Rattan root morphology under different inorganic nitrogen fertilization

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Abstract: Input such as fertilizer highly affects the root response, and can change the root morphology and architecture, which will have an impact on the nutrient uptake and biomass production. This research investigates changes in root architecture and morphology, nitrogen uptake and biomass production of the Noko rattan seed in the treatment of inorganic (N) fertilizers from different sources fertilization (N-Urea ((CO (NH₂)₂), and N-ZA ((NH₄)₂.SO₄)), and different application method. The research results showed that treatment without N+P fertilizer (control treatment) led to more intensive root growth, which was shown in dry biomass of root, higher amount of root branches and root hairs compared to N-urea or N-ZA fertilization. N-Urea fertilization affects the root hairs of rattan becomes shorter and finer, decreases the dry weight of the roots, but increases the roots surfaces compared to the roots in the control treatment and N-ZA fertilization. Localized (in hole) fertilization causes root physiological stress and change the root architecture, causing a decrease in N uptake but otherwise increase the P uptake, but the biomass production is lower than the homogeneous fertilization.

1. Introduction
Rattan root is generally geotropic and Apo geotropic [1]. However, there is little research on rattan rooting, especially in relation to the input given. Inputs such as fertilizers can affect the physico-chemical of the rhizosphere which may alter the root morphology and architecture as well as the nutrients absorption by the roots. Some research on root morphological changes due to nitrogen fertilization (N) have been done, such as the application of NO₃ fertilizer shows root length, also higher ratio of root hair: root length than control and application of NH₄⁺ fertilizer on populous sp. [2] and Crypto Mena japonica plants [3]. Also, several different studies show that root growth and biomass production, and total N absorption are increased by NH₄⁺ treatment compared to NO₃ treatment [4]. There is maximum root hair production in soil with high concentration of organic nitrogen and mineral and high phosphorus, while the lowest root production is in soil with poor nitrogen [5]. Nitrogen and phosphorus deprivation increase the number of root branches and architecture of Pistacialencitiscus seeds [6]. On the other hand, it was found
that in Korean pine, there is negative correlation between N-nitrate content and N-total available in root hair biomass and neuromas of topsoil [7].

Plasticity of root development of every plant species be their response towards the heterogeneity of nutrition distribution by increasing the root system in nutrition rich area [8-10]. Nutrition heterogeneity in the soil can also affects the plant roots architecture and morphology. The heterogeneity of nutrients due to localization of the fertilizer will increase the root mass in the fertilization area [11]. Root distribution depends on plant species, but plant roots are generally concentrated in parts of the soil where nutrient sources are abundant, and water are available [5,12,13].

Localized fertilization in one area can affect the absorption of plant nutrients and depends on the physiological adaptation of the plant. The adaptation of plants for higher absorption at the distribution of non-homogeneous nutrients is done by altering root architecture, for example by increasing root surface area and root length to the fertilization area [11,14,15]. The ability of root plasticity in response to the availability of nutrients in the soil is essential for optimizing nutrient absorption [16].

Localization of nutrition is closely related to forest plants such as rattan, where soil forest nutrients usually accumulate in the topsoil. In this research the response of rattan seedlings are investigated, especially Noko varieties (Daemonorops sp) to N fertilizers from different sources, also homogeneous and localized fertilization methods, their effects on N and P uptake, and rattan plant biomass production.

2. Materials and Methods

2.1. Experimental details

This research used three months old Noko rattan seedlings (Daemonorops sp.) to be planted in Rhizobox for 4 months for investigation. Rhizobox was made of acrylic of 26 cm height, 16 cm width and 2.5 cm thick. The bulk density is 1.2 g/cm, each rhizobox filled with 1.260 g of soil. Rhizobox is wrapped in black plastic and placed on a rack, tilted at 45° (Figure 1).

The dosage of fertilizer used in this research was Urea fertilizer equal to 100 kg N Ha⁻¹ + SP36 equal to 50 kg P Ha⁻¹, ZA fertilizer equal 100 kg N Ha⁻¹+SP36 equivalent 50 kg P Ha⁻¹. K fertilizer was used as a basic fertilizer and mixed homogeneously for all treatments.

Experiment design: fifteen rhizobox (5x3) was built in Green House. These rhizobox are set as a randomized group design for 5 (five) treatments: (i) control (without N & P fertilizers); (ii) homogeneous fertilization N-Urea 100kg N Ha⁻¹+SP36 50kg P Ha⁻¹; (iii) homogeneous fertilization N-ZA 100kg N Ha⁻¹+SP36 50kg P Ha⁻¹; (iv) in hole fertilization of N-Urea 100kg N Ha⁻¹+SP36 50kg P Ha⁻¹; (v) in hole fertilization of N-ZA 100kg N Ha⁻¹+SP36 50kg P Ha⁻¹, with three groups as replication for each treatment.

The soil used has a pH of 5.6, and after the soil was cleaned, it was sieved with a 0.5 cm sieve. The homogeneous fertilization treatment was done by dissolving N and P fertilizers with the doses according
to the treatment with the aquadest, and then mixed with soil until completely homogeneous, then the soil sieved with 0.5 cm sieve. In whole fertilization treatment was done by placing the fertilizer according to the dose of treatment on the hole located on the right and left of the root at a 16 cm depth from the soil surface. Watering was done every 2 days with weighing method for addition of water, so it can restore moisture in 70% capacity of soil.

Investigation of root morphology was performed at the end of the study, root washed away from all impurities by washing in water that flowed slowly for 30 minutes, after which it was rinsed with aquadest. Primary and secondary root diameter measurements were performed by making thin transverse incisions (about 0.5-1 mm) from each root section located 3 cm from the base of the root. While the diameter analysis of root hairs, and the number of root hairs/cm was counted from samples of 2 cm length, were taken uniformly from roots located at 3 until 5 cm from the base of the root. Observation of primary root diameter, root hair diameter, and number of root hairs was conducted using Microscope type Carton, SCW.PF + PC and Scope Image 9.0 exe. Root surface area analysis was done by taking 3 cm of all parts of the roots located starting 5 cm from the base of the root. Measurement of root surface area was conducted using CID Bio-Science Area Meter.

The production of crown and root biomass is the constant weight of crown and root after being dried by oven at 70°C for 2x24 hours. N uptake analysis was performed by Nitrogen (N) tissue analysis using Dry Burning method with CN Combustion of Yanaco Macro Corder JM1000CN type. Meanwhile, P analysis was performed using Spectrophotometry.

3. Result and Discussion

3.1 The effect of N fertilizer from different sources on root morphology

In this study, the results showed that treatment without the application of N+P fertilizers (control treatment) increases root growth, which shown by higher root branches, root hairs, and dry biomass of root than N-Urea or N-ZA treatment, as shown in Figure 2 and 3.

![Figure 2. Rattan roots conditions under treatments: control, homogeneous fertilization of N-Urea, and N-ZA, and in hole fertilization of N-urea and N-ZA.](image)

Plant roots respond to N deficiency by increasing root branching, root length, root hair, root hair density, and can also support root growth rate [17, 18]. On the other hand, that addition of N fertilizer decreases specific root length [SRL] and specific root area (SRA) [19].
In this research, localized fertilization (in hole fertilization) significantly affect the root architecture that causes the formation of branches only on the area of N and P fertilizers are placed, so that the dry biomass of root, the number of roots of hair and root branches, root diameter and surface area is smaller than homogeneous fertilization (Figure 4.5). Localized fertilization will increase the root mass in the fertilization area [11,19], but the total mass of the roots may not be significantly different [11].

However, the results of this research are in contrast with the results of the research that suggest that P-layered fertilizer applied showed a significantly higher root ratio than the homogenous P fertilization in Pinus plants [15]. Plants usually allocate more of their photosynthetic products in their root systems when plants lack of P, although these phenomena vary for each plant species [20,21]. The localized fertilization of P at one point increases the growth, root penetration depth, main root mass, but does not significantly increase the fine root mass of the Eucalyptus grandis plant [11]. In this research, treatment of N-Urea causes finer root hairs, reduces diameter and number of root hairs, also inhibits the formation of root branches, so that there are very few tertiary branches and no quartier branch. While the control and N-ZA treatment form many tertiary and quartier root branches, the number of root hair and root hair diameter is greater than N-Urea treatment (Figure 5 and 6). However, in this research the finer root hairs of the N-Urea treatment increased root surface area compared with control and N-ZA treatment (Figure 6).
Figure 5. [A] Root hairs length condition, [b] Diameter and length of root hairs, and [c] Primary roots diameter. The [a], [b], [c] is rattan root condition under control, N-urea and N-ZA treatment.

Figure 6. (a) Roots hairs diameter (mm), (b) Root hairs units/cm, (c) Root surface area (mm²) under different N sources, and homogenous or in hole fertilization methods

N fertilizer significantly affects root morphology; decreases root specific length, and root specific area of pea plants (*Pisum sativum* L.) [22]. A low nitrate concentration will cause lateral root elongation, or high nitrate concentration will inhibit root development [23]. However, the results of this research is in contrast with the results of some investigators that shows that inorganic administration of N plus protein [BSA] in *Arabidopsis* plants results in higher root dry weight, thicker roots, and longer root hair [24]. N fertilization increases soil inorganic N levels and decreases pH of soil, increases root hair elongation, but root hair diameter is not affected by N fertilization. However, the effects of changes in soil N content are less clear for the phenology and morphology of root hairs in Sugi plants (*Cryptomeria japonica*) [3]. However, N and P fertilization treatments significantly affect plant growth and root morphology of *Acer mono* seedlings. Treatment without fertilizers shows lower root diameter, root length, and root length,
whereas maximum root growth was achieved when *Acer mono* seeds were fertilized at 10 g N per plant [25]. Ammonium stimulates lateral root formation and branch formation [26, 27].

### 3.2 Morphology effect to N and P uptake, and production of biomass

This study shows that different N sources affect of N and P uptake. N-Urea causes finer root hairs, higher of root surface area (RSA) so increase the N uptake or N-tissue, and higher biomass production (Figure 7 and 11) b. The effect of N fertilizer not only affects root architecture [root structure physically] but also affects root function [2]. The size and architecture of the root system is a major factor in nutrient uptake efficiency, to determine the total volume of soil that the plant can explore and the total surface area of the exchange between the roots and the soil [28]. The fine root [diameter <2mm] plays an important role in nutrient and water absorption, and respiration [29, 30]. The nitrate treatment affects the development of fine roots, as well as easier water absorption. *Cryptomeria japonica* can respond to increased inorganic N levels in the soil by increasing the production and lifespan of fine roots [3]. However, in contrast to pea plants [*Pisum sativum* L.], it shows that genetic factors affecting root distribution patterns more than environmental factors [22]. The addition of nitrogen can decrease the concentration of N, P, K and Mn of bamboo root tissue [*Pleioblastus amarus*] [19].

This research shows that fertilization methods also affect of N and P uptake. Localized fertilization [in hole fertilization] causes the physiological stress of plant roots, changing the architecture and morphology of rattan root, which roots are concentrated in the area around the fertilization hole, decreasing the distribution of roots in the soil, reducing N uptake and biomass production lower than homogeneous fertilization.

![Figure 7](image)

**Figure 7.** Compare % N and % P status of rattan shoot tissue with dry biomass of rattan shoot [g] under different N sources, and homogeneous and hole fertilization methods.

On the other hand, in hole fertilization causes higher P uptake than homogeneous fertilization, but does not have a significant effect on the production of rattan seed biomass [figure 7]. Nitrate is relatively mobile in the soil, both horizontally and vertically, the spatial distribution of roots in the soil is important for the absorption of N by the corn root [31]. In contrast to *Eucalyptus grandis* plants, localization of P fertilizer shows higher plant growth, higher P absorption, as well as better assimilation of N than homogeneous fertilization [11]. The localization of P + ammonium fertilizer increases the content of P and N –tissue, and products of corn [14]. The application of fertilizer at one point near the root can increase production, since localization of the fertilizer will reduce the contact of the fertilizer to the soil, so that phosphate immobilization to form become unavailable, becomes lower [32]. The adaptation of plants to the localized fertilizer techniques also depends on the crops genetic. Some of the *Masson pine* plant...
genotypes show better growth with localized fertilization, although some are genetically indicative of better adaptation to homogeneous fertilization [15].

4. Conclusions
This research shows that the Noko rattan roots responds to the biochemical changes and have good plasticity ability to the condition of rhizosphere. N absorption and biomass production of rattan seedlings are more influenced by N sources, but it is different from P uptake which is favoured by fertilization methods and has little effect on the production of rattan seed biomass.

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