Changes in Root Anatomy Due to Different Watering Supply in the Growth of Capsicum frutescens L. on Verticulture Technique

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ABSTRACT

The rapid increase in human population reduces land productivity in urban areas. Verticulture is a way of planting in a vertical arrangement, such as for Capsicum frutescens L. This study aimed to examine the root anatomy and growth responses of C. frutescens L due to different watering supply on verticulture technique. Fourteen days old seedlings were grown in polybags on the ground and into vertical pots made of paralon pipe. Watering supply was given for every day, once in 3 days, and once in 6 days for 35 days, at height of 0 cm, 25 cm, 50 cm and 75 cm. The decreases in root diameter, stele diameter, and xylem diameter, but increase the portion of cortex occurred in plants at 75 cm height on paralon pipe with 6 days time interval of watering supply compared to control plants grown at 0 cm height. Growth parameters showed the same trends, where plants at 75 cm height at the paralon received watering supply once in 6 days had less growth compared to control plants by decreasing in plant height, leaf number, length-width of leaves, fresh and dry weight of roots and shoots, while chlorophyll content and root length did not change. The optimal time interval of watering supply for growth on verticulture technique is 3 days, while the optimal height at the paralon was approximately 50 cm. The xylem was less developed in plants at higher position compared to the lower position on the paralon pipe and this was more detected in plants with less time interval of watering supply. Decreasing plant growth occurs in conditions of water shortage and the higher position of plant on paralon.

1. Introduction

Population increases have an impact on improving the conversion of agricultural land into residential land (Wartapa et al., 2010), especially in urban areas. The use of narrow field in urban area should be optimized in order to remain productive to support the population survival. Verticulture is the good choice for farming in this area.

Verticulture is a technique of farming in a vertical arrangement towards the free air space with an array of vertically growing media (Wartapa et al., 2010). This technique is very appropriate to be applied in limited and narrow space (BPTP, 2006) with tin cans, plastic gutter PVC, bamboo or wooden planks arranged in stages as cultivation medium (BPTP, 2006; Wartapa et al., 2010). Verticulture arrangement can be rectangular, triangular, or shaped like a staircase with several terraces (Lukman, 2012).

Plants usually grown in verticulture technique are seasonal vegetables with a maximum height of one meter, for example cayenne peppers, lettuce, spinach, basil and tomato (Lukman, 2012). Pepper plant has high economic value with a variety of benefits, such as for spices, vegetables, medicinal and ornamental plants (Afifah et al., 2009). According to Central Bureau of Statistics (BPS, 2012), chili is one of the most sought vegetable commodities of Indonesian people after the onion, with yields decreased in every area, especially in big cities in Java Island. In 2012, the production of chili in Indonesia reached 1,650,831 tons (BPS, 2013). Chilies have a complete nutrient content, including protein, fat, carbohydrates, minerals (calcium, phosphorus, and iron), vitamins (A, B1, B2, B3, and C), flavonoids, anti-oxidants, ash,
crude fiber, and astringent useful as a drug (Bosland et al., 2012).

Planting on verticulture technique requires watering supply with optimal intensity so that water remains available to meet the needs of the plant considering the growing media are in a vertical arrangement. To obtain the optimal growth, plants need sufficient water supply. Limited water is a major constraint on growth and development of pepper plants, especially in dry land. Water is the main component in the growth of plants, because 70-90% of the body parts of plants containing water (Widiyono and Hidayati, 2005). Water deficit is the biggest factor affecting the growth and productivity of plants (Shao et al., 2008) that can affect physiological, biochemical, anatomical and morphological processes (Song Ai and Banyo, 2011).

By growing cayenne pepper in verticulture technique with different watering supply will give a different response in anatomy, morphology and physiology of plants than those grown without verticulture technique. Different soil properties also give different results, as found by Syukur et al. (2010) that the Capsicum frutescens L. hybrid planted in different places with different soil and environmental conditions give different results. In Rembang, Boyolali and Subang produced a better yield than that planted in Bogor. Those three areas have lower altitude and rainfall compared to those in Bogor. This possibility cannot be separated from the differences in temperature, soil type, moisture and other physical factors. Therefore, this study was conducted to observe root anatomy and growth of cayenne pepper grown on verticulture technique with different watering supply.

2. Materials and Methods

2.1. Plants and Cultivation Media Source

Cayenne pepper (C. frutescens L.) seeds obtained from ripe fruits were sun-dried for a week, soaked for one night and germinated in a square tray containing soil as medium. Plant cultivation was maintained in the greenhouse of Faculty of Agriculture, Universitas Gadjah Mada, while anatomical slides preparation was done in Laboratory of Plant Structure and Development, Faculty of Biology, University of Gadjah Mada, Yogyakarta, Indonesia in March to July 2013.

2.2. Treatment

Fourteen days old seedlings with 4-5 leaves were transferred-planted into polybags (size 10x15 cm) containing a mixture of cultivation medium that consisted of soil, compost, and rice husk (in ratio of 1: 1: 1) and grown on the ground as a control (K) and into each hole on the paralon pipe (9 inch in diameter and 1 m height) as a treatment of verticulture technique. Three holes were made on each pipe, one for each plant, positioned at different heights from the ground (Figure 1), which were 25 cm (T1), 50 cm (T2), and 75 cm (T3). Each treatment was with five replications. Plants were watered with different watering supply, which were a daily basis (P1), once in every three days (P2), and once in every six days (P3) and watering application was stopped after 35 days.

2.3. Growth Parameters

The growth parameters in terms of plant height, number of leaves, length and width of leaves and roots length were counted and measured at the end of the treatment. Fresh and dry weight of roots and shoots were measured at the end of treatment.

2.4. Chlorophyll measurement

The fifth leaf of each treated plant was used for chlorophyll measurement. About 0.1 g leaves was weighted and grounded on a mortar containing 10 mL of 80% acetone. Leaves extract was filtered using a Whatman paper No.1 and the chlorophyll content was measured spectrophotometrically at wavelength of 646 and 663 nm and calculated according a method by Harborne (1987).

2.5. Roots Anatomy

Root anatomical parameters included a description of the internal structure of roots as well as the measurements of cortical thickness, xylem tissue and stele diameter from the root base. Roots sections were cut from ± 1 cm of primary roots at about 7 cm distance from the root base. Roots anatomical overview was documented with Optilab connected to a microscope and a computer. Measurement of anatomical parameters was done with Raster Image Program.

2.6. Data Analysis

Quantitative data were analyzed by analysis of variance (ANOVA) and if there is a significant difference, was followed by DMRT at the 5% significance level to locate the significant difference between treatments. Qualitative data were presented descriptively.

3. Results and Discussion

3.1. Root Anatomy

Anatomically, a cross section of cayenne pepper root
those of P1T3. In condition of less time interval of watering supply, plant tried to drain water into the leaves with efforts to widen the trachea cells, but the xylem cells reduced in their formation and differentiation. Plants grown in PVC pipe, with the less time interval of watering supply, showed reduced xylem formation with smaller cells and this was particularly visible on the plants at the higher position (P2T3 and P3T3). Those two treatments produced apparent narrower vascular bundles area with a narrow xylem tissue, while the portion of the cortex appeared larger. These results are in line with research by Shao et al. (2008) that from the viewpoint of anatomy, drought causes root diameter decreases due to reduced volume and cell number. Qualitatively, reduction on cells size were also observed, especially xylem tissue from less time interval of watering supply at higher position plants. Plant tissues experienced water shortage conditions generally decreased their cell size and increased cell wall thickness (Makbul et al., 2011). The higher the plant position at the PVC pipe, the higher the chances of water in the soil was reduced due to flowing into T2 and T1 position. Anatomically, the root from control plants on the ground were similar to the roots from treated plants P1 at the position level of T1, T2, and T3, while the plant of P2 and P3 showed slower in the development of xylem tissue than P1, in all positions, at T1, T2 or T3 (Figure 1).

Quantitatively, Table 1 showed that the root diameter of P3T3 plants (with every 6 days watering and at 75 cm height on the paralon pipe) was significantly smaller that those in P1, P2 in combination with T1, T2, T3. Meanwhile, in K plants, daily watering plants (P1) had significant smaller root diameter than in P2 or P3 with no different in cortex thickness. Cortex thickness from plants under less time interval of watering supply (P3) at paralon were smaller compared to ground plants at all watering supply (P1, P2, P3). From Table 1 can also be seen that stele and xylem diameter decreased due to the lower of watering supply. In this study, P2T3 and P3T3 plants produced narrower stele and xylem tissue than that in the P1 plants. The smaller diameter of vascular tissue under drought also occurred in soybean (Makbul et al., 2011) and Phaseolus vulgaris (Holste et al., 2006). Under water shortage, stele diameter reduction was due to decreasing a number of trachea number as found in Anarthrophyllum chilense, Lycium rigidum (Durante et al., 2011) and a decrease of xylem diameter (Sinaga, 2007). This can be related to the process of absorption of water and nutrients from the soil; due to the water shortage condition xylem diameter was smaller and more efficient than the larger diameter of the xylem. Xylem structure with smaller

**Figure 1.** Model of pots on verticulture used in the study
pores is very helpful because it will be more resistant to drought. Anatomical structure change is the adaptation of plants to drought and to keep the water content in the plant body remains balanced. In dry conditions, species of acacia less tolerant of drought perform xylem with increased tracheal diameter, but the frequency is low, while the drought tolerant acacia species produced a smaller tracheal diameter but more in number (Aref et al., 2013). Under water stress plants can changes the xylem anatomy, by increasing the proportion of small tracheas that may contribute to prevent roots from collapse risk, without compromising plant growth (Guijarro-Real et al., 2014). Anatomy of the xylem majority shows the efficiency of water transport of species and correlated with the capacity of tolerance to drought stress (Lens et al., 2011). It can be said that the size of the diameter and density of the cells of the xylem shows variations between species inter and spacious. Based on the research results by Aref et al. (2013), it can be assumed that the cayenne pepper species used in this study is less tolerant of the water deficit.

3.2. Plant growth

Plant growth and biomass allocation are two most fundamental processes being remarkably influenced by environmental variables including water changing factor. From Table 2, it appears that the higher the watering supply (P1), the plants tend to be higher than that in plants received less time interval of watering supply (P2, P3). The higher the plant position on PVC, the shorter the plants resulted, especially for P2T3 and P3T3 plants. This relates to the availability of lower water supply on plants at a higher level (T3), as water poured into paralon pipe will flow downward, because in this study between T1, T2 and T3 there was no insulation for limiting soil media in paralon. The number, length and width of leaves declined as plants received less time interval of watering supply (Table 2). Outside the K plants, P3 plants, with P3 watering supply showed shorter and narrower leaves compared to other treatments. The less the watering supply, plants leaves grew shorter and narrower, and it is as one of the response of plants in the face of drought stress conditions. The inhibition of shoot and root growth is a common response of water stress (Jaleel et al.,

Figure 2. Cross section of Capsicum frutescens L. root after 35 days treated with different watering supply on verticulture technique. e, epidermis; c, cortex; p, phloem; x, xylem tissue; vc, vessel/trachea cell. Xylem tissue was mostly less developed in P3T3, plants with watered every 6 days at 75 cm position on the paralon pipe. Bar: 200 mm
2009). Under water deficit in the soil, water availability to plant cells is also restricted, and as the first response, cells try to save available water by avoiding active growth. In addition, the fewer leaves formation was an effort to reduce transpiration of plants so that the water balance in the body of the plant is maintained. The limitation of water will reduce the leaf organ development. Effect of water deficit during the vegetative level is the formation of smaller leaves, which impact on the lack of light absorption. Shao et al. (2008) stated that in the condition of water deficiency, the plant has fewer leaves with smaller cell size, and composition of its location on the trunk was denser. This could be a strategy for this species to be better adapted to less water availability. Facing with low water availability, the plant develops a number of mechanisms, namely: (1) getting out of the stress by complete its life cycle before the critical water shortages occur, (2) ovoiding stress by increasing the capacity for the absorption of water by forming extensive root system, reducing the leaf area and restricting transpiration; (3) tolerance to stress by increasing the osmotic and increases the elasticity of the cell and resistance to stress by interrupting the metabolic pathways so that plants can survive under extrem stress conditions (e.g., increase antioxidant metabolism (Xu et al., 2010; Claeyts and Inzé, 2013; Lawlor, 2013).

3.3 Leaves chlorophyll content and biomass

Total chlorophyll content of leaves increased with less time interval of watering supply (Table 3) and there was no significant difference among the treatments. Watering supply once every 3 or 6 days did not significantly change the chlorophyll content. This is in contrast to *Pisum sativum* L. whereas levels of chlorophyll a, b and total decreased along with the time interval watering of 6 days, 9 days and 12 days (Emblale et al., 2016). According Hendriyani and Setiari (2009), the factors that affect the synthesis of chlorophyll are light, carbohydrates, water, temperature, genetic, and elements such as N, Mg, Fe, Mn, Cu, Zn, Su, and O2. In the Solanaceae plants such as potato, chlorophyll content varies and does not reflect the sensitivity or tolerance to drought clearly (Song Ai and Banyo, 2011). Even though, there was a reduction on leaves number and size, but there was no chlorophyll decrease in all treatments, meaning that photosynthesis can still take place optimally. Further stress of water deficit will cause a reduction in the rate of photosynthesis (Khaerana et al., 2008), taking nutrients; assimilate production and yield (Neumann, 2008; Jaleel et al., 2009).

### Table 1. The characters of root anatomy of *Capsicum frutescens* L. after 35 days treated with different watering supply on verticulture technique.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root Diameter (µm)</th>
<th>Cortical Tissue Thickness (µm)</th>
<th>Stele Diameter (µm)</th>
<th>Xylem Tissue Diameter (µm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>K</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
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<tr>
<td>P1</td>
<td>636.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>726.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1168.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>750.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>P2</td>
<td>710.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1017.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>876.16&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>551.58&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>647.47&lt;sup&gt;c&lt;/sup&gt;</td>
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Values followed by same letters in the same column and rows in all treatments for each parameter, or in the same row or column for the average, did not differ significantly at p ≤ 5%
parameters for selection of plant resistance to drought in treated with drought. Biomass is one of the most important plants will decrease the dry weight of the root place (Sinaga, 2007). Shao et al. (2008) stated that some absorb the water increased and photosynthesis can still take at the expense of the canopy, so the capacity of the roots to change the distribution of assimilates to support root growth to improve the status of water in times of drought, crop deposit of biomass in root cells or more root growth. In order cayenne pepper was observed, though it was not significant. less time interval of watering supply, the longer the roots of leaves number and size (Table 2). Table 3 also shows that the of biomass accumulation could be due to the decrease of expansion and enlargement of existing organs. The decrease biomass accumulation could be due to the decrease of leaves number and size (Table 2). Table 3 also shows that the less time interval of watering supply, the longer the roots of cayenne pepper was observed, though it was not significant. less time interval of watering supply, the longer the roots of leaves number and size (Table 2). Table 3 also shows that the of biomass accumulation could be due to the decrease of expansion and enlargement of existing organs. The decrease stress, can reduce the differentiation of new organs, lowest weight was found in P3 plants (Table 3). Higher water shoots and roots were observed in P1 plants, while the weight of roots and shoots. The highest fresh weight of the supply declined the biomass, as measured by fresh and dry content, this study shows that less time interval of watering L. after 35 days treated with different watering supply on verticulture technique. The growth of Capsicum frutescens L. after 35 days treated with different watering supply on verticulture technique.

<table>
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<th>T2</th>
<th>T3</th>
<th>Average</th>
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<td>31.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.07&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>6.52&lt;sup&gt;x&lt;/sup&gt;</td>
<td>7.45</td>
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Values followed by same letters in the same column and rows in all treatments for each parameter, or in the same row or column for the average, did not differ significantly at p ≤ 5%

Although there was no reduction in chlorophyll content, this study shows that less time interval of watering supply declined the biomass, as measured by fresh and dry weight of roots and shoots. The highest fresh weight of the shoots and roots were observed in P1 plants, while the lowest weight was found in P3 plants (Table 3). Higher water stress, can reduce the differentiation of new organs, expansion and enlargement of existing organs. The decrease of biomass accumulation could be due to the decrease of leaves number and size (Table 2). Table 3 also shows that the less time interval of watering supply, the longer the roots of cayenne pepper was observed, though it was not significant. This means that the root elongation is not followed by the deposit of biomass in root cells or more root growth. In order to improve the status of water in times of drought, crop change the distribution of assimilates to support root growth at the expense of the canopy, so the capacity of the roots to absorb the water increased and photosynthesis can still take place (Sinaga, 2007). Shao et al. (2008) stated that some plants will decrease the dry weight of the root-shoot when treated with drought. Biomass is one of the most important parameters for selection of plant resistance to drought in eggplant (Fita et al., 2015). The decline in leaf area provides a lower leaf surface area and low ability to absorb light (Morse et al., 2002). Similar results were reported in the olive crop ‘Zalmati’ (Boughalleb, and Hajlaoui, 2011) and jatropha (Krishnamurthy et al., 2012). The decrease of root biomass could be due to less roots produced, not due to shorter roots. Root elongation in dry conditions, could be a possible effort to find the source of water. Roots become increasingly important role in dry soil conditions. One effort to meet the water needs of plants in her body that is through increasing the length of the roots to expand the field of water absorption. The allocation of biomass is lower to the stress root with less trachea cells, and it enables the roots to absorb water and nutrients slower. Meanwhile, increasing position of plants on paralon pipe, did not significantly change the growth and biomass, meaning that the availability of water in higher position of paralon pipe remained sufficient for plant growth, so that the water deficit after the treatment is not that extreme condition for this species. From these results, it can be assumed that the cayenne pepper plants avoid dryness with a conservative strategy of root system.
Table 3. Leaves chlorophyll content and plant biomass of *Capsicum frutescens* L. after 35 days treated with different watering supply on verticulture technique.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K</th>
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Values followed by same letters in the same column and rows in all treatments for each parameter, or in the same row or column for the average, did not differ significantly at p ≤ 5%

4. Conclusions

The decreases in the diameter of root, stele, and xylem, but increase the portion of cortex occurred in plants at 75 cm height on paralon pipe with 6 days time interval of watering compared to control plants grown at 0 cm height. Plants at 75 cm height at the paralon received watering supply once in 6 days had less growth compared to control plants by decreasing in plant height, leaf number, length-width of leaves, fresh and dry weight of roots and shoots, while chlorophyll content and root length did not change. The optimum time interval of watering supply for growth on verticulture technique was 3 days, while the optimum height at the paralon was 50 cm.

Acknowledgments

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