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Stomatal Distribution in Vegetative Growth of Peranggi Chili (*Capsicum annuum* L. var Chinensis) Grown in Different Growing Media

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Abstract:
Peranggi chili (*Capsicum annum* L. var chinensis) is a local plant of West Kalimantan. Vegetative growth and changes in the number of stomata and stomatal distribution are not yet known in different growing media. The purpose of this study was to determine the vegetative growth of peranggi chili and changes in stomatal distribution due to differences in growing media as an intensification effort. The research was conducted with experimental design of Randomized Completely Block Design with 3 blocks. Treatments were 4 different growing media, namely P1: alluvial soil: sand: cow manure compost (1:1:1); P2: alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3: alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); P4: alluvial soil: sand: compost: burnt husk (1:1:1:0.5). Data on vegetative growth variables measured were dry weight of roots, stems and leaves, number and area of leaves, root length, stem height, number of stomata and stomatal distribution calculated at 40, 50, 60, and 70 DAT. Data were analyzed using ANOVA SAS. All plant variables were not affected by differences in the composition of growing media, as well as the average number of stomata and stomatal distribution up to 60 DAT. However, P3 treatment, which was the addition of burnt husk to a mixture of alluvial soil: sand: cow manure compost, increased all growth variables at 60 DAT compared to other treatments, although not statistically significant. The increased due to P3 treatment increased all variables at 70 DAT significantly. The number of stomata and stomatal distribution was highest on day 40 DAT and decreased due to more mature leaf growth at 50 DAT. However, the average number of stomata and stomatal distribution were not affected by different growing media during the first-70 day periode.

Keywords: peranggi chili, vegetative growth, stomatal distribution, growing media

1. Introduction

Peranggi chili (*Capsicum annuum* L. var Chinensis) is a local chili of West Kalimantan, Indonesia. Peranggi chili has a spicy taste and unique aroma so that its use is only needed in small quantities in a dish. However, the availability of peranggi chilies in the market is limited. This is because peranggi chili has not been cultivated on a large scale. Extensification and intensification cultivation efforts are needed to be done to increase production and availability in the market. Limited production can be improved by modifying the growing environment, one of which is a growing medium that is more suitable and supports its growth (Putri et al., 2023).

One of the influential growing environments is the growing medium of a plant. Peranggi chilies are usually planted in alluvial soil with a mixture of sand and cow manure compost. However, the results of vegetative growth of peranggi chili in the composition of alluvial soil: sand: cow manure compost with a ratio of 2:1:1 were not optimal (Daningsih, 2024). Whereas vegetative growth will affect the reproductive phase. The use of growing media composition is reported to increased growth such as in strawberries (Madhavi et al., 2021), salads (Sisriana, Suryani & Sholihah, 2021), and pakcoy (Pare et al., 2023). From the results of various studies, the ratio and type of media varied for optimal growth (Febriani, Gunawan & Gafur, 2021) for each species. Peranggi chilies are generally grown on alluvial soils. This intensification effort can use different growing media in the hope that vegetative growth is faster and larger so as to produce high production. According to Mantovani (2022), vegetative growth will determine the reproduction of a plant. Vegetative growth is also accompanied by plant enlargement and results in more mature plant organs including leaves. Leaves are vital organs in the photosynthesis process involving stomata, which are located on the lower epidermis and/or upper epidermis depending on the species. The number of stomata and stomatal distribution determine the exchange of carbon dioxide and oxygen gases for photosynthesis and water vapor for transpiration. Therefore, the presence of the number of stomata and the distribution of stomata can also be influenced by the environment of a plant including the growing medium. Fitriani et al., (2006) stated that the number of potato stomata differs between those grown in the highlands near sulfur sources and those far from sulfur sources. This difference shows the adaptive attitude of potatoes to different environments. But there is also no change in the number of stomata when planted in different environments (Cai et al., 2024). Peranggi chili has not yet known its stomatal response to environmental changes. Therefore, the purpose of this study was to determine the vegetative growth, stomatal changes and stomatal distribution of peranggi chili against different growing media.

2. Material dan Method

This research was an experimental study with a Randomized Completely Block Design (RCBD) using three blocks with four kinds of growing media. Sampling of three plants was done four times and observed at 40, 50, 60, and 70 days after transfer (DAT). The materials used were peranggi chili seeds, alluvial soil, sand, cow manure compost, burn husk, perlite, compost, water, NPK fertilizer, fungicides, and pesticides. Watering with 200 ml of water was done every day. Watering was not done on rainy days. Fertilizers and pesticides/fungicides were applied when needed. Pots were placed under 70% paranet shade. Soil temperature, humidity, and wind speed were measured daily.

3. Procedure

3.1 Procedure for Making Growing Media

The growing medium was prepared by mixing different ingradients according to the treatment. All media were dried, pulverized and filtered through a soil sieve. All media materials that have been mixed were then homogenized and put into a 35 cm diameter pot. The preparation of growing media was divided into 4 treatments with the ratio as followed P1: Alluvial soil, sand, cow manure compost (1:1:1), P2: Alluvial soil, sand, cow manure compost, perlite (1:1:1:0.25), P3: Alluvial soil, sand, cow manure compost, burnt husk (1:1:1:0.5), and P4: Alluvial soil, sand, compost, burnt husk (1:1:1:0.5). The media was put into pots and labeled and given a small pipe to avoid excess water on the soil surface. Growing media were analyzed for nutrient and biophysical content for each treatment. Soil analysis for each growing media was conducted at the Soil Chemistry and Fertility Laboratory, Faculty of Agriculture, Tanjungpura University.

3.2 Seeding Procedure

Seeding was done by soaking the seeds in warm water for 2 hours to allow imbibition, germination and ensure the release of chemicals if any. Then the seeds were spreaded on soil survace for germination until they grew 3-5 leaves. Watering was done daily.

3.3 Planting and Maintenance Procedures

Planting was done when the seedlings having 3-5 leaves into the pot media that had been labeled. Each pot was planted with five plants and watered with 200 mls of water. Then, plant maintenance was carried out by weeding the plants every week and embroidering if any plant died. NPK fertilizer was applied at 1/3 of the recommended rate once every 2 weeks. Pesticide or fungicide were applied if needed.

3.4 Plant Observation Procedure

Growth observations were made destructively at 40, 50, 60, and 70 DAT. Plant observations included measurements of vegetative growth in the form of dry weight of roots, stems, and leaves; number and area of leaves; root length, and stem height. Making stomatal preparations using replica method to calculate the number of stomata and stomatal distribution wes also done at 40, 50, 60, and 70 DAT.

3.5 Vegetative Growth Measurement Procedure

Measurement procedure of plant vegetative in the form of dry weight measurements of roots, stems, and leaves; number and area of leaves; root length, and stem height. The dry weight at the beginning of growth was done with an analytical balance type Ohaus Carat series to get more accurate data since the water content fluctuated in the plant fresh weight. Dry weight was obtained by drying the plants in an oven at 80° C until constant weight.

3.6 Distribusion Stomata Calculation Procedure

Stomatal preservation preparations were made using the replica method. Leaf samples were taken from the fourth leaf from the tip of the stalk branch. Preparation was repeated three times for each treatment from the upper and lower surfaces of the leaf. The sample preparations were observed under a microscope with a magnification size of 10x10 and connected to an optilab using Image Reaster aplication. The calculation of stomatal distribution refers to Meidner & Mainsflied in Avci (2014) by measuring the number of stomatal cells and the number of epidermal cells per unit area using the formula:

$$SD = \frac{\text{Number of stomatal cells per unit}}{\text{Number of stomatal cells per unit + number of epidermal cells per unit}} \text{ x } 100\%$$

Description:

SD = Stomatal distribution

3.7 Data Analysis

All response variables namely dry weight of roots, stems, leaves, number and area of leaves, root length, and stem height were analyzed using ANOVA SAS. If there was a significant treatment, it was followed by LSD test at $\alpha = 0.05$. The average number of stomata and stomatal distribution were also analyzed using ANOVA SAS. The increase or decrease in the number of stomatal growth and distribution was observed from 40 to 70 DAT.

4. Results and Discussion

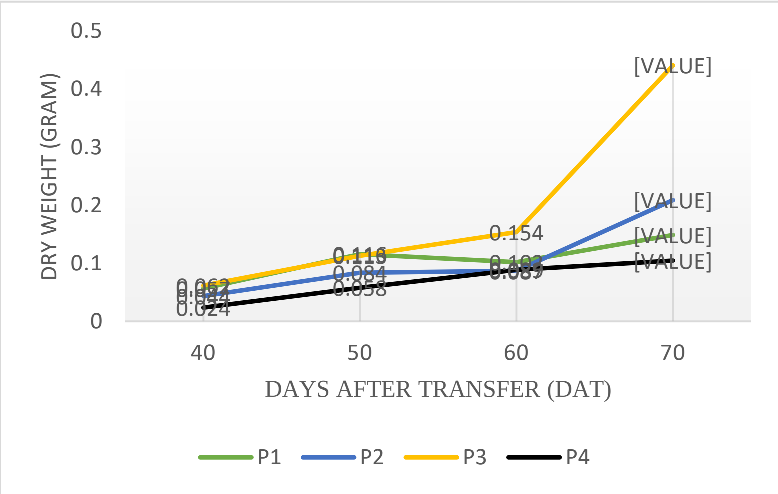
4.1 Results

Observations of vegetative growth of peranggi chili on different growing media (P1, P2, P3, and P4) showed almost the same response among treatments. The results of the analysis of dry weight measurements of roots, stems and leaves, number and area of leaves, root length, stem height, number and distribution of stomata can be seen in the following table and figures.

Table 4.1 Significance of treatment effects on growth variables on days 40, 50, and 70 DAT

Days	Treatment	Variable					
		Root Dry Weight (gram)	Stem Dry Weight (gram)	Dry Weight of Leaves (gram)	Number of Leaves	Leaf Area (cm^2)	Stem Height (cm)
40	P1			0.2089 a		10.490 a	
	P2			0.1456 a b		5.976 a b	
	P3			0.2658 a		10.657 a	
	P4			0.0668 b		2.281 b	
50	P1						12.011 a
	P2						10.500 a b
	P3						12.044 a
	P4						7.922 b
70	P1	0.1491 b	0.3378 b		10.111 b	32.209 a b	17.278 a b
	P2	0.2087 b	0.2080 b		10.778 b	22.048 b	13.722 b c
	P3	0.4415 a	0.5454 a		17.778 a	45.800 a	20.478 a
	P4	0.1047 b	0.1681 b		8.111 b	15.793 b	12.856 c

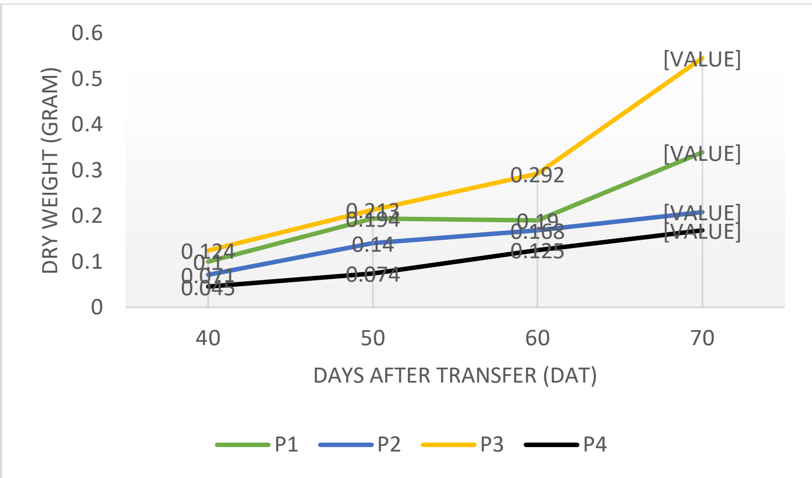
Several vegetative growth variables were significantly affected at 40, 50 and 70 DAT, although the variables were different. At 40 DAT, leaf dry weight (gram) and leaf area (cm^2) were significantly affected by the treatments (Table 4.1). The P3 treatment produced the highest leaf dry weight and leaf area but was not statistically significantly different from P1 and P2, while the P4 treatment produced the lowest effect from the other treatments. At 50 DAT only stem height variables were significantly affected by the treatments. P3 treatment still had the highest effect and P4 the lowest to stem height. However, at 70 DAT almost all variables were affected by the treatments significantly, except the dry weight of the leaves. P3 treatment had the highest effect on all variables, whereas P1 and P2 effects varied; nonetheless, P4 treatment has the lowest effect on the stem height.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 1: Root dry weight at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

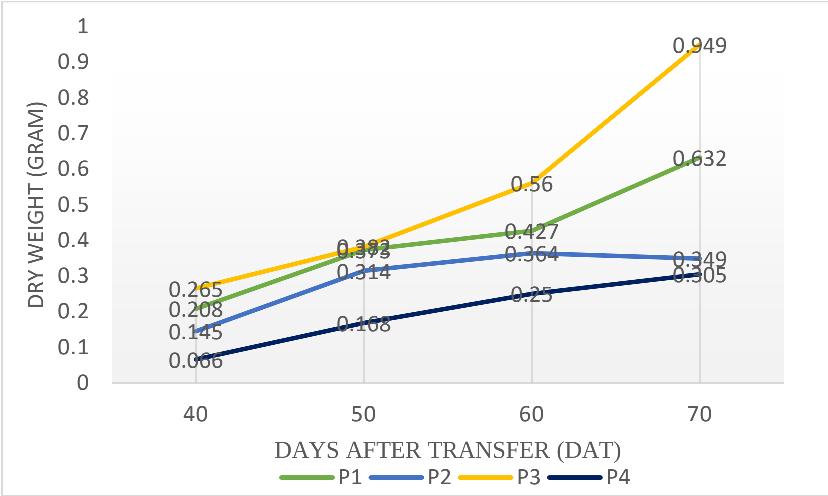
All treatments increased root dry weight from 40 to 70 DAT; however, the treatment was significant from 40 to 50 DAT only. At 60 DAT the P3 treatment increased root dry weight higher than the other treatments. Only P1 treatment reduced root dry weight slightly. However all treatments were not significantly different statistically. At 70 DAT P3 treatment continued increasing higher significantly than the other treatment. The 3 others treatments increased root dry weight; however, they were not significantly different to each other (Figure 1).



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 2: Stem dry weight at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

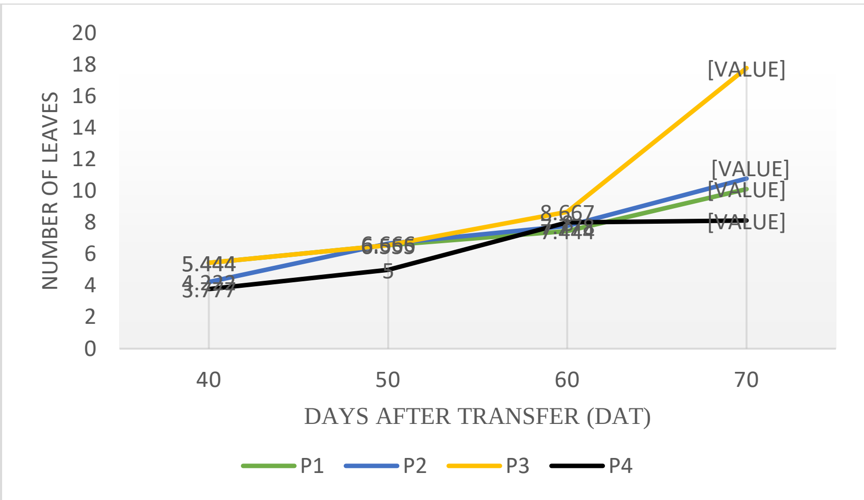
The stem dry weight of peranggi chili plants in all growing media (P1, P2, P3, and P4) increased from 40 to 70 DAT (Figure 2), except for P1 which experienced a slight decrease at 60 DAT. The dry weight of chili stem in P3 treatment was highest significantly than the other treatments at 70 DAT. The other treatments (P1, P2, and P4) also increased stem dry weight but they were similar to each other.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 3: Dry weight of leaves at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

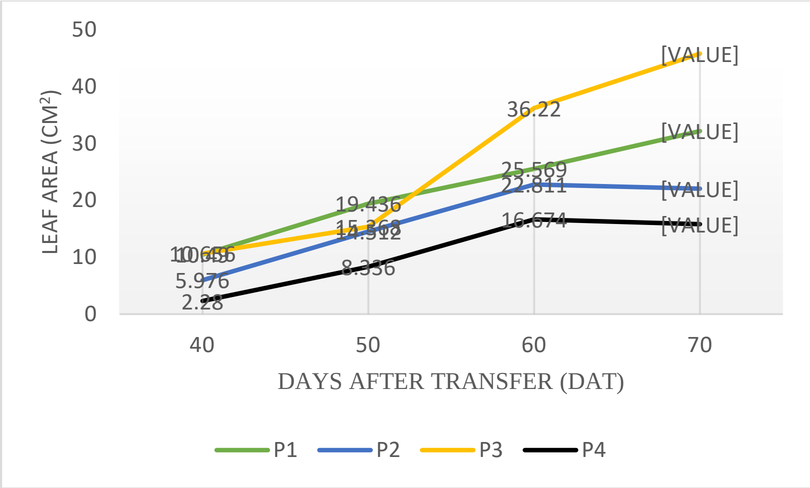
The dry weight of peranggi chili leaves in all growing media (P1, P2, P3, and P4) increased from 40 to 70 DAT (Figure 3). Statistically at 40 DAT dry weight of leaves showed a significant difference. However, it was not significantly different on days 50, 60, and 70. The dry weight of peranggi chili leaves in the P3 treatment was highest than the other three treatments, while the P4 treatment was relatively lower than the other treatments. The dry weight of peranggi chili leaves at 70 DAT remained increased at P1 and P3 treatments; however, treatments P2 and P4 only increased slightly. The significance was only found at p = 0.0758.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 4: The number of leaves at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

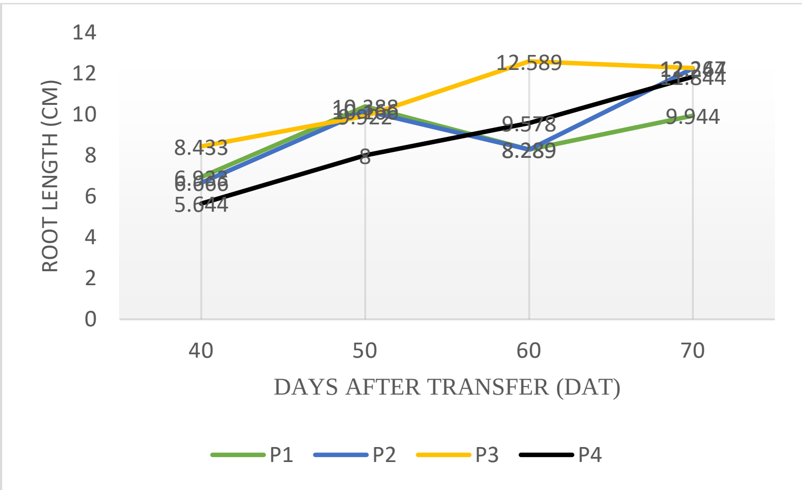
The leaf number of peranggi chili on growing media P1, P2, P3 and P4 increased from 40 to 70 DAT (Figure 4). Statistically, at the 70 DAT treatments affected significantly on the number of peranggi leaves. Treatment P3 increased the number of leaves significantly highest compared to other treatments.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 5: Leaf area of peranggi chili from 40 to 70 DAT with different growing media.

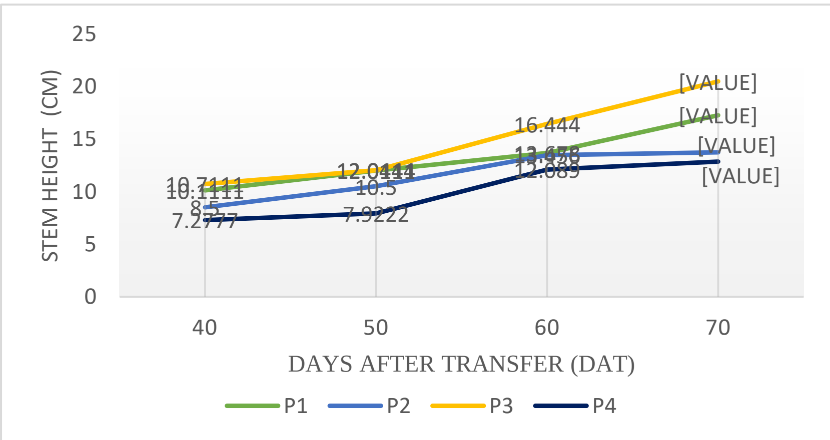
The leaf area of peranggi chili increased in all treatments as the leaf age increased. Each treatment showed a consistent pattern of increase from 40 to 60 DAT, but the rate of increase varied. P3 showed the greatest increase compared to other treatments at 50 to 60 DAT, while P4 had the lowest increase; however, it still showed growth. At 70 DAT, the treatment of P3 increased leaf area of peranggi chili significantly.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 6: Root length at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

The different types of growing media did not significantly affect root length from 40 DAT to 70 DAT. However, root length increased from 40 to 70 DAT.

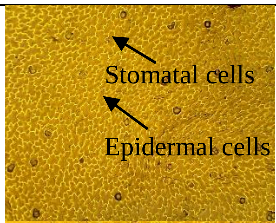


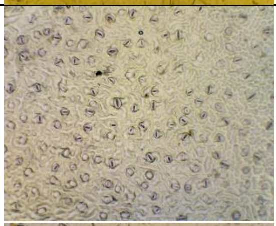
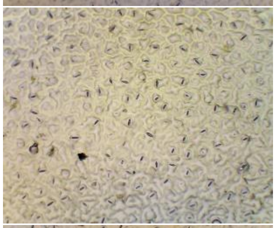
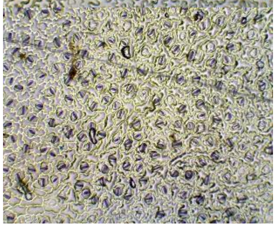
Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

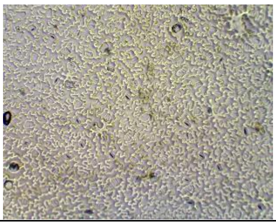
Figure 7: Stem height at vegetative growth of peranggi chili from 40 to 70 DAT with different growing media.

Stem height in all treatments increased from 40 to 70 DAT. The significances ware found at 50 and 70 DAT. At 50 DAT the lowest stem height was found at P4 treatment, although it was not different from P2 treatment. At 70 DAT both P3 and P1 treatments increased significantly than the other to treatments (P2 and P4). P4 treatment produced the lowest stem height significantly but not different from stem height of P2 treatment.

Table 4.2 The results of the analysis of the average number of stomata and the stomatal distribution of peranggi chili affected by different growing media at 40, 50, 60 and 70 DAT.

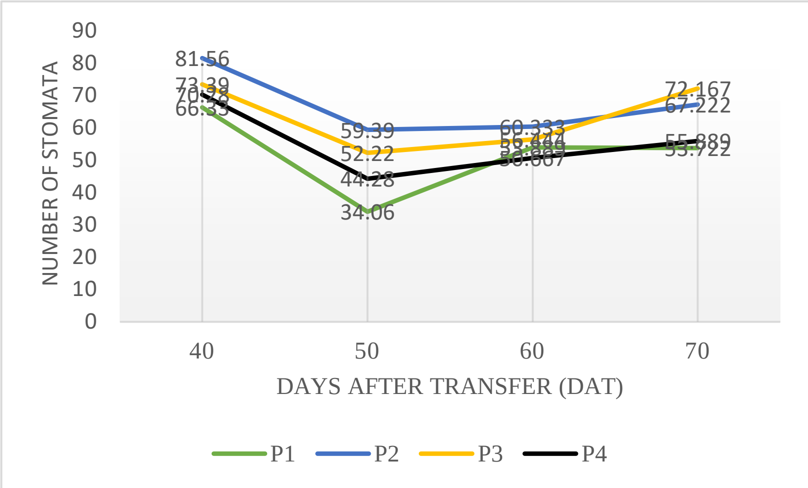
Day	Treatment	Stomata		Stomatal Distribution		Picture
		Number	Significance	%	Significance	
40	P1	66.33		16.78		
	P2	81.56		21.56		
	P3	73.39	0.6815	22.63	0.1603	
	P4	70.28		25.91		
50	P1	34.06		11.68		
	P2	59.39	0.1322	18.71	0.1713	

	P3	52.22		15.56		
	P4	44.28		13.93		
60	P1	53.889		13.21		
	P2	60.333		15.89		
	P3	56.444	0.8305	14.27	0.6181	
	P4	50.667		14.95		
70	P1	53.722		15.489		
	P2	67.222	0.3558	17.878	0.8173	
	P3	72.167		17.622		

P4	55.889	16.989	

Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5); SD= Stomatal Distribution.

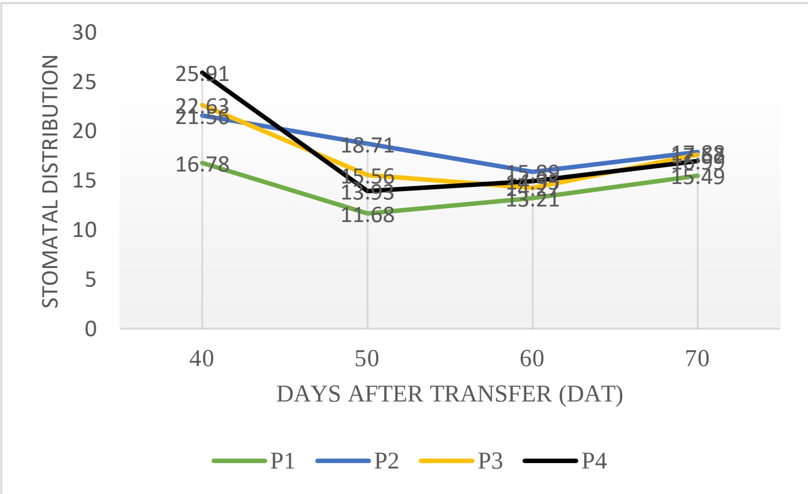
Stomata number and stomatal distribution were not afected significantly by different growing media from 40 to 70 DAT (Table 4.2).



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 8: Stomata number of peranggi chili from 40 to 70 DAT with different growing media.

The average stomata number of peranggi chili leaves planted in different growing media did not show any significant differences from 40 to 70 DAT. The average stomata number decreased from 40 to 50 DAT, but increased slightly for each treatment at 60 DAT and 70 DAT. However they were not significantly different.



Description: P1= alluvial soil: sand: cow manure compost (1:1:1); P2= alluvial soil: sand: cow manure compost: perlite (1:1:1:0.25); P3= alluvial soil: sand: cow manure compost: burnt husk (1:1:1:0.5); and P4= alluvial soil: sand: compost: burnt husk (1:1:1:0.5).

Figure 9: Stomatal distribution of peranggi chili from 40 to 70 DAT with different growing media.

The stomatal distribution of peranggi chili leaves grown in different media did not show any significant differences. Stomatal distribution decreased from 40 to 50 DAT, but increased slightly at 60 DAT and 70 DAT. All stomatal distribution of peranggi chilies at different growing media treatment increased slightly insignificantly.

Environmental factors in the form of soil temperature, humidity, and wind speed that affect the growth of peranggi chili from 40 to 70 DAT grown on different media were illustrated in Figures 10, 11 and 12.

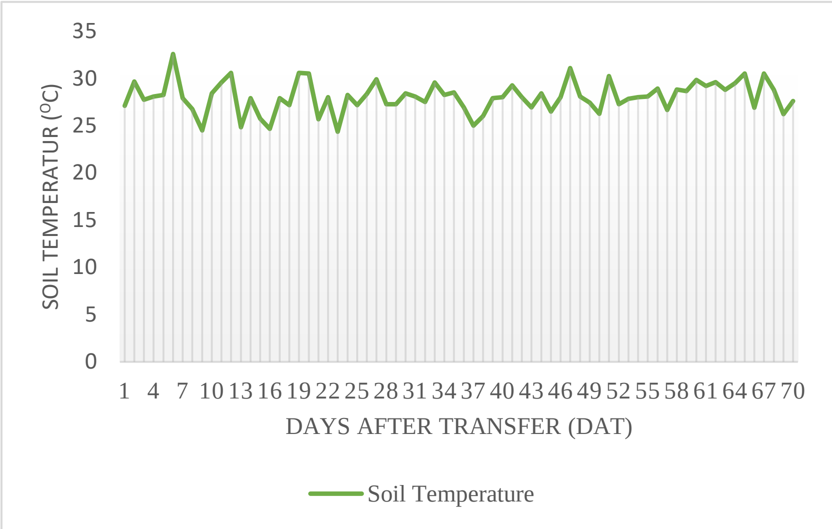


Figure 10: Soil temperature measurement results during 70 DAT

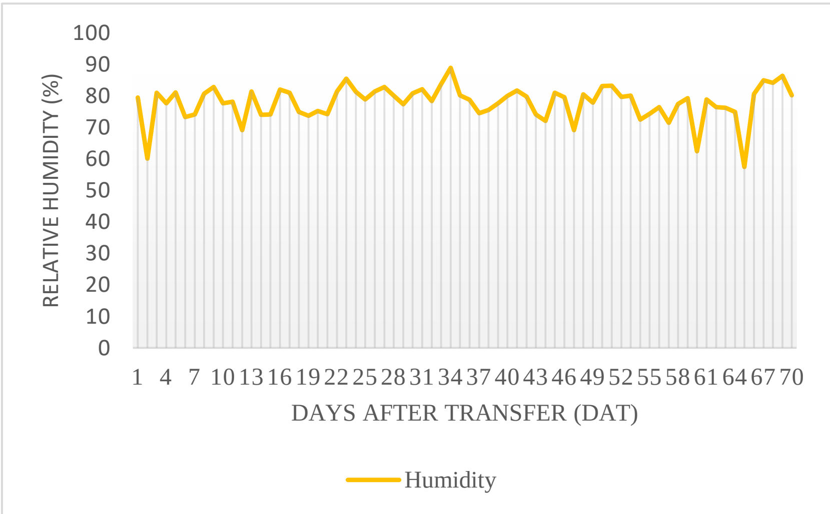


Figure 11: Humidity measurement results during 70 DAT

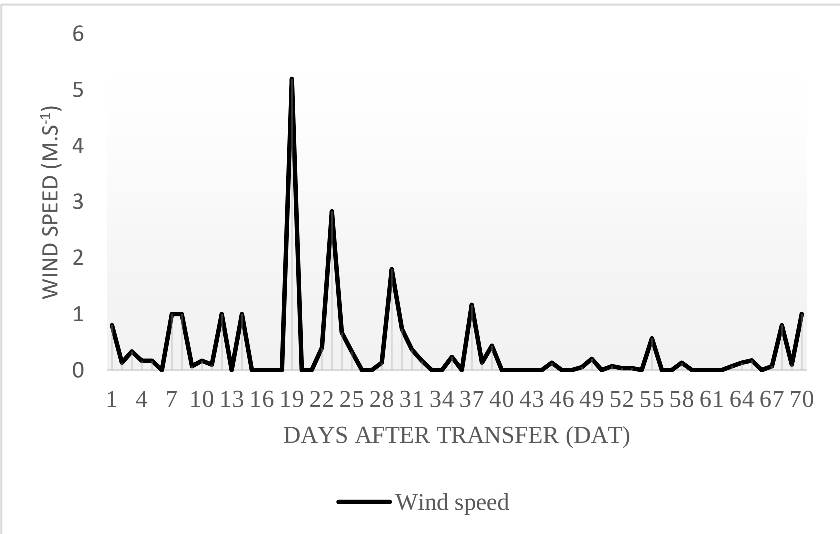


Figure 12: Wind speed measurement results during 70 DAT

4.2 Discussion

Growing media can affect the growth of a plant. Different types of growing media provide different functions and roles. Growing media can function as nutrient providers, porosity (structure and aeration), and water retention. The mixing of growing media such as soil, sand, and organic materials (husks and manure) will greatly affect the optimal growth of plants. Therefore, many researchers are looking for the composition or ratio of media that can optimize plant growth so that production is expected to increase.

Widiarta, Mayun & Astiningsih (2021) used soil composition with different additions of cow manure compost, chicken compost to produce the best cayenne pepper seeds. The addition of a mixture of cow manure compost or chicken manure compost increased plant height significantly while the number of leaves was only influenced by mixing cow manure compost on day 42 after planting. Wet and dry weight and root length also increased significantly due to the effect of mixing with cow manure compost. The effect of this growing medium not only affects cayenne pepper seeds but also adult cayenne pepper plants. Zeni & Delita (2023) showed that growing media had a significant effect on root dry weight and flowering time and liquid fertilizer had an effect on other variables such as the number of fruits, fruit weight and root dry weight. The ratio of top soil, burnt husk and cow manure compost as much as 3:2:1 with a mixture of 3ml.L^{-1} of liquid organic fertilizer water gave the best results on growth and production.

The results using 4 different composition ratios of growing media in the curent study showed almost the same results when observed at 40, 50 and 60 days after transfer. At 70 DAT the impact of P3 treatment gave the highest increase of growth variables, except leaf dry weight.

4.2.a. Vegetative growth of peranggi chili on different growing media

Growth is an indicator of change due to environmental influences on a plant. Wet or dry weight of leaves, stems and roots; root length and plant height, stem diameter are growth variables that respond to their environment (Zahro et al., 2024). Measurements can be destructive or non-destructive with advantages and disadvantages in each method. In addition, measurements can use absolute or relative values to suit the research objectives (Lopez et al., 2007).

Peranggi chili is usually grown on alluvial soils (Abdurrafi, 2021) in equatorial lowlands. Its growth and production have not been documented (Daningsih, 2024), so its production is not optimal. This can be seen in its limited availability.

Optimizing peranggi chili production can be cultivated on more conducive soils. Alluvial soil as a base material has poor nutrients so it needs to be modified with a mixture of other media that can add nutrients, aeration and water retention.

Peranggi chilies were grown on four different media compositions. Three treatments (P1, P2 and P3) contained alluvial soil, sand and cow manure compost in the ratio of 1:1:1. While P4 only contained alluvial soil and sand and replaced cow manure compost with compost. Alluvial soil as the base material. Sand as a medium that can help porosity and cow manure compost as a source of nutrients for plants. The fourth difference in the composition of growing media is the addition of perlite (P2), burnt husk (P3), and compost and burnt husk (P4). Vegetative growth of peranggi chili at 40, 50, and 60 days after transfer (DAT) reached almost the same magnitude and was not statistically different except for root dry weight on day 40. Peranggi chili roots at 40, 50, and 60 DAT were not affected by the composition of different growing media. Root weight is measured to observe how the roots develop. Its ability to absorb nutrients and water (Sun et al., 2019) meets the needs of plants for growth. The base composition ratio of alluvial soil: sand: cow manure compost gave the same effect. In P1, P2, and P3 for growth. The addition of perlite as a media agent for water retention (P2) was not significant. During growth, watering was done regularly so as to avoid drought that might occur. Therefore, the availability of water in all pots of growing media was sufficient and the function of perlite was minimal (Giuffrida & Consoli, 2016). However, based on the growth graph from day 40 to day 70 after transfer, dry weight increased faster in chili plants with P3 treatment.

The increase in root dry weight began to occur at 60 DAT even though it was not statistically different. The addition of burnt husk to the growing medium can contribute not only water retention but also provide nutrients and aeration while providing an environment for the growth of microorganisms (Li et al., 2024) which is good for soil biology. The combination of cow manure compost and burnt husk provides more opportunities for water absorption, aeration, and water availability to the roots. The application of burnt husk is reported to increase mustard greens (Huang et al., 2024; Saranya et al., 2018) in wet and dry weight of root growth followed by growth in the leaf crown, as part of the crown, is an important organ because of its function in photosynthesis (Wijaya et al., 2024). Its growth depends on the fulfillment of water and nutrients in the growing media, all treatments of different ratios of growing media composition did not affect the dry weight of leaves significantly except for the dry weight at 40 DAT, but differences in leaf dry weight at 40 DAT were not detected in other days of observation. Just like root weight, leaf dry weight also increased higher in P3 growth (Figure 3). The addition of burnt husk accelerated the increase in leaf dry weight. Burnt husk contributes to the growth of P3 around the roots and this causes more availability of nutrients needed by plants even though there is no significance among treatments, the addition of burnt husk to the composition ratio of alluvial soil, sand, and cow manure compost (P3) resulted in an increase in the dry weight of peranggi chili leaves (Figure 3). The same thing is also seen with the dry weight of chili peranggi stems even though the measured stem height is significant at 50 DAT and again not significant among treatments at 60 DAT.

Root length and number of leaves of peranggi chili were also not affected by differences in growing media. Dry weight of roots, stems and leaves increased higher from day 50 DAT to 60 DAT in P3 treatment. This phenomenon is also seen in other vegetative growth variables of peranggi chili. P3 treatment (alluvial soil: sand: cow manure compost: burnt husk) increased root length, number of leaves, leaf area, and stem height at 60 DAT although statistically insignificant (Figures 1, 4, 5 and 7). The addition of burnt husk and cow manure compost provided a nutrient-rich growing medium from animal and plant sources. In contrast to cow manure compost, burnt husk provides a firmer texture because it comes from plant cells and causes a looser soil texture and makes it easier for roots to move and take nutrients and water. According to Pakerti et al., (2021) loose soil due to the addition of burnt husk increases growth. Loose soil facilitates air exchange into the soil pores. As a result, soil pH is also better (Alamanis & Chouliaras, 2018; Ungurau & Ungureanu, 2012).

The results of soil analysis in the laboratory (data not shown) showed that the addition of burnt husk and perlite reached a pH of 6.48 and 6.41, respectively. Horticultural crops such as chili plants require soil pH between 5.5 to 6.5 (Adeoluwa et al., 2022). Thus, the addition of perlite (P2) and burnt husk (P3) is ideal for the growth of peranggi chili plants. Morever, the addition of burnt husk (P3) also increased the availability of phosphate in the soil needed for the roots (data not shown).

The growth of peranggi chili started responding significantly at 70 DAT. All variables, except leaf dry weight, were highest in the P3 treatment (alluvial soil, sand, cow manure compost and burnt husk), whereas all variables were the lowest in the P4 treatment (alluvial soil, sand, compost and burnt husk). Meanwhile, the P2 and P1 treatments gave results that varied between P3 and P4. The addition of compost and burnt husk to alluvial soil and sand resulted in the lowest pH (pH < 5), which is unsuitable for plants, especially horticultural crops. The response of plant growth was faster significantly at 70 DAT. It was possible that the plants reached a mature age that optimized all conditions for faster growth (Farida et al., 2023).

4.2.b Number and distribution of stomata in peranggi chili

Stomata are part of the upper and lower epidermis of the leaf. Its function is for the traffic of water vapor associated with transpiration and the entry of carbon dioxide as a basic material for photosynthesis and the exit of oxygen which is the by product of photosynthesis. Stomata are generally located on the lower epidermis of the leaf surface to avoid exposure to sunlight, avoiding rapid transpiration rates (Taulavuori et al., 2016). However, there are species that are also equipped with stomata on the epidermis of the upper leaf surface so that the number of stomata is a combination of upper and lower surfaces. Peranggi chilies are equipped with stomata on both sides of the leaf surface. From the results of previous studies, the number of stomata is not affected by environmental conditions that lack of water (Personal Communication, 2025). However, many studies report that stomatal number can be affected by environmental changes such as temperature (Bertolino et al., 2019), drought (Pitaloka et al., 2022), altitude (Wang et al., 2014), or changes in soil environmental conditions (Chua & Lau, 2024). Changing the ratio of growing media composition altered soil pH and nutrient composition in this study (data not shown).

Changes in the growing media environment due to differences in the composition of the growing media did not affect the number of stomata or the distribution of stomata. Changes in the average number of stomata from 40 DAT to 50 DAT decreased (Figure 8). This decrease can occur because the cells enlarge so that when observed on the field of view under a microscope lens the number of stomatal cells decreases. At 40 DAT leaf growth is not yet optimal so that stomatal cells and epidermal cells are still small and the number is in accordance with their genetic properties (Sultana et al., 2024). but on day 50 stomatal and epidermal cells enlarge due to leaf development so that the number of stomatal cells is seen to fewer in the field of view under a microscope. As a result, both the average stomatal cells and stomatal distribution decreased on day 50. On day 60 and 70 DAT the number of stomata increased slightly. The increase in the number of stomata can be an effort of plants responding to the momentary environmental conditions. The decrease in humidity from 77.88% during 40 DAT to 50 DAT to 73.71% during 50 DAT to 60 DAT (Figure 11) decreased a little water vapor in the air. Meanwhile, the wind speed increased from 0.04 $m.s^{-1}$ at 40 DAT to 50 DAT to 0.1 $m.s^{-1}$ at 50 DAT to 60 DAT. The rising wind speed occurred continuously up to the average of 0.23 $m.s^{-1}$ during 60 to 70 DAT. This condition results in accelerated transpiration so that plants try to regulate it by shrinking the stomatal pores resulting in an increase in the number of stomata under the field of view a microscope (Hasanuzzaman et al., 2023). Soil temperature also increased from 27.99 ^oC at 40 to 50 DAT to 28.43 ^oC at 50 DAT to 60 DAT. The average of soil temperature reaced 28.7 ^oC from 60 to 70 DAT.

The same response occurred in stomatal distribution (Figure 9). The decrease in the number of stomata and the percentage of stomatal distribution indicates that the number of stomata and stomatal distribution occurs at 40 DAT genetically. When the growth of peranggi chili gets bigger, the number of stomata decreases and then adapts little or insignificantly to changes in the environment and its surroundings. The number of stomata and stomatal distribution that does not change due to growth or the environment indicates that the species is resistant to change (Cai et al., 2024).

5 Conclusion

Giving different ratios of growing media composition does not significantly affect the vegetative growth of peranggi chili until 60 DAT. At 70 DAT, growing media started affecting several variables of vegetative growth. The addition of burnt husk to the ratio of alluvial soil: sand: cow manure compost (1:1:1) had given the highest impact on root dry weight, stem dry weight, number of leaves, leaf area, and stem height. On the other hand, the use of growing media using alluvial soil: sand: compost: and burnt husk (1:1:1:0.5) always produced the lowest of all variables from 40 to 70 DAT. The other two treatments gave various results from 40 to 70 DAT. Therefore, it is recommended to use the composition of alluvial soil: sand: cow manure compost: burnt husk to get optimal vegetative growth of peranggi chili. The number of stomata and stomatal distribution of peranggi chili were not affected by any growing media, indicating peranggi stomata were more influenced genetically. The peranggi chili produced the highest number of stomata and stomatal distribution at 40 DAT; however, these stomatal number and stomatal distribution reduced at 50 DAT followed by a slight increase at 60 and 70 DAT meaning the leaf cell has achieved meture leaf at 50 DAT indicated by biggers size of epidermal and stomatal cell.

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