



지베렐린(GA₃) 경엽처리에 따른 두 겨자(*Brassica juncea* L.) 품종의 형태생리학적 반응 및 주요 플라보놀 배당체에 대한 정성 분석

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Morphophysiological Responses of Two Mustard (*Brassica juncea* L.) Cultivars to Foliar Application of Gibberellic Acid (GA₃) and Qualitative Analysis of Major Flavonol Glycosides

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ABSTRACT

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Background: Mustard (*Brassica juncea* L.) is a medicinal oilseed crop of the Brassicaceae family, globally used as a food spice and folk medicine. Although extensive research has already been conducted on gibberellin (GA) application to mustard, studies focusing on specific cultivars such as ‘Asia Jeok’, used as a leafy vegetable and kimchi ingredient, and ‘Red Frill’, used as a spice, remain limited. Therefore, in this study, we investigated the morphophysiological responses of these two cultivars to GA treatment and performed a qualitative analysis of flavonol glycosides among their bioactive compounds. **Methods and Results:** We performed a single foliar application of GA₃ at 0 (control), 50, 100, 150, or 200 mg·ℓ⁻¹ to four-week-old seedlings of two cultivars, ‘Asia Jeok’ (AJ) and ‘Red Frill’ (RF), and evaluated their morphological traits, biomass accumulation and allocation, plant quality indices, remote sensing-based vegetation indices, and OJIP chlorophyll fluorescence parameters four weeks later. GA₃ promoted shoot width and leaf expansion in both cultivars, with the strongest responses generally observed at 50 mg·ℓ⁻¹. Thereafter, cultivar responses diverged. In AJ plants, GA₃ at 200 mg·ℓ⁻¹ enhanced shoot biomass and yielded higher values for compactness and Dickson quality index, indicating coordinated growth and structural quality improvements. In RF plants, morphological performance was most favorable at 50 mg·ℓ⁻¹, whereas treatment with 150 mg·ℓ⁻¹ GA₃ coincided with higher energy dissipation and lower performance index on an absorption basis than those in the control, suggesting a shift toward less favorable photochemical adjustment at intermediate-to-high concentrations. In addition, chemical profiling through UHPLC-PDA-ESI-MS/MS identified 11 major flavonol glycosides with distinct dominant cultivar-specific peaks. **Conclusions:** Our results support cultivar-specific GA₃ application to mustard crops and indicate that intermediate-to-high concentrations might unfavorably alter photochemistry.

Key Words: *Brassica juncea*, Biomass Allocation, Chlorophyll Fluorescence, Flavonol Glycosides, OJIP Transient, Photosystem II, Plant Growth Regulator, Remote Sensing Vegetation Indices

INTRODUCTION

Recent advances in crop production have increasingly

underscored the importance of growth management strategies that enable precise, stage-specific regulation of plant traits and reliable attainment of desired production targets (Gebbers and

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Adamchuk, 2010; Rezaei *et al.*, 2017). Within this framework, plant growth regulators (PGRs) have been used as practical tools to directly modulate plant growth and development (Rademacher, 2015; Lee *et al.*, 2025a), and their application has been evaluated across a wide range of crops under specific management objectives (Rajala, 2004; Lee *et al.*, 2020; Lee *et al.*, 2024; Wu *et al.*, 2024).

Among the PGRs, gibberellins (GAs) are representative phytohormones that regulate multiple growth and developmental processes, including shoot elongation, leaf expansion, and the transition to reproductive development, and exogenous application is well documented to elicit pronounced changes in plant morphology (Hauvermale *et al.*, 2012; Davière and Achar, 2013). In addition to morphological responses, gibberellic acid (GA₃) treatment can also be accompanied by shifts in photosynthetic performance, plant water status, biomass accumulation, and resource allocation patterns (Iqbal *et al.*, 2011; Fu *et al.*, 2023). Accordingly, a rigorous assessment of the effects of GA₃ would be facilitated by an integrated morphophysiological framework that considers physiological indicators along with growth and allocation traits.

In this framework, GA₃-induced morphological expansion and biomass allocation are expected to alter canopy structure and light interception (Hedden and Thomas, 2012), thereby potentially influencing PSII energy partitioning, as reflected by chlorophyll fluorescence (Kramer *et al.*, 2004). In addition, cultivar-specific antioxidant/photoprotective metabolite backgrounds may modulate physiological stability under rapid growth stimulation (Agati *et al.*, 2013); thus, qualitative metabolite profiling can provide a supportive baseline context for interpreting cultivar-dependent photochemical responses to GA₃.

Mustard (*Brassica juncea* L.) is an economically important vegetable and oil crop with recognized nutraceutical and medicinal relevance. Mustard plants have been traditionally used for the treatment of various diseases, including cancer, obesity, depression, and diabetes. Furthermore, diverse biological activities, including anticancer, antioxidant, antiviral, and antidiabetic effects, have been reported, attributable to bioactive phytochemicals such as glucosinolates and phenolic compounds (Tian and Deng, 2020; Zhang *et al.*, 2023; Hu and Yan, 2025).

Interestingly, *B. juncea* cultivars exhibit substantial diversity in morphological traits and growth habits (Vinu *et al.*, 2013). Even under identical PGR regimes, responses can differ as a function of genetic background and cultivar-specific characteristics

(McCabe and Burke, 2021; Dick and VanderWeide, 2025). More broadly, such cultivar-level variation can extend to responsiveness to exogenous hormone applications, such that the morphophysiological outcomes elicited by the same GA₃ concentration may differ among cultivars (Pavlista *et al.*, 2012; Elahi *et al.*, 2022). Therefore, an experimental comparison of cultivar-specific responses to the foliar application of GA₃ is warranted.

Foliar application is operationally straightforward and allows for a relatively flexible selection of application timing and concentration, making it a practical approach for inducing and regulating target traits in a stage-specific manner (Niu *et al.*, 2021). Direct delivery through the leaf surface is highly amenable to field applications, and both the application intensity and frequency can be adjusted in a management-oriented fashion to accommodate the prevailing production conditions, thereby facilitating a stepwise growth management strategy (Noack *et al.*, 2010; Lovatt, 2013).

To evaluate the effects of foliar-applied GA₃ from a physiological perspective, employing metrics that are suitable for use in the field is important. Chlorophyll fluorescence provides a non-destructive (or non-invasive) proxy for photosystem II (PSII) photochemical efficiency and stress-related responses (Jang *et al.*, 2023), enabling sensitive detection of treatment-associated shifts in photochemical performance (Maxwell and Johnson, 2000; Kim *et al.*, 2024a). In addition, remote sensing-based vegetation indices are useful for inferring the pigment status and optical properties of leaves and thus can support the rapid assessment of plant conditions *in situ* (Xue and Su, 2017; Lee *et al.*, 2025b). Collectively, the integration of these approaches is expected to strengthen the interpretation of physiological responses to exogenous GA₃ and provide an empirical basis for linking morphological and physiological responses within a unified framework.

The physiological effects of GA foliar application are variable across mustard cultivars. Composition and content of bioactive compounds also show variation between cultivars (Arena *et al.*, 2020; Ibrahim *et al.*, 2023) and those differences may be critical determinants of plant quality and medicinal value of mustard crops. Although *B. juncea* is primarily known as an oilseed crop, its aerial parts are widely consumed as food and used as medicine, such as in 'ssam' (wraps) or 'kimchi', particularly in Korea. Therefore, the identification and quantification of bioactive components in the aerial parts of different mustard cultivars are beneficial for evaluating their

medicinal value. Despite general acknowledgement of biochemical differences among mustard cultivars, studies in which the composition and content of these compounds have been compared across cultivars are limited.

Accordingly, we not only examined the effects of gibberellin on the morphophysiological characteristics of different cultivars but also performed a qualitative analysis of bioactive compounds in each cultivar. The results of this study serve as a basic reference to support future quantitative evaluations of bioactive compounds following GA treatment. Given that functional secondary metabolites, including glucosinolates and flavonoids, have been reported in *B. juncea*, our qualitative analysis specifically focused on flavonol glycosides as a major compound group closely associated with antioxidant activity (Heim *et al.*, 2002; Ahn *et al.*, 2007; Huang *et al.*, 2022).

Thus, the tandem objectives of this study were to a) quantify the key morphological traits and physiological responses (specifically, chlorophyll fluorescence and vegetation indices) of two mustard cultivars in response to foliar-applied GA₃ and b) provide complementary chemical baseline information by conducting UHPLC-PDA-ESI-MS/MS-based qualitative profiling to identify the major flavonol glycoside peaks in the two cultivars.

MATERIALS AND METHODS

1. Preparation of plant materials

Seeds of two mustard (*B. juncea*) cultivars, ‘Asia Jeok’ (AJ) and ‘Red Frill’ (RF), were obtained from a seed company (Asia Seed, Seoul, Korea). A propagation medium was prepared by mixing a non-fertilized horticultural substrate (Hanareumsangto, Shinsung Mineral, Goesan-gun, Korea), perlite (Ecolite Perlite, Homansaneob, Jeongeup, Korea), and vermiculite (Ecolite Jilseog, Homansaneob, Jeongeup, Korea) at a 1:1:1 (v/v/v) ratio, and the mixture was filled into 105-cell plug trays. Seeds were sown at three seeds per cell.

After sowing, the trays were transferred to a closed nursery system in an experimental greenhouse at the Department of Environmental Horticulture, Sahmyook University (Seoul, Korea). The seedlings were grown on nursery benches (1.2 × 0.7 × 0.6 m; width × length × height). White light-emitting diodes at color temperature 4100 K (T5 LED, Zhong Shan

Jinsung Electronic, Zhōngshān, China) were used as the light source, and photosynthetic photon flux density was maintained at 100 μmol·m⁻²·s⁻¹. During the nursery period, air temperature and relative humidity were maintained at 20 ± 1°C and 62.3 ± 16.9%, respectively. Following the method described by Lee *et al.* (2025b), a nutrient solution was supplied twice weekly via sub-irrigation. The nutrient solution was prepared by dissolving 15 g of a 4-18-38 premix (Masterblend International-Tyler Enterprises, Morris, IL, USA), 7.5 g of MgSO₄ (Smartrio MgS, Busan, Korea), and 15 g of Ca(NO₃)₂ (Smartrio CAL, Gijang-gun, Busan, Korea) in 20 ℓ of purified water. Approximately four weeks after sowing, the seedlings were thinned to one uniformly developed plant per cell. The selected seedlings were then hardened for 72 h prior to use in the experiment.

2. Experimental design and environmental location

The field experiment was conducted at the Sahmyook Green Education Practice Center, Sahmyook University, Seoul, Korea (37°38'16"N, 127°06'30"E) for four weeks, from September 16 to October 15, 2024. Typically, leaf mustard is harvested 35 to 40 days after sowing in the summer and 60 to 70 days in the autumn and winter seasons (Assefā *et al.*, 2023). Several studies on leaf mustard have evaluated morphophysiological traits by harvesting plants 28 to 35 days after transplanting seedlings. Accordingly, we selected four weeks after transplantation as the time point for evaluating the responses to GA₃ treatment (Maršić *et al.*, 2021; Janah *et al.*, 2023).

Exogenous GA₃ (CAS No. 77-06-5; Sigma-Aldrich, St. Louis, MO, USA) was applied as a foliar spray at five concentrations: 0 (control), 50, 100, 150, or 200 mg·ℓ⁻¹. GA₃ was first dissolved in 10 ml of 95% EtOH and then diluted with purified water to the final concentrations. Foliar applications were performed 72 h after transplantation. In each cultivar × GA₃ treatment, a total of 1 ℓ of the prepared solution was applied using a hand sprayer, equally distributed across the five replicates (200 ml per replicate), to ensure full coverage of the plant canopies.

The GA₃ application rates were based on those used in previous studies of GA₃ application to *Brassica* crops (Elahi *et al.*, 2022; Prodhan *et al.*, 2022; Shahi *et al.*, 2022). The selected range of GA₃ concentrations allows for a practical

Table 1. Soil physicochemical characteristics of the experimental field.

pH (1:5)	EC (dS/m)	CEC (cmol/kg)	Organic matter (%)	T-N (%)	T-P (mg/kg)	Total K (mg/kg)	Ca (mg/kg)
7.1	1.17	20.5	6.2	0.351	2406.0	3184.5	7482.9

foliar spray concentration window, enabling the assessment of concentration-dependent responses.

During the experimental period, ambient air temperature, relative humidity, and mean cloudiness averaged $27.8 \pm 3.8^\circ\text{C}$, $70.4 \pm 13.7\%$, and 5.4 ± 1.3 okta, respectively. The physicochemical properties of the experimental field soils are listed in Table 1.

3. Parameters and plant quality indices

The following growth- and yield-related parameters were measured: shoot height, shoot width, leaf length, leaf width, number of leaves, leaf area, stem diameter, root length, main root thickness, and the fresh and dry weights of shoots and roots. The chlorophyll content (SPAD units) was determined using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Tokyo, Japan). Compactness (Eq. 1), the Dickson quality index (DQI; Eq. 2) and relative moisture content (RMC; Eq. 3) were calculated following Hong *et al.* (2025), Dickson *et al.* (1960), and Lee and Nam (2024).

$$\text{Compactness} = \text{SDW}/\text{SH} \quad (\text{Eq. 1})$$

$$\text{DQI} = \text{TDW}/(\text{SH}/\text{SD} + \text{SDW}/\text{RDW}) \quad (\text{Eq. 2})$$

$$\text{RMC} = [(\text{FW} - \text{DW})/\text{FW}] \times 100 \quad (\text{Eq. 3})$$

(abbreviation; SDW: shoot dry weight; SH: shoot height; TDW: total dry weight; SD: stem diameter; RDW: root dry weight; FW: fresh weight; and DW: dry weight)

Leaf color characteristics were evaluated using the Commission Internationale de l'Eclairage Lab (CIELAB) color space. A spectrophotometer (CM-2600d, Konica Minolta, Tokyo, Japan) was set to D65/10° and operated in the specular component included (SCI) mode to record L^* , a^* , and b^* values. The measurement conditions and procedures were the same as those described by Lee (2023).

The mean CIELAB coordinates (L^* , a^* , and b^*) for each treatment were converted into their corresponding digital color representations using Converting Colors (Zettl, 2026). The resulting color chips were used to support a qualitative visual assessment of leaf color differences among the treatments.

4. Remote sensing vegetation indices and chlorophyll fluorescence

Remote sensing-based vegetation indices were measured

using a portable spectroradiometer (PolyPen RP410; Photon Systems Instruments, Drásov, Czech Republic). The anthocyanin reflectance index 2 (ARI2; Eq. 4), and carotenoid reflectance index 2 (CRI2; Eq. 5), normalized difference vegetation index (NDVI; Eq. 6), photochemical reflectance index (PRI; Eq. 7), and modified chlorophyll absorption ratio index (MCARI; Eq. 8) were calculated using the equations given by Lee *et al.* (2025b).

$$\text{ARI2} = \rho_{800} \times [(1/\rho_{550}) - (1/\rho_{700})] \quad (\text{Eq. 4})$$

$$\text{CRI2} = (1/\rho_{510}) - (1/\rho_{700}) \quad (\text{Eq. 5})$$

$$\text{NDVI} = (\rho_{\text{NIR}} - \rho_{\text{Red}}) / (\rho_{\text{NIR}} + \rho_{\text{Red}}) \quad (\text{Eq. 6})$$

$$\text{PRI} = (\rho_{531} - \rho_{570}) / (\rho_{531} + \rho_{570}) \quad (\text{Eq. 7})$$

$$\text{MCARI} = [(\rho_{700} - \rho_{670}) - 0.2 \times (\rho_{700} - \rho_{550})] \times (\rho_{700} / \rho_{670}) \quad (\text{Eq. 8})$$

Physiological responses were evaluated by measuring chlorophyll fluorescence using a portable fluorometer (FluorPen FP 110/D; Photon Systems Instruments, Drásov, Czech Republic). Measurements were conducted at night (22:00-04:00) to ensure full dark adaptation in accordance with the manufacturer's guidelines (PSI, 2026). The measurement protocol was based on that described by Shin *et al.* (2024). Briefly, the excitation wavelength was set to 455 nm and F_m for the JIP-test was induced by applying saturating pulse at $1,500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, corresponding to 50% of the super pulse. The selected fluorescence parameters were V_j (Eq. 9), V_i (Eq. 10), F_v/F_m (Eq. 11), M_o (Eq. 12), PI_{ABS} (Eq. 13), Φ_{P_o} (Eq. 14), Ψ_o (Eq. 15), Φ_{E_o} (Eq. 16), Φ_{D_o} (Eq. 17), ABS/RC (Eq. 18), TR_o/RC (Eq. 19), ET_o/RC (Eq. 20), and DI_o/RC (Eq. 21). The equations, as given by Kim *et al.* (2024a) and Lee *et al.* (2025b) are as follows:

$$V_j = (F_j - F_o) / (F_m - F_o) \quad (\text{Eq. 9})$$

$$V_i = (F_i - F_o) / (F_m - F_o) \quad (\text{Eq. 10})$$

$$F_v / F_m = (F_m - F_o) / F_m \quad (\text{Eq. 11})$$

$$M_o = (\Delta V / \Delta t)_o = 4 \text{ ms}^{-1} \times (F_{300\mu\text{s}} - F_o) / (F_m - F_o) \quad (\text{Eq. 12})$$

$$PI_{\text{ABS}} = (\text{RC}/\text{ABS}) \times [\Phi_{\text{P}_o} / (1 - \Phi_{\text{P}_o})] \times [\Psi_o / (1 - \Psi_o)] \quad (\text{Eq. 13})$$

$$\Phi_{Po} = TR_o/ABS = 1 - (F_o/F_m) \text{ (or } F_v/F_m) \quad (\text{Eq. 14})$$

$$\Psi_o = ET_o/TR_o = 1 - V_j \quad (\text{Eq. 15})$$

$$\Phi_{Eo} = ET_o/ABS = [1 - (F_o/F_m)] \times \Psi_o \quad (\text{Eq. 16})$$

$$\Phi_{Do} = 1 - \Phi_{Po} = (F_o/F_m) \quad (\text{Eq. 17})$$

$$ABS/RC = M_o \times (1/V_j) \times (1/\Phi_{Po}) \quad (\text{Eq. 18})$$

$$TR_o/RC = M_o \times (1/V_j) \quad (\text{Eq. 19})$$

$$ET_o/RC = M_o \times (1/V_j) \times \Psi_o \quad (\text{Eq. 20})$$

$$DI_o/RC = (ABS/RC) - (TR_o/RC) \quad (\text{Eq. 21})$$

5. Preparation of samples for UHPLC-PDA-ESI-MS/MS analysis

The leaves of the mustard plants were hot-air dried at 120°C and then pulverized. Powdered samples (0.5 g) were extracted with 50% MeOH (3 × 5 ml for 1 h each) at 45°C. After the extract was concentrated using a nitrogen evaporator, an appropriate amount of MeOH was added, and the resultant extract solution was filtered to make an HPLC sample with a concentration of 1 mg·ml⁻¹.

6. UHPLC-PDA-ESI-MS/MS analysis

The total extracts from the mustard plants were analyzed using a UHPLC system (Vanquish Flex; Thermo Fisher Scientific, Waltham, MA, USA) coupled with a quadrupole time-of-flight (qTOF) mass spectrometer (Q-TOF 5600; AB SCIEX, Framingham, MA, USA) and a photodiode array detector (Ultimate 3000 PDA detector; Thermo Fisher Scientific, Waltham, MA, USA). Liquid chromatographic analyses were

Table 2. Mobile phase conditions for qualitative analysis of major flavonol glycosides in mustard (*B. juncea*).

Time (min)	Gradient (%)		Flow (ml·min ⁻¹)
	DDW w/ 0.1% FA ¹⁾	ACN	
0.0	97	3	0.25
15.0	85	15	
50.0	0	100	
55.0	0	100	
55.1	97	3	
60.0	97	3	

¹⁾FA: formic acid

performed on a C₁₈ reverse-phase LC column (Waters Cortecs T3, 1.6 μm, 2.1 × 150 mm). The mobile phase conditions are listed in Table 2. The temperatures for the column oven and sample controller were kept at 45°C and 10°C, respectively. The volume of each sample injected was 1 μl. To ensure efficient and accurate identification of major flavonoids, the ultraviolet (UV) wavelength was set to 330 nm.

7. Data analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). The experiment was arranged in a completely randomized design (CRD) with five replicates per cultivar × treatment (GA₃ concentration). Within each replicate, six plants were measured and averaged to obtain a replicate mean, which was used for statistical analysis (n = 5). Thirty plants were assessed for each treatment combination. The main factors were cultivar and treatment, and a two-way analysis of variance (ANOVA) was conducted to evaluate the effects of these factors and their interaction. Post-hoc comparisons were performed using Duncan's Multiple Range Test (DMRT) at p < 0.05. Although Tukey's Honestly Significant Difference (HSD) is effective in controlling Type I errors, it is often considered too conservative for the analysis of data in agricultural studies, potentially increasing the risk of Type II errors (missing true differences). Therefore, DMRT was selected to maximize the statistical power for detecting significant differences among treatments (Carmer and Swanson, 1971).

RESULTS

1. Analysis of morphological traits, plant quality indices, and chlorophyll content

The two mustard cultivars examined in this study responded differently to foliar application of GA₃ at different concentrations (Fig. 1 and Fig. 2). At four weeks after foliar GA₃ application, shoot height and width varied significantly among treatments (p < 0.001), whereas leaf length and width were affected at p < 0.01 and p < 0.05, respectively. Similarly, leaf number differed significantly among treatments (p < 0.01). In contrast, chlorophyll content (SPAD units) did not differ significantly among the treatments.

In a cultivar-specific comparison, the AJ cultivar exhibited the greatest shoot height at 200 mg·l⁻¹ (38.84 cm), whereas the RF cultivar reached its maximum shoot height at 50 mg·l⁻¹ (33.03 cm). Shoot width was maximized at 50 mg·l⁻¹ in both

GA₃ 경엽처리가 겨자 품종에 미치는 영향

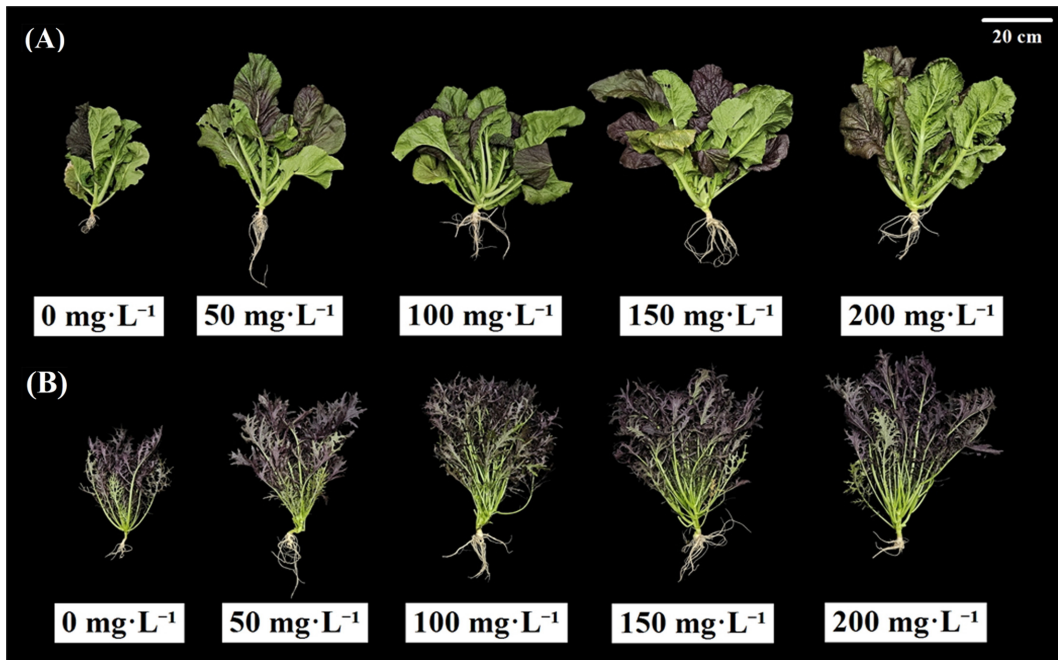


Fig. 1. Representative photographs of mustard plants (*B. juncea*) at four weeks after foliar application of gibberellic acid (GA₃) at different concentrations. (A) *B. juncea* cv. Asia Jeok. (B) *B. juncea* cv. Red Frill.

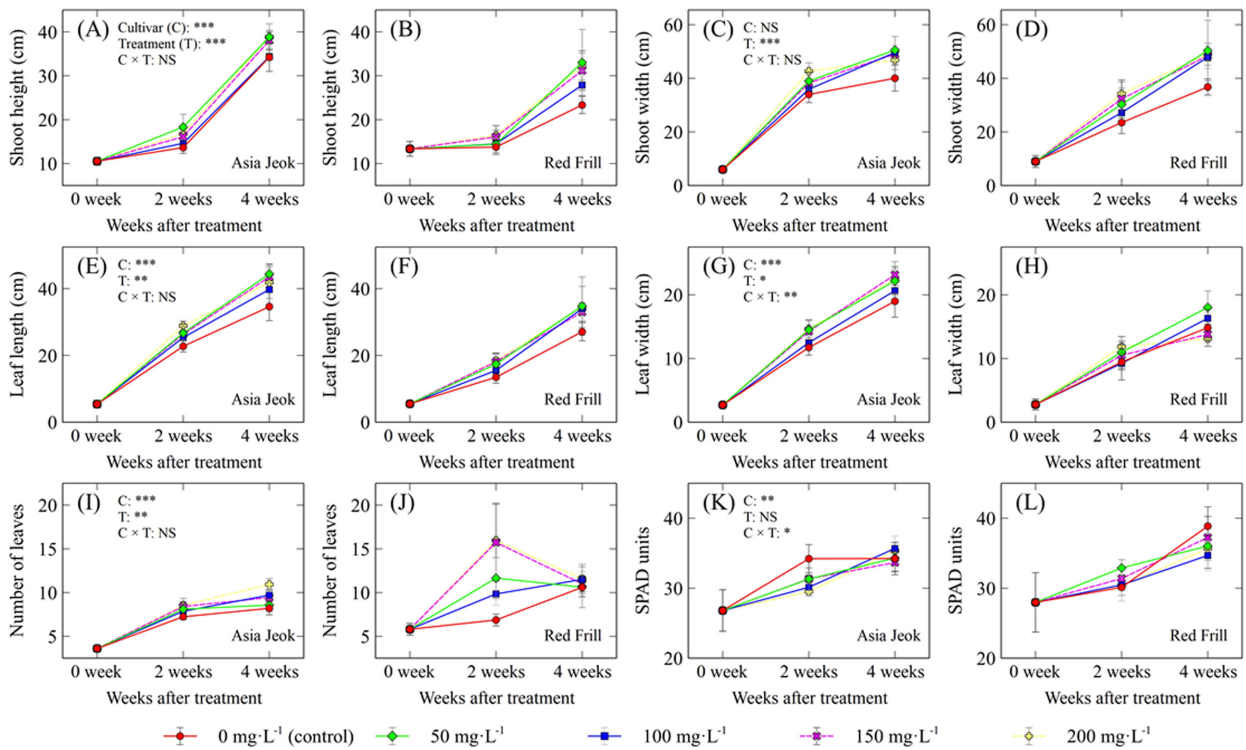


Fig. 2. Plant morphological traits and chlorophyll content (SPAD units) of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') measured at four weeks after foliar application of gibberellic acid (GA₃) at different concentrations. Panels show (A, B) shoot height, (C, D) shoot width, (E, F) leaf length, (G, H) leaf width, (I, J) number of leaves, and (K, L) chlorophyll content (SPAD units), with the left panel of each pair representing 'Asia Jeok' and the right panel representing 'Red Frill'. Values are means \pm standard deviation (SD), ($n = 5$). NS, non-significant; *, **, and *** indicate significance at $p < 0.05$, 0.01 , and 0.001 , respectively (at four weeks after treatment).

Table 3. Plant morphological traits, compactness, and Dickson quality index (DQI) of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') at four weeks after foliar application of gibberellic acid (GA₃) at different concentrations.

Cultivars	GA ₃ concentrations (mg·l ⁻¹)	Stem diameter (cm)	Ground cover (cm ²)	Leaf thickness (mm)	Leaf area (cm ²)	Root length (cm)	Main root thickness (cm)	Compactness (g·cm ⁻¹)	DQI
Asia Jeok	0 (control)	1.12 ± 0.18 ^b	1620 ± 381 ^b	0.69 ± 0.04 ^a	665.2 ± 158 ^b	16.69 ± 2.23 ^a	0.97 ± 0.19 ^c	0.380 ± 0.07 ^c	0.271 ± 0.06 ^b
	50	1.36 ± 0.11 ^a	2575 ± 526 ^a	0.63 ± 0.06 ^a	987.8 ± 126 ^a	17.82 ± 3.27 ^a	1.22 ± 0.19 ^b	0.491 ± 0.12 ^{bc}	0.417 ± 0.11 ^a
	100	1.39 ± 0.11 ^a	2467 ± 273 ^a	0.64 ± 0.08 ^a	824.1 ± 121 ^a	17.17 ± 3.38 ^a	1.12 ± 0.18 ^{bc}	0.473 ± 0.09 ^{bc}	0.396 ± 0.10 ^{ab}
	150	1.39 ± 0.16 ^a	2406 ± 159 ^a	0.66 ± 0.01 ^a	1011.2 ± 141 ^a	18.11 ± 1.49 ^a	1.48 ± 0.14 ^a	0.567 ± 0.07 ^{ab}	0.457 ± 0.09 ^a
	200	1.41 ± 0.14 ^a	2203 ± 328 ^a	0.69 ± 0.06 ^a	949.8 ± 185 ^a	17.91 ± 1.53 ^a	1.53 ± 0.14 ^a	0.645 ± 0.10 ^a	0.521 ± 0.11 ^a
Red Frill	0 (control)	0.79 ± 0.10 ^b	1358 ± 210 ^b	0.80 ± 0.04 ^a	408.2 ± 118 ^b	11.82 ± 1.54 ^b	0.45 ± 0.03 ^b	0.283 ± 0.03 ^a	0.128 ± 0.03 ^b
	50	1.03 ± 0.13 ^{ab}	2636 ± 1071 ^a	0.65 ± 0.03 ^b	643.1 ± 230 ^a	14.90 ± 1.50 ^a	0.90 ± 0.17 ^a	0.368 ± 0.09 ^a	0.273 ± 0.06 ^{ab}
	100	0.98 ± 0.21 ^{ab}	2310 ± 437 ^a	0.66 ± 0.07 ^b	554.2 ± 27 ^{ab}	9.69 ± 2.20 ^c	0.82 ± 0.24 ^a	0.323 ± 0.16 ^a	0.248 ± 0.18 ^{ab}
	150	1.14 ± 0.19 ^a	2348 ± 194 ^a	0.66 ± 0.05 ^b	455.5 ± 57 ^b	13.92 ± 1.47 ^{ab}	0.87 ± 0.14 ^a	0.403 ± 0.15 ^a	0.328 ± 0.15 ^a
	200	1.00 ± 0.20 ^{ab}	2414 ± 405 ^a	0.51 ± 0.01 ^c	454.0 ± 85 ^b	13.65 ± 0.84 ^{ab}	0.81 ± 0.32 ^a	0.386 ± 0.12 ^a	0.304 ± 0.15 ^{ab}
Significance ¹⁾	Cultivar (C)	***	NS	NS	***	***	***	***	***
	Treatment (T)	**	***	***	**	*	***	**	**
	(C) × (T)	NS	NS	***	NS	NS	NS	NS	NS

Means ± standard deviation (SD), (n = 5). ^aWithin each cultivar, means were separated using Duncan's multiple range test (DMRT) at p < 0.05. Means followed by different lowercase letters differ significantly. ¹⁾NS, non-significant; *, **, and *** indicate significance at p < 0.05, 0.01, and 0.001, respectively.

cultivars, reaching 50.54 cm in AJ and 50.33 cm in RF. Leaf length and leaf width followed a similar pattern, with the highest mean values observed at 50 mg·l⁻¹. Leaf number peaked at 200 mg·l⁻¹, with mean values of 10.9 and 11.6 leaves in AJ and RF, respectively.

As shown in Table 3, stem diameter in AJ increased under GA₃ treatments of 50-200 mg·l⁻¹, ranging from 1.36 to 1.41 cm, whereas in RF the thickest stems were obtained at 150 mg·l⁻¹ (1.14 cm), which was greater than the corresponding value in the control (0.79 cm). Ground cover expanded markedly in both cultivars at GA₃ concentrations ranging from 50 to 200 mg·l⁻¹ and was significantly greater than that of the control.

Leaf thickness did not differ significantly among the treatments in AJ; however, RF exhibited the greatest leaf thickness in the control (0.80 mm). Leaf area responses to GA₃ differed by cultivar and concentration: in AJ, all GA₃ treatments from 50 to 200 mg·l⁻¹ resulted in larger leaf areas (824.1-1011.2 cm²) than was found in the control (665.2 cm²), whereas in RF, the largest leaf area occurred at 50 mg·l⁻¹ (643.1 cm²), compared with 408.2 cm² in the control.

Root length did not differ significantly among treatments in AJ, whereas in RF the longest roots were obtained at 50 mg·l⁻¹ GA₃ (14.90 cm), exceeding those at 100 mg·l⁻¹ (9.69 cm). Main

root thickness in AJ was the greatest at 150-200 mg·l⁻¹ (1.48-1.53 cm), whereas in RF, GA₃ treatments of 50-200 mg·l⁻¹ increased main root thickness to 0.81-0.90 cm relative to the control (0.45 cm).

With respect to plant quality indices, compactness in AJ was the highest at 200 mg·l⁻¹ GA₃ (0.645), exceeding the control (0.380), whereas compactness in RF did not differ significantly among treatments. As for DQI, the AJ cultivar showed higher values than did the control (0.271) at 50, 150, and 200 mg·l⁻¹ GA₃ (0.417, 0.457, and 0.521, respectively). In the RF cultivar, DQI was relatively higher at 150 mg·l⁻¹ (0.328) than in the control (0.128).

2. Analysis of biomass and relative moisture content

Regarding biomass, shoot fresh weight increased in both cultivars at 200 mg·l⁻¹ GA₃, reaching 379.7 g in AJ and 157.0 g in RF, compared with the respective values in the controls (202.5 and 73.7 g) (Table 4). Shoot dry weight in AJ was also highest at 200 mg·l⁻¹ (25.09 g), exceeding the control (13.19 g). However, shoot dry weight in RF did not differ significantly among treatments.

Root fresh weight in AJ was greater under 150-200 mg·l⁻¹ GA₃ (10.94-13.43 g) than under lower concentrations. In RF,

Table 4. Biomass components (fresh and dry weights) and relative moisture content of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') at four weeks after foliar application of gibberellic acid (GA₃) at different concentrations.

Cultivars	GA ₃ concentrations (mg·ℓ ⁻¹)	Shoot weights (g)		Root weights (g)		Relative moisture content (%)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Shoot part	Root part
Asia Jeok	0 (control)	202.5 ± 59.9 ^d	13.19 ± 3.66 ^c	6.27 ± 1.22 ^b	0.65 ± 0.12 ^b	93.4 ± 0.44 ^a	89.4 ± 2.05 ^a
	50	294.1 ± 48.8 ^{bc}	18.95 ± 4.44 ^b	6.11 ± 1.57 ^b	0.96 ± 0.19 ^a	93.6 ± 0.65 ^a	83.9 ± 1.28 ^b
	100	241.4 ± 32.0 ^{cd}	16.23 ± 2.87 ^{bc}	6.22 ± 2.64 ^b	0.88 ± 0.27 ^{ab}	93.3 ± 0.49 ^a	85.2 ± 2.78 ^b
	150	358.4 ± 61.6 ^{ab}	21.70 ± 3.79 ^{ab}	10.94 ± 1.50 ^a	0.97 ± 0.19 ^a	93.9 ± 0.38 ^a	91.1 ± 1.01 ^a
	200	379.7 ± 88.0 ^a	25.09 ± 4.93 ^a	13.43 ± 2.61 ^a	1.11 ± 0.29 ^a	93.3 ± 0.71 ^a	91.7 ± 0.97 ^a
Red Frill	0 (control)	73.7 ± 12.1 ^b	6.64 ± 1.18 ^a	1.24 ± 0.43 ^b	0.27 ± 0.09 ^b	90.9 ± 0.55 ^a	77.3 ± 5.87 ^b
	50	126.6 ± 46.9 ^{ab}	11.94 ± 3.66 ^a	5.96 ± 1.77 ^a	0.89 ± 0.37 ^a	90.2 ± 2.08 ^a	84.9 ± 6.03 ^a
	100	130.2 ± 58.1 ^{ab}	11.14 ± 5.04 ^a	5.14 ± 2.70 ^a	0.89 ± 0.48 ^a	91.7 ± 0.38 ^a	83.3 ± 0.92 ^a
	150	144.3 ± 60.3 ^{ab}	12.58 ± 4.77 ^a	5.70 ± 2.17 ^a	0.88 ± 0.36 ^a	91.1 ± 1.14 ^a	84.6 ± 2.86 ^a
	200	157.0 ± 70.8 ^a	12.64 ± 5.16 ^a	6.38 ± 3.37 ^a	0.93 ± 0.37 ^a	91.8 ± 0.77 ^a	84.8 ± 1.48 ^a
Significance ¹⁾	Cultivar (C)	***	***	***	*	***	***
	Treatment (T)	***	***	***	**	NS	**
	(C) × (T)	NS	NS	**	NS	NS	***

Means ± standard deviation (SD), (n = 5). ^aWithin each cultivar, means were separated using Duncan's multiple range test (DMRT) at p < 0.05. Means followed by different lowercase letters differ significantly. ¹⁾NS, non-significant; *, **, and *** indicate significance at p < 0.05, 0.01, and 0.001, respectively.

root fresh weight increased across 50-200 mg·ℓ⁻¹ (5.14-6.38 g) relative to the control (1.24 g). Root dry weight in AJ ranged from 0.97 to 1.11 g at 150-200 mg·ℓ⁻¹, which was higher than the corresponding value in the control (0.65 g), but was not significantly different from the values observed at 50 and 100 mg·ℓ⁻¹ (0.96 and 0.88 g, respectively). In RF, root dry weight under 50-200 mg·ℓ⁻¹ ranged from 0.88 to 0.93 g, which was greater than the control (0.27 g).

The relative moisture content of the shoots did not differ significantly among treatments in either cultivar. In contrast, in AJ, the relative moisture content of the root decreased at 50-100 mg·ℓ⁻¹ compared with the control, whereas the values at 150-200 mg·ℓ⁻¹ were statistically comparable to the control. In RF, the relative moisture content of the root was significantly higher under 50-200 mg·ℓ⁻¹ (83.3-84.9%) than in the control group (77.3%).

3. Analysis of shoot external quality and leaf pigments

Among external quality attributes, the CIELAB lightness coordinate (*L*^{*}) did not differ significantly among treatments in AJ; however, in RF, *L*^{*} was highest at 200 mg·ℓ⁻¹ GA₃ (28.03) (Table 5). In AJ, the *a*^{*} coordinate (green-red opponent axis) showed a pattern similar to *L*^{*}, with no significant differences











among treatments. In RF, however, this pattern was not observed; *a*^{*} was greatest in the control (5.72) and lowest at 200 mg·ℓ⁻¹ (3.99). As for the *b*^{*} coordinate (blue-yellow opponent axis), AJ exhibited a higher value at 150 mg·ℓ⁻¹ (5.70) than at 200 mg·ℓ⁻¹ (1.15), whereas RF showed the highest *b*^{*} at 200 mg·ℓ⁻¹ (3.52) relative to the other treatments.

Among the remote sensing-based vegetation indices, anthocyanin reflectance index 2 (ARI2), used as a proxy for leaf anthocyanin status, did not differ significantly among the treatments in either cultivar. Similarly, the carotenoid reflectance index 2 (CRI2), an index associated with carotenoid-related reflectance characteristics, showed no significant treatment effects in either cultivar.

4. Analysis of remote sensing vegetation indices and chlorophyll fluorescence responses

With respect to the remote sensing-based vegetation indices, the NDVI, an indicator of overall plant vigor, tended to be higher in the 100 and 200 mg·ℓ⁻¹ GA₃ treatments than in the control in AJ and RF, respectively (Table 6). Specifically, NDVI increased from 0.694 (control) to 0.716 at 100 mg·ℓ⁻¹ in AJ, and from 0.703 (control) to 0.726 at 200 mg·ℓ⁻¹ in RF. The PRI, a parameter associated with photochemical efficiency,

Table 5. CIELAB color coordinates and pigment-related vegetation indices of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') at four weeks after foliar GA₃ application at different concentrations, including anthocyanin reflectance index 2 (ARI2) and carotenoid reflectance index 2 (CRI2).

Cultivars	GA ₃ concentrations (mg·ℓ ⁻¹)	Leaf color reading values of CIELAB			Converted colors ¹⁾ (color chip)	Pigment content indices ²⁾	
		L*	a*	b*		ARI2	CRI2
Asia Jeok	0 (control)	29.30 ± 1.73 ^a	4.04 ± 1.54 ^a	5.21 ± 1.93 ^{ab}		4.23 ± 0.60 ^a	9.62 ± 0.62 ^a
	50	28.83 ± 2.49 ^a	4.09 ± 1.26 ^a	4.93 ± 2.54 ^{ab}		4.26 ± 0.70 ^a	10.36 ± 0.96 ^a
	100	29.40 ± 2.44 ^a	3.30 ± 1.90 ^a	5.05 ± 2.62 ^{ab}		4.54 ± 0.60 ^a	10.09 ± 0.98 ^a
	150	29.25 ± 2.72 ^a	3.17 ± 2.15 ^a	5.70 ± 3.14 ^a		3.60 ± 1.03 ^a	9.47 ± 1.49 ^a
	200	27.48 ± 2.66 ^a	4.13 ± 2.14 ^a	1.15 ± 3.88 ^b		3.95 ± 0.62 ^a	9.22 ± 0.71 ^a
Red Frill	0 (control)	26.35 ± 0.40 ^b	5.72 ± 0.19 ^a	-0.85 ± 0.33 ^b		5.03 ± 0.89 ^a	9.39 ± 0.88 ^a
	50	26.32 ± 0.30 ^b	5.01 ± 0.38 ^b	-0.18 ± 0.46 ^b		5.08 ± 0.82 ^a	9.82 ± 0.57 ^a
	100	26.69 ± 0.67 ^b	4.88 ± 0.50 ^b	-0.09 ± 0.85 ^b		5.02 ± 0.84 ^a	9.40 ± 0.87 ^a
	150	26.99 ± 0.39 ^b	5.14 ± 0.39 ^{ab}	0.41 ± 1.23 ^b		4.81 ± 0.45 ^a	9.22 ± 0.62 ^a
	200	28.03 ± 1.28 ^a	3.99 ± 0.67 ^c	3.52 ± 1.46 ^a		5.54 ± 0.15 ^a	10.13 ± 0.55 ^a
Significance ³⁾	Cultivar (C)	***	**	***		***	NS
	Treatment (T)	NS	NS	NS		NS	NS
	(C) × (T)	NS	NS	***		NS	NS

Means ± standard deviation (SD), (n = 5). *Within each cultivar, means were separated using Duncan's multiple range test (DMRT) at *p* < 0.05. Means followed by different lowercase letters differ significantly. ¹⁾Colors converted using Commission Internationale de l'Eclairage Lab (CIELAB) color space values (L*, a*, and b*) ²⁾ARI2: anthocyanin reflectance index 2, an index sensitive to leaf anthocyanin accumulation (often associated with photoprotection and stress responses); and CRI2: carotenoid reflectance index 2, an index related to leaf carotenoid content. ³⁾NS, non-significant; ** and *** indicate significance at *p* < 0.01 and 0.001, respectively.

did not differ significantly among treatments in AJ; however, in RF, PRI increased to 0.008 at both 100 and 200 mg·ℓ⁻¹ compared with the result in the control group (0.003). MCARI, which is closely related to the leaf chlorophyll status, did not show significant treatment effects in either cultivar.

In terms of chlorophyll fluorescence parameters, relative variable fluorescence at the J-step (V_j) was higher in AJ under 150-200 mg·ℓ⁻¹ GA₃ (0.292-0.297), and in RF it was the highest at 150 mg·ℓ⁻¹ (0.307) relative to the control (0.266). Relative variable fluorescence at the I-step (V_i) did not differ significantly among treatments in AJ, whereas in RF, V_i was higher across 50-200 mg·ℓ⁻¹ (0.587-0.601). The maximum quantum yield of PSII (F_v/F_m) in AJ reached 0.854 at 100 mg·ℓ⁻¹, which was significantly higher than the value at 200 mg·ℓ⁻¹ (0.849). In RF, F_v/F_m remained relatively high in the control and under 50-100 mg·ℓ⁻¹ (0.848-0.849), but decreased to 0.844 at 150 mg·ℓ⁻¹, indicating a statistically lower mean. The initial slope of the fluorescence induction curve (M₀) was relatively elevated in AJ under 150-200 mg·ℓ⁻¹ (0.336-0.340), whereas in RF, M₀ increased to 0.356 at 150 mg·ℓ⁻¹ compared

with the control (0.265).

The performance index on an absorption basis (PI_{ABS}) in AJ was higher under 50-100 mg·ℓ⁻¹ (12.86-13.13) than under 200 mg·ℓ⁻¹ (10.51). In RF, PI_{ABS} was the highest in the control, with a value of 13.56.

According to the chlorophyll fluorescence parameters associated with quantum yields, foliar application of GA₃ elicited a broad spectrum of physiological responses, and all analyzed variables showed significant treatment effects, with *p*-values ranging from *p* < 0.01-0.001 (Fig. 3 and Table 7). The maximum quantum yield parameter, Φ_{Po}, was identical to the pattern observed for F_v/F_m. The probability that a trapped exciton drives electron transfer beyond Q_A into the electron transport chain (Ψ_o) was the highest in the AJ cultivar under 50 mg·ℓ⁻¹ (0.733) and in the RF cultivar in the control (0.733). Similarly, the quantum yield of electron transport (Φ_{Eo}) reached its maximum in AJ at 50 mg·ℓ⁻¹ (0.619), whereas RF exhibited the highest Φ_{Eo} in the control (0.623). By contrast, the quantum yield of energy dissipation (Φ_{Do}), representing the probability that absorbed excitation energy is dissipated, was the highest in AJ at 200

GA₃ 경엽처리가 겨자 품종에 미치는 영향

Table 6. Remote sensing vegetation indices of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') at four weeks after foliar application of GA₃ at different concentrations, including the normalized difference vegetation index (NDVI), photochemical reflectance index (PRI), and modified chlorophyll absorption ratio index (MCARI), together with technical chlorophyll fluorescence parameters and the performance index on an absorption basis (PI_{ABS}).

Cultivars	GA ₃ concentrations (mg·ℓ ⁻¹)	Remote sensing vegetation indices ¹⁾			Technical fluorescence parameters ²⁾				Performance index ³⁾ (PI _{ABS})
		NDVI	PRI	MCARI	V _j	V _i	F _v /F _m	M ₀	
Asia Jeok	0 (control)	0.694 ± 0.006 ^b	-0.008 ± 0.004 ^a	0.285 ± 0.030 ^a	0.278 ± 0.003 ^b	0.567 ± 0.007 ^a	0.852 ± 0.001 ^{ab}	0.297 ± 0.01 ^b	12.58 ± 0.9 ^{ab}
	50	0.700 ± 0.015 ^{ab}	-0.007 ± 0.005 ^a	0.287 ± 0.033 ^a	0.274 ± 0.010 ^b	0.558 ± 0.012 ^a	0.853 ± 0.003 ^{ab}	0.289 ± 0.02 ^b	13.13 ± 1.9 ^a
	100	0.716 ± 0.006 ^a	-0.008 ± 0.003 ^a	0.284 ± 0.021 ^a	0.278 ± 0.007 ^b	0.547 ± 0.014 ^a	0.854 ± 0.002 ^a	0.293 ± 0.01 ^b	12.86 ± 0.7 ^a
	150	0.696 ± 0.013 ^b	-0.005 ± 0.007 ^a	0.307 ± 0.035 ^a	0.297 ± 0.009 ^a	0.564 ± 0.018 ^a	0.851 ± 0.002 ^{ab}	0.340 ± 0.02 ^a	10.82 ± 1.2 ^{bc}
	200	0.709 ± 0.019 ^{ab}	-0.003 ± 0.003 ^a	0.284 ± 0.025 ^a	0.292 ± 0.014 ^a	0.549 ± 0.016 ^a	0.849 ± 0.004 ^b	0.336 ± 0.03 ^a	10.51 ± 1.5 ^c
Red Frill	0 (control)	0.703 ± 0.017 ^b	0.003 ± 0.002 ^b	0.236 ± 0.032 ^a	0.266 ± 0.010 ^c	0.560 ± 0.014 ^b	0.849 ± 0.001 ^a	0.265 ± 0.02 ^c	13.56 ± 1.5 ^a
	50	0.713 ± 0.009 ^{ab}	0.006 ± 0.003 ^{ab}	0.247 ± 0.024 ^a	0.287 ± 0.014 ^b	0.590 ± 0.011 ^a	0.848 ± 0.002 ^a	0.306 ± 0.02 ^b	11.46 ± 1.4 ^{bc}
	100	0.718 ± 0.008 ^{ab}	0.008 ± 0.004 ^a	0.255 ± 0.016 ^a	0.291 ± 0.007 ^b	0.587 ± 0.006 ^a	0.849 ± 0.002 ^a	0.305 ± 0.01 ^b	11.77 ± 0.7 ^b
	150	0.704 ± 0.011 ^b	0.007 ± 0.002 ^{ab}	0.253 ± 0.018 ^a	0.307 ± 0.008 ^a	0.601 ± 0.009 ^a	0.844 ± 0.003 ^b	0.356 ± 0.03 ^a	9.27 ± 1.0 ^d
	200	0.726 ± 0.002 ^a	0.008 ± 0.002 ^a	0.258 ± 0.018 ^a	0.301 ± 0.007 ^{ab}	0.590 ± 0.011 ^a	0.847 ± 0.003 ^{ab}	0.336 ± 0.02 ^b	10.06 ± 1.0 ^{cd}
Significance ⁴⁾	Cultivar (C)	**	***	***	*	***	***	NS	*
	Treatment (T)	**	NS	NS	***	*	**	***	***
	(C) × (T)	NS	NS	NS	*	***	NS	NS	NS

Means ± standard deviation (SD), (n = 5). *Within each cultivar, means were separated using Duncan's multiple range test (DMRT) at p < 0.05. Means followed by different lowercase letters differ significantly. ¹⁾NDVI: normalized difference vegetation index, a greenness index associated with canopy vigor; PRI: photochemical reflectance index, related to photosynthetic light-use efficiency and xanthophyll cycle activity; and MCARI: modified chlorophyll absorption ratio index, sensitive to leaf chlorophyll content while minimizing background effects. ²⁾V_j: relative variable fluorescence at the J-step; V_i: relative variable fluorescence at the I-step; F_v/F_m: maximum quantum yield of PSII; and M₀: slope at the beginning of the transient F₀ → F_m, maximal fractional rate of photochemistry (Kim et al., 2024a). ³⁾Performance index (PI_{ABS}); performance index (PI) on an absorption (ABS) basis. ⁴⁾NS, non-significant; *, **, and *** indicate significance at p < 0.05, 0.01, and 0.001, respectively.

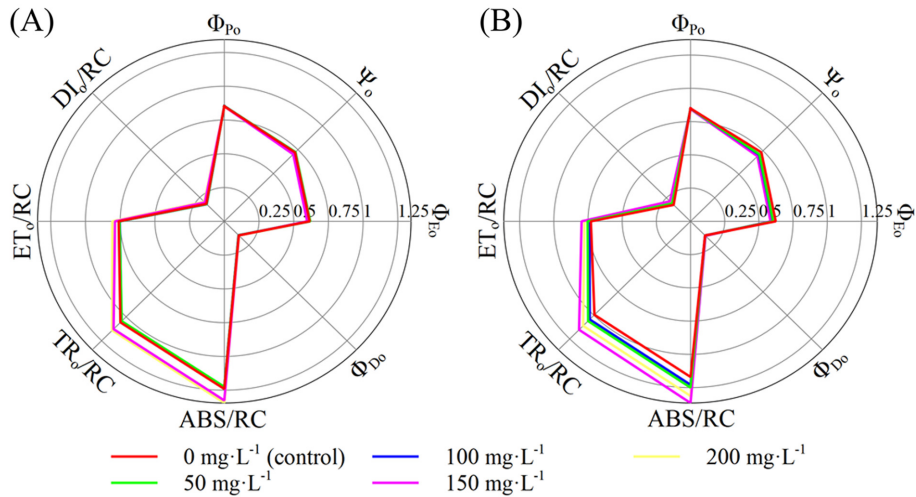


Fig. 3. Chlorophyll fluorescence-derived quantum efficiencies and specific energy flux indices of two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') at four weeks after foliar application of GA₃ at different concentrations. (A, B) quantum efficiency parameters and specific energy flux indices of mustard cultivar 'Asia Jeok' and 'Red Frill', respectively.

mg·ℓ⁻¹ (0.150) and in RF at 150 mg·ℓ⁻¹ (0.155).

With respect to specific energy fluxes per reaction center (RC), ABS/RC, which reflects the absorption flux per RC, was

the greatest in AJ at 200 mg·ℓ⁻¹ and in RF at 150 mg·ℓ⁻¹ (1.34 and 1.36, respectively). The trapped energy flux per RC (TR_o/RC), electron transport flux from Q_A to Q_B per RC (ET_o/RC),

Table 7. Significance levels for OJIP fluorescence-derived parameters in two mustard (*B. juncea*) cultivars ('Asia Jeok' and 'Red Frill') measured four weeks after foliar application of GA₃ at different concentrations, including quantum efficiencies (Φ_{Po} , Ψ_o , Φ_{Eo} , and Φ_{Do}) and specific energy fluxes per reaction center (ABS/RC, TR_o/RC, ET_o/RC, and DI_o/RC).

Significance ¹⁾	Quantum yields of photosystem II				Specific energy fluxes per reaction center (RC)			
	Φ_{Po}	Ψ_o	Φ_{Eo}	Φ_{Do}	ABS/RC	TR _o /RC	ET _o /RC	DI _o /RC
Cultivar (C)	***	*	**	***	NS	NS	NS	NS
Treatment (T)	**	***	***	**	***	***	***	***
(C) × (T)	NS	*	NS	NS	NS	NS	NS	NS

¹⁾NS, non-significant; *, **, and *** indicate significance at $p < 0.05$, 0.01 , and 0.001 , respectively.

and dissipated energy flux per RC (DI_o/RC) exhibited the same overall patterns as those of ABS/RC.

5. Qualitative analysis of total extracts of *B. juncea* using UHPLC-PDA-ESI-MS/MS

Based on the UV chromatogram at 330 nm, a representative wavelength that flavonoids absorb, major peaks were surveyed for their mass spectra with the key product MS² ions in positive mode (Table 8). The mass spectrum of compound 2 exhibited a protonated ion [M + H]⁺ at m/z 773.2164 and key diagnostic product MS² ions at m/z 611.1647, 449.1087, and

287.0564. The MS² ion at m/z 287.0564 indicated the presence of kaempferol as an aglycone. Starting with the outermost substituents of the molecule, the MS² ions at m/z 611.1647 and 449.1087 were likely formed by detachment of a glucosyl group at C-7 (m/z difference: 162.0517) and an additional glucosyl group at C-3 (m/z difference: 162.0560).

Compound 5 was determined to be a flavonol glycoside with hydroxycinnamic acid (HCA) derivatives; the mass spectrum showed MS² ions at m/z 611.1625, indicating the detachment of one glucose unit at C-7 (m/z difference: 162.0552) and subsequent hydroxyferulic acid (m/z difference: 192.0410). The

Table 8. UHPLC-PDA-ESI MS/MS data and putative identification of major flavonol glycosides in two cultivars ('Asia Jeok' and 'Red Frill') of mustard (*B. juncea*).

Cpd ¹⁾	t _R (min)	[M + H] ⁺	Error (ppm)	Key product MS ² ions ²⁾	Formula	Tentative identification ³⁾
1	9.66	1113.2990	0.1	951 (-glc), 465 (-glc, -hyfer, -2 × glc), 303 (aglycone)	C ₄₈ H ₅₆ O ₃₀	qn 3-caffeoylsophorotrioside-7-glucoside*
2	10.19	773.2164	0.2	611 (-glc), 449 (-2 × glc), 287 (aglycone)	C ₃₃ H ₄₀ O ₂₁	km 3-sophoroside-7-glucoside*
3	10.61	951.2448	-0.1	789 (-glc), 627 (-glc, -caf), 465 (-glc, -caf, -glc), 303 (aglycone)	C ₄₂ H ₄₆ O ₂₅	qn 3-caffeoylsophoroside-7-glucoside*
4	10.79	1097.3045	0.3	773 (-glc, -caf), 611 (-glc, -caf, -glc), 449 (-glc, -caf, -2 × glc), 287 (aglycone)	C ₄₈ H ₅₆ O ₂₉	km 3-caffeoylsophorotrioside-7-glucoside*
5	11.46	965.2570	1.2	611 (-glc, -hyfer), 449 (-glc, -hyfer, -glc), 355, 287 (aglycone)	C ₄₃ H ₄₈ O ₂₅	km 3-hydroxyferuloylsophoroside-7-glucoside*
6	12.34	995.2711	0.1	627 (-glc, -sin), 465 (-glc, -sin, -glc), 369, 303 (aglycone)	C ₄₄ H ₅₀ O ₂₆	qn 3-sinapoylsophoroside-7-glucoside*
7	13.46	979.2750	0.1	611 (-glc, -sin), 449 (-glc, -sin, -glc), 369, 287 (aglycone)	C ₄₄ H ₅₀ O ₂₅	km 3-sinapoylsophoroside-7-glucoside*
8	13.85	949.2639	-0.5	611 (-glc, -fer), 449 (-glc, -fer, -glc), 339, 287 (aglycone)	C ₄₃ H ₄₈ O ₂₄	km 3-feruloylsophoroside-7-glucoside*
9	13.97	919.2518	1.5	611 (-glc, -p-co), 449 (-glc, -p-co, -glc), 287 (aglycone)	C ₄₂ H ₄₆ O ₂₃	km 3-p-coumaroylsophoroside-7-glucoside*
10	14.18	641.1728	0.1	479 (-glc), 317 (aglycone)	C ₂₈ H ₃₂ O ₁₇	is 7-sophoroside*
11	20.65	479.1193	-0.1	317 (aglycone)	C ₂₂ H ₂₂ O ₁₂	is 3-glucoside*

¹⁾Cpd: compound; ²⁾glc: glucose, *p*-co: *p*-coumaric acid, caf: caffeic acid, fer: ferulic acid, hyfer: hydroxyferulic acid, sin: sinapic acid; ³⁾km: kaempferol, qn: quercetin, is: isorhamnetin, *: identified flavonoid from *B. juncea*

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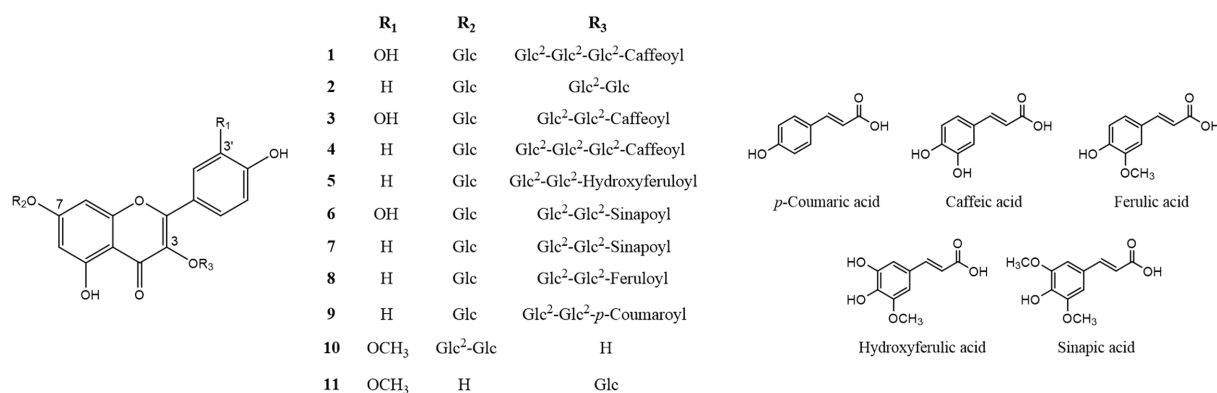


Fig. 4. Structures of major flavonoid glycosides from two cultivars ('Asia Jeok' and 'Red Frill') of mustard (*B. juncea*).

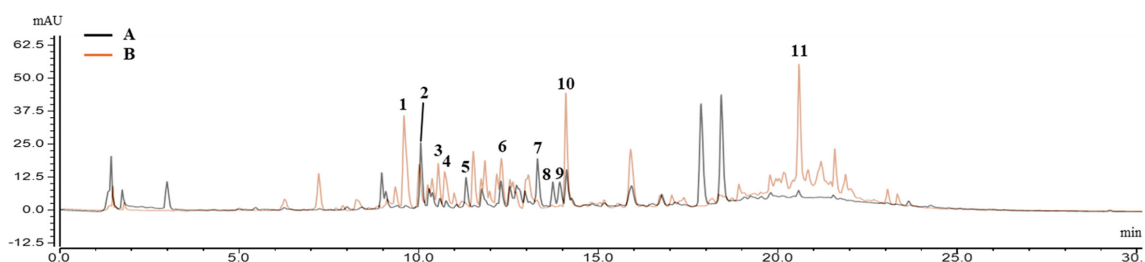


Fig. 5. Ultraviolet (UV) chromatograms (330 nm) of total extracts from two mustard (*B. juncea*) cultivars. (A) *B. juncea* cv. Asia Jeok and (B) *B. juncea* cv. Red Frill.

presence of hydroxyferulic acid was supported by a UV absorption maximum at 330.5 nm in additional UV analysis. Similarly, the remaining compounds were tentatively identified, as shown in Table 8 and Figure 4. Interestingly, compounds 2, 5, 7, 8, and 9 were found to be the major flavonol glycosides in AJ, whereas compounds 1, 3, 4, 6, 10, and 11 were the major flavonol glycosides in RF (Fig. 5).

DISCUSSION

GAs are among the principal phytohormones governing morphogenetic processes during the vegetative growth stage (Yamaguchi, 2008). Responses to exogenous GA₃ application can vary depending on species- and cultivar-specific sensitivity, as well as the applied concentration, and GA₃-induced morphological changes may, in turn, trigger cascading effects on biomass accumulation, resource allocation patterns, and broader physiological traits (Pavlista *et al.*, 2012; Shaddad *et al.*, 2013; Rademacher, 2015).

In the present study, we conducted an integrated assessment of the effects of foliar-applied GA₃ on two mustard cultivars, including morphological responses, biomass accumulation and

partitioning, external quality attributes, and physiological responses, which were quantified using chlorophyll fluorescence and remote sensing-based vegetation indices. Overall, our results indicate that GA₃ treatment elicited significant changes in several morphological traits and selected photochemical parameters, with the magnitude and significance depending on cultivar, GA₃ concentration, and the parameter evaluated.

Four weeks after foliar GA₃ application, shoot height and width differed significantly among the treatments; leaf length, width, and number also showed significant treatment-dependent variation. These findings are consistent with those reported in previous studies, which demonstrated that foliar GA₃ application can promote cell elongation in leaves and stems, thereby inducing visually discernible morphological changes within a relatively short time frame (Wenzel *et al.*, 2000; Robil *et al.*, 2025).

Notably, shoot width, leaf length, and leaf width exhibited relatively high mean values in both cultivars under the 50 mg·l⁻¹ treatment. This pattern suggests that, with respect to horizontal expansion, a comparatively low GA₃ concentration (50 mg·l⁻¹) may be effective for the two cultivars examined here, whereas higher concentrations tended to be associated with a modest reduction in plant size. Collectively, these results

imply a nonlinear concentration-response relationship of growth parameters with GA₃ concentration. Accordingly, an optimum may occur within a certain GA₃ concentration range, beyond which supra-optimal concentrations can lead to unfavorable outcomes for specific traits (Tomer, 1984; Islam *et al.*, 2021), highlighting the importance of careful concentration selection.

With respect to shoot height, the AJ cultivar attained its maximum value at 200 mg·ℓ⁻¹, whereas the RF cultivar peaked at 50 mg·ℓ⁻¹. This cultivar-specific optimum is consistent with previous reports indicating that sensitivity to exogenous GA₃ (i.e., responsiveness that may reflect differences in uptake and/or downstream signaling) can vary among cultivars, even within the same species (Muniandi *et al.*, 2018; Ibrahim *et al.*, 2019; Sari, 2024). Taken together, these findings suggest that cultivar-specific recommended concentration ranges should be considered even when the management goal is to improve morphological performance.

In terms of biomass, the AJ cultivar exhibited pronounced increases in shoot fresh weight and shoot dry weight at 200 mg·ℓ⁻¹, indicating that relatively high GA₃ concentrations promoted assimilate accumulation and overall biomass production in this cultivar. In contrast, RF did not show clear treatment-dependent differences in shoot dry weight, suggesting that despite an increase in apparent plant size following foliar GA₃ application, its conversion efficiency from morphological expansion to dry matter accumulation may be comparatively limited relative to AJ.

Regarding belowground traits, both the fresh and dry weight of the roots tended to be higher in the GA₃-treated plants than in the control for the RF cultivar. Previous studies have shown that exogenous GA₃ can induce morphological changes in shoots and increase biomass accumulation (Castro-Camba *et al.*, 2022). Although the effects of GA are often described as more pronounced in shoots than in roots (Martins *et al.*, 2019; Alam *et al.*, 2022; Lee *et al.*, 2023; Omena-Garcia *et al.*, 2025), our results suggest that GA₃-driven leaf expansion and canopy enlargement may have increased light interception and carbon assimilation, which may have translated into enhanced root growth under the conditions of this study.

From the perspective of plant quality indices, the AJ cultivar showed clear improvements in structural quality following foliar GA₃ application, as compactness reached its highest value at 200 mg·ℓ⁻¹ and DQI exceeded the control at 50, 150, and 200 mg·ℓ⁻¹. This pattern indicates that GA₃ treatment did not merely stimulate shoot elongation but was accompanied by

coordinated changes in traits linked to stem robustness, shoot assimilate accumulation, and shoot-root biomass partitioning. Because the DQI integrates both structural stability and the balance of shoot-to-root allocation (Dickson *et al.*, 1960), these improvements suggest that GA₃ application effectively enhances the overall plant quality status in AJ. By contrast, the RF cultivar showed a more limited quality-index response: compactness did not differ significantly among treatments, and DQI was relatively higher at 150 mg·ℓ⁻¹ than in the control. Collectively, these results indicate that the extent to which foliar-applied GA₃ translates visible morphological responses into integrated quality gains can differ substantially, depending on cultivar identity.

The SPAD units did not differ significantly among treatments. This result indicates that under the conditions applied in the present study, foliar GA₃ treatment exerted relatively limited effects on leaf chlorophyll status four weeks after application. Moreover, the primary effects of GA₃ appeared to be expressed more strongly through the regulation of morphological and physiological traits than through pigment accumulation, which was supported by the absence of significant treatment effects, not only for SPAD units, but also for ARI2 and CRI2. In a previous study on faba bean (*Vicia faba*), exogenous GA₃ similarly failed to induce significant changes in chlorophyll and carotenoid content (Alam *et al.*, 2022), which is consistent with our observations.

In contrast, NDVI showed an increasing tendency at selected concentrations depending on cultivar (e.g., 100 and 200 mg·ℓ⁻¹). Because NDVI is jointly influenced by leaf area and canopy density (Carlson and Ripley, 1997), the GA₃-driven increases in ground cover and leaf expansion observed in this study may have contributed to the higher NDVI values. In comparison, MCARI is an index that indirectly reflects chlorophyll status; thus, when treatment effects on SPAD units are limited, the corresponding changes in MCARI may also be constrained. Overall, our results suggest that foliar GA₃ application in these mustard cultivars modulated morphological attributes to a greater degree than leaf pigment status. In this context, the present findings align with previous reports indicating that gibberellin effects are often more evident in quantitative growth-related outcomes (e.g., growth and biomass accumulation) than in qualitative traits, such as pigment regulation (Othman *et al.*, 2021; Zhang *et al.*, 2024).

Chlorophyll fluorescence parameters exhibited significant treatment effects for most variables, indicating that GA₃ application influenced not only plant morphology, but also

energy fluxes within PSII. In particular, treatment-associated variation in quantum-yield-related parameters— Φ_{P_0} (F_v/F_m) as well as Ψ_0 , Φ_{E_0} , and Φ_{D_0} —suggests that foliar GA₃ application partially reconfigured PSII photochemical efficiency, the probability of electron transfer beyond Q_A, and the balance between photochemical energy utilization and energy dissipation (Stirbet and Govindjee, 2011).

Among the two mustard cultivars, the AJ cultivar exhibited the highest Ψ_0 and Φ_{E_0} values at 50 mg·ℓ⁻¹, suggesting that electron-transport efficiency was comparatively favored under this relatively low GA₃ concentration. This pattern is also consistent with the morphological results, in which 50 mg·ℓ⁻¹ was advantageous for leaf expansion and increased shoot width, indicating that, in AJ, this concentration may represent a practical range in which improvements in morphological traits and photochemical efficiency can be achieved concurrently. In contrast, the 200 mg·ℓ⁻¹ treatment in AJ was associated with an increase in Φ_{D_0} , implying a tendency toward a higher fraction of absorbed energy being dissipated rather than utilized photochemically. Nevertheless, considering the values of F_v/F_m , which reflects the maximum quantum yield of PSII, both cultivars maintained relatively high values (0.844-0.854). Although these values were higher than the commonly cited reference range for many healthy, non-stressed higher plants (0.78-0.84) (Muniz *et al.*, 2014; Kim *et al.*, 2024b; Lee *et al.*, 2025b), reference values can vary depending on species, measurement protocol, and instrument settings. Therefore, under the present experimental conditions, the plants were interpreted as not being under pronounced PSII stress, while still showing treatment-related photochemical adjustments in other OJIP fluorescence parameters.

In the RF cultivar, Ψ_0 and Φ_{E_0} were relatively high in the control, and PI_{ABS} was also greatest in the control. Notably, the 150 mg·ℓ⁻¹ treatment was accompanied by an increase in Φ_{D_0} , suggesting that, at this concentration, a greater acceptor-side load and/or functional adjustment of PSII reaction centers may have occurred (Stirbet and Govindjee, 2011). Furthermore, the concurrent increases in ABS/RC , TR_0/RC , ET_0/RC , and DI_0/RC at a specific concentration are consistent with the interpretation that a larger fraction of reaction centers shift toward a functionally inactivated state, thereby increasing the apparent energy fluxes per remaining active reaction center (Kim *et al.*, 2024b; Lee *et al.*, 2025b). Collectively, these results imply that meaningful improvement in morphological traits in the RF cultivar is possible under a relatively low GA₃ concentration (50 mg·ℓ⁻¹),

whereas higher concentrations—particularly 150 mg·ℓ⁻¹, and to a lesser extent 200 mg·ℓ⁻¹—may shift photochemical performance in an unfavorable direction, warranting caution in concentration selection. From a food-crop management perspective, the present results suggest that foliar GA₃ application should be managed with cultivar-specific, conservative concentration selection, considering that supra-optimal concentrations can impose a physiological trade-off even when visually discernible growth promotion is observed. Accordingly, applying the minimum concentration required to achieve the desired morphological outcome may be a practical strategy to avoid unintended declines in photochemical efficiency.

In the present study, vegetative growth and PSII performance were assessed four weeks after a single foliar GA₃ application; however, GA signaling can exert systemic effects that extend beyond shoot vegetative growth and may influence reproductive sink development and seed oil accumulation in species of *Brassica*. Foliar application of GA₃ has been used in canola to test concentration-dependent shifts in yield components and seed oil content, especially under drought stress (Elahi *et al.*, 2022). Moreover, a field study combined foliar GA₃ with mineral nutrition management to evaluate changes in seed yield and oil percentage (Aslam *et al.*, 2023). At the mechanistic level, GA signaling is linked to the transcriptional control of fatty-acid biosynthesis during early seed development, and GA₃ treatment of siliques has been shown to increase seed size and induce fatty-acid biosynthetic gene expression (Yan *et al.*, 2021). Therefore, although seed traits were beyond the scope of the present work, future studies should verify whether the concentration window that improved vegetative traits also translates to reproductive performance and oil productivity in these cultivars.

Although the identification of mustard flavonols in this study was initially hampered by challenges in securing reference standards and performing direct isolation, relatively accurate identification was achieved through precise mass determination via HRMS, MS/MS fragmentation pattern analysis, MS library matching, analysis of well-established flavonoid UV patterns, and comparisons with published spectroscopic data for flavonol glycosides. Regarding the aglycone identification, given that kaempferol, quercetin, and isorhamnetin are reported as the predominant aglycones in species of *Brassica* (Kim *et al.*, 2002; Lin *et al.*, 2011; Neugart and Bumke-Vogt, 2021), excluding the possibility of rhamnetin is reasonable. As for the positioning of the glycosyl groups, a previously published

extensive qualitative analysis of phenols in red mustard greens indicates that flavonol 3,7-diglucoside loses its 7-glycosyl group first to form the major MS² product ion (Lin *et al.*, 2011). Kaempferol 3-sophorotrioside has never been detected in *B. juncea*, and only a few cases in which triglycosylation occurs at C-3 without glycosylation at C-7 have been reported. These previous results, combined with our finding that compound 2 is one of the major peaks in the AJ cultivar, allowed us to rule out the possibility that compound 2 is kaempferol 3-sophorotrioside.

In summary, qualitative UHPLC-PDA-ESI-MS/MS profiling resulted in the putative identification of 11 major flavonol glycosides, and clear cultivar-dependent differences in the dominant flavonol glycoside peak patterns were observed between the two cultivars. These findings indicate that the composition relative contents of flavonoid glycosides can vary in a cultivar-dependent manner.

Notwithstanding the results of the qualitative analysis, it is difficult to directly correlate GA₃ treatment with cultivar-specific variations in flavonol glycoside content in the same way as the morphophysiological changes observed in this study. Nevertheless, the alterations in secondary metabolite content induced by GA₃ treatment vary significantly depending on the crop species and cultivar (Park *et al.*, 2017; Sun *et al.*, 2021; Khalil *et al.*, 2023). In light of these findings, conducting quantitative analysis across various mustard cultivars would be useful to elucidate the cultivar-specific effects of GA₃ on secondary metabolite biosynthesis.

To summarize the key findings of our study, foliar application of GA₃ effectively improved the morphological traits of both mustard cultivars; however, the concentrations of GA₃ that resulted in optimal overall growth, plant quality indices, and photochemical responses differed by cultivar. In AJ, the practical optimum depended on the target trait: a relatively low GA₃ concentration (50 mg·ℓ⁻¹) was favorable for leaf expansion and photochemical performance, whereas a relatively high concentration (200 mg·ℓ⁻¹) improved biomass accumulation and DQI. Thus, cultivar-specific recommendations for AJ should be objective-dependent. In contrast, RF tended to exhibit improved morphological traits at a relatively low concentration (50 mg·ℓ⁻¹), whereas higher concentrations, particularly 150 mg·ℓ⁻¹, may adversely affect photochemical performance.

To build on these findings, we recommend that future studies be designed with the aim of testing alternative application

strategies, including GA delivery via root drenches, different timing and frequency of application, and interactions with other plant growth regulators. Lastly, because flavonol glycoside analysis in the current study was limited to qualitative identification, the accumulation of quantitative datasets is necessary to test the relationships between GA₃ treatment effects and functional metabolite profiles.

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