



Biofortification of calcium on mustard (*Brassica juncea* L.) and lettuce (*Lactuca sativa*) cultivated in floating hydroponic system

Fitra Gustiar¹, Munandar^{2*}, Sekar Wahyu Ningsih¹, Muhammad Ammar¹

¹ Agrotechnology Study Program, Agricultural Faculty, Sriwijaya University, Indonesia

² Agronomy Study Program, Agricultural Faculty, Sriwijaya University, Indonesia

Article Information

Received: May, 29, 2020

Accepted: June 30, 2020

*Corresponding author

munandar.unsri@gmail.com

Keywords:
biofortification,
hydroponic,
calcium,
mustard,
lettuce

ABSTRACT

Calcium (Ca) is one of the essential macrominerals needed by the human body as a major component in the formation of bones and teeth. Calcium is fulfilled by eating calcium-rich foods, both animal and vegetable. Mustard and lettuce are vegetables that can be a source of Ca. Efforts to increase the Ca content in plants can be done through increasing the concentration of Ca given through fertilizer or in nutrient solution. However, excessive Ca application is not recommended because it will be toxic to plants. This study aims to determine the effect of various Ca concentrations in hydroponic nutrient solutions on the growth and yield of mustard vegetables (*Brassica juncea* L.) and lettuce (*Lactuca sativa* L.). This study used a floating hydroponic system with a completely randomized design. Treatment of Ca concentrations of hydroponic nutrient solutions that were tried were 0, 100, 200, 300, and 400 ppm. The parameters observed were plant height, number of leaves, level of greenness of leaves, fresh and dry weight, and concentration of calcium in the leaves. The results showed that Ca treatment significantly affected the number of leaves and the level of leaf greenness. Application of 400 ppm Ca in hydroponic nutrient solution is the highest Ca concentration that could increase Ca content in mustard and lettuce plants. Application of Ca 300 ppm is the highest concentration of hydroponic nutrient solution that can increase the Ca content of plants without causing a decrease in plant biomass, and therefore the treatment of Ca 300 ppm can be used for biofortification of Ca by hydroponic in mustard and lettuce plants.

© 2020 The author(s). This paper is an open-access article licensed under a [Creative Commons Attribution 4.0 International](https://creativecommons.org/licenses/by/4.0/) published by Universitas Prof Dr Hazairin SH

INTRODUCTION

The human body needs enough minerals as a raw material for enzyme activity for metabolic processes. Calcium is one of the essential macro minerals as a major component for the formation of bones and teeth, and maintains the rigidity of the body's skeleton. Needs per person every day of Ca is 535 mg, depending on age category (Wrzosek,

Woźniak, Koziół-Kaczorek, & Włodarek, 2019). Pregnant women need a higher calcium intake, at least 1300 mg every day, therefore calcium supplementation in pregnant women is important. Hidden hunger is a condition of the human body that lacks of nutrients and minerals. This deficiency can occur when the quality of food consumed cannot meet needs adequately, so that micronutrients are

lacking to support body growth and development (Hofmeyr, Lawrie, Atallah, & Torloni, 2018). Consuming calcium-rich foods is an alternative to meeting calcium needs. Sources of calcium are divided into two, namely animal and vegetable. Calcium content in vegetables is not as much as in animals, but the ability of vegetables to provide Ca can be increased through biofortification in plant tissue (Galera, et al 2010).

Mustard and lettuce are green vegetables which have high mineral content and high economic value. Natesh, Abbey, and Asiedu (2017) reported that horticultural crops, especially leafy vegetables play an important role because they contain more vitamins and minerals than other types of vegetables. Conventionally cultivated vegetables and lettuce leaves each contain 103 mg and 35 mg of calcium per 100 g (Zand, LaValle, & Spreen, 1999).

Biofortification of plant Ca is an effort to increase the concentration of Ca in plants by increasing the application of Ca through fertilizer or plant nutrients. Thus, the amount of Ca absorbed by plants will increase. This method is difficult to do in conventional cultivation systems that utilize soil as a growing medium. The availability of calcium in the soil is influenced by soil reactions, interactions with other elements, and the activity of microorganisms. Therefore, biofortification is more friendly by implementing a hydroponic system (Lenni, Suhardiyanto, Seminar, & Setiawan, 2020). The advantages of a hydroponic system include efficient land use, nutrient in growing solution media could be controlled, free of pesticide poisons, more efficient use of fertilizers and water, and easier to control pests and diseases (Sharma, Acharya, Kumar, Singh, & Chaurasia, 2019). The floating hydroponic system is currently popular as a floating hydroponic farming technique. The floating hydroponic technique emphasizes the method of cultivating plants in styrofoam tray that float on the surface of containers containing nutrient solutions. This technique allows plant roots to be submerged in nutrient solution.

Increasing Ca concentration of nutrient solution up to 300 ppm results in an increase in calcium content in fresh lettuce leaves from an average of 179 mg (100 g)⁻¹ to 229 mg (100 g)⁻¹

(Neeser, Savidov, & Driedger, 2007). High Ca absorption efficiency followed by an increase in Ca content in plant tissue (vegetables) can be implemented by increasing the concentration of Ca in nutrient solution. However, excessive application of Ca in plants is not recommended because it will be toxic and dangerous for plant growth. Therefore, this study aimed to determine the highest concentration of Ca in hydroponic nutrient solution that can increase the Ca content of plants but not to inhibit the growth and yield of mustard and lettuce plants.

MATERIAL AND METHODS

Research Design

The research was carried out at the Hydroponic House of the Agronomy Study Program of the Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, and South Sumatra, Indonesia in June - November 2019. The study used a completely randomized design method with 2 types of plants and 5 levels of Ca concentration in the AB Mix nutrient solution. Two type of crops were mustard greens and lettuce. The Ca concentration of culture solution consisted of 0 ppm as a control plant, 100, 200, 300, and 400 ppm. Each treatment was repeated four times, so there were a total of 20 experimental units. Each unit experiment consisted of a container containing 10 liters of AB Mix nutrient culture solution on which was placed the styrofoam panel planted with 2 mustard plants and 2 lettuce plants.

Plant Preparation

The mustard and lettuce seed were sown on rockwool media for 3 weeks until the seedling had 3-4 leaves. The seedlings were then put into a netpot that functions as a planting medium. The net pot was then transplanted into a floating panel on a container filled with 10 of AB mix nutrient solutions. The concentration of Ca in the solution was modified according to treatment by adding stock solution of CaCl₂. Every 5 days, the nutrient solution for all treatment concentrations was replaced with a new nutrient solution. The pH and electronic conductivity (EC) of the solution were measured at the beginning and end before the solution was replaced. The observed parameters were plant

height (cm), number of leaves, the level of leaf greenness, plant fresh weight (g), plant dry weight (g) and calcium (Ca) concentration in leaves.

Analysis of calcium content

Two grams of leaves and stems of mustard and lettuce samples were dried and finely ground, then burned in a furnace at 500°C for 4 hours. After becoming ash, the sample was cooled in a desiccator. Then it was put into the beaker and add 20 ml of HCl (1+1). After that, the glass was covered using a watch glass and heated on a heater until it boiled for 10 minutes. The glass was taken from the heater, allowed to cool and added 20 ml of distilled water. The solution was put into a 50 ml glass beaker, then filtered using filter paper. The residue left in the glass was rinsed 2 times using distilled water. Then

the remaining residue in filter paper was washed using distilled water and diluted to 50 ml using distilled water (Mardiah, 2017). The dilution solution was used to be analyzed for Ca solution using Atomic Absorption Spectrophotometry.

RESULTS AND DISCUSSION

Results

Analysis of variance on observed growth and yield parameters showed that the concentration of calcium (Ca) in nutrient culture solutions in the cultivation of mustard and lettuce plants had a very significant effect on the parameters of the number of leaves and the level of greening of the leaves, but had no significant effect on the parameters of plant height, weight fresh and dry plant weights (Table 1).

Table 5.1. The results of the variance analysis on observed parameters

No.	Observed Parameters	Level of signifiyacny		Coefficient of variance (%)	
		Mustard	Lettuce	Mustard	Lettuce
1	Plant height	2.81ns	2.15ns	4.16	5.02
2	Number of leaves	413.72**	256.72**	7.37	8.89
3	The level of leaves greenness	7.23**	7.08**	4.41	4.39
4	Plant fresh weight	2.72ns	2.59ns	0.8	2.23
5	Plant dry weight	2.46ns	2.59ns	0.82	2.23
F Table 5%		3.06			
F Table 1%		4.89			

Notes: ** is highly significantly difference, * is significantly difference, ns is not significant difference

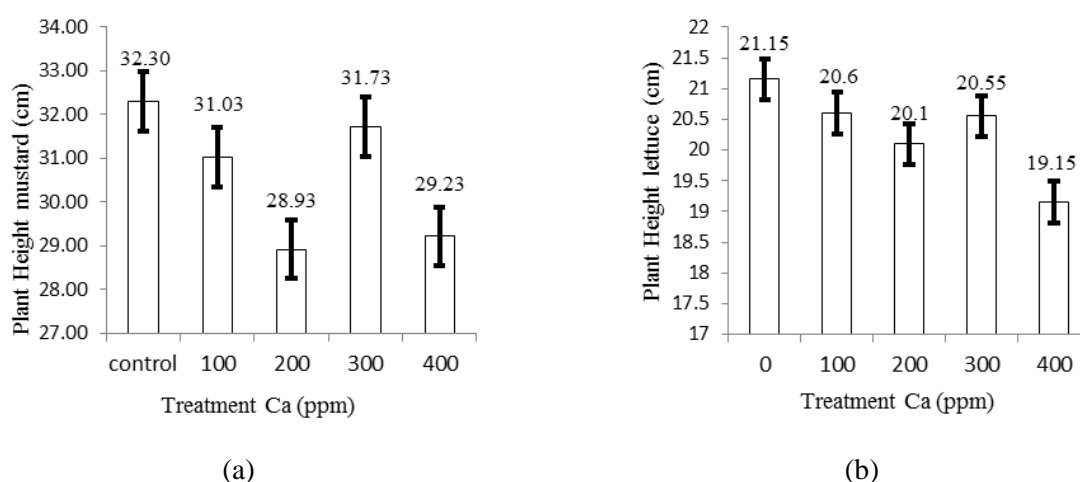


Figure 1. Effect of Ca concentration in hydroponic nutrient solution on plant height of mustard (a) and lettuce (b)

Plant Height

Plant height showed that increasing Ca concentration did not significantly influence the

height of mustard and lettuce (Figure 1), but plant height tended to decrease with higher Ca concentration of nutrient solution. The highest plant

height was shown in the control treatment of 32.3 cm in mustard plants and 19.15 cm in lettuce. The lowest plant height was shown in the concentration of Ca 400 ppm, which was 29.55 cm in mustard and 19.15 cm in lettuce.

Table 2. Leaves number of mustard and letuce treated with various Ca concentration

Treatment	Means	
	Mustard	Lettuce
0 ppm	9.75 a	9.75 a
100 ppm	11.25 b	10.50 ab
200 ppm	12.25 bc	11.00 bc
300 ppm	13.00 d	12.00 cd
400 ppm	15.00 e	12.50 d
LSD 0.05	1.12	1.22

Table 3. The leaves greenness of mustard and letuce treated with various Ca concentration

Treatment	Means	
	Mustard	Lettuce
0 ppm	33.60 a	32.00 a
100 ppm	34.67 ab	33.47 ab
200 ppm	36.70 bc	35.02 bc
300 ppm	39.1 e	36.45 cd
400 ppm	37.05 d	36.85d
LSD 0.05	1.98	1.89

The results of the analysis of variance showed that differences in the concentration of calcium (Ca) significantly affected the number of mustard and lettuce leaves (Table 2). Increased Ca concentration in nutrient culture solution increases the number of leaves in both mustard and lettuce plants. The highest number of leaves was produced in the treatment of 400 ppm Ca and the lowest was in the control treatment for both plants. The highest number of leaves was 15 in mustard plants and 12.5 in lettuce, while the lowest number of leaves is 9.75 for both mustard and lettuce plants.

Level of greenness of leaves

Increasing the concentration of Ca in culture solution significantly increases the level of

greenness of leaves in plants and lettuce. The highest level of greenness of leaves was indicated by mustard plants obtained in the treatment of 300 ppm (39.1) and was significantly different with the treatment of 400 ppm Ca (37.05), whereas in lettuce plants, application of Ca 400 ppm resulted in the highest level of greenness of leaves 36, 85, not significantly different with the treatment of 400 ppm Ca (36.85). The lowest level of greenness of the leaves is shown in the control treatment that is 33.6 in mustard greens and 32 in lettuce.

Plant fresh weight

Although statistically different concentrations of calcium (Ca) in hydroponic nutrient solutions did not significantly affect the fresh weight of mustard and lettuce plants, but the higher Ca concentrations tends to reduce the fresh weight of plants. The control treatment of 0 ppm Ca produced the highest fresh weight in mustard and lettuce plants respectively 32.82 g and 38.72 g. The fresh weight of the plant slowly decreased from in control treatment to a Ca concentration of 400 ppm, each producing a fresh weight of 38.2 g in mustard greens and 37.07 g in lettuce. Fresh weight reduction due to addition of Ca nutrient solution is sharper in mustard compared to lettuce.

Plant dry weight,

Although the analysis of variance did not show the significant effect of Ca increasing concentrations in nutrient solutions on plant dry weight, it appeared that the dry weight tended to decrease slightly with the addition of higher Ca concentrations. Fresh weight reduction due to the addition of Ca was sharper in mustard plants compared with lettuce. In the lettuce, a reduction occurred in the plants treated with Ca 400 ppm, whereas in the reduction of dry weight in mustard plants had occurred in plants treated with Ca 100 ppm (Figure 3). The highest dry weight of 1.94 g respectively in mustard greens and lettuce in the control treatment and decreased to 1.91 g in mustard greens and to 1.85 g in lettuce plants as Ca concentration increase up to 400 ppm.

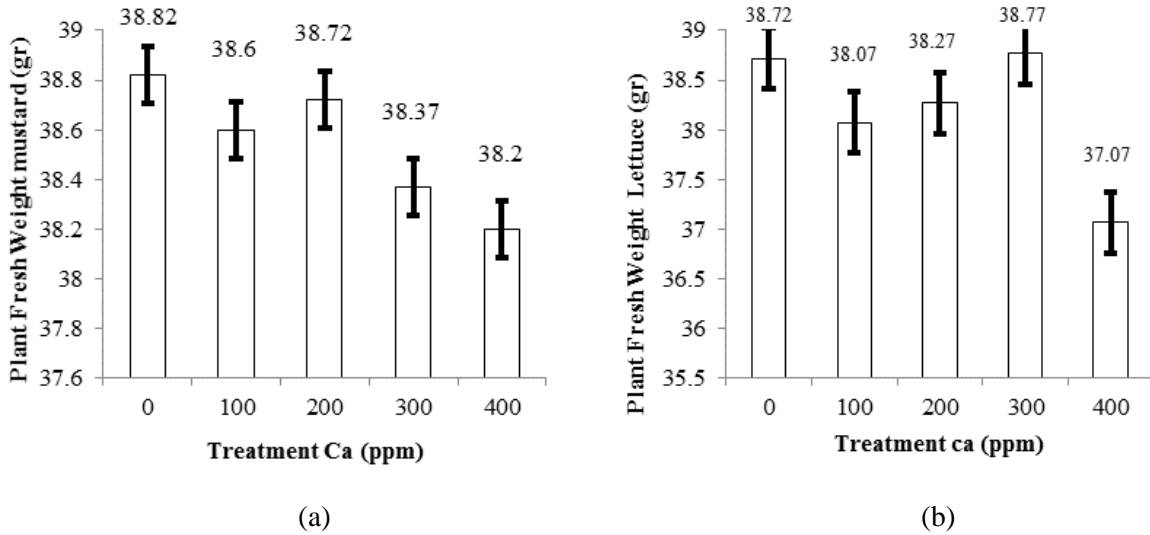


Figure 2. Fresh weight of mustard (a) and lettuce (b) treated with various level of Ca concentration in nutrient culture solution

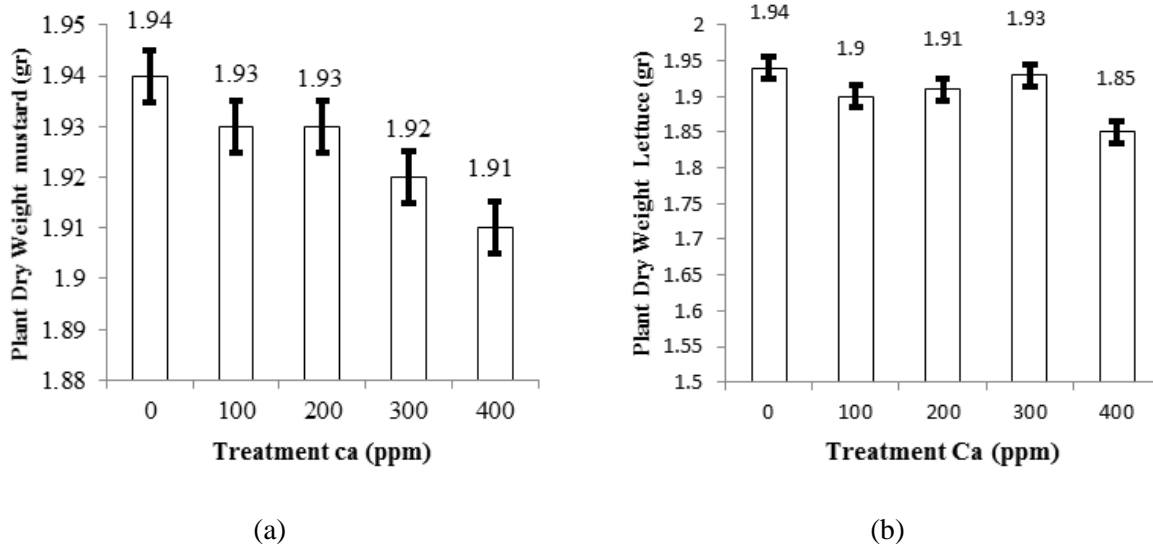


Figure 3. Dry weight of mustard (a) and lettuce (b) treated with various level of Ca concentration in nutrient culture solution

Leaf Ca content

Ca content at all levels of Ca application was higher in mustard than in lettuce. The response pattern of leaf Ca content to the increase in Ca application in nutrient solutions was relatively same in both plants. The increase in Ca in culture solution from 0 ppm to 300 ppm caused the mustard leaf Ca content to increase from 3.43% to 4.99%, or increase by 31.3%, and after that it only increased to 3.03% in Ca 400 ppm. In lettuce, leaf Ca content increased from 1.77% to 2.97% or increased to 40.4%, as Ca culture solution increased from 0 ppm

to 300 ppm. After that, addition of Ca to 400 ppm only increased leaf Ca content to 3.03%. The concentration of Ca in nutrient solution for both plants may have reached saturation level at a concentration of 400 ppm.

DISCUSSION

The results of this study indicated that increasing Ca concentrations in hydroponic nutrient solutions increased the Ca nutrient content in plants. The leaf Ca content increased to 4.99% in mustard plants and up to 2.97% in lettuce plants as 300 ppm

when CaCl₂ was added. Increased leaf Ca content affected several growth components of the two plants. The correlation and effect of increasing leaf Ca content with observed growth parameters are shown in Table 4. Leaf Ca was positively correlated

with the number and degree of greenness of the leaves, and negatively correlated with plant height, and did not correlate with wet weight and dry weight.

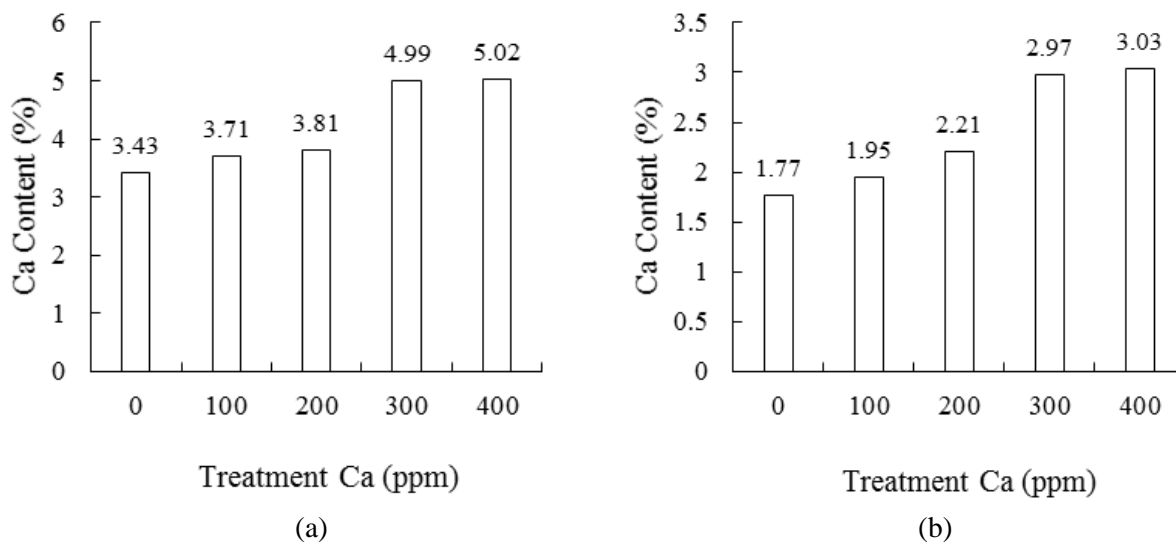


Figure 4. Leaf Ca content in mustard (a) and lettuce (b) treated with various level of Ca concentration in nutrient culture solution

Table 4: Correlation value *r* between leaf N content with various observed growth parameters.

No	Parameter	Mustard	Lettuce
1	Plant height	-0.499*	-0.465*
2	Number of leaves	0.834**	0.638**
3	Dry weight	-0.086 ^{ns}	-0.155 ^{ns}
4	Fresh weight	-0.318 ^{ns}	-0.281 ^{ns}
5	Level of leaf greenish	0.730**	0.868**

Level of significant at 1% = 0.444; 5% = 0.561; ** is highly significant; * is Significant; and ns is not significant

High Ca uptake caused a higher level of greenness and number of leaves, lower plant height and no effect on plant weight and plant weight. One of the roles of calcium is to control the development of chloroplasts. The application of calcium can increase the efficiency of the use of nitrogen which is utilized to help increase photosynthesis (Ayyub *et al.*, 2012). The same research results have been reported by Hernández *et al.*, (2003) and Lenni *et al.*, (2020) that calcium ions play a role in gene expression by controlling light (phytochromes), and therefore calcium application increases photosyn-

thetic pigments, nitrogen use efficiency and increase photosynthetic rate

This study also showed that the addition of calcium concentrations up to 400 ppm did not increase the height of mustard and lettuce plants, instead the growth of plant height was hampered by the increase in plant Ca content. This is in line with research conducted by Rachmah, Nawawi, and Koesriharti (2017) which showed that the application of calcium and gibberellins fertilizer had no significant effect on plant height, fresh fruit weight per plant, fruit diameter, and fruit quality of tomato plants. These results are different from the research reported by Parvin, Ahamed, Islam, & Haque (2015) in tomato plants that an increase in Ca nutrient concentrations causes stunted plant growth was inhibited.

The number of leaves is a very important character for plant growth and development because the leaves are the main photosynthetic organ. In this study the number of leaves increased with the addition of Ca nutrient solution. Similar research results reported by Hernández *et al.*, (2003), that Ca concentration has a significant effect on the number of leaves and number of branches of tomato plants

by increasing calcium to 5 mM. Likewise, research conducted by Ayyub *et al.*, (2012) who reported that the application of calcium fertilizer sourced from CaCl_2 at different growth stages could increase the number of compound leaves in tomato plants.

In both mustard and lettuce plants, leaf Ca content increased to reach 4.99% in mustard plants and up to 2.97% in lettuce plants at the addition of 300 ppm CaCl_2 , and relatively did not increase again despite the addition of CaCl_2 concentration to 400 ppm (Figure 4.) Furthermore, although the wet weight and dry weight of plants decreased with increasing concentrations of Ca in nutrient solution up to 300 ppm, but the decrease in wet weight and dry weight was not significantly different than at the addition 0 ppm concentrations (Figures 2 and 3).

From this fact it can be concluded that the treatment of 300 ppm CaCl_2 concentration is a concentration level of nutrient Ca in a hydroponic growing media solution that can increase the Ca content of plants several times the normal, and does not cause inhibition of growth of mustard plants and lettuce. Increasing the concentration of 300 ppm CaCl_2 nutrient solution in the cultivation of floating raft hydroponic systems can be recommended as a biofortification technique for increasing Ca mineral mustard and lettuce plants. This result is in line with the results of Rohmaniyah *et al.*, (2015) research which stated that the calcium content in plant leaf tissue will be higher if the concentration of Ca applied to the nutrient solution is higher.

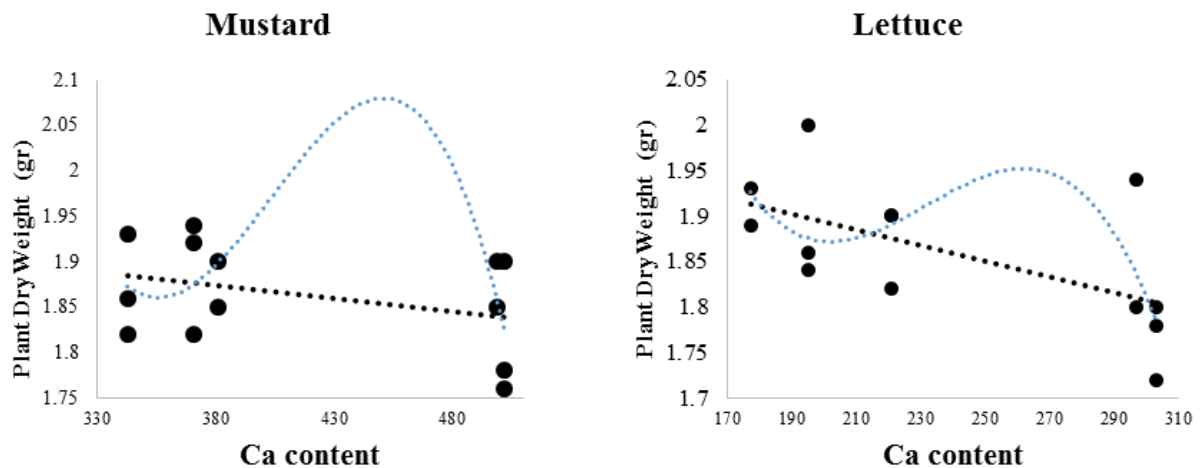


Figure 5. The relationship of Ca concentration in hydroponic solution with Ca contents in leaves tissue of mustard and lettuce

Calcium is an important and essential macro nutrient that is absorbed in the form of Ca^{++} . Calcium in plants cannot move or cannot be translocated to other parts of the plant (Marschner, 2012). Most calcium found in lamella of cell wall as calcium pectate. Calcium acts as a structural component of cell membranes, maintains membrane stability and cell integrity (regulating ion absorption selectivity, regulating membrane permeability and preventing leakage of solution in cells). Calcium stimulates the formation of root hairs, harden plant stems, neutralize organic acids produced in metabolism, and acts as calmodulin (CaM) enzyme regulation and control gene

expression (second messenger). The increase in gene expression activity and membrane stability will increase the ability of plants to deal with environmental stress (Marschner, 2012).

The results of this study also showed that the range in which the plant was deficient, sufficient and toxic or poisoning by Ca nutrients differed between mustard and lettuce plants. Deficient levels Ca nutrient in mustard and lettuce respectively were below 450 mg $(100 \text{ g})^{-1}$ FW and 250 mg $(100 \text{ g})^{-1}$ FW, sufficient range in mustard was between 450-480 mg $(100 \text{ g})^{-1}$ FW, in lettuce between 250-280 and the level of toxic or toxicity occurs if the content Ca more than 480 mg $(100 \text{ g})^{-1}$ FW for mustard and

more than 280 mg (100 g)⁻¹ FW for lettuce (Figure 5).

The absorption of Ca follows apoplastic pathway mechanism which allows Ca to enter xylem without affecting in cell activities and the plants were able to absorb Ca with the concentration up to 400 ppm and increase their Ca content (White & Broadley, 2003). The 0 ppm Ca control treatment is a treatment with optimal nutrient concentration, so various growth parameters will show the best value. In the sufficient zone, an increase in Ca increases dry weight, then increasing the concentration of Ca nutrients to 400 ppm causing several growth parameters to decrease. This shows that at concentration of 400 ppm Ca uptake of Ca by plants has reached the toxic zone. These results are

in line with research conducted by Domingues *et al.*, (2016) which stated that increasing the concentration of calcium can increase plant dry weight because with the addition of calcium the ability of plants to absorb and use photosynthate results becomes more efficient. The addition of calcium in plants below the level of toxic zone will affect the structure of plant cells for improving the strength and thickness by forming cross-links in the polysaccharide pectin matrix (Easterwood, 2002). These results were in line with the study of Domingues *et al.*, (2016) which revealed that increasing concentration of calcium can increase plant biomass as calcium addition improving the ability to absorb and utilizing photosynthate more efficiently.

Table 5. Percentage of Ca mineral sufficiency by mustard and lettuce biofortified by Ca in culture solution.

Biofortification of hydroponic media	Mustard		Lettuce	
	Ca content (mg (100gr) ⁻¹)	% Ca sufficiency *	Ca content (mg (100gr) ⁻¹)	% Ca sufficiency *
0 ppm	343	28.6	177	14.8
100 ppm	371	30.9	195	16.3
200 ppm	381	31.8	221	18.4
300 ppm	499	41.6	297	24.8
400 ppm	502	41.8	303	25.3

Adequacy standards for Ca needs: 1200 mg day⁻¹ person⁻¹ (Centeno, Díaz De Barboza, Marchionatti, Rodríguez, & Tolosa De Talamoni, 2009)

Calcium is one of the important mineral elements and is needed by the human body. The average calcium intake of Indonesian people is only around 254 mg day⁻¹ person⁻¹, which is only 21.2% of the international standard requirement for calcium adequacy, the human body that requires calcium as much as 1200 mg day⁻¹ person⁻¹ (Centeno *et al.*, 2009).

Consumption of 100 g of mustard plants cultivated at 0 ppm Ca hydroponics, will be able to meet 28.58% of the daily requirement of Ca, whereas if consuming mustard plants resulting from biofortification of 300 ppm Ca will be able to meet the daily calcium requirements up to 41.6%. In lettuce, consumption of 100 g of Ca 0 ppm hydroponic plants can only meet 14.75% of the daily calcium requirement, whereas if consuming 100 g of lettuce produced in Ca 300 ppm

biofortification will be able to meet the daily calcium needs up to 24.8% (Table 3). Hydroponically biofortification of mustard and lettuce with 300 ppm Ca can be an alternative way to meet the daily needs of Ca. In order to meet the recommended daily Ca requirements of 1200 mg, people need to consume ± 240.5 g of mustard or ± 404 g of lettuce per day.

CONCLUSION AND RECOMMENDATIONS

Concentration 300 ppm of Ca is an optimum concentration for hydroponically biofortification of Ca in mustard and lettuce. In mustard plants, increasing Ca concentration of hydroponic nutrient solution increases Ca content from 343 mg (100 g)⁻¹ FW at 0 ppm Ca to 499 mg (100 g)⁻¹ FW at 300 ppm Ca; in lettuce plants Ca content increasing from 177 mg (100 g)⁻¹ FW at 0 ppm Ca to 297 mg (100

g)⁻¹ FW at Ca 300 ppm. Consumption of 100 grams mustard and lettuce produced hydroponically biofortified in 300 ppm Ca, respectively can fulfill 41.6% and 24.8% of the daily Ca mineral needs. Increased absorption of Ca nutrients by hydroponically cultivated mustard and lettuce plants increases the number of leaves, the level of greenness of leaves, and reduces the height. The range of deficiency, sufficient and toxic zones of Ca nutrient in mustard plants are higher compared with in lettuce plants.

ACKNOWLEDGMENT

We thank to the Sriwijaya University Rector, Head of the Research and Service Institute, Dean of the Faculty of Agriculture, University of Sriwijaya for permission and the granting of the 2019 Competition research grant.

REFERENCES

- Ayyub, C. M., Pervez, M. A., Shaheen, M. R., Ashraf, M. I., Haider, M. W., Hussain, S., & Mahmood, N. (2012). Assessment of various growth and yield attributes of tomato in response to pre-harvest applications of calcium chloride. *Pakistan Journal of Life and Social Sciences*, 10(2), 102–105.
- Centeno, V., Díaz De Barboza, G., Marchionatti, A., Rodríguez, V., & Tolosa De Talamoni, N. (2009). Molecular mechanisms triggered by low-calcium diets. *Nutrition Research Reviews*, 22(2), 163–174.
- Domingues, L. da S., Ribeiro, N. D., Andriolo, J. L., Possobom, M. T. D. F., & 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), 207–217.
- Easterwood, G. W. (2002). Calcium's role in plant nutrition. *Fluid Journal*, Winter 200, 1–3.
- Galera. S.G., E. Rojas, D. Sudhakar, C. Zhu, A.M. Pelacho, T. C. dan P. C. (2010). Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Res*, 19: 165-180.
- Hernández, J. A., Aguilar, A. B., Portillo, B., López-Gómez, E., Beneyto, J. M., & García-Legaz, M. F. (2003). The effect of calcium on the antioxidant enzymes from salt-treated loquat and anger plants. *Functional Plant Biology*, 30(11), 1127–1137.
- Hofmeyr, G. J., Lawrie, T. A., Atallah, Á. N., & Torloni, M. R. (2018). Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database of Systematic Reviews*, 10.
- Janet Zand, Allan N. Spreen, J. B. L.-M. (1999). *Smart Medicine for Healthier Living*. Avery Publishing Group.
- Lenni, Suhardiyanto, H., Seminar, K. B., & Setiawan, R. P. A. (2020). Photosynthetic rate of lettuce cultivated on floating raft hydroponic with controlled nutrient solution. *HAYATI Journal of Biosciences*, 27(1), 31–36.
- Mardiah. (2017). Analisa kadar kalsium (Ca) Pada daun kelor (*Moringa oleifera*). *Jurnal Ilmu Alam Dan Lingkungan*, 8(15), 49–52.
- Marschner H. (2012). *Mineral Nutrition of Higher Plants. Ed ke-3*. London: Academic-Pr.
- Natesh N, H., SK, A., & L, A. (2017). An overview of nutritional and anti nutritional factors in green leafy vegetables. *Horticulture International Journal*, 1(2), 58–65.
- Neeser, C., Savidov, N., & Driedger, D. (2007). Production of hydroponically grown calcium fortified lettuce. *Acta Horticulturae*, 744, 317–322.
- Parvin, K., Ahamed, K. U., Islam, M. M., & Haque, M. N. (2015). Response of tomato plant under salt stress: Role of exogenous calcium. *Journal of Plant Sciences*, 10(6), 222–233.
- Rachmah, C., Nawawi, M., & Koesriharti. (2017). Pengaruh aplikasi pupuk Kalsium (CaCO₃) dan Giberilin terhadap pertumbuhan, hasil dan kualitas buah pada tanaman tomat (*Lycopersicon esculentum* Mill). *Jurnal Produksi Tanaman*, 5(3), 515–520.
- Rohmaniyah L. K., Indradewa, D., Putra, E.T.S. (2015). Tanggapan tanaman kangkung (*Ipomea reptans* Poir.), bayam (*Amaranthus tricolor* L.), dan selada (*Lactuca sativa* L.) terhadap pengayaan kalsium secara hidroponik. *Vegetalika*, 4(12), 10–14.
- Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaurasia, O. (2019). Hydroponics as an

- advanced technique for vegetable production: An overview. *Journal of Soil and Water Conservation*, 17, 364–371.
- White, P. J., & Broadley, M. R. (2003). Calcium in plants. *Annals of Botany*, 92(4), 487–511.
- Wrzosek, M., Woźniak, J., Koziół-Kaczorek, D., & Włodarek, D. (2019). The assessment of the supply of calcium and vitamin d in the diet of women regularly practicing sport. *Journal of Osteoporosis*, 2019, 1–8.