Ilmu Pertanian (Agricultural Science)
http://journal.ugm.ac.id/jip
Vol. 7 No. 2 August, 2022: 68–74 | DOI: doi.org/10.22146/ipas.69842

Anatomical characteristics of cocoa plant roots as affected by the levels of calcium fertilization

Sutan Tarmizi Lubis*, Eka Tarwaca Susila Putra, and Budiastuti Kurniasih

Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia
*Corresponding author: sutantarmizi8@gmail.com

Article Info

Received : 19th November 2021
Revised : 12th January 2022
Accepted: 3rd February 2022

Keywords:
$CaCl_2$, performance, and RCC 71

Abstract

Cocoa plant (*Theobroma cacao* L.) is one of good annual crops grown in Indonesia. The disadvantages and advantages of Ca in plants can affect morphology and anatomy in plants. This research aimed to identify the effect of Ca on the anatomical performance of the roots of cocoa plants and determine the optimal dose of Ca for the anatomical characteristics of the roots of cocoa plant. The research was conducted at the cocoa plantation owned by PT. Pagilaran at North Segayung Production Unit, Tulis Subdistrict, Batang Regency, Central Java, in October 2019 – February 2021. The observation of root anatomy was carried out by making preparations, and the plant roots were taken destructively and sliced transversely. The field experiment was arranged using a single factor randomized complete block design. The treatments consisted of without fertilizer application and calcium fertilization at a dose of 100 g/tree/year, 200 g/tree/year, 300 g/tree/year, and 400 g/tree/year. The cocoa clone used was clone RCC 71. The results showed that an increase in the dose of $CaCl_2$ fertilizer up to 400 g/tree/year was generally followed by an increase in the size of the xylem and phloem diameters, the thickness of the cork layer, the thickness of secondary cortex, and the diameter of the stele. The quadratic effect of the dose of $CaCl_2$ fertilizer was observed only on the thickness of the root cambium tissues. Thus, it is necessary to conduct further research to determine the optimal dose of Ca fertilizer for the anatomical characteristics of cocoa roots.

INTRODUCTION

Cocoa plant (*Theobroma cacao* L.) is one of the annual crops that are quite good growing in tropical climates (Badrie et al., 2014) and plantation commodities that have a fairly important role for the Indonesian economy. The development of cocoa bean production from 2017–2020 is quite fluctuating, but in general, the trend is decreasing. In 2017, cocoa bean production amounted to 590,684 tons, increased in 2019 to 734,796 tons but in 2020, decreased to 713,378 tons or decreased by -4.23 % (Directorate General of Estate, 2020).

In Indonesia, plantation that have been built still have the opportunity to increase productivity because the average productivity is currently less than 50% of its potential (Directorate General of Estate, 2020). The decrease in cocoa productivity and quality of cocoa beans is caused by several factors, such as the age of old plants, planting materials, disease pest attacks, decreased soil fertility, and improper cultivation techniques. One of the less precise cultivation techniques is fertilizing plants. According to Vliet and Giller (2017), plants need a variety of ions as essential nutrients, which are taken from the soil and distributed throughout the plant (Grusak et al., 2016). Calcium

How to cite: Lubis, S.T., Putra, E.T.S., and Kurniasih, B. (2022). Anatomical characteristics of cocoa plant roots as affected by the level of calcium fertilization. *Ilmu Pertanian (Agricultural Science)*, 7(2), pp. 68–74.

ISSN 0126-4214 (print) ISSN 2527-7162 (online)

(Ca) is an important element in plant growth and development (Aras and Bozkurt, 2021). In addition, the presence of Ca is also important for cell lengthening, cell division, and regulation of cations absorption (Dorozhkin, 2017). Ca also plays a central role in plant physiology. Ca is involved in the structure and permeability of plant cell walls, thus providing strength to plants (Riveras et al., 2015; Yang et al., 2015). Showing unique behavior as signaling molecules in the cytosols (Saito and Uozumi, 2020), Ca is also involved in the structure and permeability of plant cell walls, thus giving strength to plants (Bothe, 2015; Domingues et al.,2016).

The presence of Ca is also important for cell lengthening, cell division, and regulation of cations absorption in plant rooting (Dorozhkin, 2017). The provision of Ca fertilizer in cocoa farmland can increase the production of cocoa beans and fruit. According to the Indonesian Coffee and Cocoa Research Center (2015), the recommendation of fertilization based on the results of analysis of cocoa plant tissue at several stages of growth is 300 $kg.ha^{-1}$ Ca to support the growth and development of plants to produce fruit. There have been several studies on the effect of Ca fertilization on improving the performance of cocoa plants and increasing their production.

The deficiencies and advantages of Ca in plants can be viewed from various responses to the growth, development and yield of plants, which were previously preceded by biochemical, physiological, anatomical, and morphological responses. According to Riveras et al. (2015), one of the impacts of Ca deficiency is the failure of the formation of new shoots and disruption of the development of apical cells at the root end so that the distribution of nutrients through xylem in root can be disrupted (Sturião et al., 2020; Atkinson, 2014; Du et al., 2017). Therefore, efforts to increase the content of soil Ca is one way that can be done. At this time, the study of Ca contribution to anatomical characteristics and cocoa crop yields has not been widely carried out.

The study of plant anatomy is needed in studying the growth, development and differentiation of cells in a wide variety of cultivated plants. Anatomical observations of meristematic regions such as at the root end become the first step in identifying tissue growth and differentiation patterns as well as plant tissue specifications (McCormack et al., 2015). Roots play an important role for plants. In general, roots are composed of three types of tissues, namely protective tissues, including the epidermis and its derivates, basic tissue, consisting of parenchyma, cholenchyma and sclerenchyma, and transport tissues, including xylem and phloem (Maiti et al., 2012).

Based on the description above, research on the effect of Ca fertilization on anatomical characteristics and cocoa yields is necessary because the information is quite limited. Besides, the cocoa plantation of PT. Pagilaran at North Segayung Production Unit has never been fertilized with Ca despite its essential roles to support plant growth and production. Ca deficiency can inhibit the function of meristematic tissues, such as root ends, plant top growing points, and storage organs. The direct or indirect effects of Ca nutrient management on the anatomical characteristics and cocoa yields have not been examined.

Research related to the effects of Ca on cocoa farming is still rarely done, especially in Indonesia, so the information is quite limited. Studies related to Ca contribution to plants have more to do with growth and yield variables (Carr, 2012; Vliet and Giller, 2017). The detailed study related to Ca's contribution to the anatomical character of root tissue is quite limited, especially to cocoa commodities. The aim of the study was to determine the effects of Ca on the anatomical characteristics of the roots of the cocoa plant and to determine the optimal dose of Ca for the anatomical characteristics of the roots of the cocoa plant.

MATERIALS AND METHODS

Field research was conducted at cocoa plantations owned by PT. Pagilaran in North Segayung Production Unit, Tulis Subdistrict, Batang Regency, Central Java Province in October 2020–February 2021. The destructive observation and soil nutrient content analysis activities were conducted in Sub-Laboratory of Plant Science, Sub-Laboratory of Plant Management and Production, Sub-Laboratory of Kuningan Soil Science, Faculty of Agriculture, in Soil Laboratory of BPTP, Maguwo, Yogyakarta, and in Laboratory of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

The study was conducted at cocoa plantation locations with low Ca availability levels. The determination of the location of the plantation with low Ca level was carried out using initial soil analysis of all blocks in the North Segayung Production Unit. In each field block,

a composite soil sample was taken to determine the Ca levels (low, medium, and high).

According to the Soil Research Center (2009), the Ca content of <2 me/100 g, 2–5 me/100 g, 6–10 me/100 g, 11–20 me/100 g, and >20 me/100 g is categorized as very low, low, medium, high, and very high, respectively. Selected field blocks used as research sites are field blocks with low Ca levels. If there are several plantation blocks with low Ca content, then the next selected as the research site is the plantation block dominated by cocoa plants of RCC 71 clones. RCC 71 clones aged ± 20 years were selected as research objects because they are superior clones with high production. In selected field blocks with low Ca content and dominant RCC 71 clones, a map of the field used as the location of the research plot was made. On each block, 15 cocoa plants of RCC 71 clone were selected. Selected plants cannot be joined directly together, with at least one line of plants as a detractor. The 15 plants were then fertilized with Ca according to the doses to be tested. The total of samples was three plants per block per dose of Ca.

Field experiments were arranged using a single factor randomized complete block design, with Ca fertilization doses as treatments. Ca fertilizer was given in the form of $CaCl_2$ by immersing it in the root area of the cocoa plants. The treatment consisted of without fertilizer (C0) and Ca fertilization at the doses of 100 grams/tree/year (C1), 200 grams /tree /year (C2), 300 grams/tree/year (C3), and 400 grams/tree/year (C4). Ca was given once, which was in September 2020 during the dry season.

Root anatomy observation was carried out by making preparations, and the plant roots were taken destructively and sliced transversely. The preparations were made by the paraffin embedding method. The transverse incision preparations for the root organs were analyzed for anatomical structure using an Olympus digital binocular microscope (Germany) 40 × 10 and optilab viewer. The length and width values were measured using the Image Raster application in units (µm).

The anatomical responses of Ca plant roots to Ca fertilization were indicated by several observed characteristic variables, including xylem and phloem diameter, thickness of cork layer, thickness of cambium tissue, and stele diameter. The data of anatomical characteristics of cocoa roots were displayed in graph and analysis of variance (ANOVA) at a α=5% confidence level. If the ANOVA results showed significant difference between treatments, the data were then tested using a polynomial (regression) test at α=5% confidence level to determine the optimal Ca dose for the development of the anatomical characteristics of the cocoa roots. Data analysis was performed using SAS program version 6.12.

RESULTS AND DISCUSSION

Based on the results of regression analysis, there were significant effects of the Ca fertilization on the anatomical characteristics of the roots, including xylem and phloem diameter, thickness of cork layer, secondary cortex thickness and stele diameter. Meanwhile, there was no significant effect was observed on the thickness of the cambium tissue.

Regression analysis results showed that Ca fertilization affected the anatomical characteristics of the cocoa roots in the form of xylem and phloem diameter in a linearly positive manner. Each increase in the dose of Ca fertilizer up to 400 g/plant/year is always followed by an increase in the diameter of xylem and phloem. Ca fertilization in cocoa plants affected the diameter of xylem by 84% (R^2 = 0.8497), with a regression equation of y = 0.0428x + 36.922 (Figure 1). Meanwhile, Ca fertilization in cocoa plants affected the diameter of phloem by 82% (R^2 = 0.8253), with a regression equation of y = 0.0056x + 4.8 (Figure 1).

Availability of Ca in soil can respond to meristematic tissues, such as root ends in plants (Cacho et al., 2013), and Ca will target cytoskeletons in cytosols, which will later encourage cell division, cell wall biosynthesis and increased root end expansion (Naeem et al., 2018; Yang et al., 2015). Root changes caused by Ca affect water absorption, nutrients, and hormone production, which will eventually improve the morphology and anatomy of a plant (Vasconcelos et al., 2020).

The increase in the diameter of xylem and phloem (Figure 4) is often associated with the transport of Ca from the roots to the top of the plant through xylem following transpiration flow, and the presence of Ca is found in many plant phloem tissues (He et al., 2014). According to Liu et al. (2011), Ca administration to cocoa plants undergoes buildup on the root surface, on the cell wall (apoplast) and on the exterior surface of the plasma membrane. In xylem vessels, many

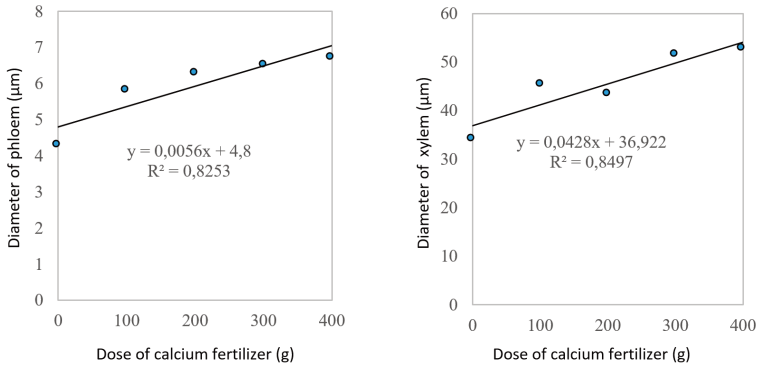

Figure 1. Relationship of xylem and phloem diameter with the dose of calcium fertilizer

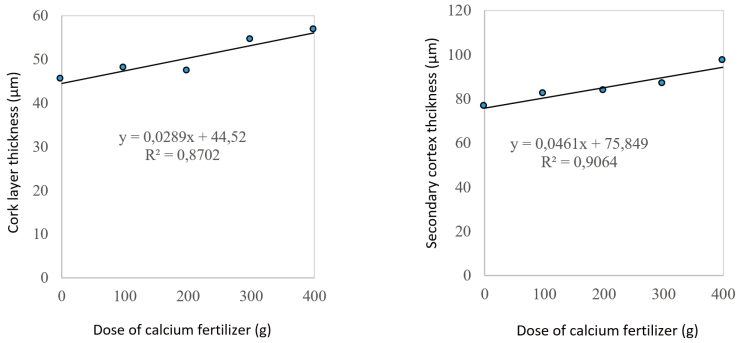

Figure 2. The relationship of cork layer and secondary cortex thickness with the dose of calcium fertilizer

nutrients move passively into the shoots together with water following transpiration flow or by root pressure.

Regression test results showed that Ca fertilization in cocoa plants affected the anatomical characteristics of the cocoa roots in the form of cork layer and secondary cortex thickness in a linearly positive manner (Figure 2), in which each increase in the dose of Ca fertilizer up to 400 g/plant/year is always followed by an increase in cork layer and secondary cortex thickness of cocoa roots. Ca fertilization in cocoa plants affected the thickness of the cork layer by 87% (R^2 = 0.8702), with a regression equation of y = 0.0289x + 44.52. Meanwhile, Ca fertilization in cocoa plants affected the thickness of the secondary cortex by 90% (R^2 = 0.9064), with a regression equation of y = 0.0461x + 75.849 (Figure 2).

This is thought to be because Ca administration can stimulate secondary meristem activity "Cambium", leading to the development of roots (Figure 4). This makes the epidermal layer separated into rupture and peeled off. The protective role of the epidermis is then replaced by cork tissue formed from the cork cambium located on the outer side of the trunk cortex (Li et al., 2018). Cork tissue is a tissue found at the edge of the roots of plants and is composed of cork parenchyma cells. Cork tissue contains suberin and cutin that are stronger than the epidermis. Cork tissue is also composed of cork cells (Ni et al., 2019). This tissue serves to protect the network below it from losing too much water (Inácio et al., 2018).

The availability of Ca in plants can have a positive impact on the development of plant tissues, such as on the thickness of the secondary cortex of cocoa roots. Increasing the dose of Ca fertilizer applied up to a concentration of 400 g results in the increase of the thickness of the root secondary cortex by 96.84 µm (Figure 4). This is in accordance with Atkinson (2014), stating that Ca can be mobilized and redistributed from the younger tissue to the older tissue through phloem, which in turn depends on unidirectional transpiration flow, which then affects the development of plant roots.

The results of regression analysis showed that Ca fertilization in cocoa plants had a quadratic effect on the anatomical characteristics of the roots, which was cambium tissue thickness (Figure 4). Increasing the fertilizer dose Ca up to the optimal dose (270 g/plant/year) is followed by an increase in the thickness

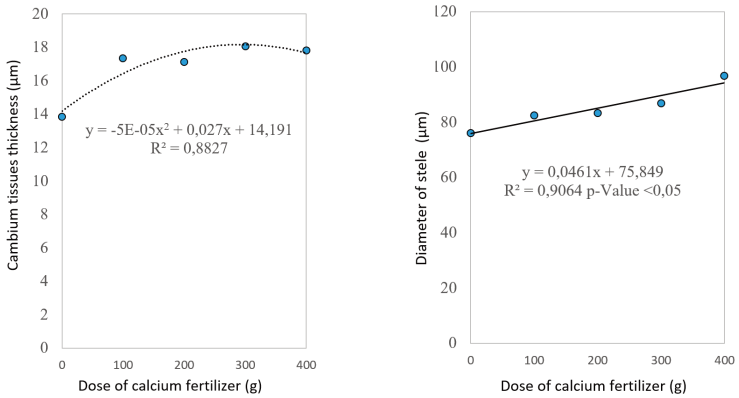

Figure 3. The relationship of the thickness of the cambium tissues and the stele diameter of the cocoa root with the dose of calcium fertilizer

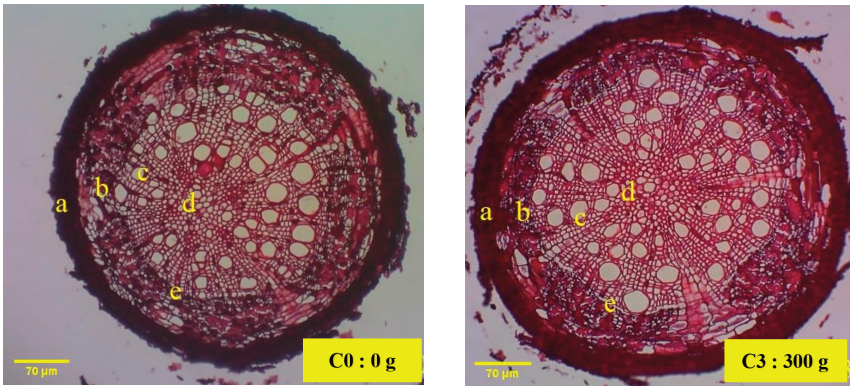

Figure 4. Cross section (10 × 10 magnification) of roots of cocoa plants in various applications of calcium fertilizer sources; a) the cork layer; b) phloem; c) xylem; d) stele; e) cambium, Scale bar: a = 70 µm.

of cambium tissue to 17.84 (µm). However, if the dose of Ca fertilizer continues to be raised until it passes the optimal dose, it actually causes a decrease in the thickness of the root cambium tissue to 17.81(µm). Ca fertilization in cocoa plants affected the thickness of cambium tissue by 88% (R^2= 0.8827), with a regression equation of y = -5E-05x^2 + 0.027x + 14.191 (Figure 3). This result is thought to be due to the excessive Ca that can inhibit the absorption and utilization of other nutrients, such as B, K, and Mg in plants (Charpentier & Oldroyd, 2013).

Ca is used to control soil pH, help the formation of soil aggregates, and function as the main ion in the soil cation exchange complex (Sturião et al., 2020; Atkinson, 2014). Gas chromatography-mass spectrometry produces excess Ca so that the primary metabolism in the roots becomes more active, thereby stimulating the activity of the secondary meristem "Cambium" (Bauer et al., 2011).

Regression analysis results showed that Ca fertilization in cocoa plants positively affected the diameter of the stele. The increase in the dose of Ca fertilizer up to 400 g/plant/year is always followed by an increase in the diameter of the cocoa root stele. Ca fertilization in cocoa plants affected the stele diameter by 90% (R^2 = 0.9064), with a regression equation of y = 0.0461x + 75.849 (Figure 3).

Ca also spurs cell lengthening in plants, causing organ tissues in roots and stems to develop, thereby spurring the development of vascular tissue and encouraging cell division that causes cambium tissue to develop (Grusak et al., 2016). Ca has several different roles in high-level plants, including controlling membrane, enzymes, cell walls, and phytohormones (Freitas et al., 2015). Song et al., (2018) state that Ca administration positively impacts larger cell size (hypodermis internal parenchyma cells, external parenchyma cells and stele diameter).

CONCLUSIONS

The effect of Ca on the anatomical performance of cacao roots was generally followed by an increase in the size of the xylem and phloem diameters, the thickness of the cork layer, the thickness of the secondary cortex and the diameter of the stele. Quadratic effect of the dose of $CaCl_2$ fertilizer was found only on the thickness of the root cambium tissues. Therefore, related to the anatomical performance of the roots, the optimal dose of $CaCl_2$ fertilizer has not been determined.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Research, Technology, and Higher Education Republic of Indonesia for funding this research.

REFERENCES

Aras, S., Keles, H., and Bozkurt, E. (2021). Physiological and histological responses of peach plants grafted onto different rootstocks under calcium deficiency conditions. *Sci. Hortic.*, 281, pp. 109967.

Atkinson, C.J. (2014). Is xylem sap calcium responsible for reducing stomatal conductance after soil liming?. *Plant Soil*, 382, pp. 349–356.

Badrie, N., Bekele, F., Sikora, E., and Sikora, M. (2014). Cocoa agronomy, quality, nutritional, and health aspects. *Critical Reviews in Food Science and Nutrition*, 55(5), pp. 620–659.

Soil Research Center. (2009). Technical instructions: chemical analysis of soil, plants, water, and fertilizers. 2nd ed. Bogor: Soil Research Center, pp. 362–368.

Bauer, P., Elbaum, R., and Weiss, I.M. (2011). Calcium and silicon mineralization in land plants: transport, structure and function. *Plant Science*, 180(6), pp. 746–756.

Bothe, H. (2015). The lime-silicate question. *Soil biology & biochemistry*, 89, pp. 172–183.

Cacho, M., Dominguez, A.T., and Elena-Rosselló, J.A. (2013). Role of polyamines in regulating silymarin production in *Silybum marianum* (L.) Gaertn (Asteraceae) cell cultures under conditions of calcium deficiency. *Journal of Plant Physiology*, 170, pp. 1344–1348.

Carr, M.K.V. (2012). *Advances in irrigation agronomy plantation crops*. Cambridge: University Press.

Charpentier, M. and Oldroyd, G.E.D. (2013). Nuclear calcium signaling in plants 1. *Plant Physiology,* 163(2), pp. 496–503.

Directorate General of Estate. (2020). Cocoa Production by Province in Indonesia 2017-2020. Available at: https://www.google.com/search?q=produksi+kakao+di+indonesia+2020&oq=produksi+kakao+2019&aqs=chrome.2.69i57j0i2i30l5.10053j0j7&sourceid=chrome&ie=UTF-8 [Accessed 25 Juni 2021].

Domingues, L.S., Ribeiro, N.D., Andriolo, J.L., Possobom, M.T.D.F., and Zemolin, A.E.M. (2016). Growth, grain yield and calcium, potassium and magnesium accumulation in common bean plants as related to calcium nutrition. *Acta Scientiarum. Agronomy,* 38(2), pp. 207–217.

Dorozhkin, S.,V. (2017). Les orthophosphates den calcium (CaPO4): occurrence etpropriétés. *Morphologie,* 101(334), pp. 125–142.

Du, E., Dong, D., Zeng, X., Sun, Z., Jiang, X., and de Vries, W. (2017). Direct effect of acid rain on leaf chlorophyll content of terrestrial plants in China. Science of the Total Environment, 605–606, pp. 764–769.

Freitas, S.T., Amarante, C.V.T., and Mitcham, E.J. 2015. Mechanisms regulating apple cultivar susceptibility to bitter pit. *Scientia Horticulturae*, 186, pp. 54–60.

Grusak, M.A., Broadley, M.R., and White, P.J. (2016). Plant macro- and micronutrient minerals. *Ecyclopedia of Life Science*, pp. 1–6.

He, H., Veneklaas, E.J., Kuo, J., and Lambers, H. (2014). Physiological and ecological significance of biomineralization in plants. *Trends in Plant Science,* 19(3), pp. 166–174.

Inácio, V., Martins, M. T., Graça, J., and Cecílio, L. M. (2018). Cork oak young and traumatic periderms show PCD typical chromation patterns but different chromation-modifying genes expression. *Frontiers in Plants Science*, 9(1194), pp. 1–18.

Indonesian Coffee and Cocoa Research Center. (2015). *Buku pintar budidaya kakao*. 1st ed. Jakarta: Agromedia Pustaka, pp. 46–51.

Li, R.Q., Yin, M.Z., Yang, M., Chu, S.S., Han, X.J., Wang, M.J., and Peng, H.S. (2018). Developmental anatomy of anomalous structure and classification of commercial specifications and grades of the *Astragalus membranaceus* var. Mongholicus. *Microscopy Research and Technique*, 81(10), pp. 1165–1172.

Liu, T.W., Wu F.H., Wang W.H., Chen J., Li Z.J., Dong X.J., Patton J., Pei Z.M., and Zheng H.L. (2011) Effects of calcium on seed germination, seedling growth and photosynthesis of six

forest tree species under simulated acid rain. *Tree Physiology*, 31(4), pp. 402–413.

Maiti, R.P., Satya., and Ramaswamy, A. (2012). *Crop plant anatomy.* 1st ed. Jakarta: GPI Group, pp.14–18.

McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmisaari, H.S., Hobbie, E.A., Iversen, C.M., and Jackson, R.B. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phyto logist* 207(3), pp. 505–518.

Naeem, M., Naeem, M.S., and Ahmad, R. (2018). Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. *Archives of Agronomy and Soil Science,* 64(1), pp 116–131.

Ni, X.L., Gui, M.Y., Tan, L.L., Zhu, Q., Liu, W.Z., and Li, C.X. (2019). Programmed cell death and Aerenchyma formation in water-logged sunflower stems and its promotion by ethylene and ROS. *Frontiers and in Plant Sciences*, 9(1928), pp. 1–16.

Riveras, E., Alvarez, J.M., and Vidal, E.A. (2015). The calcium ion is a second messenger in the nitrate signaling pathway of Arabidopsis. *Plant Physiology*, 169(2), pp. 1397–1404.

Saito, S., and Uozumi, N. (2020). Calcium-regulated phosphorylation systems controlling uptake and balance of plant nutrients. *Front. Plant. Sci.,* 11(44), pp. 1–11.

Song, W. Yim, J., Kurniadinata, O.F., Wang, H., and Huang, X. (2018). Linking fruit Ca uptake capacity to fruit growth and pedicel anatomy, a cross-species study. *Frontiers in Plant Science* 9(575), pp. 1–11.

Sturião, W. P., Martinez, H.E.P., Oliveira, L.A., Jezler, C,N., de Jesus Pereira, L., and Ventrella, M.C. (2020). Deficiency of calcium affects anatomical, biometry and nutritional status of cherry tomato. *Afr. J. Bot*., 132(1), pp. 346–354.

Vasconcelos, C.V., Costa, A.C., Müller, C., Castoldi, G., Costa, A.M., Barbosa, K.D.P., Rodrigues, A.A., and Silv, A.A.D.L. (2020). Potential of calcium nitrate to mitigate the aluminum toxicity in *Phaseolus vulgaris*: effects on morphoanatomical traits, mineral nutrition and photosynthesis. *Ecotoxicology,* 29(2), pp. 203–216.

Vliet, J.A.V. and Giller, K.E. (2017). Chapter five mineral nutrition of cocoa: a review. *Adv. Agron*., 141, pp. 185–270.

Yang, S., Wang, F., and Guo, F. (2015). Calcium contributes to photoprotection and repair of photosystem II in peanut leaves during heat and high irradiance. J*ournal of Integrative Plant Biology*, 57(5), pp. 486–495.