ผลของโซเดียมคลอไรด์ต่อการเจริญเติบโตและการต้านอนุมูลอิสระในต้นอ่อนทานตะวัน (*Helianthus annuus* L.)

Effects of Sodium Chloride on Growth and Antioxidant Activity in Sunflower (*Helianthus annuus* L.) Sprouts

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บทคัดย่อ

จากการศึกษาผลของโซเดียมคลอไรด์ต่อการเจริญเติบโตและการต้านอนุมูลอิสระในต้นอ่อนทานตะวัน โดยวิเคราะห์การเจริญเติบโต ปริมาณรงควัตถุในกระบวนการสังเคราะห์ด้วยแสง การเกิดลิพิดเพอร์ออกซิเดชัน และการต้านอนุมูลอิสระ โดยใช้โซเดียมคลอไรด์ที่ความเข้มข้นต่าง ๆ ผลการศึกษาพบว่าโซเดียมคลอไรด์ความเข้มข้นสูง ทำให้ความยาวและน้ำหนักสดลำต้นลดลง คิดเป็นร้อยละ 25.76 และ 18.34 ตามลำดับเมื่อเทียบกับกลุ่มควบคุม ($p < .05$) แต่ไม่มีผลต่อความยาวราก น้ำหนักสดราก ปริมาณคลอโรฟิลล์*เอ*และ*บี* ความเข้มเข้มข้นของโซเดียมคลอไรด์ 800 มิลลิโมลาร์ส่งผลให้ปริมาณแคโรทีนอยด์เพิ่มขึ้นเมื่อเทียบกับกลุ่มควบคุม ($p < .05$) และความเข้มข้นของโซเดียมคลอไรด์ที่สูงขึ้นส่งผลให้ปริมาณ MDA และการต้านอนุมูลอิสระของพืชเพิ่มสูงขึ้น นอกจากนี้ปริมาณโซเดียมคลอไรด์ที่เพิ่มขึ้น ยังทำให้การเจริญเติบโตของพืชลดลงแต่ส่งผลต่อการกระตุ้นการต้านอนุมูลอิสระของพืช

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Abstract

The effects of NaCl on growth and antioxidant activity in sunflower sprouts were examined by analysis of the growth, photosynthetic pigments, lipid peroxidation, and antioxidant activity. The results showed that the total shoot length and total fresh weight of sprouts in high NaCl concentration were significantly decreased by 25.76% and 18.34%, respectively, when compared with control ($p < .05$), while NaCl did not affect to the total root length, total fresh weight of root, and chlorophyll *a* and *b* contents. However, the carotenoid content of sprouts, treated with 800 mM NaCl, was significantly higher than control ($p < .05$). Malonyldialdehyde (MDA) content and antioxidant activity were increased by NaCl treatment. NaCl treatment affected the growth of sunflower sprouts negatively, but it tended to induce the antioxidant activity.

Keywords: Sodium chloride, Growth, Antioxidant activity, Sunflower, Sprouts

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1. Introduction

Nowadays, the trends of healthy consumption are dispersedly suggested especially vegetables and fruits. Sprouts are one of the popular vegetable that can be consumed as a healthy food. Sprouts contain many nutrients and minerals such as protein, potassium, fibers. Besides, sprouts are a good source of antioxidants including carotenoid, flavonoid, vitamin A, vitamin E, and vitamin C. These nutrients and antioxidants can improve health and protect the body against chronic diseases, reduce blood cholesterol level, reduce the risk of heart disease and prevent of cancer (Slavin & Lioyd, 2012).

Plant growth and development depends on the derived environmental factors such as water, light, temperature, nutrients and minerals. In particular, sodium chloride is abiotic factor which limits crop productivity. The increase of NaCl concentration in soil and water induces abiotic stress of plants, e.g., osmotic stress, nutrient deficiency, limit water and nutrient uptake from soil, and oxidative stress, thus these irregular conditions decrease the germination rate, fresh weight, dry weight, total root length and shoot length (Shrivastava & Kumar, 2015). Plants respond to salinity stress by accumulation of secondary metabolites including polyphenol, flavonoid, terpene, alkaloid, polyamine and jasmonic acid, which protect plant from abiotic stress. Moreover, secondary metabolites are not only a significant component of plants' living, but also act as the supportive substance for human health (Akula & Ravishankar, 2011).

Previous studies had shown that high concentration of NaCl affected growth and antioxidant activity in several plants, for example, romaine lettuce (Kim et al., 2008), maize (Hichem et al., 2009), radish sprout (Yuan et al., 2010), buckwheat sprout (Lim et al., 2012), broccoli (Guo et al., 2013), and lentil sprout (Swieca, 2014). Therefore, the objective of this study was to determine the effect of NaCl on growth and antioxidant activity in sunflower sprout by using various concentrations of NaCl.

2. Materials and methods

2.1 Sunflower sprout cultivation

Sunflower seeds were washed and soaked in distilled water at room temperature for 10 hours. The seeds were wrapped with moisten cheesecloth at room temperature for 24 hours. After that, 40 germinated sunflower seeds were transferred into plastic pots (7×17×6 cm) which filled with soil/coconut fiber mixture. Six plastic pots were placed in sprout cultivator. Water was irrigated once a day (100 ml/ pot) at 8.30 am each day. This experiment was performed under controlled condition for 7 days.

2.2 Sodium chloride treatment

NaCl solution was prepared at various concentrations (0, 200, 400, 600, 800 and 1,000 mM). Fifty ml of solution at each concentration was sprayed on sprouts in each pot at 3, 5, and 7 days after germination at 10.30 am each day.

2.3 Determination of the growth of sunflower sprouts

Three sprouts were randomly sampled from each treatment. Seven day after germination and fresh weight and length of both shoot and root were determined.

2.4 Determination of photosynthetic pigment content in sun flower sprouts

The chlorophyll *a, b* and carotenoid content were measured using acetone solvent according to the method of Sumanta et al. (2014). 0.5 gram of sprouts were cut into small pieces and 10 mL of 80% acetone were added and then homogenized. The homogenate was centrifuged at 10,000× *g* for 5 minutes. The supernatant was measured at 645, 663, and 470 nm, respectively. After that, the chlorophyll *a, b* and carotenoid contents were calculated as follows:

Chlorophyll *a* $= 12.25\ (A_{663}) - 2.79\ (A_{645})$

Chlorophyll *b* $= 21.5\ (A_{645}) - 5.1\ (A_{663})$

Carotenoid $= (1000A_{470} - 1.82C_a - 85.02C_b)\ /198$

2.5 Lipid peroxidation

Lipid peroxidation was estimated by determining the malonyldialdehyde (MDA) content. The MDA content in sunflower sprouts was determined by using thiobarbituric acid (TBA) reaction method according to the method of Gao et al. (2009). One gram of sunflower sprout was cut into small pieces and 4 mL 10% trichloroacetic acid (TCA) were added and then homogenized. The homogenate was centrifuged at 1,200× *g* for 10 minutes. 2 ml of the supernatant were added 2 mL of 0.62% TBA in 10% TCA. The reaction mixture was incubated at 98°C in water bath for 20 minutes. Then, the mixture was placed in ice bath. After that, the mixture was centrifuged at 1,200× *g* for 10 minutes. The supernatant was measured at 450, 532, and 600 nm respectively. The MDA content was calculated as follows:

1) MDA concentration ($\mu molL^{-1}$) $= 6.45\ (A_{532} - A_{600}) - 0.56\ A_{450}$

2) MDA content ($nmolg^{-1}$ fresh weight, FW) = MDA concentration ($\mu molL^{-1}$) × homogenate volume (ml) / FW (g)

2.6 Determination of antioxidant activity in sunflower sprout

2.6.1 Extraction of antioxidant activity

Two grams of sprouts were cut into small pieces and 20 mL of 80% methanol were added and then homogenized. The homogenate was centrifuged at 10,000× *g* for 10 minutes. After that, supernatant was used for determination of antioxidant activity.

2.6.2 Determination of DPPH free radical scavenging activity

The antioxidant activity of the sprout was measured using DPPH free radical scavenging activity assay according to the method of Enujiugha et al. (2012). The reaction mixture which contained 0.2 mL of the extract sample and mixed with 3.8 mL of 0.1 mM DPPH solution was incubated in the dark at room temperature for 30 minutes. The absorbance of reaction mixture was measured at 517 nm. The scavenging activity of DPPH free radical was calculated using a formula:

Scavenging activity (%) = [(1 – absorbance of sample at 517 nm / absorbance of control at 517 nm)] × 100.

2.6.3 Determination of ferric reducing antioxidant power (FRAP)

The antioxidant of the sprout was measured by using FRAP assay according to the method of Szollosi et al. (2002). The reaction mixture consisted of 0.5 ml of extract sample and mixed with 3 ml of FRAP working solution (FRAP reagent was contained 25 mL of acetate buffer pH 3.6 plus 2.5 mL of 10 mM 2,4,6-tripyridyl-*s*-triazine solution in 40 mM HCl and 2.5 mL of 20 mM Iron (III) chloride hexahydrate and was prepared freshly). The absorbance of reaction mixture was measured at 593 nm. The ferric reducing antioxidant power in the extract was compared with the standard curve of Trolox.

3. Results and discussion

3.1 Effect of sodium chloride on growth of sunflower sprouts

The growth of sunflower sprouts, evaluated as total shoot, root length and fresh weight were shown in Table 1. The total shoot length, treated with 200 and 400 mM NaCl, was not significantly different in sprouts compared with control. NaCl in 600, 800 and 1,000 mM affected the total shoot length significant decreasing by 13.65%, 15.87%, and 25.76% in comparison with control, respectively. Consequently, the increasing NaCl concentration tended to induce the decreasing of total shoot length. In treatment with 200, 400, 600 and 800 mM NaCl, the fresh weight of shoot was not significantly different in comparison with control but declined significantly in 1000 mM NaCl. NaCl slightly affected total length and fresh weight of root since the spray of NaCl on leaves was introduced in short time period. The results corresponded with the previous studies, which showed that NaCl treatment decreases the shoot length and fresh weight of some genotypes in two species of jute (Abass & Latif, 2005), safflower cultivars (Motamedi & Farhoudi, 2010), bean *Vicia faba* L. (Qados, 2011), canola (Farhoudi, 2010) and coriander cultivars (Kaur & Kumar, 2017). The increase of NaCl concentration affected nutrition deficiency, oxidative stress, osmotic stress, limited water and nutrition uptake from soil which cause of decreased fresh weight, and shoot length in plants (Shrivastava & Kumar, 2015).

3.2 Effect of sodium chloride on photosynthetic pigment content in sunflower sprouts

Table 2 illustrated the effect of NaCl on photosynthetic pigment content. The chlorophyll *a* and *b* were not significantly affected by NaCl treatment. The carotenoid content increased significantly in 800 mM NaCl, while it was not different in treatment of 200, 400 and 600 mM NaCl when compared with control. The findings were similarly with a previous study in buckwheat sprouts. High NaCl concentration induce abscisic acid from carotenoid by mevalonic acid pathway (Lim et al., 2012). Therefore, low light intensity caused low photosynthesis pigment content.

3.3 Effect of sodium chloride on lipid peroxidation in sunflower sprouts

Lipid peroxidation in sunflower sprouts was determined by the content of MDA and shown in Table 3. The result indicated that, the MDA content was significantly increased by 54.39% and 71.92% in sprout treated with 800, and 1000 mM NaCl, respectively, in comparison with control, whereas these were not affected by low NaCl concentrations (200, 400, and 600 mM). The SOD decrease of activity in high NaCl concentration affected the accumulation in reactive oxygen species (ROS) in rice seedling, the damaged membrane induced

the increase of MDA content from the oxidized polyunsaturated fatty acid in cell membrane by ROS (Dionisio-sese & Tobita, 1998). Moreover, Abd El-baky et al., (2003), Sairam; et al. (2005), Motamedi & Farhoudi (2010), and Astorga & Melendez (2010) concluded the increase of NaCl concentration related to the rise of value in MDA content of different plant species.

3.4 Effect of sodium chloride on antioxidant activity of sunflower sprouts

The effect of NaCl on the antioxidant activity in sunflower sprout was determined by DPPH free radical scavenging activity and ferric reducing antioxidant power. Result are shown in Table 4. In the DPPH assay, the levels of antioxidant capacity in sprouts treated with 200, 400, 600, 800, and 1000 mM NaCl were 18.83%, 26.30%, 26.89%, 31.87%, and 31.19% which were higher than control, respectively. The FRAP value, similarly, showed the same trend with DPPH assay. The results agreed with previous studies which antioxidant potential rise with an increase in concentration of NaCl in maize, radish sprout and buckwheat sprouts (Kim et al., 2009; Yuan et al., 2010; Lim et al., 2012). Due to the result of antioxidant activity under salinity stress from this research, antioxidant, for instance, polyphenol, flavonoid, terpene and alkaloid, were synthesized in order to be protective and survival factors (Akula; & Ravishankar, 2011).

Table 1 Effect of various NaCl concentrations on growth in sunflower sprouts

NaCl treatment (mM)	Shoot length (cm)	Shoot fresh weight (g)	Root length (cm)	Root fresh weight (g)
Control	18.722 ± 0.338ab	0.878 ± 0.038a	5.778 ± 0.516a	0.075 ± 0.065a
200	19.967 ± 0.170a	0.889 ± 0.021a	6.306± 0.460a	0.065 ± 0.004a
400	18.250 ± 0.642b	0.895 ± 0.044a	6.467 ± 0.305a	0.083 ± 0.010a
600	16.167 ± 0.529c	0.860 ± 0.007a	6.278 ± 0.346a	0.089 ± 0.008a
800	15.750 ± 0.294c	0.817 ± 0.053ab	6.567 ± 0.417a	0.091 ± 0.009a
1000	13.900 ± 0.296d	0.717 ± 0.030b	5.695 ± 0.353a	0.072 ± 0.008a

Each value is given as mean ± SE

[a-d] Means with different superscripts within a column indicate significant differences ($p < 0.05$).

Table 2 Effect of various NaCl concentrations on photosynthetic pigment content in sunflower sprouts

NaCl treatment (mM)	Chlorophyll *a*	Chlorophyll *b*	Carotenoid
Control	1.671 ±0.139a	0.624 ± 0.139a	0.699 ± 0.019a
200	1.551 ± 0.132a	0.410 ± 0.186a	0.747 ± 0.041ab
400	1.613 ± 0.108a	0.455 ± 0.288a	0.740 ± 0.035ab
600	1.758 ± 0.280a	0.836 ± 0.248a	0.6430 ± 0.054a
800	1.874 ± 0.191a	0.395 ± 0.145a	0.866 ± 0.068b
1000	1.731 ± 0.229a	0.558 ± 0.162a	0.724 ± 0.055ab

Each value is given as mean ± SE

[a-d] Means with different superscripts within a column indicate significant differences ($p < 0.05$).

Table 3 Effect of various NaCl concentrations on MDA content in sunflower sprouts

NaCl treatment (mM)	MDA content ($nmolg^{-1}FW$)
Control	0.057 ± 0.008a
200	0.065 ± 0.005a
400	0.058 ± 0.001a
600	0.069 ± 0.008ab
800	0.088 ± 0.008b
1000	0.098 ± 0.003b

Each value is given as mean ± S.E.

[a-b] Mean with different superscripts within a column indicate significant differences ($p < 0.05$).

Table 4 Effect of various NaCl concentrations on antioxidant activity in sunflower sprouts.

NaCl treatment (mM)	% Antioxidant activity	FRAP value
Control	18.066 ± 0.897a	0.023 ± 0.001a
200	18.828 ± 2.036ab	0.0256 ± 0.003ab
400	26.302 ±1.770abc	0.032 ± 0.003abc
600	26.895 ± 1.388bc	0.036 ± 0.005bc
800	31.870 ± 5.303c	0.041 ± 0.006c
1000	31.194 ± 2.233c	0.040 ± 0.005c

Each value is given as mean ± SE

[a-b] Means with different superscripts within a column indicate significant differences ($p < 0.05$).

4. Conclusion

NaCl treatment at different concentrations had different effects on growth, photosynthetic pigments, MDA content and antioxidant activity in sunflower sprouts. High NaCl concentration significantly decreased the total shoot length and fresh weight of shoot sprouts, but not affected in total root length and root fresh weight. Similarly, the MDA content and antioxidant activity tended to rise when concentration of NaCl increased. On the contrary, NaCl treatment did not affect the photosynthetic pigments (chlorophyll *a* and *b*) content, except carotenoid content was significantly increased by 800 mM NaCl. This work is a prior research study about antioxidant activity of sunflower sprout under salinity stress, thus antioxidant production should be examined in the similar condition.

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