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Research article

The Response of Yield and Accumulated Iodine in Melon Affected by Biofortification with Potassium Iodide in Variance Rates under Greenhouse Conditions

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Abstract

This study examined the impact of potassium iodide (KI) application on iodine accumulation, growth rate, and yield quality in melons. A 2 × 5 factorial experiment was conducted in a completely randomized design with three replications, examining two factors: (1) melon cultivar (Fujisawa, designated as Fuj, and Sweet D25, designated as Swe) and (2) KI application rates (0.0, 4.5, 9.0, 13.5, and 18.0 mg/plant/time). The results showed that KI application at 9.0 mg/plant/time resulted in the highest fresh fruit weight and largest fruit size (both width and length) for both cultivars. In contrast, KI concentrations of 13.5 and 18.0 mg/plant/time reduced yield and fruit size, likely due to excessive iodine. Iodine accumulation was highest at 9.0 mg/plant/time, reaching 108.99 µg/100 g FW in Fuj and 62.22 µg/100 g FW in Swe, with these levels meeting the recommended daily iodine intake for humans. Besides, iodine and nutrient accumulation varied between cultivars: in Fuj, iodine was negatively correlated with potassium, whereas in Swe, iodine showed a positive correlation with potassium. These findings may inform biofortification strategies with iodine in melons and other plant species.

Keywords: Potassium iodide; iodine; melon; cultivar

1. Introduction

Iodine is a critical micronutrient essential for human health, supporting various physiological functions, particularly those related to the nervous system, brain development, and overall bodily functions. As a key component of thyroid hormones—thyroxine (T4) and triiodothyronine (T3)—iodine plays a vital role in regulating metabolic processes and maintaining homeostasis (Caffagni et al., 2012). According to the World Health Organization, the recommended daily iodine intake is 150 µg for adolescents and adults, 200 µg for pregnant and lactating women, and 250 µg specifically for pregnant

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women (WHO Secretariat et al., 2007). Iodine deficiency, which arises from insufficient intake, can lead to a range of disorders that disrupt thyroid hormone production. Such imbalance often results in hypothyroidism, a condition that can have serious health consequences, including cognitive impairments and developmental delays (Lisco et al., 2023). To address iodine deficiency, salt iodization has long been a widely adopted public health strategy, providing a cost-effective means of ensuring adequate iodine intake in many countries. Nonetheless, the rising global concern over the health risks associated with excessive salt consumption, particularly cardiovascular disease and hypertension, has led numerous nations to implement policies aimed at reducing salt intake (Gonzali et al., 2017). As a result, there is an increasing need to identify alternative strategies for boosting iodine levels in the diet without exacerbating the risks of excessive salt consumption.

Biofortification is an innovative approach to agriculture that reduces hidden hunger worldwide by increasing the nutritional value of staple food crops through genetic modification and agricultural techniques (Ashoka et al., 2023). Iodine biofortification of cultivated plants has emerged as a promising strategy to address iodine deficiency, offering a sustainable alternative to salt iodization, which has become increasingly restricted due to health risks associated with excessive salt intake (Winder et al., 2022; Ciriello et al., 2023). Research demonstrates that the application of potassium iodide and potassium iodate to crops, such as wheat, rice, and maize, enhances iodine accumulation in seeds, with higher iodine concentrations leading to greater uptake (Cakmak et al., 2017). Somma et al. (2024) found that applying potassium iodate at 1 mM to cherry tomatoes resulted in the highest iodine levels, sufficient to meet the requirements of both adults and pregnant women. Besides, Srichan (2015) discovered that applying potassium iodate at 5 mg/L as a foliar spray, and to the soil, significantly raised the iodine content in cucumbers. The foliar spray produced 825.34 µg/100 g dry weight, and the soil application produced 832.19 µg/100 g dry weight, compared to 558.85 µg/100 g dry weight in untreated cucumbers. Cucumbers showed the highest iodine accumulation among the horticultural crops studied (Melgoza et al., 2022). Given its potential to enhance iodine levels in commonly consumed fruits, iodine biofortification presents a viable solution for improving iodine intake in regions such as Thailand, where iodine deficiency remains a public health concern.

Melon (*Cucumis melo* L.) is an economically important crop that is cultivated in many regions worldwide due to its remarkable adaptability to diverse soil types and climatic conditions. The demand for its production and commercialization remains consistently high. Melon is a widely consumed fruit prized for its sweet flavor and pleasant aroma. The fruit's pulp contains approximately 90% water, along with 0.90 g of protein, 0.30 g of fat, 8.4 g of carbohydrates, 0.4 g of fiber, 11 mg of calcium, 17 mg of phosphorus, 309 mg of potassium, 0.21 mg of iron, 9 mg of sodium, 26 mg of vitamin C, and provides 345 kcal per 100 g (Maynard & Hockmuth, 2007). Farmers cultivate melons either as a primary crop or a supplementary crop, providing them with an additional source of income, especially when the yield is of high quality and can command a premium price. Recent studies have investigated the potential of biofortification to increase iodine content in melons, demonstrating that optimizing the fortification rate can significantly enhance iodine accumulation (Melgoza et al., 2022; Andrade-Sifuentes et al., 2024). Compared to potassium iodate, potassium iodide results in greater iodine accumulation in melons, with soil application proving more effective than foliar spray for iodine uptake (Melgoza et al., 2022).

In Thailand, melons represent a promising alternative crop to combat iodine deficiency while providing a viable income for farmers. Two popular cultivars, *C. melo* var. *reticulatus* Naudin (netted melon) and *C. melo* var. *inodorus* Naudin (cantaloupe), are widely grown, each exhibiting distinct characteristics such as fruit size, texture, and nutrient

accumulation (Thongket, 2002). These differences are critical, as they affect the nutritional composition of the fruit, including its iodine content, as well as the cultivation practices required for optimal yield (Khomphet et al., 2023; Nur et al., 2024). Nevertheless, the iodine biofortification potential of these cultivars has not been extensively studied. Therefore, a comparative investigation of iodine fortification across various melon cultivars is necessary to establish best practices for enhancing their nutritional value and to maximize the economic benefits for farmers. This study aims to determine the optimal iodine levels for melon growth and evaluate iodine concentrations in melon yields, thereby providing guidelines for addressing iodine deficiency and increasing the commercial appeal of melons as a functional food option for consumers.

2. Materials and Methods

2.1 Experimental design

The experiment was conducted in 2 x 5 factorial in a completely randomized design, with three replications. Each replication involved one melon plant per pot, resulting in a total of 30 pots. The study investigated two factors: (1) two melon cultivars, Fujisawa (Fuj) and Sweet D25 (Swe), and (2) five potassium iodide (KI; Analytical reagent brand KEMAUS) application rates (0, 4.5, 9.0, 13.5, and 18.0 mg/plant/time). The experiment was conducted in a greenhouse at the Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, from 12 November 2023 to 25 January 2024. Potassium iodide (KI, molecular weight = 214 g/mol) was prepared by dissolving varying amounts (0.102 g, 0.203 g, 0.304 g, and 0.405 g) in 1 L of distilled water to achieve the concentrations of five application rates as mentioned above. Starting 15 days after transplantation, the KI solution was applied along with the nutrient solution through the growing medium, with ten applications spaced 5 days apart, continuing until harvest at 70 days after sowing. The total number of applications was ten, made at 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 days after sowing.

2.2 Cultivation and maintenance

Ten-day-old seedlings were transplanted into 14-inch plastic pots, each containing 6 kg of coconut coir as the growing medium. One plant was placed per pot, with 30 cm spacing between pots within a row and 1.0 m between rows. Drip fertigation was used to supply a standard plant nutrient solution, Enshi Solution (Shinohara & Suzuki, 1988), which consists of two components: Solution A, containing $Ca(NO_2)_{3\cdot4}H_2O$ and Fe-EDTA, and Solution B, containing KNO_3, $NH_4H_2PO_4$, $MgSO_4\cdot7H_2O$, and essential micronutrients (H_3BO_3, $MnSO_4\cdot4H_2O$, $ZnSO_4\cdot7H_2O$, $CuSO_4\cdot5H_2O$, $NaMoO_4\cdot2H_2O$). During the first 35 days after transplanting, Solutions A and B were mixed with filtered water, and the electrical conductivity (EC) was maintained between 1.7 and 1.8 dS/m. For the next 28 days, the EC was adjusted to 2.5 dS/m by mixing solutions A and B. For the final growth phase, the EC was adjusted to 2.0 dS/m until harvest (Chanthaprom, 2015). Throughout the experiment, each plant received an equal amount of water and fertilizers. Irrigation was initially set at 0.5 L per plant per day. During the flowering and fruiting stages, this amount was increased to 2 L per plant per day.

To ensure proper plant development, nodes 1 through 8 were pruned, while nodes 9 through 12 were retained for pollination. Pollination occurs daily between 6:00 a.m. and 10:00 a.m. Once each plant produced 25 true leaves, the main vine was pruned at the top,

and one fruit was selected per plant. Harvesting for both melon cultivars was conducted 75 days after sowing (40-45 days post-pollination), as specified by the experimental treatments. In preparation for harvest, watering was gradually reduced starting one week before harvest and minimized in the final two days until slight wilting was observed during the day.

2.3 Data collection

The yield and its components were evaluated by measuring fresh fruit weight and dimensions using both transverse (width) and longitudinal (length) diameters. After postharvest, nutrient accumulation in the melon plants was assessed. The fruits were harvested, dried at 70°C for 72 h, and subsequently ground before undergoing wet digestion. The nutrient content of the samples was analyzed using standard methods: N content was determined via the Kjeldahl method (Jones et al., 1991), P content was quantified using the colorimetric method (Murphy & Riley, 1962), K content was measured through atomic absorption spectrophotometry (FS220, Agilent), and I content was analyzed using the colorimetric method (Jopke et al., 1996).

2.4 Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the differences in fresh fruit weight, dimensions (width and length), macronutrient content (N, P and K), and accumulated I content in the melon flesh. Post hoc comparisons of treatment means were conducted using Duncan's New Multiple Range Test at a 95% confidence level. Moreover, Principal Component Analysis (PCA) was utilized to explore the relationships among various factors influencing melon growth, including fruit weight, dimensions, macronutrient content, and I accumulation.

3. Results and Discussion

3.1 Fresh weight and size of melons

Significant differences in fresh fruit weight were observed between the two melon cultivars (Table 1). The Fuj melon cultivar exhibited the highest average fresh fruit weight at 1,527 g, followed by the Swe cultivar with an average weight of 1,060 g. These findings are consistent with those of Chikh-Rouhou et al. (2019), who indicated that melon yield quality was influenced by a combination of genetic factors, plant physiology, and environmental conditions. Thus, growth parameters such as fresh fruit weight, size, pulp thickness, and soluble solids content can vary significantly between cultivars. Furthermore, the application of varying KI levels resulted in statistically significant differences in melon fruit weight. The highest average fruit weight, 1,322 g, was observed in melons treated with 9.0 mg KI/plant/time, followed by 1,305 g, 1,305 g, and 1,296 g for melons treated with 0, 13.5, and 4.5 mg KI/plant/time, respectively. In contrast, melons treated with 18.0 mg KI/plant/time exhibited the lowest average fruit weight of 1,243 g. This outcome aligns with the findings of Melgoza et al. (2022), who reported that excessive iodide and iodate concentrations above 1 mM (≈ 18.0 mg/plant/application) resulted in a decrease in melon weight, likely due to toxicity. In the current study, similar reductions in fruit weight were observed at the 18.0 mg/plant/time iodide concentration, confirming the potential for iodide toxicity at high application rates. Furthermore, the study identified potential toxicity symptoms

Table 1. Fresh fruit weight (g) and fruit size (cm) of different melon cultivars treated with different levels of potassium iodide at the harvesting stage

Factor	Fruit Weight (g)	Fruit Size (cm)	
		Fruit Width (cm)	Fruit Length (cm)
Melon Cultivars (C)			
Fuj	1527A	13.91A	14.15A
Swe	1060B	10.93B	12.58B
F-test	**	**	**
Application of potassium iodide with nutrients solution (KI: mg/plant/time.)			
0	1305AB	12.57	13.58
4.5	1296B	12.33	13.59
9	1322A	12.85	13.68
13.5	1305AB	12.43	13.23
18	1243C	11.93	12.77
F-test	**	ns	ns
Interaction (CxKI)			
Fuj*0	1537	14.50	14.47
Fuj*4.5	1517	14.17	14.25
Fuj*9.0	1558	14.27	14.57
Fuj*13.5	1552	13.93	13.93
Fuj*18.0	1475	12.70	13.53
Swej*0	1074	10.63	12.70
Swe*4.5	1075	10.50	12.87
Swe*9.0	1085	11.43	12.80
Swe*13.5	1058	10.93	12.53
Swe*18.0	1010	11.17	12.00
F-test	ns	ns	ns

Means followed by different letters are significantly different by DMRT, ** = significant at p≤0.01; ns = Not significant at p>0.05

even at a KI concentration of 13.5 mg/plant/time, suggesting a new threshold for iodide toxicity in melons.

Melon sizes, measured in both width and length, differed significantly between the two cultivars (Table 1). The Fuj cultivar produced the widest fruit, with an average width of 13.91 cm, while the Swe cultivar had a smaller average width of 10.93 cm. Similarly, in terms of length, the Fuj cultivar yielded the longest fruit at 14.15 cm, compared to 12.58

cm for the Swe cultivar. Although varying KI application rates did not affect melon fruit size, it is possible that other nutrients, such as calcium and boron (Ca-B), played a role in influencing these dimensions. Saengngam et al. (2022) reported that melons treated with different levels of Ca-B exhibited statistically significant differences in both fruit width and length, suggesting that these nutrients may contribute to variations in melon size.

3.2 The macronutrients and accumulated iodine in melon

The study revealed significant differences in the N, P, K, and I content in the melon flesh, influenced by varying KI application rates, with notable interactions between the two factors (Table 2). The first factor, melon cultivar, showed that the Fujisawa cultivar accumulated significantly higher levels of N (7.13%), P (0.54%), K (2.21%), and I (83.04 mg/kg) compared to the Swe cultivar, which accumulated 6.25%, 0.47%, 1.92%, and 46.2 mg/kg, respectively. The difference can be attributed to the larger physiological size of the Fuj cultivar, which enhances nutrient absorption and facilitates greater cell division, thus promoting superior fruit growth (Saengngam et al., 2025). The second factor, KI application rate, also had a significant effect on nutrient content in the fruit flesh. The 9.0 mg/plant/time application rate resulted in the highest N accumulation (3.62%), which was significantly different from other application rates. The 9.0 mg/plant/time rate also led to the highest accumulation of K (2.70%) and I (99.65 mg/kg) in the melon flesh.

In contrast to the other nutrients, P accumulation exhibited a different pattern, with the highest accumulation observed at the KI application rate of 18 mg/plant/time (0.651%). Conversely, the absence of KI application (0 mg/plant/time) resulted in the lowest accumulation of N (2.44%), P (0.39%), and I (17.99 mg/kg). Interestingly, K accumulation was lowest at the highest KI application rate (18 mg/plant/time). The effects of KI application on N, P, K and I accumulation varied, with each nutrient being influenced differently by the treatment levels. Such a variability aligns with the findings of Blasco et al. (2012) and Flores et al. (2016), who reported that iodine application led to distinct patterns of nutrient accumulation in plants. These patterns can be attributed to the fact that excessive I application can be toxic to plants, whereas appropriate levels of I can provide beneficial effects. As noted by Duborska et al. (2020), the impact of I on plant nutrient accumulation depends on several factors, including the plant species, application rate, method of application, and the plant's sensitivity to I.

Overall, nutrient accumulation patterns largely reflected the trend observed in the fresh fruit weight of melons, with nutrient levels peaking at a KI concentration of 9 mg/plant/time and subsequently decreasing at higher concentrations. This trend is consistent with the findings of Melgoza et al. (2022), who suggested that excessive KI application could induce yield decreased. The results imply the possibility of antagonistic interactions between I and other nutrients, as reported by Blasco et al. (2012).

When examining the interaction between melon cultivar and KI application rates, the Swe cultivar, when treated with 9 mg/plant/time of KI, exhibited the highest N content in the fruit flesh (3.93%), significantly higher than other cultivars and treatment rates. For P, the highest accumulation was observed in the Fuj cultivar at a KI rate of 18 mg/plant/time (0.685%), which was not statistically different from Swe (0.617%). Interestingly, K accumulation was highest in the Fuj cultivar without KI application (3.37%), and this level was not significantly different from that in Swe treated with 9 mg/plant/time of KI.

I accumulation followed a distinct pattern, with the highest levels observed in the Fuj cultivar treated with 9 mg/plant/time of KI (130.74 mg/kg). In general, I accumulation in

Table 2. Content of nitrogen, phosphorus, potassium, and iodine in flesh fruit of different melon cultivars (%) under different potassium iodide application rates at harvest stage

Factor	N Concentration in Flesh (%)	P Concentration in Flesh (%)	K Concentration in Flesh (%)	I Concentration in Flesh (mg l/kg)
Melon Cultivars (C)				
Fuj	7.13A	0.54A	2.21A	83.04A
Swe	6.25B	0.47B	1.92B	46.20B
F-test	**	*	**	**
Application of potassium iodide with nutrients solution (KI: mg/plant/time)				
0	2.44c	0.39d	2.08b	17.99d
4.5	2.69c	0.44d	2.15b	40.41c
9.0	3.62a	0.49c	2.70a	99.65a
13.5	3.21b	0.56b	1.76c	84.47b
18.0	2.45c	0.65a	1.66c	80.69b
F-test	**	**	**	**
Interaction (CxKI)				
Fuj*0	2.77*d-e*	0.40e	3.37*a*	20.04*f*
Fuj*4.5	2.88*b-e*	0.49*de*	3.01*b*	45.82*e*
Fuj*9	3.29*b*	0.54b*c*	2.12*c*	130.74*a*
Fuj* 13.5	3.16*b-d*	0.59*b*	1.31*d*	109.47*b*
Fuj*18	2.84*c-e*	0.68*a*	1.25*d*	109.15*b*
Swe*0	2.10*f*	0.37e	0.78*e*	15.93*f*
Swe*4.5	2.51*e*	0.38*e*	1.29*d*	34.97*e*
Swe*9	3.93*a*	0.44*de*	3.29*a*	68.38*c*
Swe* 13.5	3.25*b-c*	0.53b*c*	2.19*c*	59.47*d*
Swe*18	2.06*f*	0.62*ab*	2.07*c*	52.25*d*
F-test	**	**	**	**

Means followed by different letters are significantly different by DMRT, ** = significant at p≤0.01; ns = Not significant at p>0.05

both cultivars showed a similar trend: it increased with KI concentrations of 0, 4.5, and 9.0 mg/plant/time, but declined when the concentration exceeded 9.0 mg/plant/time (at 13.5 and 18.0 mg/plant/time), as illustrated in Figure 1. This suggests that while moderate levels of KI promote I uptake, higher concentrations may be detrimental to its accumulation, further supporting the idea that excessive KI application may lead to toxicity or interfere with nutrient balance in melon plants.

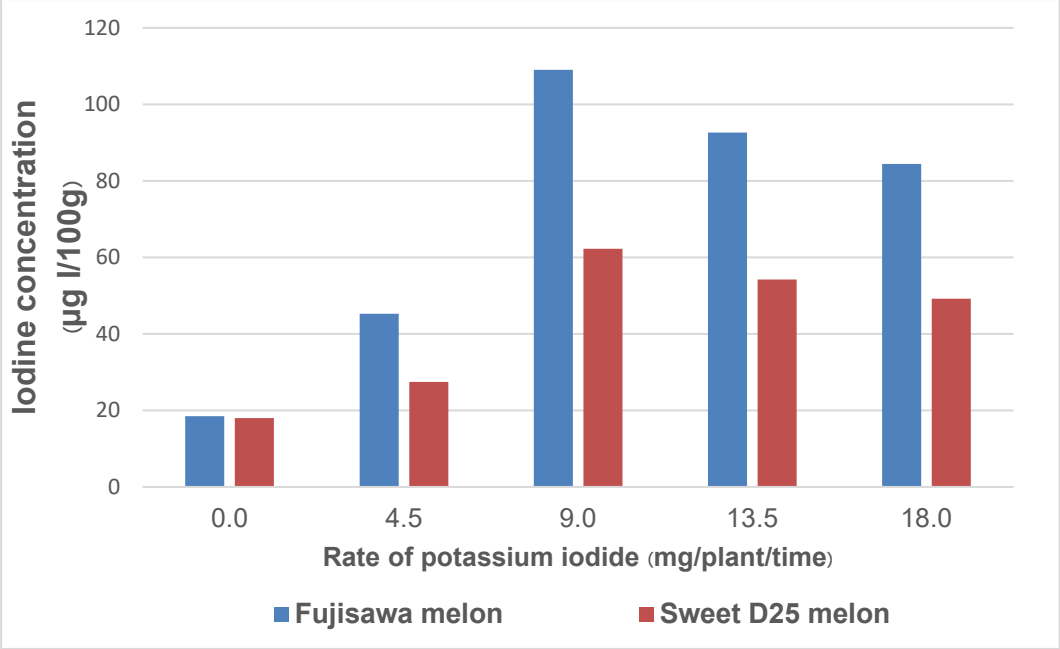


Figure 1. Iodine concentrations in fresh fruit for consumption of two melon cultivars treated with different levels of potassium iodide

This finding aligns with the fresh fruit weight values presented in Table 1, which showed a decline at a KI concentration of 13.5 mg/plant/time. The trends in I accumulation and fresh fruit weight observed in this study are consistent with the results of Melgoza et al. (2022), who reported an increase in I accumulation with rising KI concentrations, followed by a decline at excessively high concentrations. The decline is attributed to the toxic effects of excessive I application on plants (Duborska et al., 2020). In the current study, toxicity symptoms were observed at a concentration of 13.5 mg/plant/time, which was lower than the threshold reported by Melgoza et al. (2022), suggesting that a KI concentration of 13.5 mg/plant/time may already be detrimental to melon plants. The accumulation of primary macronutrients (N, P, and K) varied across different cultivars, a finding consistent with the work of Mackowiak and Grossl (1999), who explained that I uptake in plants was influenced by several factors, including genetic variation, plant species, environmental conditions, and the quantity and form of I available in the root zone. This accentuates the complex interplay between nutrient availability and plant physiology, underscoring the need to carefully consider I application rates in relation to cultivar-specific characteristics and environmental factors.

The results demonstrate that increasing KI concentration in the growing medium leads to higher I accumulation in both melon cultivars. The I levels reached in these melons are sufficient to contribute significantly to the daily iodine intake requirements for human consumption. According to the European Food Safety Authority (EFSA, 2006), the recommended daily I intake for adults is 150 µg. At a KI application rate of 9.0 mg/plant/time, which yielded the highest I accumulation in both cultivars (Figure 1), consumers who eat Fuj melons, which contain 108.99 µg of I per 100 g of fresh weight, or Swe melons, which contain 62.22 µg per 100 g, can meet a substantial portion of their daily

I needs. In contrast, melons without iodine supplementation, such as Fuj and Swe, which accumulate only 18.22 and 17.97 µg of I per 100 g of fresh weight, respectively, provide much lower amounts of I. These findings indicate that consuming either the Fuj or Swe melons, when supplemented with appropriate I levels, could offer a practical dietary solution to help prevent I deficiency disorders. By incorporating these melons into a normal dietary routine, consumers could address I deficiencies while maintaining a balanced, everyday diet. This stresses the potential of biofortified melons as a valuable dietary source of I, contributing to public health initiatives aimed at preventing iodine deficiency.

3.3 Correlation between yield components, macronutrient content, and iodine accumulation in melons

The principal component analysis (PCA) results revealed correlations between various melon components, highlighting both similarities and differences between the two cultivars. For the Fuj cultivar (Figure 2a), the first two principal components (PC1 and PC2) together accounted for 85.1% of the total variance, with 56.5% of the variance explained by PC1 and 28.6% by PC2. A strong positive correlation was observed among most yield components, particularly fruit weight and size (width and length). However, no significant correlation was found between these yield components and the P and K content. Regarding macronutrients, both positive and negative correlations with iodine accumulation were noted. Specifically, P and N exhibited a positive correlation with I, whereas K showed a negative correlation.

In contrast, for the Swe cultivar (Figure 2b), PC1 and PC2 together explained 78.2% of the total variance, with 42.2% attributed to PC1 and 36.0% to PC2. Like the Fuj cultivar, yield components (fruit weight, width, and length) in Swe were positively correlated. However, I accumulation in Swe was positively correlated with K, which contrasted with the results for Fuj. Nevertheless, I accumulation in both cultivars remained positively correlated with N and P, indicating that while the pattern of nutrient accumulation in response to I application varied between the two cultivars, the general trend of I being positively associated with certain macronutrients (N and P) was consistent.

These findings indicate that while both cultivars show similar relationships between yield components and certain nutrients, their responses to I application differ, particularly in terms of the interaction between I and K. It depicts the cultivar-specific nutrient dynamics influenced by I supplementation.

4. Conclusions

This study investigated the effects of different melon cultivars and varying KI application rates on growth, yield, and nutrient accumulation. The results indicated that the Fuj cultivar exhibited superior fruit weight and size (both width and length) compared to the Swe cultivar. When both cultivars were treated with a KI application rate of 9.0 mg/plant/time, the highest I accumulation in the fruit fresh was observed, with 108.99 µg per 100 g of fresh weight for Fuj and 62.22 µg for Swe. However, when the KI application rate exceeded 9.0 mg/plant/time, signs of negative impact of I were observed, which led to reduced yield, lower macronutrient accumulation, and a decrease in iodine content.

Moreover, nutrient accumulation patterns varied between the two cultivars. In Fuj, I accumulation was negatively correlated with K, while in Swe, I accumulation was positively correlated with K. These differential responses suggest that cultivar-specific

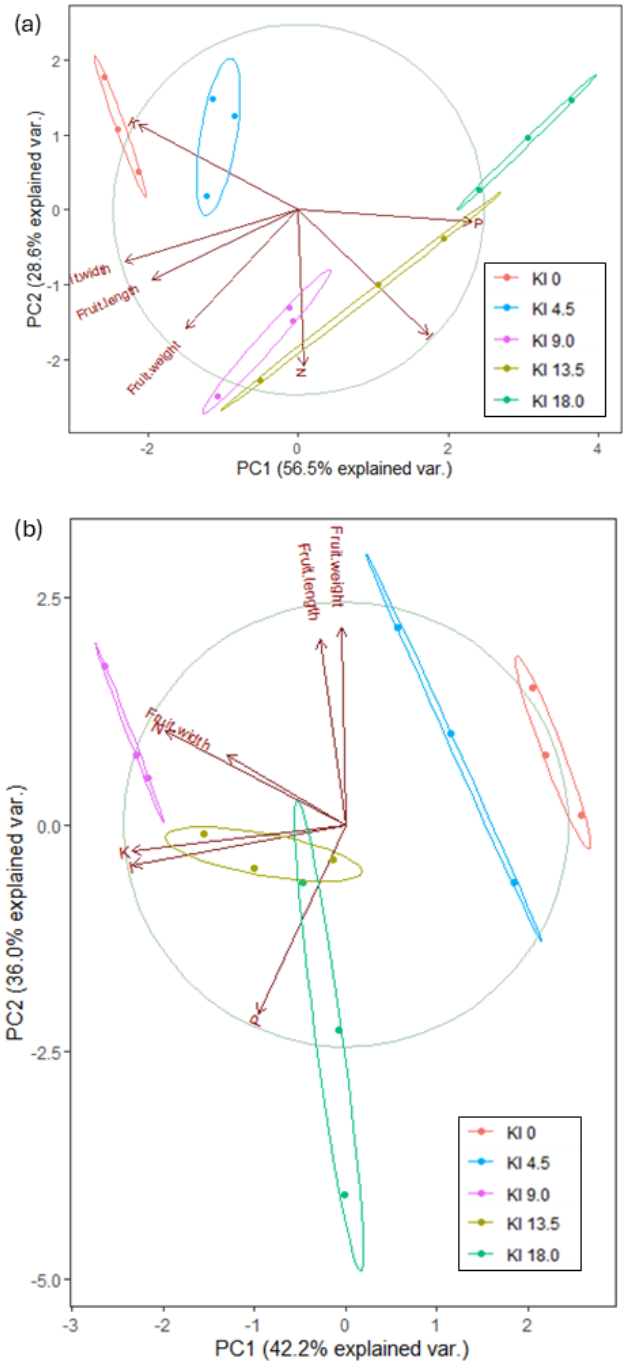


Figure 2. Principal component analysis of yield components, plant nutrients, and iodine in melons across all treatments for (a) Fuj and (b) Swe cultivar.

strategies may be necessary for optimizing I supplementation in melons and potentially in other plant species. This study contributes valuable insights into the management of iodine levels in crop production and highlights the importance of cultivar selection and nutrient balance for achieving optimal yield and I content. However, further research is needed in other areas and seasons, including both greenhouse and field systems, to provide more comprehensive data.

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6. Authors' Contributions

Thamthawat Saengngam: experimental design, performed the experiment, data collection and analysis, and prepared original manuscript. Tawatchai Inboonchuay: suggested the concept idea and improved experimental design and manuscript. Sirisuda Bootpetch: prepared and performed chemical analysis, suggested an idea for experimental design and improved manuscript. Chaisit Thongjoo: supervised and conceived this research, reviewed and improved manuscript. Pongpet Pongsivapai: improved experimental design, data analysis, reviewed and edited final draft of manuscript.

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7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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