygen for respiration and normal cellular activity, excess water limits oxygen availability, causing root function to decline, reducing mineral and water absorption, and ultimately leading to plant wilting or root death [2].

3.2 Root Count

The number of roots is closely related to the components that make up the root architecture. Root number determines the amount of water and nutrients absorbed by the plant. Under normal conditions, a greater number of roots allows for higher absorption of both water and nutrients [1]. Conversely, under flooding stress conditions, plants tend to reduce the number of roots to limit excessive water uptake, which could otherwise disrupt other physiological functions [2].

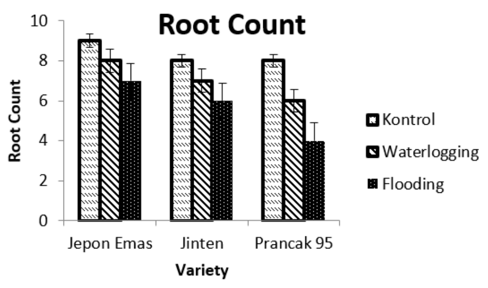

Fig. 3. Root Count of *Nicotiana tabacum* L.

Based on **Fig. 3.**, root number was influenced by both water stress and varietal differences, with Jepon Emas showing the highest number of roots under control conditions (9 roots), followed by Jinten (7 roots) and Prancak 95 (6 roots). Under waterlogging stress, all varieties experienced reduced root numbers, with the greatest decrease observed in Prancak 95 (25%, 6 roots), followed by Jinten (12%, 7 roots) and Jepon Emas (11%, 8 roots).

When exposed to more severe flooding stress, root number continued to decline, with Prancak 95 showing the largest reduction (33%, 4 roots), followed by Jinten (14%, 6 roots) and Jepon Emas (12%, 7 roots). This trend aligns with previous findings that flooding significantly reduces root morphological parameters such as root number and length, thereby limiting water and nutrient uptake due to hypoxia, which inhibits mitochondrial respiration and ATP synthesis [2]. The more pronounced reduction in Prancak 95 is consistent with reports identifying this variety as highly sensitive to flooding, showing greater root damage than Jinten, Jepon, and Kemloko [6]. This sensitivity is associated with its lower production of adventitious roots, which normally compensate for damaged primary roots and play essential roles in oxygen acquisition, nutrient absorption, and energy production needed for root development and cell division [1]. Consequently, limited adventitious root formation results in reduced energy availability and inhibited root growth, leading to a lower total root number in Prancak 95 compared to the other varieties.

3.3 Root Diameter

Root diameter is the result of the activity of meristematic root cells that divide and undergo cell elongation. Cell elongation occurs in the elongation zone, located just behind the cell division zone, as a result of cell division. The elongated cells then differentiate and initiate secondary growth, forming the root diameter [7].

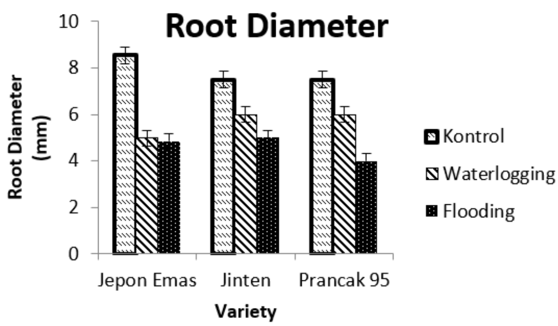

Fig. 4. Root Diameter of *Nicotiana tabacum* L.

Based on **Fig. 4**, root diameter in the Jepon Emas, Jinten, and Prancak 95 varieties was influenced by both stress and varietal factors. Under control conditions, Jepon Emas had the largest diameter (8.5 mm), followed by Jinten and Prancak 95 (7.5 mm). Waterlogging caused the greatest reduction in Jepon Emas (42%, 5 mm), while Jinten and Prancak 95 decreased by 20% (6 mm). Flooding led to further declines, with Prancak 95 showing the largest decrease (33%, 4 mm), followed by Jinten (17%, 5 mm) and Jepon Emas (3%, 4.8 mm), consistent with Sakazono et al. (2014). The sharper reduction in Jepon Emas under waterlogging but milder change under flooding suggests distinct adaptive responses, where Jepon Emas may enlarge root tissues while Jinten and Prancak 95 reduce tissue size as tolerance strategies [2,5,8].

3.4 Number of Adventitious Roots

Adventitious roots are roots that usually form at the base of the stem or in plant parts rich in lenticels, growing laterally parallel to the water or soil surface. These roots function to replace the main roots that are damaged or decayed under water and to improve aeration [3].

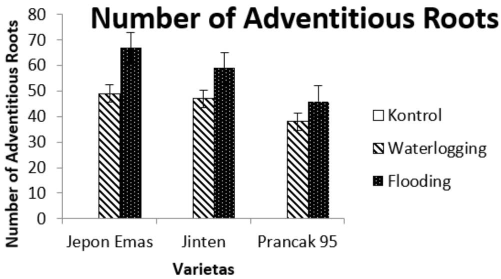

Fig. 5. Number of Adventitious Roots of *Nicotiana tabacum* L.

Based on **Fig. 5**, the number of adventitious roots is influenced by stress and varietal factors. As flooding intensity and duration increase from waterlogging to full flooding the number of adventitious roots also increases. Under normal conditions, no adventitious roots appear since tobacco is a terrestrial plant [2]. After waterlogging treatment, the highest increase occurred in Jepon Emas (49 roots, +49%), followed by Jinten (47 roots, +47%) and Prancak 95 (38 roots, +38%). Under flooding, all varieties showed further increases: Jepon Emas (+27%, 67 roots), Jinten (+20%, 59 roots), and Prancak 95 (+17%, 46 roots). Adventitious roots appeared within 24 hours in Jepon Emas and Prancak 95, and after 2–4 days in Jinten. This increase is linked to auxin accumulation near the stem base, as auxin transport to roots is inhibited, triggering adventitious root formation [3].

3.5 Fraction of Adventitious Roots

Root fraction defined as the proportion of horizontal and vertical roots to total root number [4] was strongly influenced by flooding intensity and varietal differences, consistently decreasing from waterlogging to flooding (**Fig. 6a–b**). Prancak 95 showed the highest horizontal fraction under normal conditions but experienced the greatest decline under submergence, confirming its high sensitivity to flooding stress [5], as lateral roots are rapidly degraded in oxygen-depleted upper soil layers [8]. Vertical fractions were initially highest in Jepon Emas and, despite reductions under stress, remained more stable due to adventitious root formation that maintains oxygen uptake and aerobic respiration [4]. Overall, Prancak 95 exhibited the most severe reductions in both fractions, while Jepon Emas showed more adaptive root restructuring, consistent with the principle that tolerant genotypes develop structural and hormonal adjustments to reduce hypoxic damage [2].

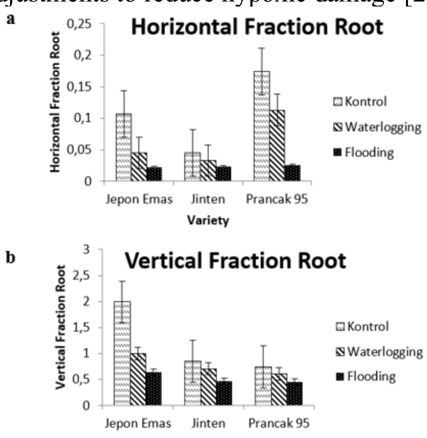

Fig. 6. a. Horizontal Fraction Roots Graph. **b.** Vertical Fraction Roots of *Nicotiana tabacum* L.

3.6 RAI (Root Anchorage Index) dan RGA (Root Grip Index)

Root fraction is the ratio between the number of horizontal roots and vertical roots to the total number of roots [3]. Based on **Fig. 6a** and **6b**, both horizontal and vertical root fractions of *Nicotiana tabacum* var. Jepon Emas, Jinten, and Prancak 95 were significantly influenced by flooding stress intensity and varietal characteristics.

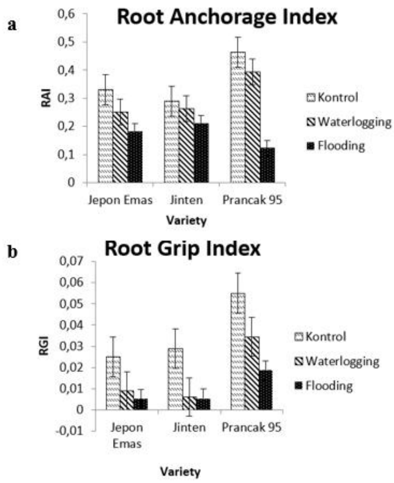

Fig. 7. **a.** RAI (Root Anchorage Index), **b.** RGA (Root Grip Index) of *Nicotiana tabacum* L.

Based on **Fig. 7a** and **7b**, both the Root Anchorage Index (RAI) and Root Grip Index (RGI) of Jepon Emas, Jinten, and Prancak 95 were strongly influenced by flooding intensity and varietal traits, with values consistently decreasing from normal to waterlogging and flooding conditions. Under control conditions, Prancak 95 showed the highest RAI (0.46), indicating stronger vertical rooting, while Jepon Emas experienced the greatest decline under waterlogging (24%), and Prancak 95 showed the sharpest drop under flooding (67%), with all varieties remaining within the moderate anchorage category (0.1–1) as described by [2] reflecting well-developed vertical roots [8]. For RGI, Prancak 95 had the highest initial value (0.054), but waterlogging caused the largest reduction in Jinten (79%), and further flooding produced the highest decline in Prancak 95 (46%). All varieties exhibited RGI values below 1.5, indicating a low grip index category [3], associated with limited horizontal root development and reduced resistance to soil movement [4]. The substantial RAI and RGI declines in Prancak 95 align with its reduced root diameter under severe stress, consistent with its previously noted sensitivity.

4 Conclusion

Flooding stress significantly altered the root architecture of *Nicotiana tabacum*, shown by the largest reduction in root length in Jinten and the smallest in Jepon Emas, while Prancak 95 experienced the greatest decreases in both root number and root diameter. Adventitious roots increased most in Jepon Emas, indicating strong adaptive responses to hypoxia. The horizontal root fraction declined most in Prancak 95, whereas the vertical root fraction decreased most in Jepon Emas. The shoot–root ratio increased most in Jepon Emas, and both the Root Anchorage Index (RAI) and Root Grip Index (RGI) showed the largest reductions in Prancak 95. Overall, flooding stress suppressed vertical and horizontal root growth while enhancing adventitious root formation as the primary adaptive mechanism.

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