ISSN: 1412-4718, eISSN: 2581-138x

Zahro RS · Faozi K · Ulinnuha Z . Sarjito A

Agronomic biofortification of calcium in pak choy (*Brassica rapa* subsp. *chinensis*) via hydroponic nutrient film technique

Abstract. Pak choy is rich in calcium, magnesium, phosphorus, iron, and vitamin K, all essential for health. Pak choy requires a proper balance of nutrients for optimal growth. In a hydroponic system, any imbalance in nutrient levels can negatively impact plant growth and reduce the nutritional value stored in the tissues. Selecting suitable varieties and providing appropriate calcium treatment can help increase calcium content and enhance productivity. This study aims to determine the effect of calcium concentration on growth, yield, and Ca content and the most responsive varieties to increased calcium concentration. The research design used was a Split Plot Design with two factors, namely calcium nitrate concentration (k) as the main plot consisting of k_0 = without addition of $Ca(NO_3)_2$, k_1 = addition of 300 mg/L $Ca(NO_3)_2$, k_2 = addition of 600 mg/L $Ca(NO_3)_2$, k_3 = addition of 900 mg/L $Ca(NO_3)_2$, and k_4 = addition of 1200 mg/L $Ca(NO_3)_2$. Varieties (v) as subplots consist of v_1 = Masbro, v_2 = Nauli F1, and v_3 = Flamingo. The results showed that the Nauli F1 variety had the best effect on the number of leaves, growth rate index, and fresh weight of shoots. Adding 900 mg/L of calcium nitrate had the best impact on plant growth, while adding 1200 mg/L of calcium nitrate caused plant poisoning. The Masbro variety was responsive to Ca biofortification.

Keywords: Biofortification · Growth · Macro nutrients · Toxicity

Submitted: 2 October 2024, Accepted: 23 November 2024, Published: 15 December 2024
DOI: https://doi.org/10.24198/kultivasi.v23i3.56760

Zahro RS[1] · Faozi K[2] · Ulinnuha Z[2*] . Sarjito A[2]
[1] Undergraduate Program of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University, Dr. Soeparno Street No.63, Karang wangkal, Purwokerto Utara, Banyumas Regency 53122, Indonesia
[2] Department of of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University, Dr. Soeparno Street No.63, Karang wangkal, Purwokerto Utara, Banyumas Regency 53122, Indonesia
*Correspondence: zulfaulinnuha@unsoed.ac.id

Zahro RS, Faozi K, Ulinnuha Z, Sarjito A. 2024. Agronomic biofortification of calcium in pak choy (*Brassica rapa* subsp. *chinensis*) via hydroponic nutrient film technique. Jurnal Kultivasi, 23(3): 299-309.

Jurnal Kultivasi Vol. 23 (3) December 2024
ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Brassicaceae vegetables are popular globally, with Pak choy (*Brassica rapa* subsp. *Chinensis*) being widely utilized in diverse culinary preparations worldwide. From Asian stir-fries to international fusion cuisine, peppery flavor and crunchy texture make it a favored ingredient. Pak choy is a highly nutritious vegetable, offering 13 kcal per 100 grams, with 95% of its weight coming from water. It also contains 1.5 grams of protein, 2.2 grams of carbohydrates, 0.2 grams of fat, 105 mg of calcium, 0.8 mg of iron, 19 mg of magnesium, 41 mg of phosphorus, and 252 mg of potassium.

The calcium given to pak choy benefits consumer health and plant growth and development. Calcium is a secondary macronutrient, although it is needed in smaller amounts compared to primary macronutrients. Calcium is used to control the pH of nutrient solutions. As the divalent cation (Ca^{2+}), it is required for structural roles in the cell wall and membranes, as a counter-cation for inorganic, and as an intracellular messenger in the cytosol (Ahmad et al. 2016). However, the calcium content in pak choy is relatively lower compared to other leafy greens like collard greens, which provide 232 mg of calcium per 100 g, and kale, which offers 150 mg per 100 g.

Efforts to increase the concentration of certain nutrients in plants by providing fertilizers can be achieved through agronomic biofortification (Farid & Ulinnuha, 2022). This process allows for the targeted accumulation of essential minerals, such as calcium, in plant tissues, enhancing their nutritional content. However, biofortification in conventional soil-based systems presents significant challenges. Soil reactions, interactions between nutrients, and the activity of microorganisms all influence the availability and uptake of minerals like calcium (Ceccherini et al. 2024). These complex factors make it difficult to control and optimize nutrient absorption in traditional agricultural settings, limiting the effectiveness of biofortification in soil-based systems. As a result, alternative approaches, such as hydroponic systems or more controlled soil management practices, may be necessary to achieve consistent and reliable nutrient enhancement in crops.

One of the popular hydroponic systems, namely the Nutrient Film Technique (NFT), can be defined as a type of closed-loop system that offers several advantages, particularly in biofortification. In an NFT system, a thin film of nutrient solution continuously flows over the plant roots, ensuring efficient nutrient uptake while minimizing water and nutrient wastage. This closed system significantly conserves nutrient availability, allowing for precise control over the concentration of essential elements, including those targeted for biofortification (Galić et al., 2021).

Agronomic biofortification using a hydroponic medium has been used several times, namely in the Zn biofortification of lettuce (de Lima et al., 2023), iodine on lettuce (de Figueiredo et al., 2022), iron on *Eruca sativa* L. (Ceccherini et al., 2024). Agronomic biofortification is expected to overcome nutritional deficiencies in various regions, improve public health, and provide better access to nutritious food. Also, agronomic biofortification can increase agricultural productivity and food security in the long term. So, this research aims to determine the effect of variety and calcium concentration on growth, yield, and Ca content in pak choy plant tissue.

Materials and Methods

Research activities have been conducted at the Screen House, Faculty of Agriculture, Jenderal Soedirman University, from December 2022 to March 2023. The materials in this research are Masbro pak choy variety, Nauli F1 pak choy variety, Flamingo pak choy variety, $Ca(NO_3)_2$, rockwool, water, plastic bucket, plastic tray, AB Mix, water pump, and NFT hydroponic installation. The tools used are a 1-liter measuring cup, knife, cutter, stationery, analytical scales, lux meter, thermo-hygrometer, spectrophotometer, and oven.

The experimental design used in this research was a Split Plot Design using two factors, namely calcium nitrate concentration as the main plot consisting of 5 levels, namely: k_0 = without addition of $Ca(NO_3)_2$, k_1 = addition of 300 mg /L $Ca(NO_3)_2$, k_2 = addition of 600 mg/L $Ca(NO_3)_2$, k_3 = addition of 900 mg/L $Ca(NO_3)_2$, k_4 = addition of 1200 mg/L $Ca(NO_3)_2$. Variety (v) as a subplot consists of 3 levels: v_1 = Masbro variety, v_2 = Nauli F1 variety, and v_3 = Flamingo variety. Combining the two factors resulted in 15 treatments with three replications, resulting in 45 research units. Each research unit consisted of two pak choy plants.

The parameters observed were plant height, number of leaves (pieces), leaf area (cm^2), root length (cm), root volume (mL), stem skin thickness (cm), growth rate index (g/week), fresh shoot weight (g), shoot dry weight (g), chlorophyll content, composite Ca content (%). The data obtained were analyzed using analysis of variance (ANOVA). If there were significant differences in the observed data, they were analyzed further using the Duncan Multiple Distance Test (DMRT) with a level of 5% and regression analysis on Ca content.

Results and Discussion

The following result in Table 1 is the recapitulation of the ANOVA result of numerous measured variables. Present study confirmed that the calcium treatment significantly affects all variables. This is in line with the research by Suryantini et al. (2020), which states that the addition of calcium nitrate significantly affects the growth and yield of curly lettuce plants. This indicates that the effect of calcium is significant on the development, physiology, and yield variables of pak choy plants.

Plant height. Plant height is an essential morphological and developmental phenotype that directly indicates overall plant growth and can predict plant yield and biomass (Wang et al., 2019). Calcium affects plant height, as presented in the following table (Table 2). Adding 900 mg/L of calcium nitrate produced higher plants up to 32.90 cm, but it was not significantly different with the addition of Ca at 0 and 300 mg/L. This research aligns with research (Suryantini et al., 2020), which states that the height of curly lettuce plants has increased due to the addition of $Ca(NO_3)_2$ with significantly different results. The element calcium plays a role in cell elongation and maintaining plant membrane structure (Zhang et al., 2020). Calcium-dependent protein kinases play a role in signal transduction pathways that regulate cell elongation in response to environmental cues (Yip et al. 2019). Cell elongation will produce taller plants (do Moraes et al. 2023).

Adding 1200 mg/L of calcium nitrate resulted in the lowest plant height 5 WAP was 26.13 cm. This could be an indication of calcium toxicity in pak choy plants. According to Reitz, et al. (2021), excessive calcium levels can disrupt the balance of other essential nutrients within the plant, such as potassium and magnesium. This imbalance can interfere with various physiological processes for plant growth and development.

Number of leaves. The number of leaves on a plant indicates the plant's performance. The statistical analysis shows an interaction between variety and calcium on the number of leaves at 5 WAP, which is presented in Table 3. The best interactions on the number of leaves variable, namely the Nauli F1 variety with the addition of 900 mg/L calcium nitrate (33.67 pieces) (Table 3). Environmental factors and genetic factors cause this interaction. The environmental factor in this research is the addition of calcium nitrate in hydroponic nutrients. Genetic factors play a role in plant responsiveness to the addition of calcium nitrate. In producing the number of leaves, the Nauli F1 variety was responsive to adding 900 mg/L of calcium nitrate. Plant responsibility can be seen from the calcium content data.

Table 1. Recapitulation of the Analysis of Variance (ANOVA) result of numerous measured variables as impacted by calcium concentrations, varieties and its combination.

No	Observation Variables	Variety	Calcium	Variety x Calcium
1	Plant height	ns	s	ns
2	Number of leaves	s	s	s
3	Leaf area (cm^2)	ns	s	ns
4	Root length (cm)	ns	s	ns
5	Root volume (ml)	ns	s	ns
6	Stem skim thickness (cm)	ns	s	ns
7	Growth rate index (g/week)	s	s	ns
8	Shoot Fresh Weight (g)	s	s	ns
9	Shoot Dry Weight (g)	ns	s	ns
10	Chlorophyll content (mg/g)	ns	s	ns

Note: ns: not significant; s: significant.

ISSN: 1412-4718, eISSN: 2581-138x

Table 2. Pak choy plant height in various varieties and concentrations of calcium addition.

Treatment factors	Plant height (cm)
Varieties	
Masbro (v_1)	29.09 a
Nauli F1 (v_2)	31.13 a
Flamingo (v_3)	30.09 a
CV (%)	7.27%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	30.51 a
300 mg/L (k_1)	30.71 a
600 mg/L (k_2)	30.15 ab
900 mg/L (k_3)	32.90 a
1200 mg/L (k_4)	26.13 b
CV (%)	7.27%

Note: Means on the same factor followed by the same letter are not significantly different based on the 5% DMRT.

Table 3. Number of pak choy leaves in various varieties and calcium concentrations.

Concentration of calcium nitrate addition	Masbro (V1)			Nauli F1 (V2)			Flamingo (V3)		
0 mg/L (k_0)	26.33	AB	a	27.67	BC	a	23.00	B	a
300 mg/L (k_1)	28.67	A	a	23.67	CD	b	20.00	BC	b
600 mg/L (k_2)	21.67	BC	b	30.33	AB	a	21.00	B	b
900 mg/L (k_3)	30.67	A	ab	33.67	A	a	28.33	A	b
1200 mg/L (k_4)	19.33	C	ab	22.33	D	a	15.33	C	b
CV (%)	11.31%			11.31%			11.31%		

Note: Means in the same column followed by the same capital letter are not significantly different based on the 5% DMRT; Means in the same row followed by the same lowercase letter are not significantly different based on the 5% DMRT.

Calcium (Ca) is a divalent cation and a vital element that plays a key role in maintaining the structure and permeability of cell membranes, as well as in processes such as plant cell division and elongation, carbohydrate transport, and nitrogen metabolism. During the early stages of leaf formation, an adequate calcium supply helps form new cells in the meristem (growth tissue), especially in the leaf primordia, which produces more leaves. Additionally, calcium is involved in regulating plant cell metabolism, signal transduction, and the uptake of nutrients through cell membranes (El-Beltagi & Mohamed, 2013).

Adding 1200 mg/L of calcium nitrate produced the lowest number of pak choy leaves, indicating calcium toxicity in pak choy plants. Calcium toxicity has side effects, including a deficiency of the Potassium nutrient. Potassium deficiency can hinder enzyme activity and plant photosynthesis (Divya et al. 2021). Sufficient potassium (K+) supply promotes photosynthetic assimilation, enhances nutrient uptake, and regulates leaf inclination by controlling turgor pressure. Potassium (K+) is essential in regulating stomatal opening, ensuring proper exchange of gases and water fluxes (Sardans & Peñuelas, 2021).

Leaf area. Leaf area is an essential indicator of a plant's photosynthetic capacity because it is directly related to the amount of light a plant can capture. The results of the DMRT test analysis on the effect of variety and calcium on leaf area are presented in Table 4.

Adding 300 mg/L calcium nitrate had a higher effect on leaf area, namely 85.33 cm^2, but not significantly different from adding 900 mg/L calcium nitrate, i.e., 82.56 cm^2. This is because the element calcium has a role in the formation, cell division, morphogenesis, and signaling in the formation and development of new plant organs (Himschoot et al., 2015). Calcium acts as a mitotic spindle and second messenger in the plasma membrane during the division process. Calcium is required to synthesize new cell walls, especially for the middle lamella. In the middle lamella, calcium forms electrostatic bonds with organic components (combining polygalacturonate with RCOO- groups (Huang et al., 2023). The membrane is maintained by calcium, which acts as a bridge between phosphate and carboxylate groups of phospholipids and surface proteins with the membrane (Melcrová et al., 2016).

Table 4. Leaf area of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Leaf area (cm)
Varieties	
Masbro (v_1)	71.89 a
Nauli F1 (v_2)	75.64 a
Flamingo (v_3)	78.94 a
CV (%)	23.13%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	74.92 ab
300 mg/L (k_1)	85.33 a
600 mg/L (k_2)	70.81 ab
900 mg/L (k_3)	82.56 a
1200 mg/L (k_4)	63.84 b
CV (%)	23.13%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Excessive calcium application can cause plant toxicity. This can be seen from the addition of 1200 mg/L $Ca(NO_3)_2$, which results in the lowest leaf area, i.e., 63.84 cm^2. Calcium toxicity can cause the absorption of magnesium (Mg) to be hampered. The Mg element plays a role in chlorophyll biosynthesis in plants. Hence, a decrease in Mg absorption in plants causes chlorosis, which decreases plants' photosynthesis rate (Gaj et al., 2018).

Stem skin thickness. The results of the variance analysis show that calcium influences stem skin thickness, which is presented in Table 4. Calcium treatment significantly affected stem skin thickness, as indicated by the addition of 900 mg/L calcium nitrate, which had the best impact, namely 0.12 cm, compared to other calcium treatments, which ranged from 0.08 - 011 cm. Wang et al. (2019) state that one of the benefits of calcium for plant growth is that it increases the development of plant leaves and stems. Calcium also affects cell elongation and division. Cell division and elongation will be directly proportional to the thickness of the stem skin obtained.

Adding 1200 mg/L calcium nitrate gave a lower result, namely 0.08 cm. This indicates the occurrence of toxicity in the pak choy plant. Nutrient toxicity is the level of a nutrient that can damage or be toxic to plants (Ahmad et al., 2016). Adding 1200 mg/L calcium nitrate produces lower stem thickness results than adding 900 mg/L calcium nitrate. This certainly affects the weight results that will be obtained later. High calcium content in the planting medium can harden plant cell walls, inhibit cell growth, disrupt phosphoric acid-based energy metabolism, and damage plant cytomembrane structures (Li et al., 2020).

Table 5. Stem skin thickness of pak choy in various varieties and concentrations of calcium addition

Treatment factors	Stem skin thickness (cm)
Varieties	
Masbro (v_1)	0.10 a
Nauli F1 (v_2)	0.09 a
Flamingo (v_3)	0.11 a
CV (%)	18.99%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	0.10 ab
300 mg/L (k_1)	0.11 ab
600 mg/L (k_2)	0.11 ab
900 mg/L (k_3)	0.12 a
1200 mg/L (k_4)	0.08 b
CV (%)	18.99%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Variety differences do not have a significant effect on stem skin thickness. The variety that produces the most considerable stem skin thickness is the Flamingo variety, with a stem skin thickness of 0.11 cm, but this is similar to other varieties, namely Masbro 0.10 cm and Nauli F1 0.09 cm. The thickness of the stem skin can be influenced by the variety used. Each variety has different genetic characteristics. This genetic trait influences stem thickness.

Root Length and Root Volume. Root length and volume are influenced by increasing calcium concentration. The results of the DMRT test analysis on the effect of variety and calcium on root length and root volume are presented in the Table 6.

The DMRT results stated that 900 mg/L calcium nitrate treatment gave better results in root volume, i.e., 40.89 ml, and root length, i.e., 49.47 cm. An increase in root length provides the most optimum nutrient availability for pak choy plants. Calcium ions regulate the flux of other

ISSN: 1412-4718, eISSN: 2581-138x

ions, such as potassium and chloride, across the cell membrane. This ion flux is crucial for maintaining cellular osmotic balance, cell expansion, and turgor pressure regulation (Kashtoh & Baek, 2021).

Adding 1200 mg/L calcium nitrate resulted in the lower root volume, i.e, 24.44 ml, and the lowest root length, i.e., 25.84 cm, in pak choy plants. This indicates that adding calcium nitrate that exceeds the optimum point for pak choy plant nutrition can reduce root volume and length because genetic diversity in root architecture may be limited.

Table 6. Root length (cm) and root volume (ml) of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Root length (cm)	Root volume (ml)
Varieties		
Masbro (v_1)	31.49 a	26.67 a
Nauli F1 (v_2)	37.79 a	32.93 a
Flamingo (v_3)	42.03 a	28.93 a
CV (%)	24.23%	24.06%
Concentration of calcium nitrate addition		
0 mg/L (k_0)	35.19 ab	24.89 b
300 mg/L (k_1)	41.47 ab	26.67 b
600 mg/L (k_2)	33.54 ab	30.67 ab
900 mg/L (k_3)	49.47 a	40.89 a
1200 mg/L (k_4)	25.84 b	24.44 b
CV (%)	24.23%	24.06%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Growth Rate Index. The results of the DMRT test analysis on the effect of variety and calcium on the growth rate index are presented in Table 7. Adding calcium nitrate 900 mg/L gave the best effect of 131.44 g/week. Providing calcium nitrate fertilizer can increase the absorption of calcium and nitrate, as well as increase plant yields (Seyfferth & Tsuda, 2014). The calcium element itself has a role in improving plant production. The growth rate index is based on the power value of the growth rate based on plant weight over time. The growth rate index will also be high if the plant weight is high. So, providing optimum calcium fertilizer for plants can increase the growth rate index.

Adding 1200 mg/L resulted in the lowest growth rate index of 31.28 g/week. This can be caused by giving too much calcium nitrate fertilizer, inhibiting pak choy plants' growth. According to Aryandhita & Kastono, (2021), excess calcium can inhibit plant production. The growth rate index is related to the fresh weight of the plant, so if production is not optimal, the growth rate index will be smaller.

Table 7. Growth rate index of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Growth rate index (g/week)
Varieties	
Masbro (v_1)	65.29 b
Nauli F1 (v_2)	86.05 a
Flamingo (v_3)	68.57 b
CV (%)	21.19%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	70.02 b
300 mg/L (k_1)	67.28 b
600 mg/L (k_2)	66.51 b
900 mg/L (k_3)	131.44 a
1200 mg/L (k_4)	31.28 c
CV (%)	21.19%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Variety treatment had a significant effect on the growth rate index. The variety that had the best influence on the growth rate index was the Nauli F1 variety, with an index value of 86.05 g/week. This is done by describing the varieties of each pak choy plant. In the description of plant varieties, the Nauli F1 variety has the highest productivity compared to other varieties, namely 37 – 39 tonnes/ha or 400 – 500 g/plant. This productivity is, of course, in line with the growth rate index, which is related to the fresh weight of the plant canopy. The greater the fresh weight of the canopy, the higher the growth rate index.

Shoot Fresh Weight and Shoot Dry Weight. The results of the DMRT analysis on the effect of variety and calcium on shoot fresh weight and shoot dry weight are presented in Table 8. Adding 900 mg/L calcium nitrate had the best effect on the variable fresh weight (364.15 g) and dry weight of the pak choy plant canopy (10.67 g). This is in line with research by Suryantini, et al. (2020), which stated that the addition of 90 g of calcium nitrate fertilizer (in 100 L) was able to increase production results because the availability of the nutrients provided was in a balanced state and by the needs of curly lettuce plants.

Table 8. Shoot fresh and dry weight of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Shoot fresh weight (g)	Shoot dry weight (g)
Varieties		
Masbro (v_1)	178.99 b	6.74 a
Nauli F1 (v_2)	257.96 a	8.53 a
Flamingo (v_3)	178.99 b	7.50 a
CV (%)	22.27%	23.00%
Concentration of calcium nitrate addition		
0 mg/L (k_0)	207.60 b	7.60 ab
300 mg/L (k_1)	197.42 b	7.07 ab
600 mg/L (k_2)	204.59 b	7.75 ab
900 mg/L (k_3)	364.15 a	10.67 a
1200 mg/L (k_4)	94.80 c	4.86 b
CV (%)	22.27%	23.00%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

The high fresh weight of the canopy is caused by the relatively high leaf area and number of leaves. In the leaf area analysis results, adding 300 mg/L Ca treatment produced the highest leaf area, 85.33 cm^2. However, this was not significantly different from the leaf area of the additional 900 mg/L Ca treatment, namely 82.56 cm^2. In the analysis of the number of leaves, the addition of 900 mg/L Ca treatment produced a higher number of leaves, namely 30.89, compared to the addition of 300 mg/L Ca treatment, namely 24.11. So, if the two variables are correlated, they are related to the fresh weight of the shoot produced.

In leaf vegetable commodities, an increase in the number of leaves will result in a higher fresh shoot weight. The wider the plant leaves, the wider the area to capture light processed in photosynthesis. The photosynthesis process increases with the increasing plant growth rate (Wang et al., 2019). When adding 1200 mg/L, fresh weight decreased due to the addition of excess calcium nitrate. The addition of excessive concentrations of calcium elements can inhibit the absorption of K or Mg elements. Both elements play a role in photosynthesis (Weng et al., 2022). Adding 1200 mg/L calcium nitrate resulted in the lowest dry weight of pak choy shoots. Plant dry weight reflects the nutritional status of the plant. Research conducted by Aryandhita & Kastono, (2021) stated that increasing calcium concentration can increase plant dry weight because adding calcium nutrients streamlines the plant's ability to absorb and use photosynthate. However, adding excess calcium can also inhibit plant growth and cause a decrease in yield.

The Nauli F1 variety had the best effect on the shoot's fresh weight, which was significantly different than others. Based on the description of Masbro, Nauli F1, and Flamingo varieties, it can be seen that Nauli F1 has the highest productivity compared to other varieties. The results of this research also align with the description of the variety, namely that the Nauli F1 pak choy variety has the highest productivity compared to other varieties.

Chlorophyll content. The results of the DMRT test analysis on the effect of variety and calcium on chlorophyll content are presented in Table 9. Adding 0 mg/L calcium nitrate was 15.51 mg/L chlorophyll content, significantly different from other calcium treatments. Chlorophyll is the main factor that influences photosynthesis. Photosynthesis is the process of changing inorganic compounds (CO_2 and H_2O) into organic compounds (carbohydrates) and O_2 with the help of sunlight (Ardiansyah et al., 2022).

Table 9. Chlorophyll content of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Chlorophyll content (mg/L)
Varieties	
Masbro (v_1)	9.88 a
Nauli F1 (v_2)	10.86 a
Flamingo (v_3)	11.14 a
CV (%)	22.73%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	15.51 a
300 mg/L (k_1)	9.31 bc
600 mg/L (k_2)	9.44 bc
900 mg/L (k_3)	12.49 ab
1200 mg/L (k_4)	6.39 c
CV (%)	22.73%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT Test.

The addition of calcium nutrients through fertilization has side effects in the form of deficiencies in the nutrients K and Mg when the Ca content is high in plants. K deficiency in plants can disrupt the photosynthesis process and plant enzyme activity (Aryandhita & Kastono, 2021). Photosynthesis is what will affect the chlorophyll content in plants. There is no diversity of chlorophyll in the varieties used. According to

Jurnal Kultivasi Vol. 23 (3) December 2024
ISSN: 1412-4718, eISSN: 2581-138x

Wang et al., (2023b), chlorophyll biosynthesis is carried out by specific genes in the chromosomes. This gene encodes an enzyme that will play a role in the biosynthesis pathway of tetrapyrrole (porphyrin nucleus) as the structural center of chlorophyll.

Calcium content. Calcium is a secondary macronutrient that is important in plant growth and development. Calcium is part of plant cell walls and plays a role in various metabolic processes, including activation of enzymatic systems, membrane stability, and cell integrity. The following regression graph presents the influence of variety and calcium on Ca content (Figure 1).

The results of the quadratic regression analysis (Figure 1) on V1 obtained the equation y= -3E -06x2 + 0.0043x + 4.5654. The optimum x value obtained from the quadratic equation is 716.67. So, the best calcium treatment based on the regression graph is between the 600 mg/L and 900 mg/L calcium nitrate treatments in V1. The R^2 value = 0.5508h indicates that calcium has a moderate influence on the Ca content of the Masbro variety. The results of the quadratic y= 6E-05x + 5.238. The graph shows that the linear line tends to be flat but is still increasing, even though it is small. The highest point of the linear line is at a calcium concentration of 1200 mg/L. The R^2 value = 0.0018 indicates that calcium weakly influences the Ca content of the Nauli F1 variety. The results of the quadratic regression analysis on V3 obtained the equation y = 0.0008x + 4.406. The graph shows that the linear line tends to rise and is at its highest point when the calcium concentration is 1200 mg/L. The R^2 value = 0.6381 indicates that calcium has a moderate influence on the Ca content of the Flamingo variety.

The variety that produces the highest Ca content is the Masbro variety, while the variety that produces the lowest is the Nauli F1 variety. In the variety description, the Nauli F1 variety has the highest productivity but has the lowest Ca content compared to other varieties. This can be indicated because the calcium absorption responsibility of each variety is different. The genetic characteristics of each plant variety cause this responsibility. So, even though the Nauli F1 variety has the highest productivity in the description, its responsibility for calcium nutrients is low. Different plant varieties may have inherent genetic differences that influence their ability to absorb and accumulate nutrients. Genetic variation can affect traits such as root morphology, nutrient uptake kinetics, nutrient transporters, and nutrient storage mechanisms (Griffiths et al., 2021).The study showed that adding 900 mg/L of calcium nitrate positively impacted plant growth. Specifically, the treatment led to an increase in both the number and the area of the leaves. As the number of leaves increased, the plant's ability to carry out photosynthesis was significantly enhanced. This improvement can be attributed to the fact that each additional leaf contributes to a larger overall surface area, which allows the plant to capture more sunlight. The plant can absorb more light with more leaves, increasing the production of energy-rich compounds (Zhou et al. 2023). This increased photosynthetic capacity supports faster growth and helps the plant accumulate more resources for development, ultimately improving overall plant growth.

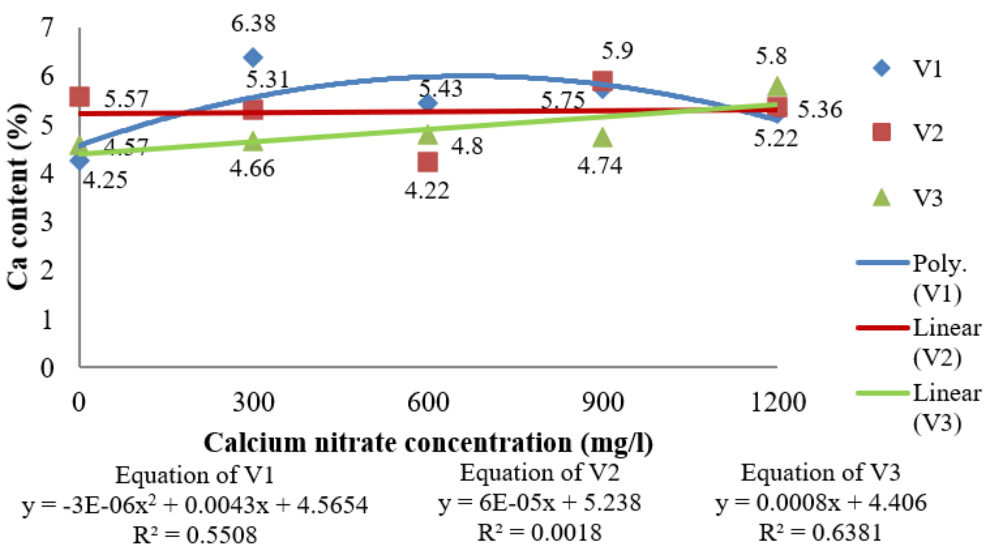

Figure 1. Ca Content of pak choy tissue in various varieties and Ca addition.

There was an increase in plant growth rate with the addition of 900 mg/L of calcium nitrate. As a result of this increased growth, the plants also showed a substantial increase in fresh weight. Additionally, the accumulation of photosynthates, sugars, and other organic compounds produced during photosynthesis was evident in the plant's dry weight. This suggests that the plants increased growth and became more efficient in storing energy, as evidenced by the increased accumulation of solid biomass. As the plants grow faster, they expand in size and devote more resources to developing structural components, such as roots, stems, and leaves. These structures store energy in the form of carbohydrates and other organic compounds produced during photosynthesis. The enhanced storage of energy in the form of solid biomass indicates that the plants could convert more of the absorbed nutrients into usable, long-term reserves. The study's findings show the significant role that calcium nitrate plays in supporting plant development. Providing calcium compounds can appear to stimulate growth processes and promote better nutrient utilization (Weng et al., 2022).

Although a 900 mg/L calcium nitrate increase significantly increased growth, it did not increase calcium accumulation in plant tissues. This is related to Calcium primarily functioning as a structural element in plant cells. Calcium stabilizes cell walls by forming pectate complexes in the middle lamella, which connect plant cells. Calcium regulates membrane structure and function and is a secondary messenger in signal transduction processes (Thor, 2019). Thus, calcium is not stored in large amounts like other nutrients but is incorporated into cellular structures and used in signaling. Although more calcium may lead to increased growth due to better cell wall structure and more vital cell membranes, the total accumulation of calcium in tissues may not increase significantly because plants use it for specific structural and regulatory roles rather than storing it in large amounts (Wdowiak et al., 2024).

Conclusion

Adding 900 mg/L calcium nitrate had the best effect on almost all variables except leaf area, chlorophyll content, and composite Ca content. However, the addition of 1200 mg/L calcium nitrate was indicated to be toxic to the growth of pak choy. The Masbro variety was responsive to increasing Ca content in the tissue, but the Nauli F1 and Flamingo varieties were not responsive to CA addition.

References

Ahmad P, Latef AAA, Abd-Allah EF, Hashem A, Sarwat M, Anjum NA, Gucel S. 2016. Calcium and potassium supplementation enhanced growth, osmolyte secondary metabolite production, and enzymatic antioxidant machinery in cadmium-exposed chickpea (*Cicer arietinum* L.). Frontiers in Plant Science, 7: 1–12.

Ardiansyah M, Nugroho B, Sa'diyah K. 2022. Estimating chlorophyll and n content in corn leaves based on chlorophyll content index. Jurnal Ilmu Tanah dan Lingkungan, 24: 53-61.

Aryandhita MI, Kastono D. 2021. Pengaruh pupuk kalsium dan kalium terhadap pertumbuhan dan kualitas hasil sawi hijau (*Brassica rapa* L.). Vegetalika, 10: 107-112.

Ceccherini GJ, de Lima BM, de Leme LT, Santucci LF, Verruma-Bernardi MR, Purquerio LFV, Sala FC. 2024. Agronomic biofortification of *Eruca sativa* L. with iron in nutrient film technique hydroponic cultivation. Australian Journal of Crop Science, 18: 145–152.

de Figueiredo MN, Martinez HEP, Fontes EAF, Milagres CDC, Silva JMD. 2022. Agronomic biofortification with iodine in lettuce plants cultivated in floating hydroponic system. Revista Ceres, 69: 210–217.

de Lima BM, Noboa CS, de Lima FM, da Costa MS, Purquerio LFV, Sala FC. 2023. Agronomic biofortification with zinc in hydroponically cultivated lettuce. Australian Journal of Crop Science, 17: 198–205.

Divya R, Sandhya V, Hitesh S. 2021. A review on effect of potassium and calcium on different parameters on plants under hydroponic condition. EPRA International Journal of Research & Development (IJRD), 1: 87–91.

do Moraes GVC, da Silva BH, Silva VFA, da Cunha FF, de Oliveira RA, de Oliveira JT, Silva PA. 2023. Influence of calcium on the development of corn plants grown in hydroponics. Agriengineering, 5: 623–630.

Jurnal Kultivasi Vol. 23 (3) December 2024
ISSN: 1412-4718, eISSN: 2581-138x

Elena CM, Cámara-Martos F, Obregón S, Rubén BF, de Haro A. 2021. Advances in breeding in vegetable brassica rapa crops. in brassica breeding and biotechnology. Intech Open. Spanish.

El-Beltagi, H. S., and Mohamed, H. I. (2013). Alleviation of cadmium toxicity in *Pisum sativum* L. seedlings by calcium chloride. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 41: 157–168.

Farid N, Ulinnuha Z. 2022. Pertumbuhan dan hasil genotipe bawang merah pada peningkatan dosis sulfur melalui sistem hidroponik nutrient film technique growth and yield of several genotypes of shallots (*Allium ascalonicum*) on increasing sulfur doses. Biofarm, 18: 102–115.

Gaj R, Budka A, Górski D, Borowiak K, Wolna-Maruwka A, Krzysztof B. 2018. Magnesium and calcium distribution in maize under differentiated doses of mineral fertilization with phosphorus and potassium. Journal of Elementology, 23: 137–150.

Gali´c L, Vinkovi´c T, Ravnjak B and Lonˇcari´c Z. 2021. Agronomic biofortification of significant cereal crops with selenium—A Review, Agronomy 11: 10-15.

Griffiths M, Roy S, Guo H, Seethepalli A, Huhman D, Ge Y, Sharp RE, Fritschi FB, York LM. 2021. A multiple ion-uptake phenotyping platform reveals shared mechanisms affecting nutrient uptake by roots. Plant Physiology, 185: 781–795.

Himschoot E, Beeckman T, Friml J, Vanneste S. 2015. Calcium is an organizer of cell polarity in plants. Biochimica et Biophysica Acta - Molecular Cell Research, 1853: 2168–2172.

Hosseini SA, Réthoré E, Pluchon S, Ali N, Billiot B, Yvin JC. 2019. Calcium application enhances drought stress tolerance in sugar beet and promotes plant biomass and beetroot sucrose concentration. International Journal of Molecular Sciences, 20: 1-22.

Huang W, Shi Y, Yan H, Wang H, Wu D, Grierson D, Chen K. 2023. The calcium-mediated homogalacturonan pectin complexation in cell walls contributes the firmness increase in loquat fruit during postharvest storage. Journal of Advanced Research, 49: 47–62.

Kashtoh H, Baek KH. 2021. Structural and functional insights into the role of guard cell ion channels in abiotic stress-induced stomatal closure. Plants, 10: 1-20.

Li F, He X, Tang M, Tang X, Liu J, Yi Y. 2020. Adaptation of plants to high-calcium content kart regions: Possible involvement of symbiotic microorganisms and underlying mechanisms. Brazilian Journal of Biology, 80: 209–214.

Melcrová A, Pokorna S, Pullanchery S, Kohagen M, Jurkiewicz P, Hof M, Jungwirth P, Cremer PS, Cwiklik L. 2016. The complex nature of calcium cation interactions with phospholipid bilayers. Scientific Reports, 6: 1–12.

Reitz NF, Shackel KA, Mitcham EJ. 2021. Differential effects of excess calcium applied to whole plants vs. excised fruit tissue on blossom-end rot in tomato. Scientia Horticulturae, 290: 1-7.

Sardans J, Peñuelas J. 2021. Potassium control of plant functions: Ecological and agricultural implications. Plants. 10: 1-31.

Seyfferth C, Tsuda K. 2014. Salicylic acid signal transduction: The initiation of biosynthesis, perception and transcriptional reprogramming. Frontiers in Plant Science, 5: 1-10.

Sirohiwal A, Neese F, Pantazis DA. 2019. Microsolvation of the redox-active tyrosine-d in photosystem ii: correlation of energetics with epr spectroscopy and oxidation-induced proton transfer. Journal of the American Chemical Society, 141: 3217–3231.

Suryantini NN, Wijaya G, Dwiyani R. 2020. Pengaruh penambahan $Ca(NO_3)_2$ terhadap hasil tanaman selada kriting (*Lactuca sativa* L.) pada sistem hidroponik deep flow technique (dft). Agrotrop : Journal on Agriculture Science, 10: 190-200.

Thor K. 2019. Calcium—nutrient and messenger. Frontiers in Plant Science, 10: 1-7.

Wang Q, Yang S, Wan S, Li X. 2019. The significance of calcium in photosynthesis. Int'l Journal of Molecular Sciences, 20: 1–14.

Wang Q, Zhang H, Wei L, Guo R, Liu X, Zhang M, Fan J, Liu S, Liao J, Huang Y, Wang Z. 2023. Yellow-green leaf 19 encoding a specific and conservative protein for photosynthetic organisms affects tetrapyrrole biosynthesis, photosynthesis, and reactive oxygen species metabolism in rice. International Journal of Molecular Sciences, 24: 1-26.

Wdowiak A, Podgórska A, Szal B. Calcium in plants: an important element of cell physiology and structure, signaling, and stress responses. Acta Physiologiae Plantarum, 46: 1-20.

Weng X, Li H, Ren C, Zhou Y, Zhu W, Zhang S, Liu L. 2022. Calcium regulates growth and nutrient absorption in poplar seedlings. Frontiers in Plant Science, 13: 1–15.

Yip DT, Boudsocq M. 2019. Properties and functions of calcium-dependent protein kinases and their relatives in *Arabidopsis thaliana*. New Phytologist, 224: 585–604.

Zhang XP, Ma CX, Sun LR, Hao FS. 2020. Roles and mechanisms of Ca^{2+} in regulating primary root growth of plants. Plant Signaling and Behavior, 15:1-6.

Zhou Z, Struik PC, Gu J, Peter ELVDP, Wang Z, Yin X, Yang J. 2023. Enhancing leaf photosynthesis from altered chlorophyll content requires optimal partitioning of nitrogen. Crop and Environment 2:24-36.