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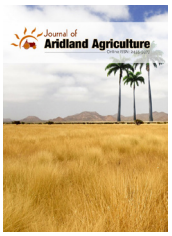

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Analysis of glucosinolate and phenylpropanoid contents in four different cultivars of radish sprouts

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ABSTRACT

Radish (*Raphanus sativus* L.) is a prominent root vegetable cultivated throughout the world because of its nutritional and bioactive components. This study is conducted for growth performance and phytochemical investigation of four different cultivars of radish sprouts i.e., white, yellow, red, and black. Growth parameters such as shoot length, root length, and fresh weight were recorded and the results showed that the red cultivar had the highest values in all aspects, showing the highest shoot length (5.72 cm), root length (12.1 cm), and fresh weight (283.7 mg). In the black cultivar, these parameters exhibited the lowest growth. Individual glucosinolates (GSLs) and phenylpropanoid compounds were also analyzed from all the cultivars. The analysis of 10-day-old radish sprouts revealed the presence of 7 glucosinolates and 10 phenylpropanoid compounds. The individual and total content of all the compounds varied significantly within cultivars. The highest amount of total glucosinolates (87.00 µmol/g DW) was detected in red whereas the lowest total was found in the yellow cultivar (60.78 µmol/g DW). The variation of glucoraphanin content among the cultivars varied largely than any other glucosinolate showing much more in the red and white cultivars. The accumulation of glucoraphasatin content was much higher irrespective of cultivars. As for phenylpropanoids, the red cultivar exhibited the greatest amounts of chlorogenic acid (56.34 µg/g DW) and benzoic acid (42.35 µg/g DW). Among all cultivars, the white cultivar had the lowest phenylpropanoid profile, especially in terms of benzoic acid. This study reveals wide variation in growth and phytochemicals among the radish cultivars cultured on soil pots the positive traits of the red cultivar showed enhanced yield and nutraceutical compounds.

KEYWORDS: *Raphanus sativus*, Growth performance, Phytochemical composition, Glucosinolates, Phenylpropanoids

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INTRODUCTION

Radish (*Raphanus sativus* L.) is an important root vegetable worldwide as an edible biennial in the Brassicaceae (cabbage) family. They are mainly grown in Asian countries, especially China, Japan, and Korea (Gancheva *et al.*, 2016; Heng *et al.*, 2022). Radish is widely consumed for its seedlings or taproots, and the seeds are used as edible oil (Mitsui *et al.*, 2015). Radish is important in human diets as a source of nutrients and antioxidants (Manivannan *et al.*, 2019; Kajszczak *et al.*, 2024). Radish, a brassicaceous crop, is cultivated primarily for its swollen taproots, often globular or tapering, but often cylindrical. Root colors may be pink, green, white, yellow, purple, red, and black, while the flesh is usually white (Yu *et al.*, 2016; Zhang *et al.*, 2021).

Radish chemical constituents consist of glucosinolates (GSLs) and sulfur-containing derivatives, phenylpropanoid sucrosides, small volumes of organic acids and derivatives, terpenoids, steroids, oligosaccharides, alkaloids, flavone glycosides, etc. (Gao *et al.*, 2022). Indeed, myrosinase of plant or intestinal microbiota transforms glucosinolates into isothiocyanates presenting diverse biological activity including anti-inflammatory, anti-tumor, metabolic disease, antioxidant, antibacterial, central nervous system protection, and anti-osteoporotic functions (Gutiérrez & Perez, 2004; Castro-Torres *et al.*, 2014).

The fundamental, well-established phenylpropanoid biosynthetic pathway not only contributes to the lignin biosynthetic pathway, but also for the production of a wide range of additional classes of equally important compounds

such as flavonoids and other coumarins, isoflavonoids, polyphenolics, phenolic acids, monolignols, phenylpropenes, stilbenoids, stilbenes, lignans, suberins, and sporopollenin (Fraser & Chapple, 2011; Ortiz & Sansinenea, 2023). The involvement of this pathway is fundamental in various plant-environment interactions and in growth and development of plants. Phenylpropanoid derivatives protect plants from UV radiation and reactive oxygen species and provide structural support or mediate the communication between plants and microorganisms (Naoumkina *et al.*, 2010; Rates & Cesarino, 2023). Phenylpropanoids have been linked to many medicinal properties, such as anti-aging, anti-hypertensive, anti-inflammatory, anti-retroviral, insulin-sensitizing activity, and the benefits of reducing the risks of chronic disease including cardiovascular cancer, disease, and osteoporosis, along with inhibition of LDL oxidation (Korkina *et al.*, 2011; Zheng *et al.*, 2012; Tohge & Fernie, 2017).

Commercially, radish sprouts have been grown in darkness and high humidity conditions and harvested well before the cotyledons unfold, i.e. usually within 3-5 days of seed hydration (Ebert, 2022). Because of their favorable nutritional composition, such as fiber, carbohydrates, minerals, amino acids, and protein (Fu *et al.*, 2001; Vidal-Valverde *et al.*, 2002; Márton *et al.*, 2010; Martinez-Villaluenga *et al.*, 2010). Their review on the bioactive compounds and antioxidant activity of broccoli and radish cultivars during germination.

In the present study, we analyzed and quantified the GSLs and phenylpropanoid content in sprouts from four different radish cultivars to facilitate a comparative analysis of these two phytochemical profiles. Additionally, profiling GSLs and phenylpropanoids in these radish cultivars will enhance our understanding of the distribution patterns of these compounds in various radish cultivars. Our findings reveal significant variations in the composition and overall concentration of GSLs and phenylpropanoids among the radish cultivars studied.

MATERIALS AND METHODS

Plant Material

The seeds of white, yellow, red, and black radish cultivars were obtained from Asia Seed Co. in South Korea and named according to their skin colors. 100 seeds were soaked in distilled water overnight. The seeds were then placed in pots of vermiculite. This experiment was founded on a comprehensive randomized design, which has already been replicated thrice for the two different cultivars. In an LED plant growth chamber (Sejong Scientific Co., Sejong, South Korea), the germination and cultivation of the seedlings were performed under a temperature range of 24.8 to 26.8 °C, photoperiod of 16/8 h, fluorescent light photon flux of 700 lux, and relative humidity of about 60-70%. Sprouts from each cultivar were collected 10 days after sowing (DAS).

Upon harvest, the sprouts were thoroughly washed and cleaned with distilled water. For growth measurements, 10 randomly selected plants from each cultivar were chosen, and various growth parameters were recorded. The shoot length (SL) and root length (RL) were measured in centimeters using a meter ruler. Fresh weight (FW) was determined by weighing the radish cultivars in milligrams using a precision balance. The non-selected sprouts were subsequently ground into powder using a mortar and pestle in the presence of liquid nitrogen and then freeze-dried using a freeze-dryer (HyperCOOL, Gyrozen Co. LTD, Gyeonggi-do, South Korea). The dehydration process was carried out for 72 h at -40 °C. The resulting dehydrated powder samples were used for subsequent analysis of GSL and phenylpropanoid content.

Extraction of Desulfo-glucosinolates (DS-GSLs)

Extraction of de-sulfated GSLs (DS-GSLs) was performed as described by Sathasivam *et al.* (2023). In brief, 100 mg of lyophilized powder was extracted with 1.5 mL of 70% (v/v) boiling methanol in a water bath at 70 °C for 5 min to inactivate endo-myrosinases and the extracts are referred to as crude GSLs. These mixtures were then centrifuged at 12,000 × g at 4 °C for 10 min with a Hanil microcentrifuge (Micro 17R, Incheon, Korea), and the supernatants were harvested to 5 mL test tubes. The residue was then extracted two additional times in the same way, and the supernatants were combined to make the respective crude GSL extracts. Next, the extracts were loaded to 1,000 µL pipet tips packed with DEAE-Sephadex A-25 (H^+ form, pre-equilibrated with 0.5 M sodium acetate, ~40 mg dry weight). After washing the columns with ultrapure water, the GSLs in the columns were desulfated by adding 75 µL of aryl sulfatase solution to the column. The DS-GSLs were then incubated overnight (16-18 hours) at ambient temperature and eluted three times with 0.5 mL of ultrapure water into 2 mL microcentrifuge tubes. The eluates were filtered through a 0.45 µm PTFE syringe filter into brown High-performance liquid chromatography (HPLC) vials and then stored immediately at 4 °C in a refrigerator until HPLC analysis.

HPLC Analysis of DS-GSLs

The separation of DS-GSLs was performed on a reversed-phase Inertsil ODS-3column (150×3.0 mm i.d., particulate, 3 µm; GL Sciences, Tokyo, Japan) coupled with Agilent Technologies 1260 series system (Palo Alto, CA, USA.). Column oven temperature was kept at 30 °C and detection wavelength was set to 229 nm. The flow rate was maintained at 0.5 mL/min. Ultrapure water (containing 20 mM ammonium acetate and 0.02% acetic acid) was used as solvent A, and solvent B as acetonitrile (containing 20 mM ammonium acetate and 0.02% acetic acid). The HPLC gradient condition and quantification were done according to the area and response factor of HPLC as determined by Sathasivam *et al.* (2023).

Extraction and Analysis of Phenylpropanoids

HPLC analysis of the phenylpropanoids was performed according to methods previously described with minor modifications (Li *et al.*, 2020). To the 100 mg of the sample,

2 mL of 80% methanol was added and then sonicated for 1 h. The supernatant was collected after being centrifuged at 1000 g for 20 min and filtered through a 0.45 µm PTFE syringe filter for HPLC analysis. The analysis was conducted using a Futecs model NS-4000 HPLC system (Daejeon, Korea) that was fitted with an UV−vis detector and autosampler. The detection wavelength was 280 nm and separation was performed with a C_{18} column (250 × 4.6 mm, 5 µm; RStech; Daejeon, Korea). Solvent (A): 0.2% acetic acid and (B) methanol were used as a mobile phase. The column temperature was kept at 30 °C, flow rate of 1.0 mL/min, and injection volume of 20 µL, and the compounds were quantified based on peak areas and expressed as representative standard compound equivalents. All data outputs are shown in µg/g dry weight (DW), analyzed by ANOVA and Tukey's post hoc test (R software (ver. 3.1.0)).

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA), with sums of square partitioned to account for trial effects, utilizing SAS Software version 9.2. Mean differences were assessed using the Duncan Multiple Range Test.

RESULTS

The shoot length, root length, and fresh weight of 10 DAS of four radish cultivars (white, yellow, red, and black) are shown in Table 1. White cultivar had a shoot length of 5.67±0.49 cm, a root length of 11.36±1.06 cm, and a fresh weight of 274.8±31.7 mg. The tall yellow cultivar recorded a short shoot length of 5.38±0.45 cm, root length of 10.7±0.85 cm, and fresh weight of 263.3±22.6 mg, respectively. The Red cultivar has the longest shoot length (5.72±0.55 cm) and root length (12.1±1.16 cm) and shows the highest knockout fresh weight (283.7±25.9 mg), indicating its superiority in growth performance than the other cultivars. Whereas the black type had the shortest shoot (4.84±0.35) and root (9.84±0.73) length and the lowest fresh weight (242.2±18.3 mg). In general, the red cultivar showed maximum growth performance while the growth of the black cultivar was minimal for all the traits.

The total amount of glucosinolates in sprouts of four radish cultivars (red, black, yellow, and white) at 10 DAS (Table 2). The total is obtained by the summation of the individual glucosinolate compounds. Among cultivars, the red had the highest total glucosinolate concentration (87.00±4.84 µmol/g DW) which was primarily followed by a significant increase in glucoraphasatin (52.16±3.54 µmol/g DW).

Table 1: Shoot length, root length, and fresh weight of four different cultivars of radish sprout determined 10 DAS in a growth chamber

Radish cultivars	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
White	5.67±0.49^{a}	11.36±1.06^{ab}	274.8±31.7^{a}
Yellow	5.38±0.45^{a}	10.7±0.85^{ab}	263.3±22.6^{a}
Red	5.72±0.55^{a}	12.1±1.16^{a}	283.7±25.9^{a}
Black	4.84±0.35^{a}	9.84±0.73^{b}	242.2±18.3^{a}

The total glucosinolate concentration for white was second highest (85.69±6.77 µmol/g DW) and was mostly comprised of glucoraphasatin (47.97±1.97 µmol/g DW) but significantly less than that in red. The total glucosinolate concentration for the yellow cultivar was 73.99±10.62 µmol/g DW, and the dominant glucosinolate glucoraphasatin (54.59±8.34 µmol/g DW) was higher than in both red and white cultivars. The total concentration of glucosinolates was lowest in black (60.78±1.13 µmol/g DW), in which glucoraphasatin was also the most abundant (49.19±1.64 µmol/g DW), although its glucosinolate content was substantially lower than the other cultivars. Values comparison shows that glucoraphasatin is the major glucosinolate in all cultivars and that total glucosinolates are higher in red and yellow than in black cultivars.

Total phenylpropanoid levels (µg/g DW) of four different cultivars of radish sprouts (black, white, yellow and red) determined at 10 DAS, and concentrations of individual phenolics, including 4-hydroxybenzoic acid, catechin hydrate, chlorogenic acid, caffeic acid, epicatechin, ferulic acid, benzoic acid, rutin, trans-cinnamic acid and kaempferol were also determined. Caffeic (16.18±4.17 µg/g DW) acid was significantly higher in the black cultivar than in yellow (3.06±2.65 µg/g DW) and red (5.35±0.05 µg/g DW) cultivars (lowest in the white cultivar (11.71±0.54 µg/g DW)), while the highest chlorogenic acid concentrations were identified in yellow and red cultivars (52.25±1.94 and 56.34±1.93 µg/g DW), on the other hand in the black cultivar (46.86±1.76 µg/g DW). The highest amounts of benzoic acid were detected in the yellow cultivar (41.12±12.02 µg/g DW) while intermediate was found in both black (21.75±8.32 µg/g DW) and red (42.35±12.69 µg/g DW) cultivars. In the white cultivar it increased two-fold (32.29±1.16 µg/g DW), whereas in the yellow cultivar it decreased by almost half (5.38±0.42 µg/g DW). Kaempferol, which was not found in both the black and red cultivars, was only detected in the white and yellow cultivars (1.65±1.46 µg/g and 10.38±1.19 µg/g DW, respectively).

DISCUSSION

The present study provides a comprehensive evaluation of 10 DAS four radish cultivars (white, yellow, red, and black) based on key growth characteristics, glucosinolate concentrations, and phenylpropanoid content. All parameters measured revealed large differences, indicating cultivar-specific characteristics, which can have a potential effect on both growth performance and phytochemical concentration.

Regarding growth, the red cultivar showed the maximum shoot length (5.72 cm), root length (12.1 cm), and fresh weight (283.7 mg), while the white cultivar was moderate in terms of these different growth parameters. These results were in accordance with previous studies that reported red varieties showed better growth traits than other cultivars (Zhang *et al.*, 2021). Slow growth was observed in the more elegant yellow cultivar that produced reasonable shoot (5.38 cm) and root (10.7 cm) lengths with fresh weight (263.3 mg). In comparison, the growth profile of the black cultivar was the least favorable

Table 2: The concentration of glucosinolates content in four different cultivars of radish sprout

Glucosinolate (μmol/g DW)	Red	Black	Yellow	White
Glucoraphanin	28.42±7.68[b]	6.18±0.52[b]	13.43±1.64[b]	30.42±4.17[a]
4-Hydroxyglucobrassicin	1.54±0.26[b]	0.69±0.04[c]	1.91±0.20[b]	3.25±0.27[a]
Glucobrassicanapin	0.20±0.00[b]	0.25±0.00[b]	0.37±0.07[a]	0.24±0.08[b]
Glucoerucin	0.87±0.01[a]	0.84±0.02[a]	0.81±0.01[a]	0.48±0.19[b]
Glucoraphasatin	52.16±3.54[a]	49.19±1.64[a]	54.49±8.34[a]	47.49±1.97[a]
Glucobrassicin	0.76±0.11[a]	0.66±0.01[ab]	0.54±0.13[bc]	0.38±0.01[c]
4-Methoxyglucobrassicin	3.06±0.31[a]	2.69±0.03[ab]	2.32±0.23[b]	2.94±0.09[b]
Total	87.00±4.84[a]	60.78±1.13[b]	73.99±10.62[a]	85.69±6.77[a]

Table 3: The concentration of phenylpropanoids content in four different cultivars of radish sprout

Phenolics (μg/g DW)	Black	White	Yellow	Red
4-hydroxybenzoic acid	nd	6.10±0.87[b]	nd	9.07±2.34[a]
Catechin hydrate	nd	nd	13.86±5.18[a]	nd
Chlorogenic acid	46.68±1.76[b]	51.91±3.67[a]	52.25±1.94[a]	56.34±1.93[a]
Caffeic acid	16.68±4.17[a]	11.71±0.54[b]	nd	17.09±0.37[a]
Epicatechin	nd	nd	nd	241.59±17.50[a]
Ferulic acid	5.22±0.36[ab]	6.13±0.44[a]	3.06±2.65[b]	5.35±0.05[ab]
Benzoic acid	21.75±8.32[b]	7.77±2.44[b]	41.12±12.02[a]	42.35±12.69[a]
Rutin	9.45±0.52[b]	32.29±1.16[a]	5.38±0.42[c]	4.97±0.60[c]
Trans-cinamic acid	0.45±0.03[b]	0.51±0.03[a]	0.41±0.03[b]	nd
Kaempferol	nd	1.65±1.46[c]	10.38±1.19[a]	5.05±0.40[b]
Total	100.23±15.16[b]	118.07±10.61[b]	126.46±23.43[b]	381.81±35.88[a]

nd=not detected

where it had the lowest mean shoot length (4.84 cm), root length (9.84 cm), and fresh weight (242.2 mg). These outcomes are consistent with Yu *et al.* (2016) when results also found genetic and environmental variation for roots and shoots of different cultivars of radish that indicates variation among the strains may be a problem in producing black radish at maximum yield and size potential for growth.

With respect to the glucosinolates, which are very important for the nutritional and health-promoting properties of radishes, again the red cultivar reached the highest total glucosinolate concentration (87.00 μmol/g). This was particularly reflected by the increased amount of glucoraphasatin (52.16 μmol/g), which agrees with previous studies that reported high levels of glucoraphasatin in radish (Gutiérrez & Perez, 2004). The glucosinolate content of the white cultivar was also relatively high (85.69 μmol/g), but its glucoraphasatin (78.43 μmol/g) (Table 3) was lower than that of the red cultivar. Interestingly, the yellow cultivar accumulated much more glucoraphasatin (54.59 μmol/g) than both red and white cultivars, even though the expressed level of total glucosinolate in yellow was not as high (73.99 μmol/g). It implies that it may have a different chemical make-up than the previously known yellow cultivar that can be investigated for its beneficial aspects. By contrast, the black cultivar only contained a total of 60.78 μmol/g of glucosinolates, with glucoraphasatin being the dominant glucosinolate (although as much lower levels than the other cultivars). According to Castro-Torres *et al.* (2014), the content of glucosinolates, particularly glucoraphasatin, is controlled by genotype and is likely a key determinant of the overall phytochemical potential of radish cultivars.

For the phenylpropanoids, the maximum concentration of chlorogenic acid (56.34 μg/g) and benzoic acid (42.35 μg/g) was also shown by the red cultivar, as these compounds with antioxidative and anti-inflammatory properties (Korkina *et al.*, 2011), respectively. Chlorogenic acid content was 52.25 μg/g and benzoic acid was 41.12 μg/g for the yellow cultivar closely followed by white and black cultivars which demonstrated decreased phenolic concentrations, which were also lowest in the black cultivar generally containing the least number of phenolic compounds. The increased phenolic contents of red and yellow cultivars are likewise to the findings of Martinez-Villaluenga *et al.* (2010) showed in the case of red and yellow radishes that they accumulate greater amounts of chlorogenic acid which is related to superiority in antioxidant activity. Chlorogenic acid and other phenolic compounds are abundant in these cultivars which indicate their potential to provide health-promoting effects like higher protection against oxidative stress and chronic diseases (Zheng *et al.*, 2012).

CONCLUSIONS

In conclusion, this study shows that there are marked differences in growth performance and phytochemical content among four different cultivars of radish sprout. In addition, the highest growth parameters, including shoot length, root length, and fresh weight, also yielded the highest concentrations of glucosinolates and phenylpropanoids, with glucoraphasatin and chlorogenic acid predominately produced by the red cultivar. In particular, the yellow cultivar, although a poor performer in terms of total yield, had the most favorable phytochemical profiles of glucoraphasatin. The black cultivar showed the least performance in relation to growth and phytochemical and can be considered less appropriate for agricultural and nutraceutical purposes in comparison with the other cultivars. Such results render the red cultivar the best model for cropping and health-related studies.

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