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The role of natural ZPT and NaCl on the phytochemical content of microgreen wheatgrass (Triticum aestivum L)

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ABSTRACT

Microgreen Wheatgrass is a young seed harvested 7-14 days after sowing that has fully developed cotyledons and a pair of true leaves. Development technology and scientific advances provide scientific evidence that some types of food plants can provide great health benefits, one of which is wheatgrass. Microgreens wheatgrass is a plant that is rich in nutrients. This study aims to determine the effect of natural ZPT from coconut water and shallots and the effect of NaCl concentration on the phytochemical content of wheatgrass (Triticum aestivum L.). This research was conducted at the Seed Technology Laboratory, Faculty of Agriculture, University of North Sumatra in March 2023 using a Completely Randomized Design (CRD) with 3 replicates and 2 factors, namely natural ZPT of shallots and coconut water, and NaCl concentration of 50 mM, 100 mM, 150 mM. The results showed that the natural ZPT treatment increased the antioxidants of microgreen wheat grass. Giving natural ZPT coconut water increased wheatgrass flavonoids. NaCl concentration increased flavonoid parameters, and antioxidants had no significant effect on chlorophyll a, b and total and carotenoids. The best NaCl treatment was best at concentrations of 100mm and 150mm. The interaction between natural ZPT and NaCl can increase flavonoids, and antioxidants.

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1. INTRODUCTION

Microgreen wheatgrass (Triticum aestivum L.) is a young plant from wheat seeds that is harvested at an early stage of growth. This plant is known to have various health benefits due to its high phytochemical content, such as flavonoids, phenols and carotenoids, which play an important role in providing antioxidant, anti-inflammatory and anticancer benefits (1–3). Increasing the phytochemical content of microgreen wheatgrass through appropriate cultivation techniques can increase its nutritional value and health benefits.

One method that can be used to increase the phytochemical content is by administering natural growth regulators (ZPT). Shallots (Allium cepa) and coconut water (Cocos nucifera) are two examples of natural PGRs that have long been used in agricultural practices. Shallots contain growth hormones such as auxin and gibberellin, which can stimulate plant growth and increase phytochemical synthesis (4). Coconut water is rich in cytokinins, which play a role in cell division and plant growth (5). The combination of these two natural PGRs has the potential to improve the quality and phytochemical content of wheatgrass microgreens.

The use of shallot extract has an effect on the root growth of teak shoot cuttings and the provision of natural PGR of shallots is also able to stimulate the growth of roots and shoots of jatropha (Jatropa curcas) (6). The use of coconut water in plant propagation can increase the performance of plants to grow, this is because there are additional carbohydrates so that food reserves that are converted into energy for growth will be fulfilled (7).

On the other hand, giving NaCl (salt) to plants is often associated with abiotic stress which can affect plant metabolism. At the right concentration, salt stress can stimulate the production of phytochemicals as a plant defense mechanism (8). However, the effect of NaCl on the phytochemical content of wheatgrass microgreens has not been widely studied. Therefore, this study aims to explore the role of providing natural ZPT from shallots and coconut water and NaCl on the phytochemical content of wheatgrass microgreens.

This research is expected to provide useful information regarding effective cultivation techniques to increase the phytochemical content of wheatgrass microgreens. With a better understanding of the role of natural PGRs and NaCl, microgreen farmers and producers can apply appropriate techniques to produce products with higher nutritional value and health benefits. Apart from that, the results of this research also have the potential to contribute to the development of sustainable agricultural practices by utilizing natural materials that are easily available and environmentally friendly.

2. RESEARCH METHOD

2.1 Place and time of research

This research was carried out in the Seed Technology Laboratory and tested in the Plant Disease Laboratory, Faculty of Agriculture, as well as in the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences and in the Phytochemistry Laboratory, Faculty of Pharmacy, University of North Sumatra, at an altitude of ± 26 meters above sea level. The research was conducted on March 2-15 2023.

2.2 Research Materials and Tools

The materials used in this research are wheatgrass seeds, coconut water and shallot extract as natural PGR, NaCl, the best planting medium which has a significant effect on plant height and wet weight of wheatgrass plant roots according to Oematan et al., 2022 (9), namely 50% top soil: 50% burnt husk charcoal (1:1), clean water, tissue paper, label paper, filter paper and other materials that support this research. The equipment used in this research is a seeding tray, sprayer, ruler, knife, scissors, cup, plastic container, blender, cloth filter, bucket, 100 ml measuring cup, stationery, camera, beacker glass, measuring flask, tube reactions, dropper pipettes, syringes, analytical scales, ovens, envelopes, vortexes, water baths, and other tools that support research.

2.3 Research design

The design used in this research was a Completely Randomized Design consisting of 2 treatment factors and 3 replications, namely: Factor I: Natural PGR (Coconut Water and Shallots) with 3 levels, namely Z0: No PGR, Normal water 100 ml / tray (Control); Z1: Shallots 100 ml / tray; Z2: Coconut Water 100 ml / tray. Factor II: NaCl (diluted with 100ml Aquadest) with 4 levels, namely N0: No NaCl (Control) / 100ml tray; N1: 50 mM (2.922 gr NaCl / L) /100ml tray; N2: 100 mM (5.844 gr NaCl / L) /100ml tray; N3: 150 mM (8.766 gr NaCl / L) /100ml tray.

2.4 Research Implementation

This research was carried out in several stages, starting from preparing the planting media, planting wheatgrass seeds, spraying application of natural PGR treatment with coconut water and shallot extract as well as NaCl treatment, harvesting wheatgrass.

2.4.1 Planting Media Preparation

The planting media used in this research were topsoil and burnt husk charcoal in a ratio of 1:1 (size 1L:1L). The two media are mixed and stirred evenly then filtered using a sand filter. The planting medium is then put into the plot tray / tray no. 4 measuring 35 cm x 26 cm x 4 cm with holes drilled in the bottom.

2.4.2 Planting Seeds

Before sowing the wheatgrass seeds, weigh 100g/tray each. Then the wheatgrass seeds are soaked in plain water for a minimum of ± 6 hours to overnight for the imbibition process and breaking of seed dormancy. Wheatgrass seeds are sown on the planting medium by spreading them evenly, and leaving a distance of 1 cm from the corner of the tray. The seeds that have been spread are slightly pressed so that they stick into the planting medium. Then sprayed with 100 ml of plain water using a spayer, then covered with tissue for 4 HSS. The aim of covering it with tissue is to prevent it from being exposed to direct sunlight or light. Then after four days the tissue cover was removed.

2.4.3 Application of Natural ZPT and NaCl

The application of Natural PGR is done by spraying a solution of 100 ml/tray each of coconut water and shallot extract. It is applied by spraying evenly on the plant canopy using a sprayer in the morning starting at 09:00 WIB every day. Spraying is done only once a day. The application of Natural PGR from coconut water and shallots is carried out on days 6, 7, 8 and 9 days after sowing (HSS). NaCl application is carried out starting from the age of 10, 11, 12 and 13 HSS. Preparation of dilution concentration solutions in attachments 41 and 42. NaCl stock solution is applied to microgreen wheatgrass according to the treatment by watering using a sprayer.

2.5 Observed parameters

2.5.1 Carotenoids (mg/g)

Carotenoid analysis was carried out at the Plant Disease Laboratory, Faculty of Agriculture, University of North Sumatra. The method used to calculate the amount of carotenoids is to use the same sample in the chlorophyll test, the difference is by using the A470 wavelength on a spectrophotometer. Carotenoids in µmol/L units are calculated using the formula: Carotenoids = {(A470+(6.10 x A665) - (20.0 x A649)} /1 A470 = absorbance of chlorophyll extract at 470 nm.

2.5.2 Flavonoids

A total of 1 ml of sample was added with 1.5 ml of ethanol then 2.8 ml of distilled water, 0.1 ml of aluminum (III) chloride and 0.1 ml of potassium acetate. Incubate for 30 minutes and measure absorbance using a UV-Vis spectrophotometer at a maximum wavelength of 439 nm. To determine flavonoid levels, the regression equation from the standard curve between absorbance and standard solution concentration is used. Systematically it can be written:

$$y=ax\pm \text{b} \quad (1)$$

Information:
y = Absorbance value
a = Intersection of the straightline curve
b = Intersection of the curve with coordinate
x = Extract concentration (mg/L)
Next, the absorbance value is substituted into the regression equation as (y) so that to determine flavonoid levels in herbal samples the equation can be used:

$$Kadar\ Flavonoid=\frac{CxV}{M} \quad (2)$$

Note:
C = Concentration (mg/L)
V = Volume of sample solution (M)
M = Mass of extract (g)

2.5.3 Antioxidants (ppm)

Weighed 10 mg of the thick extract and dissolved it with methanol to make 10 mL. obtained a solution with a concentration of 1000 ppm. Take 0.25 mL; 0.5 mL; 0.75 mL; 1 mL; 1.25 mL of 1000 ppm extract solution. then added 1 ml of DPPH solution (concentration 200 ppm) at each concentration and added with methanol to the mark (5 mL volumetric flask). concentrations of 50, 100, 150, 200, 250 ppm were

obtained. Incubated for 30 minutes then absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm.

2.6 Wheatgrass harvesting

Harvesting of wheatgrass is carried out when the plants are 14 HSS by pulling them out by the roots, and then cleaning the roots from the rest of the planting medium by dipping them in water and then measuring them. Parameters of plant length and root length and other variables.

2.7 Data Analysis

Next, the data was analyzed using Variety Print Analysis (Ansira).α= 5 %. If the results of the Variety Scan Analysis are significant then a follow-up test is carried out using the Duncan Distance Test (DMRT)α= 5 % for each observation parameter. The observation data were analyzed using the ANOVA (Analysis of Variance) test and if the results obtained showed significant differences, then further tests were carried out using the Duncan New Multiple Range Test (DNMRT) at a significance level of 5% (Hanafiah, 2014).

3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Content Carotenoids

The results of the variance show that the influence of growth and production as well as the phytochemical content of microgreen wheatgrass due to NaCl and Natural PGR treatment has no significant effect on the plant's carotenoid content, and The interaction between NaCl and natural PGR treatment also had no significant effect. The average caratenoid content of wheatgrass plants resulting from NaCl and natural PGR treatment can be seen in Table 1.

Table 1. Average effect of Natural ZPT and NaCl treatment on carotenoid content (mg/g) of microgreen wheatgrass

Treatment	Natural ZPT			
	100 ml Water (Z0)	100 ml Red Onion Extract (Z1)	100 ml Coconut Water (Z2)	Average
NaCl				
N0 (0mM)	2.35	2.33	3.30	2.66 ab
N1 (50mM)	2.67	2.17	2.37	2.41 ab
N2 (100mM)	2.23	2.70	1.35	2.09 ab
N3 (150mM)	2.19	2.54	1.01	1.91 a
Average	2.36 ab	2.44 ab	2.01 a	-

Note: Numbers followed by the same letter are not significantly different according to the Duncan 5% multiple range test.

Table 1 showed that the response to an increase in carotenoid content was due to the NaCl No treatment (control), namely, 2.66, and the lowest was in the N3 treatment (150mM), namely 1.91. In the Natural PGR treatment, the highest caratenoid content results were obtained due to the Z1 (shallot) treatment, namely 2.44, and the lowest was in the Z2 (coconut water) treatment, namely 2.01. It is suspected that the highest carotenoid content in the control treatment was due to the NaCl treatment being applied directly to the leaves or plant crowns which disrupted the photosynthesis process in plant cells. And coupled with the presence of plants grown indoors, the level of carotene in plant leaves is lower compared to plants exposed to direct sunlight. Normal capture of light energy for photosynthesis under conditions of low light intensity causes low carotenoid content, where light is an important factor in carotenoid biosynthesis.

NaCl salinity did not affect photosynthetic pigment content, and carotenoid content did not correlate with NaCl concentration under the conditions tested in the given treatments. NaCl concentration and harvest time did not affect leaf chlorophyll and carotenoid contents, while antioxidant capacity and total phenol, flavonol glycoside, and anthocyanin contents generally increased with salinity (10). Salt stress can limit water and nutrient uptake by roots and disrupt plant water relations and leaf photosynthesis (11).

Environmental factors can affect the formation of carotenoids, such as temperature, water, light and humidity.Carotenoids in photosynthesis are helping to absorb light, so that the light used for the photosynthesis process becomes greater (12). The energy absorbed by carotenoids is forwarded to chlorophyll which is then used in photosynthesis (13). Presumably, carotenoids in addition to being photosynthetic pigments also function to protect chlorophyll from high light, so that the carotenoid content in plants adjusts to the chlorophyll content. Genetic differences in each plant will affect the ability to synthesize carotenoids (14,15). Carotenoids are accessory pigments in the photosynthesis process, located in chloroplasts together with chlorophyll.

3.1.2 Content Flavonoids

The variance results show that the influence of growth and production as well as the phytochemical content of microgreen wheatgrass due to treatment with NaCl and Natural PGRs has a significant effect on the flavonoid content of the plant, as well as interactions NaCl and Natural ZPT treatments also had a significant effect. The average flavonoid content of wheatgrass plants resulting from NaCl and natural PGR treatment can be seen in Table 2.

Table 2. Average effect of natural PGR and NaCl treatment on wheatgrass microgreen flavonoid content

Treatment	Natural ZPT			
	100 ml Water (Z0)	100 ml Red Onion Extract (Z1)	100 ml Coconut Water (Z2)	Average
NaCl		mgQE/g		
N0 (0mM)	0.14 bcd	0.09 b	0.14 bcd	0.13 a
N1 (50mM)	0.12 bcd	0.11 bc	0.16 bcd	0.13 a
N2 (100mM)	0.12 bcd	0.25 d	0.13 bcd	0.16 a
N3 (150mM)	0.18 a	0.25 d	0.66 bcd	0.36 b
Average	0.14 a	0.17 a	0.27 b	-

Note: Numbers followed by the same letter are not significantly different according to the Duncan 5% multiple range test.

Table 2 showed that the response to increasing flavonoids was in the N3 (150mM) treatment, namely 0.36, and the lowest was in the No (control) and N1 (50mM) treatments with 0.13 each. Meanwhile, the highest flavonoid content was obtained in the Natural PGR treatment due to Z2 (coconut water) treatment, namely 0.27, and the lowest was in Z0 (control), namely 0.14. Providing NaCl with a concentration of 150 mm can increase the flavonoid content which is the result of secondary plant metabolites under stress conditions. Previous studies in cereal plants, as in other species, phytochemical content increased during the germination phase and early seedling growth Wheatgrass during germination is a nutritional and phytochemical powerhouse (16).

Flavonoids, polyphenols, and antioxidant activity decreased for smaller NaCl concentrations, while higher NaCl concentrations stimulated the production of active compounds. Flavonoids are often induced by abiotic stress and play a role in plant protection such as the results obtained in this study. Therefore, based on previous evidence and current data, it is to affirm that 150mM salt stress can be a viable approach to maintain, or even increase, the content of phytochemical compounds in microgreen wheatgrass. NaCl increases total flavonoid content but decreases phenolic content (17,18) while total antioxidant capacity is not affected. Plants vary widely in their phenolic composition and content also according to genetics and environmental conditions.

3.1.3 Content Antioxidant

The variance results show that the influence of growth and production as well as the pigment content of microgreen wheatgrass due to NaCl and Natural PGR treatment has a significant effect on the plant's antioxidant content, as well as interactions NaCl and Natural ZPT treatments also had a significant effect. The average antioxidant content of wheatgrass plants due to NaCl and natural PGR treatment can be seen in Table 3.

Table 3. Average effect of natural PGR and NaCl treatment on the antioxidant content of wheatgrass microgreens

Treatment	Natural ZPT			
	100 ml Water (Z0)	100 ml Red Onion Extract (Z1)	100 ml Coconut Water (Z2)	Average
NaCl		ppm		
No (0mM)	145.13 a	204.13 cdef	195.96 eph	181.74 a
N1 (50mM)	169.35 ab	228.14 eph	260.24 g	219.24 bc
N2 (100mM)	211.88 cde	278.18 h	233.74 eph	241.27 c
N3 (150mM)	194.52 bc	197.68 cds	213.67 cdef	201.96 b
Average	180.22 a	227.03 b	225.90 b	-

Note: Numbers followed by the same letter are not significantly different according to the Duncan 5% multiple range test.

Table 3 showed that the response to increasing antioxidant content was found in NaCl treatment with an N2 concentration (100mM), namely 241.27, and the lowest was at a NO concentration (control), namely 181.74. Meanwhile, the highest antioxidant content resulting from natural ZPT treatment was obtained in treatment Z1 (shallots), namely 227.03, and the lowest was in treatment Z0 (control), namely 180.22. Wheatgrass is considered a superfood because of its high antioxidant potential and other beneficial properties. Especially in recent years, wheatgrass juice and powder have been tested in vivo in clinical study models against several diseases (19)

The antioxidant activity of wheatgrass is not affected by different drying methods (20). Growing young wheat leaves indoors for seven or ten days resulted in higher free radical scavenging activity than growing outdoors (21). Devi et al., (2020) (22) showed that the antioxidant potential of wheatgrass is influenced by sunlight, which is better not exposed to direct sunlight. Salinity causes oxidative stress and excess reactive oxygen species (ROS), and plants react to salt stress by increasing the content of polyphenols and other antioxidants (23,24). For this reason, germination under salinity has been proposed as a means to increase the nutritional value of sprouts in broccoli and radish (25,26).

4. CONCLUSION

Application of ALami ZPT treatment increased flavonoids and antioxidants of microgreen wheatgrass. Giving NaCl concentration increases flavonoids, antioxidants and carotenoids. The best NaCl treatment at concentrations of 100mM and 150mM. The interaction of Natural ZPT and NaCl can increase flavonoids and antioxidants. Further research to explore the mechanism behind the increase in phytochemical content due to the application of natural ZPT and NaCl.

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