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# Evaluating three foliar-applied biostimulants for Asian leafy green production under high tunnel conditions

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## Abstract

Asian leafy greens are a group of cool-season vegetables valued for their versatility, nutritional quality, and rapid growth, and have been increasingly cultivated in various growing systems and climates. The applications of various biostimulants have been reported to increase crop productivity and alleviate stress in the production of several specialty crops. The current study evaluated plant growth, gas exchange, and mineral nutrient compositions of five *Brassica rapa* cultivars grown in a high tunnel as affected by three foliar applied biostimulants. Results revealed that the five cultivars varied in plant height, crown diameter, leaf SPAD and coloration, leaf and substrate temperatures, marketable yield, physiological parameters, and leaf mineral concentrations. 'Win Win Choi' produced the highest fresh shoot weight of 669.1 $g \cdot plant^{-1}$, and the highest marketable yield of 1.84 $kg \cdot m^{-2}$. The biostimulant treatments did not affect plant size, coloration, marketable yield, fresh or dry shoot weights, but altered physiological parameters, including leaf temperature, quantum efficiency, electron transport rate, and mineral nutrient uptake, including concentrations of phosphorus (P), potassium (K), calcium (Ca), iron (Fe), and copper (Cu). Under high tunnel conditions, the five *Brassica rapa* cultivars exhibited distinct growth, physiological responses, and nutrient uptake, which were largely attributed to genetic variation, whereas the biostimulant application could potentially yield more benefits when plants are subjected to suboptimal growing conditions with abiotic stresses.

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## Introduction

Asian leafy greens are generally cool season vegetables, thriving within a temperature range of 10 to 24 °C, making them well-suited for cultivation in diverse climates and seasons[1,2]. Due to their high nutritional values and versatile use in various cuisines, there has been increasing interest in the cultivation of *Brassica rapa* species, including *B. rapa* var. *perviridis* (komatsuna), *B. rapa* var. *chinensis* (bok choi or pak choi), and *B. rapa* var. *pekinensis* (Chinese cabbage) in the United States[3]. *B. rapa* vegetables can serve as a cash crop, and offer substantial economic benefits for vegetable growers, tapping into the growing market demand for fresh and nutritious greens[4,5]. Pak choi typically reaches marketable maturity within 40 to 50 d after seeding. They are then harvested by cutting the entire plant from the soil surface at a stage with 10 to 15 fully developed leaves, cleaned and packed for sale[4]. Other *Brassica rapa* varieties, such as *B. rapa* var. *perviridis*, may require 30 to 60 d from sowing to harvest[6]. Asian leafy greens are rich in essential minerals such as calcium, magnesium, potassium, and phosphorous[7,8]. They are also rich in health-beneficial phytochemicals, including ascorbic acid, phenolics, carotenoids ($\beta$-carotene and $\gamma$-carotene), and glucosinolates[9]. Specialty cultivars like the purple bok choi are receiving attention for the high levels of anthocyanin accumulation and its antioxidant properties[10]. The broad genetic diversity and adaptability of *Brassica rapa* cultivars allow them to be successfully cultivated across many regions worldwide, including the southeastern US[11,12].

High tunnels are generally constructed with metal frames and covered by polyethylene films with various shapes and sizes[13]. These structures lack automated heating or cooling and are passively heated by solar radiation and cooled through ventilation. High tunnels serve to increase frost free days and extend the growing season into early spring and late fall[14]. The use of high tunnels has seen a significant increase across the United States over the past several decades[15]. High tunnels allow for early planting and harvest due to elevated heat, which is advantageous for specialty crop producers as they enable off-season production that provides a competitive edge[16]. The rain exclusion in high tunnels also serves to reduce disease pressure and increase produce quality. High tunnels were reported to enhance yield and quality in a number of specialty crops, including tomato, spinach, lettuce, strawberry, and blueberries[17−19]. Spring and fall plantings of pak choi in high tunnels in Kansas were shown to have significantly higher yields when compared with field-grown plots[20]. In the southern United States, the mild winter temperatures present an opportunity for winter production of cool-season leafy greens such as Asian greens, lettuce, and spinach in a high tunnel[2,21].

Biostimulants have recently gained attention as a sustainable approach to boost plant health and alleviate abiotic stresses in agriculture. These complex, biologically derived products can enhance plant productivity through various modes of action, including stress mitigation, soil microbiome modification, metabolic activation, and improved nutrient absorption[22]. For example, seaweed extracts, derived from various marine algae, are a common biostimulant type used in horticultural production. Foliar-applied seaweed extracts were reported to increase yield and nutrient concentration in broccoli (*Brassica oleracea* var. *cymosa* L.). Such improvements were attributed to plant growth regulators like auxin and cytokinin contained in these extracts[23]. Plant growth-promoting rhizobacteria (PGPR) is another common biostimulant[24]. Generally applied to the root zone, PGPR can enhance root growth, but may reduce nutrient uptake by immobilizing inorganic nutrients in the soil[25].

Their effects on nutrient availability may vary depending on microbial strain, soil conditions, and environmental factors.

The use of biostimulants has shown tremendous potential in combating stresses such as drought, salinity, and extreme temperatures by modulating physiological and biochemical processes in plants[26]. Physiological parameters such as stomatal conductance, photosynthetic efficiency, and electron transport rate are strongly influenced by environmental and internal plant factors. In particular, stomatal conductance and electron transport rate are negatively correlated with vapor pressure deficit, and positively correlated with photosynthetically active radiation (*PAR*)[27]. Nutrient availability and temperature are key factors that modulate photosystem activity and carbon assimilation rates. Stress conditions such as drought or salinity can restrict carbon dioxide ($CO_2$) diffusion and reduce electron transport efficiency, leading to decreased photochemical performance. Protein hydrolysate biostimulants are reported to improve leaf gas exchange through pigment biosynthesis and induce an array of defense responses under adverse plant growth conditions, and thus present growth and/or yield-promoting effects[26]. Biostimulants including vitamin B complex, aspartic acid, and moringa leaf extract also significantly increased transpiration rate, stomatal conductance, and photosynthetic rate in radish[28].

Despite the increased adoption of high tunnel systems for the production of cool-season leafy greens, limited research has evaluated the role of biostimulants in mitigating abiotic stress, and enhancing the productivity of *Brassica rapa* cultivars under protected conditions. The majority of biostimulant studies on *Brassica* vegetables have been conducted under open-field environments or greenhouses, with emphasis placed on yield and nutrient concentration, lacking physiological responses such as gas exchange and photosynthetic performance[29,30]. Furthermore, high tunnel production during early spring and late fall is characterized by fluctuating temperatures, elevated vapor pressure deficit during midday, and reduced rootzone temperature, all of which can constrain nutrient uptake and carbon assimilation[31,32]. The interaction among these environmental stressors, cultivar-specific growth responses, and foliar applied biostimulants remains poorly understood, particularly for Asian leafy greens grown for fresh market consumption. Therefore, the objective of this study was to investigate the effects of three foliar-applied biostimulant treatments on plant growth, gas exchange, yield, and mineral nutrient composition of five *Brassica rapa* cultivars grown in a high tunnel production system in the southeastern US.

# Materials and methods

## Transplant preparation and plant cultivation

This experiment evaluated five non-heading Chinese cabbage cultivars, 'Win Win Choi' (*Brassica rapa* var. *chinensis*) and 'Carlton' (*Brassica rapa* var. *perviridis*) purchased from Johnny's Selected Seeds (Winslow, ME, USA), 'Nabai Spring' (*Brassica rapa* var. *chinensis*), 'QD-2 Express' (*Brassica rapa* var. *pekinensis*), and 'Chun Mei' (*Brassica rapa* var. *chinensis*) purchased from True Leaf Market (Salt Lake City, UT, USA). Transplants were prepared in a greenhouse at Mississippi State University (Mississippi State, MS, USA; lat. 33.45° N, long. 88.79° W). Seeds of each cultivar were sown into 50-cell growing trays using a soilless substrate (PRO-MIX BX General Purpose, Premier Tech Horticulture, Quebec, Canada) on January 4, 2024. One week after germination, transplants were thinned to have one plant per cell to have uniform seedlings.

The high tunnel used in this experiment measured 29.0 m long and 9.1 m wide, oriented north to south, and is located at the R.R. Foil Plant Science Research Center of Mississippi State University (Mississippi State, MS, USA; lat. 33.45° N, long. 88.79° W). The high tunnel is covered with a 6 mil (0.15 mm) clear polyethylene film, fitted with side curtains with a height of 1.5 m, and end doors with a height of 3 m (Tubular Structure, Lucedale, MS, USA). During this experiment, the side curtain and end walls were closed when air temperatures dropped below 4.4 °C, typically at night, and otherwise remained open. Four raised beds were constructed with composted pine bark in the high tunnel, spaced 1.2 m from center to center. Each raised bed measured 26 m long, 80 cm wide at the base, and 35 cm wide at the top, and had a depth of 20 cm. Granular lime at a rate of 2.96 $kg \cdot m^{-3}$ (Soil Doctor Pelletized Lawn Lime, Oldcastle, Atlanta, GA, USA) and a slow-release fertilizer 15N-3.9P-10K (Osmocote®15-9-12 plus, 3–4 months; ICL Specialty Fertilizers, Summerville, SC, USA) at a rate of 4.21 $kg \cdot m^{-3}$ were incorporated into the raised beds. Prior to mulch laying, one drip tape (15.9 mm in diameter, 0.91 $L \cdot h^{-1}$; Netafim, Tel Aviv-Yafo, Israel) was placed at the center of each raised bed with emitters spaced 30 cm apart. Each raised bed was then covered with a black plastic film (32 µm, Filmtech Corp., Allentown, PA, USA).

The microenvironment, including air temperature, relative humidity (RH), and photosynthetically active radiation (*PAR*), was monitored in the high tunnel within the experiment duration. A temperature and RH sensor (HOBO S-THB-M002; Onset Computer Corp., Bourne, MA, USA), and a quantum sensor (HOBO S-LIA-M003; Onset Computer Corp.) were connected to a micro station data logger (H21-USB; Onset Computer Corp.). The sensors were installed in the center of the high tunnel to measure air temperature, relative humidity, and *PAR* at 1-h intervals. Daily light integral (DLI) was calculated by [daily average *PAR* × 0.0864] as described by Torres & Lopez[33]. Daily growing degree days (GDDs) were calculated by (daily average temperature − base temperature). Cumulative GDDs between certain time periods were estimated by summing up daily GDDs. The base temperature used for Asian greens was 4 °C[21].

Seedlings were hardened off and transplanted into the high tunnel on February 15, 2024. On each raised bed, Asian greens were planted in two parallel rows with 25 cm spacing between plants, and 25 cm between rows. Plants were drip irrigated daily and fertigated with a water-soluble fertilizer 20N-8.7P-16.6K (Peters® Professional 20-20-20 General Purpose; ICL Specialty Fertilizers) using an injector (D14MZ2; Dosatron Intl. Inc., Clearwater, FL, USA) at 100 ppm N during establishment.

## Biostimulant treatments

Three biostimulant treatments, including Dune™ (Impello Biosciences, Loveland, CO, USA), Continuum™ (Impello Biosciences, Loveland, CO, USA), and Kelpak Maxx (Kelp Products Ltd., Cape Town, South Africa), were applied. The product Dune has an active ingredient of stabilized ortho silicic acid, which is soluble and plant available. Continuum is a microbial inoculant that contains plant growth-promoting rhizobacteria, including *Paenibacillus chitinolyticus* ($1 \times 10^6$ CFU/mL), *Bacillus subtilis* ($1 \times 10^6$ CFU/mL), *Bacillus pumilus* ($1 \times 10^6$ CFU/mL), and *Bacillus amyloliquefaciens* ($1 \times 10^6$ CFU/mL). Kelpak Maxx is a seaweed concentrate derived from a large brown kelp species *Ecklonia maxima*.

Application concentrations and frequency were selected based on manufacturer recommendations to reflect commercially application practices commonly used by specialty crop growers. Biostimulant application methodology was also consulted with manufacturer's technical support, and suggestions were made based on the experiment objective and production system.

Accordingly, all three biostimulants were applied as a foliar spray with the following concentrations: Dune at 0.53 mL·L$^{-1}$ (2 mL·gal$^{-1}$), Continuum at 0.53 mL·L$^{-1}$ (2 mL·gal$^{-1}$), and Kelpak Maxx at 1% based on manufacturer's instructions. Biostimulant applications were initiated 8 d after planting, and were applied weekly with a total of four applications. Plants in the control group were sprayed with tap water at each application timing.

## Plant growth data collection

Prior to harvest, three marketable plants from each plot consisting of 10 plants were randomly selected to measure vegetative growth, including plant height, crown diameters, and relative leaf chlorophyll content at 30 d after transplanting (DAT). Plant height was measured from the mulch surface to the tip of the plant. Crown diameters were measured from two perpendicular directions and averaged to represent the spread of a given plant. Relative leaf chlorophyll content was measured as a Soil-Plant Analysis Development (SPAD) reading. Leaf SPAD readings were taken from three fully expanded leaves of a selected plant using a chlorophyll meter (SPAD 502 Plus; Konica Minolta, Inc., Osaka, Japan). The three readings were then averaged to present the relative leaf chlorophyll content of a given plant.

Leaf surface temperatures were measured on two plants from each subplot using a handheld infrared laser thermometer (Thermo Fisher, Waltham, MA, USA). The substrate temperature in each subplot was measured at a depth of 10 cm using a digital thermometer probe (Thermo Fisher, Waltham, MA, USA), with two readings collected per subplot. Two substrate moisture readings from each subplot were measured using a soil moisture sensor (ML2x; Delta-T Devices, Cambridge, UK) at a depth of 6 cm. Leaf and substrate temperatures, as well as soil moisture, were measured at 30 DAT.

## Plant physiological parameters

Plant physiological parameters, including stomatal conductance, transpiration (E), photosynthetic efficiency of photosystem 2 (PhiPS2), and electron transport rate (ETR) were measured on three randomly selected plants from each subplot. Readings were taken from one fully expanded leaf from a given plant using a portable handheld LI-600 porometer system integrated with a fluorometer (Li-Cor Biosciences; Lincoln, NE, USA) during sunny hours between 10:00 am and 12:00 pm at 33 DAT.

## Plant harvest

Plants were harvested at 36 DAT, when marketable plants from each plot were harvested by cutting the entire plant at the crown level. Marketable yield per plot was measured as the total fresh weight of all marketable plants. The number of marketable plant per plot was also recorded. Three marketable plants from each subplot were randomly selected to measure their individual fresh shoot weight and for leaf color using a chroma meter (CR-400, Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA). These three selected plants were then oven-dried at 60 °C until a constant weight was achieved. The dry shoot weight of each selected plant was measured.

## Mineral nutrient analyses

Dry plant samples were ground to pass through a 1 mm sieve with a grinder (Wiley mini mill, Thomas Scientific, Swedesboro, NJ, USA). Combustion analysis was used to determine total nitrogen (N) concentration with 0.25 g of ground plant material and an elemental analyzer (vario MAX cube; Elementar Americas Inc., Long Island, NY, USA). The concentrations of phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) were measured using inductively coupled plasma optical emission spectrometry (SPECTROBLUE; SPECTRO Analytical Instruments, Kleve, Germany). Both macronutrient (mg·g$^{-1}$) and micronutrient (μg·g$^{-1}$) concentrations were presented on a dry weight basis. All plant samples were tested at the Mississippi State University Extension Service Soil Testing Laboratory.

## Experimental design and statistical analyses

This study was conducted using a split plot design with four replications. The biostimulant treatments served as the main plot, and cultivar served as the subplot that was randomly distributed within a main plot. Each subplot consisted of 10 individual plants. The two experimental factors were plant cultivar (5), and the biostimulant treatment (three types plus control), resulting in 20 treatment combinations. Two-way analysis of variance (ANOVA) was performed on the tested variables. Data were analyzed using the PROC GLIMMIX procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Mean separation was conducted using Tukey's Honest Significant Difference (HSD) test at $\alpha = 0.05$.

# Results

## Plant height, crown diameter, and leaf SPAD

Plant height, crown diameter, and leaf SPAD varied among the five Asian green cultivars, while the biostimulant treatment did not affect these three variables (Table 1). Among the tested cultivars, 'Carlton' had the tallest plant structure of 38.11 cm. 'Nabai Spring' was the most dwarf cultivar producing a height of 12.98 cm, shorter than 'QD2-Express', or 'Win Win Choi'. The three cultivars 'Chun Mei', 'QD2-Express', and 'Win Win Choi' had similar heights ranging from 22.06 to 26.59 cm. 'Carlton', 'QD2-Express', and 'Win Win Choi' produced plants with similar crown diameters of 42.54, 41.84, and 41.43 cm, respectively, wider than 'Chun Mei' or 'Nabai Spring. 'Nabai Spring' produced the narrowest plant form with a crown diameter of 31.78 cm. Leaf SPAD measurements showed a clear separation among cultivars with the following ranking: 'Carlton' (66.05) > 'Nabai Spring' (56.38) > 'Win Win Choi' (52.19) > 'Chun Mei' (47.13) > 'QD2-Express' (28.61).

**Table 1.** Plant height, crown diameter, and leaf SPAD varied among five Asian green cultivars were not affected by biostimulant application when grown in a high tunnel production system.

| Cultivar | Plant height[z] (cm) | Crown diameter (cm) | Leaf SPAD |
|---|---|---|---|
| 'Carlton' | 38.11 a | 42.54 a | 66.05 a |
| 'Chun Mei' | 22.06 bc | 33.75 b | 47.13 d |
| 'Nabai Spring' | 12.98 c | 31.78 c | 56.38 b |
| 'QD2-Express' | 26.59 b | 41.84 a | 28.61 e |
| 'Win Win Choi' | 26.25 b | 41.43 a | 52.19 c |
| *p*-Value | | | |
| Cultivar | < 0.0001 | < 0.0001 | < 0.0001 |
| Biostimulant | 0.41 | 0.20 | 0.34 |
| Interaction | 0.32 | 0.13 | 0.56 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD test at a $p < 0.05$.

## Substrate, leaf temperatures, and substrate moisture

Substrate temperature, leaf temperature, and substrate moisture varied among cultivars (Table 2). Leaf temperature was also affected by the main effect of the biostimulant treatment (Table 3). There was no significant interaction between cultivar and biostimulant application in substrate and leaf temperatures, and substrate moisture.

Substrate temperatures were higher in cultivars 'Chun Mei' and 'Nabai Spring', ranging from 15.68 to 15.74 °C, than 'QD-2 Express' or 'Win Win Choi', ranging from 14.95 to 15.19 °C. Leaf temperature among cultivars, ranging from 15.03 to 16.05 °C, were generally similar among cultivars except that 'Carlton' had a higher leaf temperature than 'Chun Mei' (Table 2). The biostimulant Kelpak Maxx resulted in a higher leaf temperature of 15.95 °C compared to the control with a leaf temperature of 14.96 °C, but similar to Dune or Continuum (Table 3). Higher substrate moisture was observed in the cultivars 'Nabai Spring' (15.9%) and 'Chun Mei' (15.2%) compared with 'Win Win Choi' with substrate moisture of 13.0%.

## Plant physiological parameters

Plant physiological parameters, including stomatal conductance, transpiration, quantum efficiency of photosystem 2 (PhiPS2), and electron transport rate, all varied among cultivars (Table 4). PhiPS2 and electron transport rate were also affected by the biostimulant application (Table 3). There was no significant interaction between cultivar and biostimulant treatment in any of the physiological parameters mentioned above.

Stomatal conductance in 'Chun Mei', 'Nabai Spring', and 'Win Win Choi' were similar, ranging from 0.478 to 0.492 mol·m$^{-2}$·s$^{-1}$, and higher than 'Carlton' or 'QD2-Express' with values of 0.343 and 0.306 mol·m$^{-2}$·s$^{-1}$, respectively. Transpiration rate in 'Chun Mei', 'Nabai Spring', and 'Win Win Choi' were also the highest among cultivars, ranging from 4.721 to 5.043 mmol·m$^{-2}$·s$^{-1}$, with 'QD2-Express' producing the lowest transpiration rate of 3.660 mmol·m$^{-2}$·s$^{-1}$ (Table 4). 'Chun Mei' and 'QD2-Express' exhibited similarly higher PhiPS2 of 0.659 and 0.657, respectively, compared to 'Nabai Spring' or 'Win Win Choi'. Electron transport rate was similar among cultivars (Table 4). The biostimulant Dune resulted in higher PhiPS2 than Kelpak Maxx, but similar to control or Continuum. However, Kelpak Maxx resulted in an increased electron transport rate compared to Dune or Continuum (Table 3).

## Leaf coloration

Leaf coloration measurements including lightness (L), green-red color (a*), and yellow-blue color (b*) varied among cultivars. These same parameters were not affected by the biostimulant applications (Table 5). Clear separation among cultivars were found for L, a* and b* values. 'QD2-Express' produced leaves with the highest lightness of 47.23, and the ranking of lightness among cultivars was 'QD2-Express' (47.23) > 'Chun Mei' (40.42) > 'Win Win Choi' (39.24) > 'Nabai Spring' (34.26), 'Carlton' (33.58). The ranking of green-red coloration (a*) was as follows: 'Carlton' (–7.395) > 'Nabai Spring' (–9.995) > 'Win Win Choi' (–10.97) > 'Chun Mei' (–11.97) > 'QD2-Express' (–15.29). The ranking of yellow-blue coloration (b*) followed: 'QD2-Express' (27.66) > 'Chun Mei' (17.02) > 'Win Win Choi'

**Table 2.** Substrate temperature, leaf temperature, and substrate moisture varied among five Asian green cultivars grown in a high tunnel production system.

| Cultivar | Substrate temperature[z] (°C) | Leaf temperature (°C) | Substrate moisture (%) |
|---|---|---|---|
| 'Carlton' | 15.58 ab | 16.05 a | 14.0 abc |
| 'Chun Mei' | 15.74 a | 15.03 b | 15.2 ab |
| 'Nabai Spring' | 15.68 a | 15.72 ab | 15.9 a |
| 'QD2-Express' | 15.19 bc | 15.32 ab | 13.9 bc |
| 'Win Win Choi' | 14.95 c | 15.33 ab | 13.0 c |
| *p*-Value | | | |
| Cultivar | < 0.0001 | 0.021 | 0.0005 |
| Biostimulant | 0.80 | 0.011 | 0.39 |
| Interaction | 0.70 | 0.27 | 0.54 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD test at a $p < 0.05$.

**Table 3.** Leaf temperature, quantum efficiency, and electron transport rate of five Asian green cultivars grown in a high tunnel as affected by the application of three biostimulants.

| Biostimulant | Leaf temperature[z] (°C) | PhiPS2[y] | Electron transport rate (µmol·m$^{-2}$·s$^{-1}$) |
|---|---|---|---|
| Control | 14.96 b | 0.620 ab | 198.1 ab |
| Dune | 15.49 ab | 0.654 a | 171.6 b |
| Kelpak Maxx | 15.95 a | 0.607 b | 214.8 a |
| Continuum | 15.56 ab | 0.637 ab | 183.4 b |
| *p*-Value | | | |
| Cultivar | 0.021 | < 0.0001 | 0.11 |
| Biostimulant | 0.011 | 0.023 | 0.002 |
| Interaction | 0.27 | 0.93 | 0.061 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD test at a $p < 0.05$. [y] PhiPS2 stands for PSII actual photochemical quantum yield.

**Table 4.** Stomatal conductance, transpiration, and quantum efficiency of photosystem 2 varied among five Asian green cultivars grown in a high tunnel production system.

| Cultivar | Stomatal conductance[z] (mol·m$^{-2}$·s$^{-1}$) | Transpiration (mmol·m$^{-2}$·s$^{-1}$) | PhiPS2[y] | Electron transport rate (µmol·m$^{-2}$·s$^{-1}$) |
|---|---|---|---|---|
| 'Carlton' | 0.343 b | 3.891 bc | 0.637 ab | 188.6 |
| 'Chun Mei' | 0.492 a | 4.845 ab | 0.657 a | 209.9 |
| 'Nabai Spring' | 0.485 a | 5.043 a | 0.590 b | 199.5 |
| 'QD2-Express' | 0.306 b | 3.660 c | 0.659 a | 184.5 |
| 'Win Win Choi' | 0.478 a | 4.721 ab | 0.605 b | 177.3 |
| *p*-Value | | | | |
| Cultivar | < 0. 0001 | 0.001 | < 0. 0001 | 0.11 |
| Biostimulant | 0.67 | 0.13 | 0.023 | 0.002 |
| Interaction | 0.43 | 0.68 | 0.93 | 0.061 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD test at a $p < 0.05$. [y] PhiPS2 stands for PSII actual photochemical quantum yield.

**Table 5.** Leaf coloration varied among five Asian green cultivars and was not affected by biostimulant treatment when grown in a high tunnel production system.

| Cultivar | L[z] | a* | b* |
|---|---|---|---|
| 'Carlton' | 33.58 d | −7.395 a | 8.479 e |
| 'Chun Mei' | 40.42 b | −11.97 d | 17.02 b |
| 'Nabai Spring' | 34.26 d | −9.995 b | 13.55 d |
| 'QD2-Express' | 47.23 a | −15.29 e | 27.66 a |
| 'Win Win Choi' | 39.24 c | −10.97 c | 15.14 c |
| *p*-Value | | | |
| Cultivar | < 0.0001 | < 0.0001 | < 0.0001 |
| Biostimulant | 0.096 | 0.89 | 0.86 |
| Interaction | 0.70 | 0.34 | 0.23 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD test at a $p < 0.05$.

(15.14) > 'Nabai Spring' (13.55) > 'Carlton' (8.479), suggesting the leaves of 'QD2-Express' had the highest lightness, green, and yellow hue among cultivars. The cultivar 'Carlton' had the darkest leaves, as well as the least amount of green and yellow hues in its foliage.

## Marketable yield and individual shoot weight

Total marketable yield, individual fresh and dry shoot weight varied among cultivars (Table 6). The aforementioned three variables were not affected by the biostimulant treatments. The cultivar 'Win Win Choi' produced the greatest marketable yield of 1.84 kg·m$^{-2}$. 'Carlton' and 'Nabai Spring' had the lowest marketable yields of 0.79 and 0.93 kg·m$^{-2}$, while 'QD2-Express' and 'Chun Mei' produced intermediate marketable yields of 1.52 and 1.22 kg·m$^{-2}$, respectively. The four cultivars 'Carlton', 'Chun Mei', 'QD2-Express', and 'Win Win Choi', produced a comparable number of marketable plants, ranging from 9.75 to 10 per plot, while 'Nabai Spring', producing the lowest marketable plant number of 9.38 due to its slow growth rate.

'Win Win Choi' produced the highest individual fresh shoot weight of 669.1 g·plant$^{-1}$ among cultivars, with 'QD2-Express' producing the second-highest individual fresh shoot weight of 600.5 g·plant$^{-1}$. 'Carlton' produced the lowest individual fresh shoot weight of 302.4 g·plant$^{-1}$. The ranking of individual dry shoot weight followed: 'QD2-Express' (46.58 g·plant$^{-1}$) > 'Win Win Choi' (43.91 g·plant$^{-1}$), or 'Carlton' (43.49 g·plant$^{-1}$) > 'Chun Mei' (40.46 g·plant$^{-1}$), or 'Nabai Spring' (39.64 g·plant$^{-1}$).

## Nitrogen concentration

Nitrogen concentrations varied among cultivars, whereas biostimulant application did not affect N concentration (Table 7). 'Nabai Spring' had the highest N concentration of 52.62 mg·g$^{-1}$ compared to all other cultivars in this study. 'Win Win Choi' produced the lowest N concentration of 42.86 mg·g$^{-1}$, which was significantly lower than 'QD2-Express' or 'Carlton' with N concentrations of 45.79 and 47.18 mg·g$^{-1}$, respectively, but similar to that in 'Chun Mei'.

## Phosphorous concentration

Phosphorous concentration was affected by the interaction between cultivar and the biostimulant treatment (Table 7). The two cultivars 'Chun Mei' and 'Nabai Spring' had similar P concentrations ranging from 4.60 to 5.07 mg·g$^{-1}$ regardless of biostimulant treatment. 'Carlton' had the lowest P concentrations of 3.15 to 3.25 mg·g$^{-1}$ among cultivars with each biostimulant treatment. The three biostimulants resulted in similar P concentrations in 'Carlton', 'Chun Mei', and 'Nabai Spring' compared with the control. However, Kelpak Maxx increased P concentration in 'QD2-Express' compared to Continuum. Kelpak Maxx also increased P concentration in 'Win Win Choi' compared to the control.

**Table 6.** Marketable yield, number of marketable plants per plot, individual fresh and dry shoot weight varied among five Asian green cultivars and were not affected by biostimulant application when grown in a high tunnel.

| Cultivar | Marketable yield[z] (kg·m$^{-2}$) | Number of marketable plant (per plot) | Fresh shoot weight (g·plant$^{-1}$) | Dry shoot weight (g·plant$^{-1}$) |
|---|---|---|---|---|
| 'Carlton' | 0.79 d | 9.94 a | 302.4 d | 43.49 b |
| 'Chun Mei' | 1.22 c | 9.75 ab | 442.6 c | 40.46 c |
| 'Nabai Spring' | 0.93 d | 9.38 b | 410.1 c | 39.64 c |
| 'QD2-Express' | 1.52 b | 9.75 ab | 600.5 b | 46.58 a |
| 'Win Win Choi' | 1.84 a | 10 a | 669.1 a | 43.91 b |
| *p*-Value | | | | |
| Cultivar | < 0.0001 | 0.012 | < 0.0001 | < 0.0001 |
| Biostimulant | 0.51 | 0.74 | 0.18 | 0.89 |
| Interaction | 0.99 | 0.79 | 0.085 | 0.15 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD at $p < 0.05$.

## Potassium concentration

Potassium concentration was affected by the interaction between cultivar and biostimulant treatment (Table 7). Concentrations of K ranged from 39.32 mg·g$^{-1}$ in 'Carlton' treated with Kelpak Maxx to 67.74 mg·g$^{-1}$ in 'Win Win Choi' treated with Kelpak Maxx. The biostimulant treatments resulted in similar K concentrations within each cultivar. Among cultivars, 'Win Win Choi' and 'QD2-Express' had higher K concentrations than 'Carlton' with each biostimulant treatment or control. 'Chun Mei', 'QD2-Express', and 'Win Win Choi' generally had similar K concentrations, while 'Carlton' and 'Nabai Spring' also had similar K concentrations regardless of biostimulant treatment.

## Calcium concentration

Calcium concentrations varied among cultivars, and was also affected by biostimulant treatments without interaction (Tables 7 and 8). 'Nabai Spring', 'QD2-Express', and 'Win Win Choi' produced similarly the highest Ca concentrations, ranging from 14.65 to 15.28 mg·g$^{-1}$. 'Carlton' produced an intermediate Ca concentration of 13.61 mg·g$^{-1}$, while 'Chun Mei' produced the lowest Ca concentration of 11.31 mg·g$^{-1}$ among cultivars (Table 7). When affected by the biostimulant application, Dune and Continuum resulted in lower Ca concentrations of 13.63 and 13.58 mg·g$^{-1}$ in tested Asian green cultivars compared to the control (Table 8).

## Magnesium concentration

Magnesium concentrations varied among cultivars and were not affected by the biostimulant application (Table 7). 'Win Win Choi' produced the highest Mg concentration of 2.47 mg·g$^{-1}$ among cultivars. 'QD2-Express and 'Nabai Spring' produced similarly the second highest Mg concentrations of 2.20 and 2.21 mg·g$^{-1}$, respectively, while 'Carlton' and 'Chun Mei' produced the lowest Mg concentrations of 1.83 and 2.04 mg·g$^{-1}$.

## Sulfur concentration

Sulfur concentrations varied among cultivars and were not affected by the biostimulant application (Table 7). The cultivar 'QD2-Express' produced the highest S concentration of 6.90 mg·g$^{-1}$, higher than any other cultivar. The cultivar 'Win Win Choi' produced the second highest S concentration of 5.43 mg·g$^{-1}$. 'Carlton', 'Chun Mei', and 'Nabai Spring' produced similarly the lowest S concentrations, ranging from 4.56 to 4.81 mg·g$^{-1}$.

## Boron concentration

Boron concentrations varied among the five Asian green cultivars and were not affected by biostimulant application (Tables 8 and 9). There was a clear separation of B concentrations among cultivars. The ranking of B concentration among cultivars was as follows: 'Win Win Choi' (46.02 μg·g$^{-1}$) > 'Nabai Spring' (37.82 μg·g$^{-1}$) > 'Chun Mei' (33.89 μg·g$^{-1}$) > 'QD2-Express' (30.51 μg·g$^{-1}$) > 'Carlton' (27.69 μg·g$^{-1}$).

## Iron concentration

Iron concentrations were affected by the main effects of both the cultivar and biostimulant treatment separately without interaction (Tables 8 and 9). The cultivars 'Nabai Spring', 'QD2-Express', and 'Win Win Choi' produced the highest Fe concentrations of 69.76 to 73.84 μg·g$^{-1}$, higher than those of 'Carlton' or 'Chun Mei' with similar Fe concentrations of 59.79 and 55.72 μg·g$^{-1}$, respectively (Table 9). The biostimulant Kelpak Maxx increased Fe concentration compared with Dune or Continuum, but resulted in a similar Fe concentration to control (Table 8).

## Manganese concentration

Manganese concentrations varied among cultivars and were not affected by the biostimulant treatment (Tables 8 and 9). The two cultivars 'Nabai Spring' and 'QD2-Express' had the highest Mn concentrations of 61.00 and 58.16 μg·g$^{-1}$, higher than 'Chun Mei'

**Table 7.** Macronutrient concentrations varied among five Asian green cultivars or as affected by the interaction between cultivar and biostimulant application.

| Cultivar | Biostimulant | Nitrogen[z] (mg·g$^{-1}$) | Phosphorous[y] (mg·g$^{-1}$) | Potassium (mg·g$^{-1}$) | Calcium (mg·g$^{-1}$) | Magnesium (mg·g$^{-1}$) | Sulfur (mg·g$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 'Carlton' | Control | 47.18 b | 3.22 d | 46.33 efg | 13.61 b | 1.83 d | 4.59 c |
| | Dune | | 3.25 d | 40.51 fg | | | |
| | Kelpak Maxx | | 3.21 d | 39.32 g | | | |
| | Continuum | | 3.15 d | 39.57 g | | | |
| 'Chun Mei' | Control | 44.54 cd | 5.07 abc | 57.53 a–e | 11.31 c | 2.04 c | 4.56 c |
| | Dune | | 4.60 bc | 52.4 b–e | | | |
| | Kelpak Maxx | | 4.79 bc | 51.84 b–f | | | |
| | Continuum | | 4.84 abc | 52.72 b–e | | | |
| 'Nabai Spring' | Control | 52.62 a | 4.85 abc | 50.34 c–g | 14.83 a | 2.21 b | 4.81 c |
| | Dune | | 4.77 bc | 46.73 d–g | | | |
| | Kelpak Maxx | | 4.69 bc | 46.64 efg | | | |
| | Continuum | | 4.67 bc | 46.62 efg | | | |
| 'QD2-Express' | Control | 45.79 bc | 4.96 abc | 58.03 a–d | 15.28 a | 2.20 b | 6.90 a |
| | Dune | | 5.12 abc | 58.35 abc | | | |
| | Kelpak Maxx | | 5.59 a | 62.77 ab | | | |
| | Continuum | | 4.79 bc | 55.49 b–e | | | |
| 'Win Win Choi' | Control | 42.86 d | 4.42 c | 58.4 abc | 14.65 a | 2.47 a | 5.43 b |
| | Dune | | 4.49 bc | 59.64 abc | | | |
| | Kelpak Maxx | | 5.25 ab | 67.74 a | | | |
| | Continuum | | 4.74 bc | 58.48 abc | | | |
| *p*-Value | Cultivar | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Biostimulant | 0.052 | 0.027 | 0.037 | 0.016 | 0.24 | 0.21 |
| | Interaction | 0.70 | 0.0097 | 0.0496 | 0.33 | 0.51 | 0.92 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD at $p < 0.05$. [y] Where significant, the interaction between cultivar and biostimulant was presented for concentrations of phosphorus and potassium as indicated by the *p*-values. The main effect of cultivar was presented for nitrogen, calcium, magnesium, and sulfur when there was not a significant interaction.

**Table 8.** Calcium and micronutrient concentrations of five Asian green cultivars as affected by biostimulant treatment when grown in a high tunnel.

| Biostimulant | Calcium[z] (mg·g$^{-1}$) | Boron (μg·g$^{-1}$) | Iron (μg·g$^{-1}$) | Manganese (μg·g$^{-1}$) | Zinc (μg·g$^{-1}$) | Copper (μg·g$^{-1}$) |
|---|---|---|---|---|---|---|
| Control | 14.59 a | 34.97 | 66.68 ab | 54.11 | 46.85 | 1.20 ab |
| Dune | 13.63 b | 34.76 | 60.68 b | 54.09 | 44.36 | 1.27 ab |
| Kelpak Maxx | 13.95 ab | 35.29 | 71.88 a | 56.01 | 40.90 | 1.06 b |
| Continuum | 13.58 b | 35.73 | 64.33 b | 55.33 | 42.4 | 1.34 a |
| *p*-Value | | | | | | |
| Cultivar | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0007 | 0.29 |
| Biostimulant | 0.016 | 0.72 | 0.0002 | 0.49 | 0.17 | 0.021 |
| Interaction | 0.33 | 0.72 | 0.25 | 0.89 | 0.41 | 0.40 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD at $p < 0.05$.

**Table 9.** Micronutrient concentrations varied among five Asian green cultivars when grown in a high tunnel production system.

| Cultivar | Boron[z] (μg·g$^{-1}$) | Iron (μg·g$^{-1}$) | Manganese (μg·g$^{-1}$) | Zinc (μg·g$^{-1}$) | Copper (μg·g$^{-1}$) |
|---|---|---|---|---|---|
| 'Carlton' | 27.69 e | 59.79 b | 54.63 b | 37.81 b | 1.15 |
| 'Chun Mei' | 33.89 c | 55.72 b | 44.92 c | 45.54 ab | 1.22 |
| 'Nabai Spring' | 37.82 b | 73.84 a | 61.00 a | 50.72 a | 1.30 |
| 'QD2-Express' | 30.51 d | 70.87 a | 58.16 ab | 43.70 ab | 1.29 |
| 'Win Win Choi' | 46.02 a | 69.26 a | 55.7 b | 40.36 b | 1.12 |
| *p*-Value | | | | | |
| Cultivar | < 0.0001 | < 0.0001 | < 0.0001 | 0.0007 | 0.29 |
| Biostimulant | 0.72 | 0.0002 | 0.49 | 0.17 | 0.021 |
| Interaction | 0.72 | 0.25 | 0.89 | 0.41 | 0.40 |

[z] Different lower-case letters suggest significant difference among means within a column as indicated by Tukey's HSD at $p < 0.05$.

with the lowest Mn concentration of 44.92 μg·g$^{-1}$ among cultivars. 'Carlton' and 'Win Win Choi' had intermediate Mn concentrations that were similar to 'QD2-Express' but lower than 'Nabai Spring' (Table 9).

## Zinc concentration

Zinc concentrations varied among cultivars and were not affected by the biostimulant treatment (Tables 8 and 9). The concentration of Zn ranged from 37.81 μg·g$^{-1}$ in 'Carlton' to 50.72 μg·g$^{-1}$ in 'Nabai Spring', withthe four cultivars 'Chun Mei', 'QD2-Express', 'Carlton', and 'Win Win Choi' having similar Zn concentrations. 'Carlton' or 'Win Win Choi' had lower Zn concentrations than 'Nabai Spring'.

## Copper concentration

Copper concentrations were affected by the main effect of the biostimulant treatment but were similar among cultivars (Tables 8 and 9). Plants treated with the biostimulant Continuum produced a higher Cu concentration of 1.34 μg·g$^{-1}$ compared to Kelpak Maxx with a Cu concentration of 1.06 μg·g$^{-1}$. Copper concentrations were similar among plants treated with control, Dune, and Continuum, ranging from 1.20 to 1.34 μg·g$^{-1}$ (Table 8).

# Discussion

Asian green cultivars exhibited various growth rates and morphology, including plant height, crown diameter, leaf SPAD, leaf coloration, and individual fresh and dry shoot weights. Among the cultivars evaluated, 'Carlton' exhibited the tallest plant stature while, 'Nabai Spring' was the most dwarf cultivar. 'Nabai Spring' also had the lowest number of marketable plants per plot, mainly due to its slow growth rate and undersized plants. Wider crown diameters were observed in 'Carlton', 'QD2-Express', and 'Win Win Choi' than other cultivars, indicating a morphological advantage for light interception and potential biomass accumulation, but may require wider spacing with lower planting density. The cultivar 'Carlton' had the highest leaf SPAD and darkest leaf (with the lowest L value), while 'QD2-Express' had the lowest leaf SPAD, highest lightness (L value), and yellow coloration (b* value), reflecting potential differences in chlorophyll content and nitrogen status[34]. Such results demonstrated that genetic variation among cultivars was the primary determinant of these parameters, including plant stature, crown architecture, and leaf chlorophyll status. Differences in leaf SPAD, leaf coloration, and biomass accumulation among cultivars likely reflect inherent variation in nitrogen uptake capacity, internal nitrogen allocation, and photosynthetic efficiency, which are documented traits controlled by genotype in *Brassica rapa* vegetables[35]. The lack of biostimulant effects on these parameters suggests that genetic potential for growth and nutrient efficiency outweighed any additional stimulation under the growing conditions in a high tunnel production system.

The application of biostimulants could be more helpful in alleviating stress for vegetable species under suboptimal conditions. Their beneficial effects may not be as significant when plants are grown in healthy ideal conditions as in this current study. Hirst et al.[36] revealed that biostimulant containing plant growth-promoting microbes (PGPM) with a mixture of *Bacillus*, *Pseudomonas*, and *Trichoderma* species increased the leaf area of hydroponically cultivated pak choi when subject to moderate-salinity stress from 80 mM NaCl. However, when pak choi plants were not subject to any salinity stress, the five PGPM biosimulant treatments resulted in similar or lower leaf area and shoot fresh weights compared with the no-biostimulant control. Similarly, Colla et al.[37] concluded that protein hydrolysates biostimulant had the potential to improve crop performance under environmental stress conditions.

Marketable yield of Asian greens varied among cultivars, ranging from 0.79 kg·m$^{-2}$ (7,900 kg per hectare) in 'Carlton' to 1.84 kg·m$^{-2}$ (18,400 kg per hectare) in 'Win Win Choi', and was not affected by biostimulant applications. Such yield was satisfactory and higher compared with those of five other non-heading Chinese cabbage cultivars when grown in a high tunnel under a similar climate[2]. For other leafy greens, foliar application of the seaweed extract Kelpak significantly increased marketable yield of lettuce grown in a high tunnel under three N fertilizer rates[38]. Increased marketable yield in greenhouse-grown spinach was also reported as a result of foliar application of biostimulants, including a seaweed extract[39]. In both aforementioned studies, the spinach and lettuce were direct-seeded and harvested as baby leaf salad greens, whereas Asian greens were established through transplants and harvested as whole plants in our study. A number of factors, including environmental conditions, production systems (e.g., open field, high tunnel, or greenhouse), cultural practices (e.g., direct seeding vs transplanting), and harvesting style (baby leaf vs entire plant), could alter the interaction between plants and biostimulant applications.

The marketable yield of leafy greens is to some degree a function of individual fresh shoot weight, as multiple species, including mustard, kale, and a variety of Asian brassicas, commonly use a similar spacing of 25 cm[40]. The planting density (800 plants per tunnel) used in our study is equivalent to 30,418 plants per hectare (12,309 plants per acre), in agreement with reported ranges[41]. Multiple trials also reported leafy green yield using individual plant fresh weight[12]. For example, nine Asian green cultivars showed difference in yield or fresh shoot weight when planted in East Texas in both open field and high tunnel production systems. The cultivar 'Chinese' (*B. rapa* var. *chinensis*) produced the highest yield at 180 g·plant$^{-1}$ in open-field production, while 'Purple Magic' (*B. rapa* var. *chinensis*) yielded only 63 g·plant$^{-1}$.

Heat accumulation has a significant role in individual plant weight and yield. 'Win Win Choi' produced fresh shoot weight of 318 g·plant$^{-1}$ when grown in a cooler climate in Ames, IA, USA, in a high tunnel[40], whereas 'Win Win Choi' produced fresh shoot weight of 669.1 g·plant$^{-1}$ in our study. Such differences in fresh shoot weight can be attributed to heat accumulation during the production cycle. Marketable yields of Asian leafy greens, harvested at 36 DAT, were reported to be 1.66 to 1.97 times higher when planted in February, with 352 accumulated GDDs, compared with November planting with 245 accumulated GDDs in Starkville, MS, USA[2]. Higher yield of lettuce, spinach, and Asian green cultivars were obtained from a warmer location when trialed for winter production in high tunnels in the northwest US[21].

Within the experiment duration, daily average temperature fluctuated from 6.97 to 22.1 °C, with a minimum temperature of −3.51 °C, and maximum temperature of 34.28 °C inside the high tunnel. At harvest (36 DAT), a total of 353 GDDs were accumulated using 4 °C as the base temperature. DLI ranged from 3.25 to 33.06 mol·m$^{-2}$·d$^{-1}$. Asian leafy green cultivars are known to tolerate light frost and perform best with daily average temperatures of 13–21 °C[4,11,12]. The high tunnel provided a feasible environment with elevated heat to produce the fast-growing *Brassica rapa* cultivars during early spring or even enable winter production in the southeastern US, as shown by previous research[2]. The protection from the high tunnel and lack of environmental stressors, e.g., extreme temperatures, likely shielded beneficial effects from biostimulant applications, whose benefits are more reported under suboptimal growing conditions with abiotic stresses. It should be noted that this study was conducted during a single production season and was not repeated across seasons or years. However, local temperature data and heat accumulation (GDDs) could be used as a reference to predict the seasonal yield pattern of *Brassica rapa* cultivars.

Although biostimulants did not alter plant size, leaf SPAD, marketable yield, or individual fresh and dry shoot weights in our study, the *Ecklonia maxima*-derived seaweed extract Kelpak Maxx increased overall leaf temperature and increased P and Fe concentrations overall or in certain cultivars. Cultivar-specific differences in nutrient uptake suggest an interaction between genetic background and nutrient use efficiency. The increased nutrient uptake could be driven by the beneficial effect from Kalpak Maxx on root development, observed evidently in the two fast growing cultivars, including 'Win Win Choi' and 'QD2-Express', with the highest fresh shoot weights. This pattern indicates that cultivars with greater intrinsic growth rates and nutrient demand may more effectively utilize the biostimulant-mediated improvements to root development and nutrient uptake. Previous studies have shown that seaweed extracts from various marine species can improve root growth and improve water and nutrient uptake[42,43]. Our findings are in agreement with previous reports showing that the application of *A. nodosum* and *Laminaria spp*. significantly increased absorption of macronutrients (S, Mg, and Ca) and micronutrients (Zn, Fe, B, Cu, and Mo) in maize than control[44]. Similarly, the use of *E. maxima* also increased concentrations of N, P, K, and Mg in spinach grown in high tunnels, along with increased head fresh weight, ascorbic acid, and polyphenols[45].

In this current study, biostimulant treatment also affected gas exchange in the tested Asian green cultivars. Specifically, the seaweed extract Kelpak Maxx increased electron transport rate compared with Dune or Continuum, confirming its beneficial effects in physiological processes like photosynthesis. However, this improvement to photosynthetic electron transport did not consistently translate into increased plant biomass or marketable yield. This suggests that cultivar specific genotypic traits for growth and resource allocation constrained productivity under the environmental conditions of this study. Seaweed extracts are known to contain a diverse array of bioactive molecules, including eckol with auxin-like activity, as well as brassinosteroids, which can modulate phytohormone signaling and nutrient transport[46−48]. The betaine compounds found in seaweed extracts were reported to inhibit chlorophyll degradation and suspend photosynthetic activity loss[49]. As a result, a significant increase in chlorophyll content, stomatal conductance, phytosynthetic rate, and transpiration was reported in asparagus plants treated with *A. nodosum*[50]. The extract of *E. maxima* enhanced electron transfer rates of both photosystems in willow plants[51]. When supported by appropriate research, the application of various biostimulants can potentially serve as a sustainable approach to increase productivity and alleviate stress in horticultural crop production.

# Conclusions

The five tested Asian green cultivars varied in plant size, leaf coloration, individual fresh and dry plant weight, and marketable yield. Marketable yields of tested Asian green cultivars ranged from 0.79 to 1.84 kg·m$^{-2}$, with the two fast-growing cultivars 'Win Win Choi' and 'QD2-Express' producing the highest and second-highest marketable yield and fresh shoot weight. Genetic variation among cultivars was the primary determinant of these parameters, including plant stature, biomass accumulation, and leaf coloration. The high tunnel resulted in heat accumulation of 353 GDDs within the experiment duration, and served as a feasible season extension tool for the cool-season production of Asian green cultivars from late fall to early spring in the southeastern US. The application of three biostimulants, including a seaweed extract, an ortho-silic acid, and a PGPR inoculant, did not alter plant size or marketable yield, but affected physiological parameters, including leaf temperature, electron transport rate, PhiPS2, and altered mineral nutrient uptake of P, K, Ca, Fe, and Cu. The seaweed extract Kelpak Maxx increased nutrient uptake of Fe and P overall or in certain cultivars, and increased electron transport rate and leaf temperature compared with the other biostimulant treatments, or control. The benefits of biostimulant application are highly dependent on the growing environment and may potentially be more pronounced under suboptimal conditions, including temperature extremes, high salt levels, drought, or limited nutrient availability.

## Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Li T; experiment operation and data collection: Arthur J, Li T; draft manuscript preparation: Arthur J; review and editing: Li T, Bi G, White S, Bheemanahalli R; funding acquisition, Li T, Bi G. All authors reviewed the results and approved the final version of the manuscript.

## Data availability

All data generated or analyzed during this study are included in this published article.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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