Physiological characters of four lowland chilli varieties (Capsicum annum L.) with root cutting

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Abstract. The root system plays some vital roles in overall plant development, promoting plant anchorage, absorption of nutrients and water, and hormone production. Cutting is an attempt to root regeneration and enhance the plant’s metabolism capability, including canopies and roots for high yielding. Non-hybrid chilli can be used as a model plant because Indonesia’s chilli yield has not been maximal yet. This research was aimed to determine the physiological characters of four lowland chilli varieties with root cutting. This study was designed using a randomized, complete block design (RCBD) with two factors: root cutting and varieties. The root cutting factor consisted of 4 levels: root cutting in seeding, root cutting in ridging, root cutting in seedling and ridging, and non-cutting. The varieties factor consisted of 4 levels, namely Lembang, Kencana, Tanjung, dan Ungu. The results showed that root cutting in ridging for Kencana, Lembang, and Ungu increased stomatal conductance, transpiration rate, CO₂ intercellular, chlorophyll accumulation, photosynthesis rate, and improved fruit yield per plant. However, Tanjung did not respond to root cutting treatment because it did not improve plant physiology characters and fruit yield than non-cutting treatment. It is concluded that root cutting in ridging can be improved plant physiology, which contributes to increasing yield on Kencana, Lembang, and Ungu.

1. Introduction
The root is an organ that functions to absorb water and nutrients to support vegetative and reproductive organ growth. A roots system with a wide range can access a large soil volume so it can contribute to the anthesis and fruit formation process [1]. Root could change the rizosfer zone by metabolites on the exudate affected by water, nutrient, and environment. Upper soil generally tends to higher in nutrient and dryer and prone to extreme weather compared to subsoil layers [1]. Root architecture is a spatial root system distribution [2] whose role is to support resource distribution evenly and contribute to plant productivity [3].

Root architecture can be improved by cutting roots. Cutting is a technique to reduce vegetative plant growth classified as practical and economical for stunting but can induce new roots to maintain growth [4]. Root cutting can change the growth balance, assimilation, nutrient distribution, and hormones. Increased hormone production in the meristem can initiate lateral roots [5], and the small diameter roots from where they cut can increase the water and nutrient absorption.

Cell division and cell enlargement during root formation need a massive quantity of energy and carbon. The primary carbon source comes from sucrose, which is translocated to the sink. The translocated sucrose by basipetal is used as energy for cell division and cell enlargement and a carbon
source for roots growth [6]. The acceleration of the physiology plant process can help accumulate photosynthetic products used in organ regeneration. Stomata activity, CO₂ content at the leaf, transpiration rate, and photosynthetic rate in the canopy can affect plants to produce triose phosphate, which will be used as a basic component in the complex carbohydrate molecule formation [7].

Chilli plants can act as model plants that can be used to measure success in root-cutting treatment. Chilli is an economic crop with a high demand in Indonesia that can trigger inflation [8]. National chilli productivity has reached optimal productivity that can be produced, yet [9]. This research was aimed to determine the physiological characters of four lowland chilli varieties with root cutting.

2. Methods

2.1. Experimental details
The research was conducted at Greenhouse of Agriculture Faculty, Universitas Gadjah Mada for nursery, Kebun Tridharma of Agriculture Faculty, Banguntapan, Bantul, Yogyakarta for planting local curly red chilies. The research was conducted from August 2019 – March 2020.

The material used was four non-hybrid chilli varieties, namely Lembang, Kencana, Tanjung, Ungu. Other materials used were media for nursery from soil: fertilizer: sand: cockpit with composition 1:1:1:1, media for transplanting is from the soil and manure composition, polybag, nail polish, acetone, NED, SA. The tools used were 1). Measuring instruments: analytic scale, gauge, ruler, nailed board; 2). Cultivation tools: plastic rope, sprayer, hoe, measuring cup, and a bucket; 3). Plant physiology observation tools: microscope, optical lab, photosynthetic analyzer type Li COR 6400.

This study was designed using a two-factor factorial design (4 x 4) arranged in a randomized completely block design (RCBD) with three blocks replication. The first factor is four non-hybrid chilli varieties, namely Kencana, Lembang, Tanjung, dan Ungu. The second factor is root cutting consisted of 4 levels, namely root cutting in seeding, root cutting in ridging, root cutting in seedling and ridging, and non-cutting. Each block contains 16 treatment combinations. Each block consisted of 10 plants, so in total, there were 480 chilli plants each block. Three plants selected were observed periodically and harvested sample plants; 2 plants were observed destructively. Root cutting treatment was given by lateral root cutting by 50% when seedlings were ready to be transplanted, cutting by 50% of the sides plant when planting, and transplanting them into bigger polybags.

2.2. Sampling and measurement
2.2.1. Environmental Data. Leaf environmental data include leaf temperature and light intensity observed by a leaf thermometer and lux meter.
2.2.2. Dry Weight. The root, stem, and leaf of the plant, before being oven-dried, is first let dry for 6 hours. The oven was carried out at 80 °C for three days until the weight was constant.
2.2.3. Character physiology. Measurements on sample plants were determined by one maintenance leaf as a random sample to measure photosynthesis rate, transpiration rate, leaf CO₂, photosynthetic radiation, and H₂O using the photosynthetic analyzer model Licor 6400. The photosynthetic analyzer can be used after the battery, and the chemical tube is filled, and the chamber tube clamped to leaves connected to a photosynthetic analyzer cable connection. The tool is then turned on by pressing the button and waiting for a few minutes until AC=0 (warning up). Then the sample leaves are clamped by chamber d for 5 minutes, and after the measurement number is stabilized, the chamber is removed. The monitors displayed on the monitor are the photosynthetic measurement rate, transpiration rate, leaf CO₂, PAR, and dan H₂O.
2.2.4. Chlorophyll and carotenoid contents. Leaf chlorophyll content was analyzed by weighing 1 gram of leaves ground with a mortar and then adding 20 ml acetone 80%. The mixture is filtered using a paper filter. Next, I turned on the spectrophotometer and left for 10 minutes. The 80% pure acetone solution was put into cuvet until the limit was a blank. With the button set at a wavelength of 645 nm, the absorbance is set to zero. The pigment solution sample was poured into another cuvet until the limit, and the absorbance number was recorded (A645). The step can be repeated to get an absorbance number
at a wavelength of 663 nm (A663). Chlorophyll a and chlorophyll b on leaves content were calculated using the equation [10] with units of mg g leaf fresh weight⁻¹.

Chlorophyll content a = (12.7 x A663 – 2.69 x A645) x 0.02
Chlorophyll content b = (22.9 x A645 – 4.68 x A663 )x 0.02

Carotenoid concentration (Cx+c) was calculated with a spectrophotometer at a wavelength of 470 nm and acetone 95.5% used as a blank.

Carotenoid concentration = A470 + (0.144 A663 – 0.638 A645)

2.2.5. Length and Width of Stomatal. Stomata characters were observed in the form of height, and width stomatal carried of by printed stomata using transparent nail polish smeared evenly under the leaves across 4 cm. Dried it for 5 minutes, the stained part fixed with transparent insulation. Then the insulation part is carefully peeled to get the stomata print. The isolation was affixed to the glass side under a microscope binocular Olympus (Germany) with magnification to 40x [11]. After that, take a picture of stomata used using the OptiLab Mikroskop Digital Advanced Type and the software OptilAB Viewer version 2.1. Furthermore, the number of stomata and the length and width of the stomata opening were measured using OptiLab ImageRaster software version 2.1. Measurement of the number of stomata is carried out per unit square millimetre (mm²), and the width and length of the stomata opening are expressed in units of micrometres (μm).

2.3. Statistical analysis

The observational data obtained were tested using variance (ANOVA) for factorial design arranged in a completely randomized block design. The linear model that follows this design appears in equation (1).

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \beta)_{ij} + \epsilon_{ijk} \]  

By i = 1, 2, 3, 4 shows the first factor levels; j= 1, 2, 3, 4, shows the second factor levels and k = 1, 2, 3 shows block. \( Y_{ijk} \) shows the observed data; \( \alpha \) shows the first-factor effect; \( \beta \) shows the second-factor effect; \( \alpha \beta \) shows the interaction between the first and second factor; dan \( \epsilon \) shows error (Gomez & Gomez, 1976). If there is an interaction, it is further tested with a simple effect analysis (simple effect) by post hoc honestly significant difference visualized using a table [12]. Data analyzed using software SAS version x64 3.1.2.

3. Results and discussion

Environmental factors were one of the factors that affect plant growth. The temperature and light intensities received by plants can be metabolized inside the plant's body. Temperature did affect the physiological process and the biochemical reaction rate. The optimum temperature did contribute to physiological reaction, so the simple formed sugar becomes the raw ingredients for optimum regeneration cells to happen [13]. The plants' high temperature can inhibit plant growth by suppressing enzyme activities and increasing the transpiration rate.
The characters of temperature and light intensity received by plants canopy shown in Figure 1. The plant canopy temperature between the upper and lower one of all varieties and root cutting treatment ranged from 29.5 °C - 32.25 °C. The top, middle, and bottom canopy temperature did not show any significant symptoms in the whole treatment. However, the plant temperature over 35 °C can cause symptoms that aggravate physiological conditions and plant growth [14]. Unlike the temperature case, the light intensity received by upper part plants is higher than the light intensity reached the middle and lower canopy. The middle and lower canopy showed no significant effect on the overall plant treatment. The light intensity that decreased to the canopy located at the bottom shows that the composition of the middle and lower leaves position is tighter than at the top. The light at the bottom is lower than that of the top of the canopy.

Figure 1. Leaf temperature by the leaves at the upper canopy, middle canopy, and lower canopy, n=6

Figure 2. Light intensity by the leaves at the upper canopy, middle canopy, and lower canopy, n=6

Figure 3. Organel stomata on leaves from root cutting treatment of 4 non-hybrid chilli varieties. *: significant P < 0.05

Figure 4. Chlorophyll and carotenoid content on leaves from root cutting treatment of 4 non-hybrid chilli varieties. *: significant P < 0.05
Stomata are organelle where CO$_2$, H$_2$O, and O$_2$ are exchanged, abundant at lower leaves epidermis. Stomata openings can be affected by light, temperature, and water content in leaves [15]. The stomata length of the longest diameter and the width, which is the shortest diameter, can describe the extent of gas exchange in the leaves. Based on observation, Ungu, Tanjung, and Lembang varieties had a wider opening than the length opening. Width opening, which is wider than the length opening, shows that the guard cells have turgor to open wide [16]. Ungu offers a control treatment that gives the most wider width opening. While in Tanjung and Lembang, the widest wide opening from ridging treatment and seedling. In Kencana, seedling and seedling ridging shows a much longer length opening than width opening. This indicates that the guard cells condition is not turgor making the stomata opening not maximal. Ridging and controlling Kencana had a much wider width opening than length opening, showing stomata had urgency. The stomatal width also indicated that water availability in the soil is sufficient so that the transpiration process occurred to maintain optimal body temperature. The stomata activity in chilies belongs to type C3, which is an indicator of optimal metabolism. This thing can spur the physiological processes that occurred.

The involvement of stomata in photosynthesis is supported by the mineral and water content on the leaves; also, the leaf's thickness is related to the chlorophyll content. The chlorophyll content is a measurement for the pigment photosynthesis quantity. It is located on leaves palisade tissue. The analyzed result (Figure 4.) shows that chlorophyll b content is higher than chlorophyll a. Chlorophyll b on Lembang and Tanjung shows a significant difference compared to Kencana and Ungu. In Lembang and Tanjung, root cutting treatment during ridging can increase chlorophyll b content. Chlorophyll B is found in the pigment antenna system that makes it function as a light catcher. And chlorophyll A is on the photosystem I and Photosystem II and the system in the antenna pigment [16]. The pigment-protein light harvester LHC-I from PSII has a ratio of a/b ~3, while LHC-II from PSI has a rate of a/b 1.1-1.3. The balance a/b LHC-II from PSII, which is variate, shows an adaptation response toward the light. Shade plants have a much higher LHC-II than an exposed plant, resulting in a lower a/b ratio than the exposed plant. Thus, a decreased a/b can indicate there is an enlargement antenna system on PSII. When the a/b rate is low, it also shows a perfect leaves development, classified as shading leaves and C3 plants [18]. The chlorophyll a and b ratio toward total carotenoid also indicates a greenish presence of the plants. The chlorophyll a and b with a lower carotenoid shows aging, stress, and damage to photosynthesis equipment, which means chlorophyll break down is faster than carotenoids. However, it can also happen when the ratio < 1, the plant's professional chromoplast development on ripe fruits, which changes from green to red, so the ratio keeps decreasing [19]. This indicates a chromoplast development on fruit so that the leaves' chlorophyll content is lower than carotenoids. The a/b rate is also lower due to the shading effect on the canopy shown in the light intensity (Figure 2.).

**Table 1.** Nett activity of photosynthesis (Pn), stomatal conductance (g$_s$, CO$_2$ intercellular (Ci), Transpiration rate (Tr), H$_2$O, Photosynthetic active radiation (PAR) on chili canopy

| Variety  | Cutting root       | Pn (µmol CO$_2$ m$^{-2}$ s$^{-1}$) | g$_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$) | Ci (µmol CO$_2$ mol$^{-1}$) | Tr (mmol H$_2$O m$^{-2}$ s$^{-1}$) | H$_2$O Daun (mol H$_2$O mol/air) | PAR (mol/m$^2$/s) |
|----------|--------------------|-----------------------------------|------------------------------------|----------------------------|-----------------------------------|---------------------------------|------------------|
| Kencana  | seedling           | 17.34<sup>b</sup>                | 0.0062<sup>b</sup>                | 1129.3<sup>b</sup>           | 1.36<sup>b</sup>                 | 2.56<sup>b</sup>                | 3922.5<sup>c</sup> |
|          | seedling and ridging| 20.90<sup>b</sup>                | 0.0267<sup>ab</sup>               | 1990.8<sup>b</sup>           | 5.42<sup>ab</sup>               | 6.79<sup>b</sup>                | 3983.3<sup>a</sup> |
|          | ridging            | 35.48<sup>a</sup>                | 0.0315<sup>a</sup>                | 3101.5<sup>a</sup>           | 7.01<sup>a</sup>                | 9.07<sup>a</sup>                | 4274.0<sup>b</sup> |
|          | control            | 22.05<sup>b</sup>                | 0.0285<sup>ab</sup>               | 1868.3<sup>b</sup>           | 7.27<sup>a</sup>                | 5.85<sup>ab</sup>               | 3506.3<sup>a</sup> |
| Lembang  | seedling           | 15.86<sup>b</sup>                | 0.0140<sup>b</sup>                | 1389.0<sup>b</sup>           | 3.78<sup>b</sup>                | 5.30<sup>a</sup>                | 3141.5<sup>c</sup> |
|          | seedling and ridging| 20.23<sup>b</sup>                | 0.0094<sup>b</sup>               | 2348.0<sup>b</sup>           | 2.82<sup>b</sup>                | 3.57<sup>a</sup>                | 4625.8<sup>a</sup> |
|          | ridging            | 26.40<sup>a</sup>                | 0.0284<sup>a</sup>                | 4702.7<sup>a</sup>           | 4.50<sup>a</sup>                | 5.82<sup>a</sup>                | 4729.8<sup>a</sup> |
|          | control            | 15.29<sup>b</sup>                | 0.0091<sup>b</sup>                | 2559.8<sup>b</sup>           | 2.03<sup>b</sup>                | 5.51<sup>a</sup>                | 3387.7<sup>a</sup> |
| Tanjung  | seedling           | 23.96<sup>a</sup>                | 0.0194<sup>a</sup>                | 2206.8<sup>a</sup>           | 4.96<sup>a</sup>                | 6.13<sup>b</sup>                | 4799.7<sup>a</sup> |
Notes: Means different letters were significantly different at same variety, P < 0.05 (n=9) using the Honestly significant difference

Active photosynthesis, water content on leaves, transpiration, stomata conductance, CO2 leaf concentration, and photosynthesis is a leaf physiology activity that can be observed. Photosynthesis active radiation is visible radiation used in the photosynthesis process. Where PAR is half of the total radiation received by the earth's surface. Of the total radiation, it is assumed half of it is absorbed by plant canopy, and the other half is reflected and absorbed by the earth's surface. Based on the PAR experiment received from the treatments, the plants had the same result of high PAR from the leaf that can affect the plant's stomata conductance activity. Based on the results show that the increase in stomata conductance in the root cutter is offset by the availability of H2O and CO2 in the leaves. Transpiration is the loss of water from the plant in water vapor through stomata, cuticle, and lenticel to the outside, which is not saturated with water. Transpiration functions for the plant in water absorption, nutrient transportation, and photosynthate distribution. The decrease in the transpiration rate is a physiological mechanism for the plant to maintain its water status to do the physiology mechanism. The existence of CO2 and water on cell affect the photosynthesis rate. Photosynthesis is an anabolism reaction that produced the final product, glucose. Plant production depends on the size and efficiency of the photosynthesis system. Based on the analysis result, it is shown that Kencana, Lembang, and Purple have the same photosynthesis level as the root cuts at planting time.

Figure 5. Dry weight of plant organs from root cutting treatment of 4 non-hybrid chili varieties. * : significant P < 0.05

Dry weight as a variable can describe the accumulation of assimilating, which translocated during the growth phase referred to as biologic plant weight. In this case, the dry weight plant is divided into root, stem, and leaf. The root dry weight is a parameter that explains the growth and development of the plant root system. [20] explain the growth and development of the plant root system affected by division.
rate and enlargement cell, in addition to the accumulation rate of dry weight in the root. This assimilated accumulation results from the absorption of solar energy used for the photosynthesis process [20]. Based on the analysis results, Kencana dry weight at the time of ridging and seedling and ridging had the growth of root, stem, and the other stem that dominates the other root cutting treatment. The same thing can be seen on the Lembang variety given root cutting at ridging. Root cutting at ridging had a positive effect on plant dry weight by the improved physiology process. Also, plant dry weight as a variable, fresh fruit weight, and whole fruit in the ridging treatment of Kencana, Lembang, and Ungu varieties showed a positive result. However, this does not happen on the Tanjung type.

When observed in the harvesting chili process, the Kencana variety was harvested up to 12 times, while Lembang up to 15 times. The 3rd to 8th harvest period indicates too high. It indicates at the 3rd to 8th harvest period ridging treatment the maximum results. In contrast, the Lembang variety had an optimum harvest period from the 5th, 7th, and 8th. The harvest period shows that root cutting has a maximum harvest time. Tanjung has a harvest period of up to 13th, as an Ungu until 16th. Tanjung, the optimal harvest occurred on 5th to 6th and 8th up to the 9th period. In that period, care provides the most optimal value. While in Ungu, the peak crop harvest happens in the 8th period. In this period, the yield provides the most optimal value in treatments. In the treatment of control, optimal results occur in the
7th-8th period, in the ridging treatment happened at 7th up to 9th period, in seedling happened at 9th period, while in seedling and ridging happened at 9th period.

4. Conclusion
Root cutting treatment during the planting can increase the fruit's fresh weight, and the number of fruit by increasing the plant's physiologic processes make the growth increased, with the increase of dry weight as the variable. In addition, the ridging also accelerates the harvesting time so that the fruit yields are optimal.

5. Acknowledgements
The authors would like to acknowledge the Directorate General of Higher Education's financial support under Universitas Gadjah Mada's management based on contract No. 5712/UN1.DITLIT/DIT-LIT/ LT/2018

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