Effects of seed pre-soaking on bioactive phytochemical levels of wheat and barley microgreens grown under hydroponics *versus* organic soil conditions

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Highlights

- *Pre-soaking of seeds influenced growth characteristics of wheat and barley microgreens.*
- *Growing microgreens in hydroponics versus organic soil had effects on bioactive compounds and antioxidants.*
- *Both barley and wheat microgreens grown in organic soil from pre-soaked seeds exhibited the highest levels of bioactive compounds and antioxidant activities.*

Abstract

This study was conducted to examine the effects of seed pre-soaking on bioactive phytochemicals in barley and wheat microgreens grown under two different growing media, *i.e.*, hydroponics and organic soil. Microgreens were cultivated for 12 days in a plant growth chamber consistent with the following: light-dark interval (12/12 hours), light-dark temperature (20/15°C), light intensity (150 μmol·m^{-2}·s^{-1}), and relative humidity (60%). Both wheat and barley microgreens grown in organic soil from pre-soaked seeds showed increased levels of bioactive compounds, especially carotenoids, flavonoids, phenolics, total vitamin C, and anthocyanins. Antioxidant activities [2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, nitrite scavenging activity, and superoxide dismutase (SOD)-like activity] and antioxidant enzymes (catalase activity, glutathione reductase, and guaiacol peroxidase activity) were highest in both barley and wheat microgreens grown in organic soil from pre-soaked seeds.

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Introduction

Wheat (*Triticum aestivum*, L.) and barley (*Hordeum vulgare*, L.) are important cereal crops cultivated worldwide, and their grain products are consumed globally. Sprouts (just germinated seeds) and microgreens (7–14-day old seedlings) of plant species have become increasingly popular in recent years because they are considered alternative healthful foods owing to a wide range of phytochemicals and their associated beneficial effects on human health (Benincasa *et al.*, 2015; Choe *et al.*, 2018).

Microgreens may contain higher amounts of phytochemicals than their grain counterparts (Wojdyło *et al.*, 2020). They are rich in minerals, vitamins, flavonoids, phenolics, dietary fibers, enzymes, chlorophylls, and antioxidants, which have been shown to reduce the risk of cardiovascular diseases, cancer, and hemophilia (Shewry and Hey, 2015; Zeng *et al.*, 2018).

Grain sprouting and microgreens development mainly depend on temperature, humidity, light, and water content (Benincasa *et al.*, 2019). Water uptake by seeds is essential for seed germination and growth, and various approaches have been used to improve germination and seedling establishment (Singkhornart and Ryu, 2011; Siddique and Kumar, 2018). Microgreens are usually grown in greenhouses or indoor environments using different growing media (Galieni *et al*., 2020; Toro *et al*., 2021).

The growing medium plays an important role in influencing the yield and environmental sustainability, representing one of the most critical aspects of the production process (Bulgari *et al.*, 2021). There are two possible growing methods for microgreens: i) soil or soil substitute cultivation; ii) hydroponic cultivation. Hydroponics could be a feasible replacement for soil-based culti-

vation as they reduce pesticide and fertilizer usage and areas for crop cultivation (Sharma *et al.*, 2018). Bioactive compounds can be affected by pre-soaking the seeds in water before sowing (Thaku *et al*., 2021). The present work was conducted to study the effect of seed pre-soaking or non-pre-soaking on the bioactive phytochemicals of wheat and barley microgreens grown in two different growing media, hydroponics and organic soil.

Materials and Methods

Growing environment and treatments

Wheat (*Triticum aestivum,* L. cv. 'Baegjoongmil') and barley (*Hordeum vulgare,* L. cv. 'Keunalbori') grains were collected from the National Institute of Crop Science in Suwon, Korea. 200 g of both grains and distilled water (dH_2O) were used for this experiment. Half of the grains (grain: dH_2O proportion of 1:1) were soaked for one day (24 hours) in a microgreen growth chamber at 20°C, and the rest were not. Half of the pre-soaked and non-soaked grains were transferred to the deep flow technique of hydroponics, and the rest of the grains were transferred to organic soil growing substrate in a growth chamber to obtain microgreens. The organic soil medium consisted of 0.1% guano, 2.5% vermiculite, 5% zeolite, 7.4% perlite, 25% mushroom culture, 25% granite soil, and 35% coco peat. In the organic soil medium, distilled H_2O was sprayed at approximately 200 mL/day until the microgreens were reaped. In the hydroponics system, about 200 mL of distilled H_2O per 200 g of grains were used until the microgreens were ready to be picked. The growth chamber was maintained at a light-dark interval (12/12 hours), light-dark temperature of 20/15°C (light/dark), relative humidity (60%), and light intensity (150 µmol m^{-2} s^{-1}). Eight-day-old microgreens were used to analyze bioactive phytochemicals and antioxidant activity.

Growth parameters

The germination rate, the height and the calculated yield of barley and wheat microgreens were measured to validate the effects of soaking or non-soaking in hydroponics and organic soil.

Chlorophyll content

The chlorophyll content of fresh microgreens (0.10 g) was measured after treatment with N, N-dimethylformamide (5 mL) for one day (24 hours). The absorbance of the supernatant was analyzed using an ultraviolet-visible light spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 664 nm and 647 nm to determine the total chlorophyll content (Moran, 1982).

Preparation of microgreens extract

Wheat and barley microgreens were extracted (1:4, w/v, microgreen: dH_2O) at 20°C. Approximately 10 g of each microgreen was weighed and ground using a Millstone juice maker (NEWSERA-9001, Oscar Electronics, Gimhae, Korea) in 40 mL of dH_2O. The mixture was filtered on Whatman paper No. 2 for 15 minutes at 4°C.

Carotenoid content

About 0.02 mL of the extract of microgreens was assimilated with 99.9% acetone (5 mL) and incubated in the dark at 4°C for 24 hours. The absorbance of the supernatant was analyzed at a wavelength of 510 nm.

Total phenolic content

The total phenolic content of the extract was measured according to Emmons *et al.* (1999). One milliliter of the extract was mixed with 1 N Folin-Ciocalteu reagent (0.4 mL) and 7% sodium carbonate (2 mL). The sample mixture was whirlpooled and incubated (20°C for 20 minutes). Absorbance was measured at 734 nm. Gallic acid (Sigma-Aldrich, St. Louise, MO, USA) was used as the standard (0-200 ppm).

Total flavonoid content

Total flavonoid content was analyzed using the method described by Zhishen *et al.* (1999). An aliquot of the extract (2 mL) was poured with 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), and dH_2O (2.8 mL) in a glass test tube. The mixture was allowed to react (45 min at 20°C) before measuring the absorbance at 450 nm.

Total vitamin C content

The extract (2 mL) was pooled with 5% (w/v) metaphosphoric acid (5 mL) and the vitamin C of the sample was analyzed at 525 nm.

Anthocyanin content

Anthocyanin content was analyzed at 530 nm, as described by Islam *et al.* (2019). 1 mL of extract was vortexed (24 hours; 4°C) in the dark with 5 mL of 95% ethanol/1.0 N HCl (85:15, v: v).

Guaiacol peroxidase activity (EC. 1.11.1.7)

Guaiacol peroxidase (POD) activity was analyzed as described by Putter (1974). A 0.1 M (pH 7.0) phosphate buffer (3.0 mL), 20 mM guaiacol solution (50 µL), enzyme sample (100 µL), and 12.3 mM H_2O_2 solution (30 µL) were poured into the test tube and centrifuged. The absorbance of the sample was measured at 436 nm using a UV-spectrophotometer.

Catalase activity (EC. 1.11.1.6)

Catalase activity (CAT) was analyzed according to the method described by Aebi (1983). A 100 mM (pH 7.0) phosphate buffer (1.7 mL), 150 mM H_2O_2 solution (1.4 mL), and enzyme extract (500 µL) were poured into the test tube and whirlpooled. The absorbance of the sample was measured at a wavelength of 240 nm.

Glutathione reductase (EC 1.6.4.2)

Glutathione reductase (GR) was analyzed as described by Carlberg and Mannervik (1975). A 50 mM (pH 7.6) phosphate buffer (1.8 mL), 3.0 mM EDTA disodium salt (300 µL), 0.10 mM NADPH (300 µL), 1.0 mM glutathione (300 µL), and enzyme sample (300 µL) were poured in the test tube and whirlpooled. The absorbance of the samples was measured at 340 nm.

Superoxide dismutase-like activity

The microgreen extract (0.2 mL) was mixed with 50 mM (pH 8.5) Tris-HCl buffer (3.0 mL) and pyrogallol (0.2 mL). The mixture was vortexed (10 minutes at 25°C), and then incubated (10 minutes at 25°C) in the dark. The mixture was treated with 1 N HCl (1 mL) to stop the reaction ending. The absorbance was measured at 420 nm.

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assay

A 7 mM *2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)* (ABTS) solution (3 mL, pH 7.4) was mixed with the test extract sample or standard (200 µL), and the mixed samples were stored (20°C for 2 hours) in the dark before the absorbance was analyzed at 734 nm.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The microgreen extracts (1.8 mL) were diluted with dH_2O (7.2 mL) and stirred with 0.4 mM methanol containing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (1.8 mL). The mixture was vortexed (10 minutes) and whirlpooled (10 minutes) in the dark to measure the absorbance at 525 nm.

Nitrite scavenging activity

Nitrite scavenging activity (NSA) of the microgreen extract was measured, as reported by Kato *et al.* (1987). Each extract (1 mL), 1 mM sodium nitrite (1 mL), and 0.2 M citrate buffer (8 mL, pH 3.0 and 4.2) were mixed and vortexed (37°C, 1 hour). An aliquot sample (1 mL), 2% acetic acid (2 mL), and Griess reagent (0.4 mL, 1% naphthylamine and 1% sulfanilic acid in a methanol solution bearing 30% acetic acid) were mixed (15 minutes at 20°C) to analyze the NSA at 520 nm.

Statistical analysis

For both wheat and barley, a one-way analysis of variance was applied to test (F-test) the effects of pre-soaking and growing media on all the investigated variables, followed by Tukey test to analyze statistical significance between samples using SPSS Statistics V. 25 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Growth characteristics of wheat and barley microgreens

The percent germination of pre-soaked and non-pre-soaked seeds of wheat and barley and the growth characteristics of their microgreens grown in hydroponics or in organic soil are presented in Table 1. The germination rates of wheat and barley seeds in organic soil after soaking treatment were 97 and 96%, respectively, which were the highest among the treatments. An optimum germination environment could be provided by sowing pre-soaked seeds in organic soil, which stimulated the germination of wheat seeds (Pietruszewski, 1999). Soil media have already been indicated to allow higher germination rates than hydroponics in spinach, thyme, and marjoram microgreens (Brockhagen *et al.*, 2021). Pre-soaking seeds growing in organic soil showed the maximum height, weight, and yield for both barley and wheat microgreens.

Growing in hydroponics, either starting from pre-soaked or non-soaked seeds, hampered the weight, height, and yield of both barley and wheat microgreens because of reduced germination rates and growth. Wheat microgreens showed higher weight, height, and yield than barley microgreens, which is probably due to different growth dynamics owing to the species.

Chlorophyll and carotenoid contents of microgreens

The chlorophyll and carotenoid contents of wheat and barley microgreens obtained in hydroponics or organic soil from pre-soaked or non-pre-soaked seeds are reported in Figure 1.

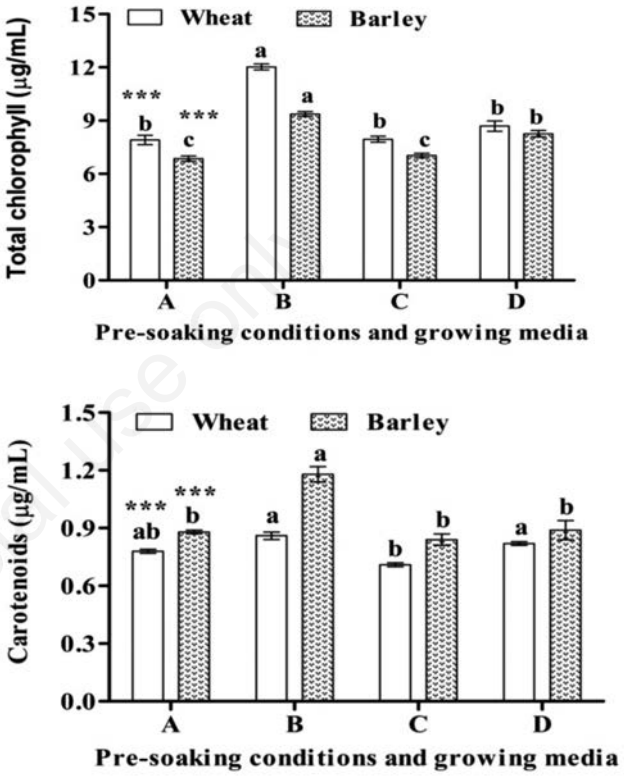

Figure 1. Chlorophyll and carotenoid contents of wheat and barley microgreens obtained with or without seed pre-soaking and grown in hydroponics or in organic soil. Means comparisons by Tukey's test (n=5 ± standard error). ***Indicates significance at P≤0.001. A) With pre-soaking and hydroponics; B) With pre-soaking and organic soil; C) Without pre-soaking and hydroponics; D) Without pre-soaking and organic soil.

Table 1. Percent germination of pre-soaked and non pre-soaked wheat and barley seeds, and individual height, weight and total yield of their microgreens grown in hydroponics or in organic soil.

Plant species	Pre-soaking condition	Growing media	Germination (%)	Microgreen height (cm)	Microgreen weight (g)	Yield (g/m^2)
Wheat	With	Hydroponics	91±0.9	9.7±0.4^{bz}	48±0.7^{b}	737±10.3^{b}
		Soil	97±0.5	16.2±0.6^{a}	116±1.4^{a}	1785±21.6^{a}
	Without	Hydroponics	93±0.7	11.5±0.3^{b}	54±1.0^{b}	826±14.7^{b}
		Soil	94±0.6	14.2±0.4^{a}	111±1.0^{a}	1712±15.9^{a}
	P		-	***	***	***
Barley	With	Hydroponics	89±0.9	9.1±0.3^{b}	39±0.6^{c}	593±9.4^{c}
		Soil	96±0.7	14.2±0.4^{a}	113±1.1^{a}	1735±17.3^{a}
	Without	Hydroponics	92±0.9	10.1±0.3^{b}	46±0.7^{b}	713±10.3^{b}
		Soil	94±0.9	12.9±0.5^{a}	108±0.9^{a}	1651±13.7^{a}
	P		-	***	***	***

$^{a-c}$Letters in the same column indicate different significance. ^{z}Indicates mean separation within columns by Tukey's test (n=5 ± standard error). ***Indicates significance at P≤0.001.

In both species, the chlorophyll content of microgreens was highest when grown in organic soil from pre-soaked seeds. The chlorophyll content usually increases due to the high rates of stomatal conductance, transpiration rate, and photosynthesis (Han *et al.,* 2019). However, hydroponics, with or without seed pre-soaking, decreased the chlorophyll levels of barley and wheat microgreen extracts. Gao *et al*. (2019) also found that hydroponics may reduce the quantum efficiency of PSII, stomatal conductance, transpiration rate, photosynthetic rate, respiration rate, and water-use capacity in plants, resulting in a decrease in chlorophyll content.

In both species, growing microgreens in organic soil starting from pre-soaked seeds caused the highest carotenoid content, which was 1.21 times higher for wheat and 1.40 times higher for barley as compared to the values recorded in microgreens grown hydroponically from non-soaked seeds. Xiao *et al.* (2012) also found that microgreens cultivated in organic soil conditions showed higher carotenoid content than those grown in hydroponics. Compared to wheat microgreens, barley microgreens had a high-grade carotenoid content, which may be due to crop and variety variations.

Bioactive compounds in microgreen extract

The bioactive phytochemicals of wheat and barley microgreens from pre-soaked and non-presoaked seeds grown in hydroponics or organic soil conditions are presented in Table 2. Compared to hydroponics, organic soil also resulted in higher flavonoid, phenolic, vitamin C, and anthocyanin content. The highest phenolic content in wheat and barley microgreen extracts was observed in organic soil, starting from pre-soaked seeds. The phenylalanine ammonia-lyase promotes the biosynthesis of polyphenols in the shikimate pathway (Santos-Sánchez *et al.*, 2019).

After pre-soaking, growing microgreens in organic soil showed 1.80-times higher vitamin C content for wheat and 2.60-times higher for barley than in hydroponics. *De novo* synthesis may increase the vitamin C content in germinated barley and wheat (Lemmens *et al.*, 2018; Benincasa *et al.*, 2019). Independent of the seed treatment (*i.e.*, pre-soaking or not), growing microgreens of both species in organic soil resulted in higher anthocyanin content as compared to hydroponics. β-Carotene is anabolic by the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway in the thylakoid membranes (intergranal thylakoids or lamellae) of the chloroplasts and is converted to anthocyanin (Phillips *et al.,* 2008). This anthocyanin biosynthesis is moderated by the phenylpropanoid pathway (Shi and Xie, 2014). Wheat and barley microgreens grown in organic soil, regardless of they were pre-soaked or not, had more bioactive compounds than those grown in hydroponics. Wheat microgreens showed higher flavonoid, phenolic, and vitamin C content than barley microgreens, except for anthocyanin content.

Table 2. Contents of bioactive compounds (phenolics, flavonoids, vitamin C and anthocyanins) of wheat and barley microgreens obtained with or without seed pre-soaking and grown in hydroponics or in organic soil.

Plant species	Pre-soaking condition	Growing media	Phenolics (µg/mL)	Flavonoids (µg/mL)	Vitamin C (µg/mL)	Anthocyanins (µg/mL)
Wheat	With	Hydroponics	119±2.5^{bz}	49±1.4^{b}	2.0±0.0^{b}	5.1±0.6^{b}
		Soil	155±1.5^{a}	98±5.1^{a}	3.6±0.1^{a}	8.7±0.6^{a}
	Without	Hydroponics	98±3.5^{c}	60±7.3^{b}	2.4±0.0^{ab}	4.6±0.7^{b}
		Soil	140±1.9^{a}	66±2.8^{ab}	3.0±0.5^{ab}	6.1±0.8^{ab}
	P		***	***	***	**
Barley	With	Hydroponics	68±1.5^{abz}	13±1.4^{b}	0.5±0.1^{b}	10.7±1.2^{b}
		Soil	76±1.9^{a}	23±3.2^{a}	1.3±0.0^{a}	17.9±1.8^{a}
	Without	Hydroponics	63±1.4^{b}	17±1.1^{ab}	0.4±0.1^{b}	10.6±1.3^{b}
		Soil	69±2.1^{ab}	19±1.4^{ab}	1.2±0.0^{a}	14.9±0.9^{ab}
	P		***	*	***	**

$^{a-c}$Letters in the same column indicate different significance. ^{z}Mean separation within columns by Tukey's test (n=5 ± standard error). *,**,***Indicate significance at P≤0.05, 0.01, and 0.001, respectively.

Table 3. Antioxidant enzymes of wheat and barley microgreens obtained with or without seed pre-soaking and grown in hydroponics or in organic soil.

Plant species	Pre-soaking condition	Growing media	POD (unit/min/mL)	CAT (unit/min/mL)	GR (unit/min/mL)
Wheat	With	Hydroponics	0.03±0.00^{bz}	134±2.4^{bc}	0.31±0.04^{ab}
		Soil	0.07±0.01^{a}	154±3.3^{a}	0.43±0.02^{a}
	Without	Hydroponics	0.03±0.00^{b}	123±2.1^{c}	0.28±0.04^{b}
		Soil	0.05±0.01^{ab}	149±3.8^{ab}	0.34±0.01^{ab}
	P		***	***	*
Barley	With	Hydroponics	0.09±0.00^{abz}	104±1.5^{c}	0.22±0.02^{b}
		Soil	0.12±0.01^{a}	138±1.2^{a}	0.34±0.03^{a}
	Without	Hydroponics	0.08±0.01^{b}	96±1.0^{d}	0.26±0.04^{ab}
		Soil	0.11±0.01^{a}	130±1.0^{b}	0.28±0.02^{ab}
	P		***	***	*

$^{a-c}$Letters in the same column indicate different significance. ^{z}Mean separation within columns by Tukey's test (n=5 ± standard error). *, ***Indicate significance at P≤0.05, and 0.001, respectively. POD, guaiacol peroxidase; CAT, catalase; GR, glutathione reductase.

Table 4. Antioxidant activity [(2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assay, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, superoxide dismutase (SOD)-like, and nitrite scavenging activity)] of wheat and barley microgreens obtained with or without seed pre-soaking and grown in hydroponics or in organic soil.

Plant species	Pre-soaking condition	Growing media	ABTS (µmol TEAC/L)	DPPH (µmol TEAC/L)	SOD-like (%)	NSA (µmol TEAC/L)
Wheat	With	Hydroponics	2266±8.8^{abz}	2341±57.7^{b}	10.3±0.5^{b}	217±41.2^{ab}
		Soil	2367±26.2^{a}	3158±52.1^{a}	18.7±0.5^{a}	434±97.2^{a}
	Without	Hydroponics	2256±23.3^{b}	1666±35.8^{c}	9.4±0.8^{b}	177±44.2^{b}
		Soil	2352±18.0^{ab}	2876±48.4^{a}	13.5±0.7^{b}	395±51.7^{ab}
	P		**	***	***	*
Barley	With	Hydroponics	1014±13.0^{bz}	313±30.4^{b}	5.9±0.50^{c}	173±15.9^{b}
		Soil	1284±14.7^{a}	566±40.6^{a}	13.5±1.0^{a}	272±15.4^{a}
	Without	Hydroponics	1070±12.3^{b}	386±29.3^{ab}	9.0±0.6^{bc}	164±13.7^{b}
		Soil	1198±11.8^{a}	495±63.8^{ab}	10.5±0.2^{ab}	227±8.8^{ab}
	P		***	**	***	***

$^{a-c}$Letters in the same column indicate different significance. ^{z}Mean separation within columns by Tukey's test (n=5 ± standard error). *, **, *** correspond to significance at P≤0.05, 0.01, and 0.001, respectively. ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; SOD-like, superoxide dismutase; NSA, nitrite scavenging activity.

Antioxidant enzymes in microgreen extract

Antioxidant enzymes in wheat and barley microgreens are reported in Table 3. In both species, growing microgreens in organic soil starting from pre-soaked seeds caused the highest levels of CAT, GR, and POD. These values were 1.15 times higher for CAT, 1.39 times higher for GR, and 2.33 times higher for POD under organic soil conditions than under hydroponics for wheat microgreens from pre-soaked seeds. This may be due to lower moisture content than in hydroponic cultivation conditions because lower moisture or a water deficit enhances antioxidant enzymes in rice seedlings (Wang *et al.*, 2019). Pre-soaked barley microgreens showed higher values of POD (1.33 fold), CAT (1.33-fold) and GR (1.55-fold) in organic soil compared to hydroponics. Wheat microgreens exhibited a higher level of antioxidant enzymes than barley microgreen extract, except for POD, which may be due to different plant species.

Antioxidant activity in microgreen extract

The effects of treatments (growing microgreens in hydroponics or soil starting from pre-soaked or non-pre-soaked seeds) on antioxidant activity are shown in Table 4. Microgreens grown in organic soil from pre-soaked seeds showed an increased level of antioxidant properties such as DPPH, ABTS, and SOD-like assays in both wheat and barley microgreen extracts compared to those grown in hydroponics without soaking (Siracusa *et al.*, 2017). In wheat microgreens obtained from pre-soaked seeds and grown in organic soil, these values were almost 2.45-times (NSA), 1.99-times (SOD-like), 1.90-times (DPPH), and 1.05-times (ABTS) higher than in microgreens obtained from non pre-soaked seeds and grown in hydroponics. Moreover, values of barley microgreens were almost 1.66-times (NSA), 1.50-times (SOD-like), 1.47-times (DPPH), and 1.20-times (ABTS) higher in organic soil than in hydroponics without pre-soaking. Plants may establish stress-responsive defensive mechanisms by enhancing enzymatic and antioxidant activities (Yao *et al.*, 2009). Wheat microgreens showed the highest level of antioxidant activity compared to barley microgreen extract in ABTS, DPPH, SOD-like, and NSA assays, which may be due to different growth dynamics owing to the species. Therefore, growing in organic soil after soaking may play a decisive role in improving the activity of antioxidants in barley and wheat microgreen extracts.

Conclusions

This study was conducted to investigate how pre-soaking wheat and barley seed and growing microgreens in hydroponics versus organic soil can affect the content of bioactive compounds. Pre-soaking seed and growing microgreens in organic soil allowed the highest levels of bioactive compounds, especially carotenoids, phenolics, flavonoids, vitamin C, and anthocyanin, in both barley and wheat microgreen extracts. Antioxidant enzymes (POD, CAT, and GR) and antioxidant activities (DPPH, ABTS, NSA, and SOD-like activity) were high in microgreens obtained from pre-soaked seed and grown in organic soil. These results may be useful for the commercial production of extracts from barley and wheat microgreens for the expansion of new functional products.

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