

Effects of Electrical Conductivity and Fertilizer Frequency on the Growth, Yield and Some Quality Attributes of Wasabi cv. 'Daruma' (*Eutrema japonicum*) in Substrate Culture System

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Abstract

This study examined the combined effects of electrical conductivity (EC) levels and fertilizer frequency on the growth, yield, and some quality attributes of wasabi (*Eutrema japonicum* cv. 'Daruma') under controlled conditions. A nine-month factorial experiment using three EC levels (1, 2, and 3 mS cm⁻¹) and two fertilizer frequencies (weekly and biweekly) revealed significant EC × fertilizer frequency interactions. The combination of moderate EC (2 mS cm⁻¹) and weekly fertilization resulted in the highest photosynthetic rate, vigorous growth, and greatest rhizome yield, while also enhancing the 2-propenyl-glucosinolate content in rhizomes during storage. In contrast, low EC with biweekly fertilization limited growth, whereas high EC (3 mS cm⁻¹) mainly promoted petiole and leaf elongation without yield improvement. Overall, optimal EC management combined with frequent fertilization synergistically improved nutrient uptake, physiological efficiency, and storability, providing an effective strategy for high-quality wasabi production.

Keywords: Brassicaceae, Glucosinolates, Wasabi, Electrical conductivity (EC), Fertilization frequency

Introduction

Wasabi (*Eutrema japonicum*), a member of the Brassicaceae family, is a traditional condiment crop from Japan, widely valued for its pungent paste used in Japanese cuisine (Douglas and Follett, 1992). Its rhizome, leaves, and petioles are the main parts utilized for culinary and commercial purposes (Giacoppo et al., 2015). Moreover, isothiocyanates (ITCs), the key flavor compounds in wasabi, have been reported to offer additional health benefits, including antioxidants and anticancer properties (Hara et al., 2003). Wasabi is classified as a high-value plant in the global market, valued at USD 0.47 billion in 2024, and the market is projected to reach USD 0.99 billion by 2033, growing at a CAGR of 8.58% during the forecast period from 2025 to 2033 (Business Research Insights, 2024). Fresh wasabi stems are highly prized in premium markets. Although commonly called rhizomes or roots, botanically they are classified as stems. Marketable stems generally range from 2 to 4 inches in diameter and 6 to 12 inches in length. These stems are typically grated to create the characteristic green paste served with sushi, sashimi, and noodle dishes. Smaller or cosmetically imperfect stems are often dried and ground into powder, which can be used in processed foods such as rice crackers or in tube-packaged wasabi pastes (Chadwick, 1990). However, the cultivation of wasabi is challenging, particularly in terms of nutrient management. The appropriate fertilizer dosage for wasabi remains uncertain (Miles and Daniels, 2019), and there are differing opinions among traditional Japanese farmers, modern growers, and global researchers on whether fertilizers are necessary at all (Lefebvre, 2020).

Postharvest management of crops is closely linked to pre-harvest practices, as the quality of produce cannot be improved once harvested, but it can be maintained through proper handling. The final market value and consumer acceptance largely depend on appropriate pre-harvest techniques, careful harvesting, and postharvest management. Among the key pre-harvest factors, plant nutrition plays a critical role in determining both the quality and postharvest life of horticultural crops. Balanced provision of essential

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nutrients is vital for optimal plant growth and development, while nutrient excess or deficiency can significantly reduce product quality and storability. Other pre-harvest factors affecting postharvest quality include irrigation frequency, use of fertilizers and growth regulators, climatic conditions, natural environment, and plant physiological status. Collectively, these factors influence the chemical composition, morphology, physiology, and long-term storability of the produce (Cuquel et al., 2011; Thokar et al., 2022).

Proper nutrient availability and favorable environmental conditions are essential for optimal plant physiology, growth, and development (Savvas et al., 2008; Signore et al., 2016). Electrical conductivity (EC) is widely used as an indicator of salt and electrolyte concentrations in solutions, with the EC of nutrient solutions reflecting the ions accessible to plants in the root zone. Since the optimal EC differs by crop and is shaped by environmental factors, proper management is essential for maintaining plant growth and productivity (Krishna and Nemali, 2002; Le Bot et al., 1998; Sonneveld and Voogt, 2009). High EC levels generally inhibit nutrient uptake by elevating the osmotic pressure of the nutrient solution, which not only wastes nutrients but also increases nutrient leaching into the environment, thereby contributing to pollution. In contrast, low EC may drastically impair plant vigor and reduce overall yield (Samarakoon et al., 2006; Signore et al., 2016). It has been reported that, three weeks before harvest, the application of $6.0 \text{ dS}\cdot\text{m}^{-1}$ results in higher shoot fresh weight, dry weight, total glucosinolate, and total phenolic content compared to an application of $1.5 \text{ dS}\cdot\text{m}^{-1}$ in *Brassica oleracea* L. (Lee et al., 2024). A previous study found that *Brassica campestris* L. ssp. *Chinensis* showed increased chlorophyll, ascorbic acid, and nitrate contents under high EC treatment compared to low EC, while crude fiber and soluble sugar contents decreased. The application of nutrient solutions at either high or low EC levels induced nutrient stress, enhanced plant antioxidant enzyme activities, and suppressed plant growth and quality (Ding et al., 2018).

Effective modification of fertilizer application strategies is a key requirement for agronomists and farmers to sustain yield levels and improve nutrient use efficiency (Hoffmann et al., 2017). Rubio-Asensio and Intrigliolo. (2024) reported that high-frequency fertigation enhanced shoot fresh weight (harvest yield) by increasing greater shoot water content in *Cichorium endivia* L. Another study found that vegetative growth, biomass accumulation, nutrient uptake, and transport efficiency were greater with six fertilizer applications than with four or eight applications in *Magnolia wufengensis* (Deng et al., 2019). In *Brassica oleracea* L. var. *capitata*, a species in the same family as wasabi, the maximum plant height, head weight, head length, head diameter, head volume, and head width were observed under the application of 100% RDF split into 12 equal doses at 5-day intervals (Nikzad et al., 2020). Nevertheless, the appropriate fertilizer frequency should be determined for each plant species. Excessive frequency may be unnecessary and ineffective; therefore, identifying the optimal frequency is essential (Cai et al., 2023).

We hypothesize that optimizing EC levels and fertilizer frequency in wasabi could promote plant growth and yield, while enhancing post-harvest quality through improved glucosinolate content. To test this hypothesis, a nine-month experiment was conducted in a controlled-environment plant factory using a substrate culture system to evaluate the effects of different EC levels and fertilizer frequencies on wasabi growth, yield, and glucosinolate content at harvest and after storage.

Materials and Methods

Plant Materials and Growth Conditions

The experiment was conducted at the H.M. The King Initiative Centre for Flower and Fruit Propagation, Chiang Mai University, Thailand. One-month-old wasabi seedlings (cv. Daruma) were transplanted into a substrate mixture of cocopeat and rice husk at a 1:3 ratio. The plants were cultivated in a plant factory

under a light intensity of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a 12-hour photoperiod and a temperature range of 15–18 °C. The treatments began two weeks after transplantation and continued until harvest. Nutrient solutions with three electrical conductivity (EC) levels (1, 2, and 3 mS cm^{-1}) and two fertilizer frequencies (weekly and biweekly) were applied manually at 100 mL per plant. The EC levels of the nutrient solutions were adjusted accordingly each week or every two weeks, depending on the treatment. Plants were watered at two-day intervals throughout the experiment. The pH of the solutions was maintained at 6.0 by adjusting with diluted HCl or NaOH. The nutrient solution composition was based on a modified Hoagland formulation. Each nutrient solution contained equal concentrations of macronutrients and micronutrients: nitrogen (N) at 300 mg L^{-1} , phosphorus (P) at 50 mg L^{-1} , potassium (K) at 100 mg L^{-1} , sulfur (S) at 100 mg L^{-1} , calcium (Ca) at 100 mg L^{-1} , magnesium (Mg) at 60 mg L^{-1} , iron (Fe) at 3.00 mg L^{-1} , boron (B) at 0.24 mg L^{-1} , manganese (Mn) at 0.02 mg L^{-1} , zinc (Zn) at 0.02 mg L^{-1} , copper (Cu) at 0.01 mg L^{-1} , and molybdenum (Mo) at 0.05 mg L^{-1} . Each treatment was replicated five times, with one wasabi plant per replicate.

At the end of the experiment, freshly harvested wasabi rhizomes were trimmed to remove leaves and roots, then cleaned thoroughly. The cleaned rhizomes were wrapped in tissue paper and placed in tightly sealed containers. The samples were stored in a refrigerator at 4 °C, and rhizomes were collected for analysis of the remaining pungency content at 3 and 7 days after storage.

Data collection

Plant height, leaf number, and leaf greenness index (measured with a SPAD-502 Plus, Spectrum Technologies Inc., Aurora, IL, USA) were recorded for each treatment at two-week intervals. Photosynthetic rate was measured monthly using a portable LCpro-SD system (ADC BioScientific Ltd., Hoddesdon, England). Both leaf greenness index and photosynthetic rate were measured on the uppermost fully expanded leaf of each plant. At harvest, rhizomes were washed with deionized water to remove surface impurities before determining fresh weight and preparing samples for subsequent chemical analysis. All measurements were taken randomly from five plants per treatment. The 2-propenyl-glucosinolate content was determined following the method of Ruamrungsri et al. (2025) using gas chromatography–mass spectrometry. For the analysis, freeze-dried rhizome samples were ground into a fine powder, and 0.1 g of the material was extracted with 60% ethanol (Dos Santos Szewczyk et al., 2023).

Statistical analysis

A factorial method within a completely randomized design was used to assess the effects of EC levels and fertilizer frequencies and their interactions on wasabi growth, yield, and 2-propenyl-glucosinolate content. Each treatment had five replications. Data were analyzed using SPSS (IBM Corp., Armonk, NY, USA) and presented as mean $\pm$ standard deviation. Statistical differences were determined using Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Growth Characteristics and Yield

The average morphological traits, including plant height, petiole length, and leaf number, at nine months after treatment (MAT) are presented in Table 1. The results showed that the interaction between EC and fertilizer frequency (EC $\times$ F) significantly affected these traits. Although plant height showed no significant difference across EC levels and fertilizer frequencies individually, their interaction revealed that plants grown under low EC combined with biweekly fertilization were significantly shorter than those under other

combinations (except medium EC combined with biweekly fertilization, EC2F2). This indicates a negative effect of limited nutrient availability, where insufficient EC and fertilizer supply restricted plant growth. In general, plant height tended to increase with rising EC levels, with mean values of 19.58, 23.08, and 24.00 cm at EC 1, 2, and 3 mS cm^{-1} , respectively. These findings are consistent with Chiloane (2012), who reported that plant height increased with EC up to 3 mS cm^{-1} in lettuce. Petiole length was generally similar across EC levels and fertilizer frequencies, except for plants grown under EC1F2, which exhibited the shortest petioles. The EC2F1 treatment increased petiole length by 56.52% compared with EC1F2, suggesting that adequate EC combined with more frequent fertilizer application promotes petiole elongation. This supports the findings of Song et al. (2022), who reported that favorable growing conditions enhanced petiole elongation in *Centella asiatica* (L.). Similarly, the number of leaves was markedly higher under EC2 and EC3 combined with weekly fertilization, increasing by 77.19% and 45.77%, respectively, compared with EC1F1. This trend suggests that optimal EC together with sufficient fertilizer frequency ensures an adequate nutrient supply, supporting photosynthetic activity and new leaf formation. Fallovo et al. (2009) and Rouphael et al. (2012) also noted that low EC levels, associated with insufficient nutrient concentrations, reduced plant growth rate. In this study, an optimal EC likely ensured sufficient nutrient supply, supporting photosynthesis and ultimately promoting the production of more leaves. Overall, the combination of higher EC levels (2–3 mS cm^{-1}) and more frequent fertilizer application enhanced plant growth by improving nutrient availability and uptake efficiency. This agrees with Padmini et al. (2023), who reported that frequent fertigation (every three days) increased nutrient absorption, plant height, and leaf production in *Capsicum annuum* L. compared with longer fertigation intervals (once every 6 days or twice every 9 days).

Table 1 Growth of wasabi cultivated in substrate culture under different EC levels and fertilizer frequencies.

Treatments	Morphological traits		
	Plant Height (cm)	Petiole Length (cm)	Leaves Number
EC1F1	22.83 ± 2.20 a	16.50 ± 1.32 a	7.67 ± 1.45 b
EC1F2	16.33 ± 0.17 b	11.50 ± 0.29 b	4.00 ± 0.58 c
EC2F1	25.33 ± 1.74 a	18.50 ± 0.76 a	11.67 ± 0.33 a
EC2F2	20.83 ± 1.01 ab	15.67 ± 0.89 a	9.00 ± 1.00 ab
EC3F1	23.17 ± 2.92 a	17.67 ± 2.19 a	8.67 ± 0.67 ab
EC3F2	24.83 ± 2.46 a	18.00 ± 1.53 a	8.33 ± 1.20 b
ANOVA			
EC	ns	ns	*
F	ns	*	*
EC × F	*	*	*

EC1, EC2, and EC3 represent electrical conductivity (EC) levels. F1 and F2 correspond to fertilizer frequencies of weekly and biweekly applications, respectively. * and ns denote significant ($p \leq 0.05$) and non-significant differences, respectively. Different lowercase letters indicate significant differences among treatments at $p \leq 0.05$ according to the LSD test.

The data on leaf color intensity, photosynthetic rate, and rhizome fresh weight are presented in Table 2. Leaf color intensity, or SPAD value, an indicator of chlorophyll content in leaves, was not significantly affected by EC or fertilizer frequency. However, a slight increase was observed with increasing EC levels, especially in more frequently fertilized treatments, possibly due to sufficient nutrient availability for chlorophyll synthesis—particularly of N, Mg, and Fe—under higher EC conditions. This trend aligns with previous findings in tomato, where higher EC levels enhanced chlorophyll concentration, resulting in darker leaf coloration (Romero-Aranda et al., 2001; Wu and Kubota, 2008). For the photosynthetic rate, a clear interaction effect

between EC and fertilizer frequency was observed. The highest photosynthetic rate was recorded in EC2F1, indicating that a moderate EC level combined with weekly fertilization enhanced photosynthetic activity. Both lower and higher EC levels, particularly when coupled with less frequent fertilization, resulted in markedly lower photosynthetic rates, suggesting that nutrient limitations under these conditions reduced photosynthetic efficiency. These results are consistent with Ding et al. (2018), who reported that optimal EC supports photosynthetic performance by maintaining a proper balance between nutrient availability and osmotic conditions. Moreover, the superior photosynthetic performance under EC2F1 corresponded with the highest rhizome fresh weight, implying that enhanced photosynthetic efficiency facilitated greater assimilate production and translocation to the storage organ, thereby promoting rhizome development in wasabi. The higher rhizome biomass under EC2F1 also suggests that frequent fertilizer supply at an optimal EC supported continuous nutrient uptake and carbon assimilation, which together improved storage organ formation. These findings agree with Hawkesford (2014) and Fallovo et al. (2009), who emphasized that sufficient and timely fertilizer application enhances nutrient uptake, chlorophyll synthesis, and photosynthetic performance, ultimately improving crop yield. Similarly, Kumar et al. (2007) reported that in Indian potato, a plant that also stores carbohydrates in underground organs, increased fertilizer application, particularly nitrogen, promoted foliage growth and photosynthetic activity, leading to greater translocation of assimilates to the tubers.

Table 2 Leaf color intensity, photosynthetic rate, and rhizome fresh weight of wasabi under different EC levels and fertilizer frequencies

Treatments	Leaf Color Intensity (SPAD Unit)	Photosynthetic Rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Rhizome Fresh Weight (g)
EC1F1	30.07 $\pm$ 1.39	0.80 $\pm$ 0.09 d	21.85 $\pm$ 3.12 c
EC1F2	29.10 $\pm$ 3.68	0.57 $\pm$ 0.55 d	17.10 $\pm$ 1.06 c
EC2F1	33.60 $\pm$ 1.23	3.93 $\pm$ 0.49 a	64.69 $\pm$ 2.60 a
EC2F2	31.87 $\pm$ 1.27	2.53 $\pm$ 0.30 b	43.29 $\pm$ 2.08 b
EC3F1	33.50 $\pm$ 1.65	1.67 $\pm$ 0.21 c	50.57 $\pm$ 3.73 b
EC3F2	33.67 $\pm$ 1.39	0.87 $\pm$ 0.09 d	49.90 $\pm$ 2.82 b
ANOVA			
EC	ns	*	*
F	ns	*	*
EC $\times$ F	ns	*	*

EC1, EC2, and EC3 represent electrical conductivity (EC) levels. F1 and F2 correspond to fertilizer frequencies of weekly and biweekly applications, respectively. * and ns denote significant ($p \leq 0.05$) and non-significant differences, respectively. Different lowercase letters indicate significant differences among treatments at $p \leq 0.05$ according to the LSD test.

2-Propenyl-Glucosinolate Content Determination

2-Propenyl-glucosinolate, a secondary metabolite, is the major compound responsible for the pungent flavor of wasabi. In general, this pungent compound is unstable and degrades over time, with higher moisture and temperature accelerating its loss (Kojima et al., 1982). Among all treatments, significantly higher 2-propenyl-glucosinolate content was observed at EC2F1 throughout both stages of the storage period. This indicates that plants grown under an appropriate EC level with more frequent fertilization can support growth and enhance the synthesis of secondary metabolites, such as 2-propenyl-glucosinolate, as observed in this study, due to sufficient nutrient availability associated with the synthesis process. The composition and concentration of glucosinolates are influenced by sulfur and nitrogen availability (Baek et al., 2019; Jeschke et

al., 2019; Katsarou et al., 2016). The production of both aliphatic and indole glucosinolates is enhanced by sulfur application. Conversely, sulfur limitation activates sulfur deficiency-induced genes (SDI1 and SDI2), with SDI1 interacting with MYB28 to suppress aliphatic glucosinolate biosynthesis (Aarabi et al., 2016; Park et al., 2018). Nitrogen is also fundamental for amino acid synthesis, which provides the precursors for glucosinolate formation, while ammonium particularly promotes this process (Marino et al., 2016). Overall, adequate EC and frequent nutrient supply may enhance 2-propenyl-glucosinolate accumulation during the growing stage, thereby providing a larger precursor pool that sustains its levels during storage.

Table 3 2-Propenyl-Glucosinolate content after 3 and 7 days of storage

Treatments	2-Propenyl-Glucosinolate Content (mg gDw ⁻¹)	
	3 DAS	7 DAS
EC1F1	19.91 ± 0.79 bc	14.33 ± 1.20 c
EC1F2	11.50 ± 1.44 d	7.00 ± 0.58 d
EC2F1	27.25 ± 1.05 a	25.00 ± 0.53 a
EC2F2	22.54 ± 1.17 b	18.67 ± 0.33 b
EC3F1	22.22 ± 1.37 b	19.00 ± 0.68 b
EC3F2	17.36 ± 0.60 c	15.63 ± 0.32 c
ANOVA		
EC	*	*
F	*	*
EC × F	*	*

EC1, EC2, and EC3 represent electrical conductivity (EC) levels. F1 and F2 correspond to fertilizer frequencies of weekly and biweekly applications, respectively. DAS indicates days after storage. * and ns denote significant ($p \leq 0.05$) and non-significant differences, respectively. Different lowercase letters indicate significant differences among treatments at $p \leq 0.05$ according to the LSD test.

Conclusions

Optimizing EC levels and fertilizer frequency is essential for maximizing the productivity and quality of wasabi cultivated in substrate systems. The combination of a medium EC level (2 mS·cm⁻¹) with weekly fertilization (EC2F1) provided the most favorable balance between growth and photosynthesis, resulting in the highest rhizome yield and the greatest accumulation of 2-propenyl-glucosinolate—the key compound responsible for wasabi's pungency. This combination effectively enhanced vegetative growth, photosynthetic activity, and metabolite synthesis, highlighting the importance of precise nutrient management for sustainable wasabi production under controlled environment agriculture.

This study emphasizes that controlling pre-harvest factors is the foundation for producing high-quality wasabi preserving its pungency during storage. Future research should focus on postharvest aspects such as optimizing storage conditions, improving packaging, and developing effective quality preservation strategies to ensure that the freshness, flavor, and commercial value of wasabi are maintained throughout the supply chain.

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