KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 2): 471-482, 2022
KSU J. Agric Nat 25 (Suppl 2): 471-482, 2022
https://doi.org/10.18016/ksutarimdoga.vi.1081099

The Investigation of the Effect of the Salicylic Acid on the Antioxidant Potential, Vitamin C Content and DNA Protection Activity of Wheatgrass (*Triticum aestivum* L.)

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ABSTRACT
Wheatgrass is considered as a superfood because of its high antioxidant potential and beneficial ingredients. Especially in recent years, wheatgrass juice and its powder have been tested *in vivo* animal models and clinical studies against some diseases. Salicylic acid (SA) one of the important phytohormones controlling plant growth is used as an exogenous elicitor to increase plant bioactive compounds. The aim of this study was to investigate antioxidant potential, vitamin C content and DNA protection of wheatgrass grown from SA pre-treated seeds. For this purpose, total antioxidant statue, total oxidant statue, oxidative stress index and vitamin C level were determined. Additionally, pUC19 plasmid was incubated with Fenton's solution to determine DNA protection activity of lyophilized wheatgrass extract. Bread wheat caryopses were imbibed in different concentrations of SA for 2 hours. Wheatgrass grown from seeds pre-treated with 10^{-8} M SA had significantly higher total antioxidant statue, vitamin C and soluble protein content than control. It was observed that wheatgrass extracts had a DNA protective role against hydroxyl radicals. It was concluded that SA pretreatment of seeds could be a good approach to increase their antioxidant potential, soluble protein content and vitamin C level of plants used as antioxidant sources by people.

Plant Physiology

Research Article

Article History
Received : 01.03.2022
Accepted : 06.05.2022

Keywords
Wheatgrass
Salicylic acid
Vitamin C
Elicitor
DNA protection

Salisilik Asitin Buğday Çiminin (*Triticum aestivum* L.) Antioksidan Potansiyeline, C Vitamini İçeriğine ve DNA Koruyucu Aktivitesine Etkisinin Araştırılması

ÖZET
Buğday çimi yüksek antioksidan potansiyeli ve yararlı içeriğinden dolayı bir süper besin olarak görülmektedir. Özellikle son yıllarda, buğday çim suyu ve tozu bazı hastalıklara karşı *in vivo* hayvan modellerinde ve klinik çalışmalarda test edilmiştir. Bitki büyümesini kontrol eden önemli bitki hormonlarından biri olan salisilik asit (SA) bitki biyoaktif maddelerini artırmak için dış kaynaklı bir elisitör olarak kullanılır. Bu çalışmanın amacı, SA ile ön uygulama yapılmış tohumlardan büyüyen buğday çiminin antioksidan potansiyelini, C vitamini içeriğini ve DNA koruyucu aktivitesini araştırmaktır. Bu amaç için, toplam antioksidan seviye, toplam oksidan seviye, oksidatif stres indeksi ve C vitamini seviyesi belirlendi. Bunlara ek olarak, liyofilize buğday çimi ekstrelerinin DNA koruyucu aktivitesini belirlemek için pUC19 plazmiti Fenton solüsyonu ile muamele edildi. Ekmeklik buğday karyopsis meyveleri farklı konsantrasyonlarda ki SA içinde 2 saat şişirildiler. 10^{-8} M SA ile şişirilen tohumlardan büyüyen buğday çimi kontrole göre anlamlı olarak daha yüksek toplam antioksidan seviyeye, C vitamini ve çözünen protein içeriğine sahiptir. Buğday çimi ekstresinin hidroksil radikallerine karşı DNA koruyucu rolüne sahip olduğu gözlendi. İnsanlar tarafından antioksidan kaynağı olarak tüketilen bitkilerin antioksidan potansiyelini, çözünen protein içeriğini ve C vitamini

Bitki Fizyolojisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 01.03.2022
Kabul Tarihi : 06.05.2022

Anahtar Kelimeler
Buğday çimi
Salisilik asit
C vitamini
Elisitör
DNA koruma

seviyesini artırmak için tohumlarına SA ön uygulamasının yapılmasının iyi bir yaklaşım olabileceği sonucuna varıldı.

Atıf Şekli: Battal A, Demir S 2022. Salisilik Asitin Buğday Çiminin (*Triticum aestivum* L.) Antioksidan Potansiyeline, C Vitamini İçeriğine ve DNA Koruyucu Aktivitesine Etkisinin Araştırılması . KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 2): 471-482. https://doi.org/10.18016/ksutarimdoga.vi.1081099

To Cite : Battal A, Demir S 2022. The Investigation of the Effect of the Salicylic Acid on the Antioxidant Potential, Vitamin C Content and DNA Protection Activity of Wheatgrass (*Triticum aestivum* L.). KSU J. Agric Nat 25 (Suppl 2): 471-482. https://doi.org/10.18016/ksutarimdoga.vi.1081099

INTRODUCTION

Wheat is one of the most important crop plants consumed by human. Bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum durum* L.) are mostly cultivated species for food source in the world. In recent years, wheatgrass juice and powder have been popular as a functional food which is used for human health (Bar-Sela et al., 2015). Wheatgrass is harvested 10-15 days after germination. It has been used for some disease treatments for several years because of its rich source of chlorophyll, vitamins, amino acids and mineral content and antioxidant potential (Rana et al., 2011; Thakur et al., 2019). Wheatgrass juice has 70 % of chlorophyll also called as green blood because of its similar chemical structure to hemoglobin, only central magnesium is present at porphyrin ring of chlorophyll instead of iron in hemoglobin (Padalia et al., 2010). Similarly, the treatment with wheatgrass is called as green blood therapy. Wheatgrass has chlorophyll, tannins, flavonoids, phytic acid, saponins, protein, crude fat, ascorbic acid, beta carotene, magnesium, calcium, iron, magnesium, selenium, zinc, copper, chromium and cobalt (Singh et al., 2012; Devi et al., 2019; Thakur et al., 2019). Harvesting of wheatgrass between seven and thirteen days after planting was suggested for high free radical scavenging activity (Devi et al., 2020).

There are several studies reporting wheatgrass use in some diseases such as cancer, oxidative stress, immunologic disorders, bone diseases, metabolic and cardiovascular diseases (Rana et al., 2011; Bar-Sela et al., 2015). Nephroprotective effect of wheatgrass juice was reported ethanol-induced oxidative damage in rats (Hebbani et al., 2020). It was reported that cereal grass juice had the wound healing potential (Karbarz et al., 2019). Oh et al., (2019) investigated that ethanolic bread wheat extract contributed to protection of liver damage in mice fed a choline deficient or high fat diet. Two novel compounds isolated from wheatgrass had the cytotoxic activities against some cancer cell lines (pancreatic, breast, colon, prostate and lung) (Save et al., 2019). It was suggested that wheatgrass might have had an important role in kids with thalassemia receiving chronic blood transfusion (Mutha et al., 2018). The healing effects of wheatgrass juice were presented in a study conducted with type 2 diabetic patients (Shakib et al., 2017). The protective role of wheatgrass on liver damage was investigated alcohol administered rats and heated polyunsaturated fatty acids (Durairaj et al., 2014).

Plant bioactive compounds also known as secondary metabolites are specific products of primary biological pathways and intermediates (Bourgaud et al., 2001). Plants and mushrooms have some bioactive compounds playing important role against biotic or abiotic environmental stresses (Dogan et al., 2018). Plants or plant cells adapt to physiological and morphological changes by producing bioactive compounds against biotic or abiotic stress factors (Isah, 2019). Additionally, plant bioactive substances can be used in pharmaceutical fields, food additives and other industries (Balandrin et al., 1985). Elicitors are used to manipulate to production of bioactive compounds presence in plants (Guerriero et al., 2018). Some chemicals such as calcium, silver nitrate, iron, magnesium, or some macromolecules such as proteins, carbohydrates and fatty acids or allelopathic relationships may have an elicitor effect (Bhatia and Bera, 2015). Chemical elicitors can be applied *in vitro* by creating callus in plant tissue culture or applied directly to plants (Dias et al., 2016). Salicylic acid, methyl salicylate, benzoic acid and chitosan are widely studied chemicals their effects on the production of phenolic acids and activity of defence enzymes (Patel and Krishnamurthy, 2013). Salicylic acid is a colourless and crystalline organic acid. Foliar spraying of common purslane (*Portulaca oleracea* L.) with different concentration with SA improved photosynthetic pigments, respiration and its bioactive content (Saheri et al., 2020). Exogenous SA treatment of wheat seeds or seedlings had protective role against different stresses (Fardus et al., 2018; Azeem et al., 2019; Loutfy et al., 2020).

In recent years, the popularity of wheatgrass is increasing as a functional food for human health. Studies about enrichment of food ingredients are also being hot topic in plant research. The aim of this study was to explore how pretreatment of wheat caryopses with different concentrations of SA affected germination rate, its antioxidant capacity (TAS and (TOS) and its vitamin C content. Additionally, another aim was to observe *in vitro* DNA protection role of lyophilized wheatgrass extracts against hydroxyl radicals generated by Fenton reaction.

Furthermore, we wondered how different concentrations of the salicylic acid imbibition of bread wheat caryopses affected to physiological parameters (length and fresh weight of shoot and root), biochemical parameters (proline, malondialdehyde, hydrogen peroxide, chlorophyll and soluble protein content) and antioxidant enzyme activities (catalase, ascorbate peroxidase and superoxide dismutase) of wheatgrass.

MATERIALS and METHOD

Plant Material

Bread wheat caryopses (*Triticum aestivum* L. 'Bezostaja-1') were used as a plant material. It was kindly provided from Transitional Zone Agricultural Research Institute, Eskişehir, Turkey.

Sterilization

Seeds were surface sterilized by 3 % of sodium hypochlorite for 5-10 minutes shaking at 200 rpm. After washing with sterile distilled water for several times, seeds were treated with 70% of ethanol for 1 minute. Washing with sterile distilled water was repeated.

Preparation of Salicylic Acid Concentrations

Different concentrations of salicylic acid (SA) were tested in this study. 10^{-2} M (SA-2), 10^{-4} M (SA-4), 10^{-6} M (SA-6), 10^{-8} M (SA-8), 10^{-10} M (SA-10) and 10^{-12} M (SA-12) salicylic acid were prepared from 1 M stock solution.

Seed Pretreatment with Salicylic Acid and Growth of Seedlings

Surface sterilized seeds were imbibed with different concentrations of SA for 2 hours. Control seeds were imbibed with sterile distilled water for 2 hours. Imbibed bread wheat caryopses were cultivated on hydroponic culture under controlled growth room (25±1 °C and 16/8 light and dark photoperiod). Hoagland's medium was used to grow wheat seedlings (Hoagland and Arnon, 1950). This study was conducted under semi-sterile conditions. Hoagland's medium was prepared from stock solutions and pH was adjusted between 5.7-5.8. After autoclaving, Fe-EDTA solution was added. Ten days old seedlings were harvested and stored ultra-freezer until analysis.

Wheatgrass Homogenization

Ten days old wheatgrass samples were homogenized with liquid nitrogen using mortar and pestle. Samples were stored at ultra-freezer. Homogenized wheatgrasses were used to determine malondialdehyde (MDA) content, proline content, hydrogen peroxide (H_2O_2) content, total antioxidant statue (TAS), total oxidant statue (TOS) oxidative stress index (OSI), vitamin C content and some antioxidant enzyme activities including ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD).

Germination Rate

Seeds were sown in petri dishes with wet two-layer filter paper for 3 days. Germination rate was calculated for SA treated and control wheat seeds according to formula given below.

Germination Rate (%) = (Germinated seeds / Cultivated Seeds) X 100

Determination of Antioxidant Capacity of Wheatgrass

The effects of salicylic acid pre-treatment of wheat seeds were evaluated for antioxidant potential of wheatgrass. Total antioxidant statue (TAS) and total oxidative statue (TOS) were measured and oxidative stress index (OSI) were calculated for this aim. Commercially available TAS and TOS kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used to determine TAS and TOS levels according to Erel (2004; 2005). TAS kit is based on reduction of dark-blue color of ABTS radical (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) to colorless form of ABTS. Extracted wheatgrass was homogenized with 140 mM potassium chloride buffer (1:10, w/v) to determine total antioxidant statue (TAS) and total oxidative statue (TOS). After vortex, samples were filtrated and centrifuged to collect supernatants. 18 µL of sample or standard (1 mmol/L Trolox) or water put into spectrophotometer cuvette. After that, 300 µL of reagent 1 (buffer solution, acetate buffer 0.4 mol/L, pH:5.8) was added into cuvette and mixed well. Absorbance 1 (Abs1) was spectrophotometrically measured at 660 nm. 45 µL of reagent 2 (pro chromogenic solution, ABTS 30 mmol/L) was put into cuvette and mixed well. Cuvette was incubated at room temperature for 10 minutes and absorbance 2 (Abs2) at 600 nm was measured. Results were presented as µmol Trolox equivalent g^{-1} FW. TAS level was calculated according to commercial kit guideline.

TOS test is based on oxidizing the ferrous ion chelator complex to ferric ion by oxidants in the sample. 45 µL of sample or standard (10 µL/L hydrogen peroxide) and 300 µL of reagent 1 (buffer solution, sulphuric acid 25mM pH:1.25) were mixed in a cuvette and measured at 530 nm for absorbance 1 (Abs1). 15 µL of reagent 2 (substrate solution, sulphuric acid 25 mM pH:1.75, ferrous ion 5mM, o-dianisidine 10nM) was added into cuvette and mixed well before incubation at room temperature for 10 minutes. After incubation, intensity of color change was spectrophotometrically measured as absorbance 2 (Abs 2) at 530 nm to determine total oxidant level in the sample or

standard. Results were presented as µmol hydrogen peroxide g^{-1} FW. TOS level and oxidative stress index (OSI) were calculated according to commercial kit guideline.

Determination of Vitamin C Content of Wheatgrass

Vitamin C content of shoot tissue was determined with colorimetric method using commercial kit protocol (E-BC-K034, Elabscience Biotechnology, USA). Samples were read at 536 nm and vitamin C content was calculated as µg g^{-1} FW.

DNA Protection Activity

Lyophilized extract preparation

3 g of shoot tissue ground by liquid nitrogen was extracted with 80 % of ethanol at a ratio 1:20. Samples were incubated in a shaker at 200 rpm for 2 hours. After that, samples were centrifuged at 3500 rpm for 10 minutes. Supernatant was collected and ethanol was evaporated. Water was evaporated by a freeze dryer. Extract yield was about 3.8 %. Lyophilized extract was used as 10 mg/mL final concentration after filter sterilization.

Bacterial growth

Escherichia coli having pUC19 plasmid was inoculated in Luria Broth (LB) medium. After overnight incubation at 37 °C, 100 µL of cultivation was spread onto nutrient agar medium. Ampicillin was added to bacterial growth media to select colonies with pUC19 plasmid having an ampicillin resistance gene. Plasmid isolation was performed using commercial plasmid isolation kit (K0502, Thermo Fisher Scientific). Plasmid was run in an agarose gel (1 %) to observe plasmid. Plasmid was digested with restriction enzymes for correction. Additionally, plasmid concentration and purity were determined by spectrophotometrically.

In vitro DNA damage protection assay

This assay is based on the principle that plasmid DNA is damaged by hydroxyl radicals formed by incubation of Fenton's solution at 37 °C and this damage is visualized on agarose gel (Lee et al., 2002; Locatelli et al., 2018). 10 mg/mL of lyophilized wheatgrass extracts obtained from bread wheat leaves grown from seeds imbibed with different concentrations of SA were tested for DNA damage protection. Fenton's solution (30 mM hydrogen peroxide, 50 mM ascorbic acid, 80 mM iron chloride) was used for *in vitro* hydroxyl radical source (Locatelli et al, 2018). Quercetin known as an antioxidant was used as a positive control as 50 µg/mL (Lee et al., 2002). 8 µL of pUC19 plasmid, 3 µL of lyophilized wheatgrass extract or quercetin and 9 µL of Fenton's solution were added into an eppendorf tube in order. 8 µL of pUC19 plasmid and 12 µL of nuclease free water were used as control. 8 µL of pUC19 plasmid, 9 µL of Fenton's solution and 3 µL of nuclease free water were used as negative control. Samples were run agarose gel (1 %) after incubation at 37 °C for 30 minutes and visualized with a transilluminator.

Physiological Parameters

The length of shoots and roots were measured with ruler. Also, fresh weight, dry weight and turgor weight were noted for shoot and root tissues. While shoots directly were weighted with analytical balance for fresh weight (FW), roots were rinsed with running water and were dried with tissue paper.

Biochemical Parameters

Malondialdehyde (MDA) content of shoots were determined. Shoot tissue was weighted and recorded. Tissue was extracted with 1 mL of 5 % trichloroacetic acid (TCA) in eppendorf tubes. Supernatants were collected after 12000 rpm centrifugation for 15 min. at room temperature. Equal amounts of supernatant and 0.5 % thiobarbituric acid (TBA) in 20 % TCA were added in a new tube. Tubes were incubated at 96°C for 25 min. in a hot block after brief vortex. After that, tubes were cooled into ice until they reached to room temperature. At the last step, tubes were centrifuged at 10000 rpm for 5 min. The absorbance of supernatant was read at 532 nm and 600 nm. 0.5 % TBA in 20 % TCA was used as blank (Ohkawa et al., 1979). The MDA content was calculated as nmol MDA g^{-1} FW.

The effect of SA imbibition of seed was evaluated for proline content. Shoot tissue was extracted wit 1 mL of 3 % sulphosalicylic acid. Extracts were transferred into eppendorf tubes and centrifuged at 14000 rpm for 5 min at 4°C. Acid ninhydrin (0.2 mL), acetic acid (0.2 mL), 0.1 mL sulphosalicylic acid and 0.1 mL of supernatant were added into a new tube in order. After briefly vortex, tubes were incubated at 96°C for 1 hour in a hot block. Toluene (1 mL) was added tubes to stop reaction at the end of the incubation. Vortexed tubes were centrifuged at 14000 rpm for 5 min at 4°C. Upper red phase was read at 520 nm absorbance against toluene (Bates et al., 1977). The proline content was calculated as µmol proline g^{-1} FW.

Shoot tissue (0.5 g) was homogenized with 100 mM K-PO_4 buffer (pH:6.8). After filtration, samples were centrifuged 18000 g at 4°C for 20 minutes to determine H_2O_2 content. Supernatant (0.25 mL) was put into a new tube and peroxidase solution (1.25 mL) including o-dianisidine (0.005 % (w/v)) and peroxidase (40 µg/mL) was added into tube. Tubes were incubated in a water bath at 30°C for 10 min. Lastly, 1N perchloric acid (0.25 mL) was added into to tubes to stop reaction. After centrifugation at 5000 g for 5

min., samples were read at 436 nm against blank which was peroxidase solution. The H_2O_2 content was calculated as nmol H_2O_2 g^{-1} FW (Bernt and Bergmeyer, 1974).

Total protein extraction was performed from ground shoot tissue. Shoot samples were extracted in 1 mL of extraction buffer (50 mM K-PO_4 including 1 mM EDTA and 2 % of PVP). Supernatants were collected after centrifugation at 13000 g for 20 min. at 4°C. Bradford method was used to determine protein concentration (Bradford 1976). Protein content of samples was calculated as mg protein g^{-1} FW. Total chlorophyll (Chl) content of wheatgrass was measured according to Lichtenthaler (1987).

Determination of Antioxidant Enzymes

Catalase activity was measured with spectrophotometer at 240 nm according to Aebi (1974). 900µL of assay solution (50 mM KH_2PO_4, pH 7.00), 100µg of crude protein and 100µL of 100 mM H_2O_2 were added into a quartz cuvette in order. Cuvette was read at 240 nm for 120 second with 10 second intervals. CAT activity of samples was calculated as U mg^{-1} protein.

Ascorbate peroxidase activity was measured according to Nakano and Asada method (1981). Quartz cuvette including (800µL of 50 mM K-PO4, pH 6.60, 100µL of 2.5 mM ascorbate, 100µg of crude protein and 100µL of 10 mM H_2O_2) was read at 290 nm for 120 second with 10 second intervals. APX activity of samples was calculated as U mg^{-1} protein.

Superoxide dismutase activity was measured with nitro blue tetrazolium (NBT) method (Govinda et al., 2017). Reaction mix was prepared with 2.9 mL of 50 mM K-PO_4 (pH: 7.80) buffer having 10 mM methionine, 168 µM NBT, 0.025 % Triton X-100 (w/v) and 1.17 µM riboflavin) and 0.1 mL of crude protein in tubes. Tubes were incubated with 15 watts fluorescent light for 15 minutes. Samples were read at 560 nm with a spectrophotometer. SOD activity of samples was calculated as U g^{-1} FW.

Statistical Analysis

Each experiment was performed at least three times. Results were presented as mean $\pm$ SEM and analyzed by one-way analysis of variance (ANOVA) using MINITAB package program. Statistically significance difference was accepted as $p < 0.05$.

RESULTS and DISCUSSION

Wheatgrass is one of the important superfoods which have protective role in human health because of its high antioxidant potential, chlorophyll content, amino acid content, vitamin and mineral content. Young wheat seedlings are used to produce wheatgrass juice or powder (Singh et al., 2012). The protective role of wheatgrass against some diseases such as cancer, diabetes, oxidative stress and thalassemia was investigated *in vitro* and *in vivo* studies (Bar-Sela et al., 2015). There are a lot of reports that SA causing increase of the secondary metabolites and antioxidant enzymes of wheatgrass had protective role in wheat plants under environmental stresses (Fardus et al, 2018; Azeem et al., 2019; Loutfy et al., 2020).

Germination Rate

While imbibition of bread seeds with SA-2 application prevented germination, germination rates of other SA concentrations were more than control (Fig. 1). However, only SA-12 application caused significantly increase (8.3 %) in germination rate compared to the control. The increase in germination rate was between 2.8 – 5.6 % for other concentrations (Fig. 1). Germination is the most important process for plants. SA priming of wheat seeds with different concentrations of SA for 12h promoted seed germination under salt stress (Azeem et al., 2019). Similarly, the positive effects of SA to germination of wheat seeds were reported under salinity stress (Fardus et al., 2018). Yanik et al., (2018) found that high concentrations of SA prevented seed germination of rye plant, while the lowest SA application promoting seed germination. We also found similar results to Yanik et al., (2018), the highest concentration of SA (SA-2) inhibited seed germination. On the other hand, the lowest SA concentration (SA-12) promoted seed germination (Fig. 1).

Antioxidant Potential

SA-8 application caused significantly increase (30.2 %) in TAS compared to the control (Fig. 2a). Additionally, we found that SA-8 application caused significantly increase in TAS compared to SA-4, SA-6 and SA-10 treatments. SA application did not cause increase in TOS (Fig. 2b). According to OSI results, SA-8 shoot tissue had the lowest OSI value and significantly lower (31.1 %) than control (Fig. 2c). Additionally, OSI of SA-8 treated wheatgrass was the importantly lower than SA-4 and SA-10 applications. Wheatgrass has high antioxidant potential because of its high content of antioxidants, vitamins (Vitamin E, C and A) and minerals (iron, magnesium and calcium) (Aydos et al., 2011). Kamat et al., (2000) demonstrated that chlorophyllin a derivative of chlorophyll had a protective effect on mitochondria against oxidative damage. Antioxidant activity of wheatgrass was not affected from different drying methods (Devi et al., 2019). Indoor growth of wheat young leaves for seven or ten days resulted with higher free radical scavenging activity than outdoor growth ones (Devi et al.,2020). Virdi et al., (2021) demonstrated that antioxidant potential of

wheatgrass was affected from different photoperiod regime and incubation media. Additionally, using spring water in cultivation of wheat seedlings resulted with increase in radical scavenging activity and phenolic content (Fortună et al., 2018). We found that SA-8 application caused significantly increase in TAS compared to the control, SA-4, SA-6 and SA-10 treatments. Additionally, SA did not cause increase in TOS. Moreover, OSI of SA-8 treated wheatgrass was the importantly lower than control, SA-4 and SA-10 (Figure 2c).

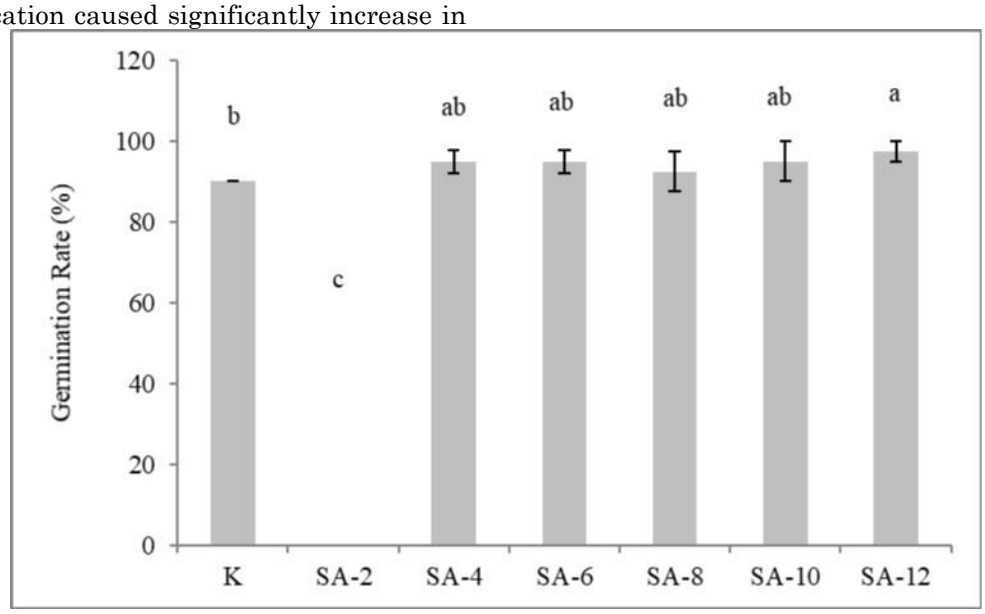

Figure 1. The effect of salicylic acid pre-treatment on germination rate. Different letters in the each column indicate significant difference (P<0.05).

Şekil 1. Salisilik asit uygulamasının çimlenme oranına etkisi. Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

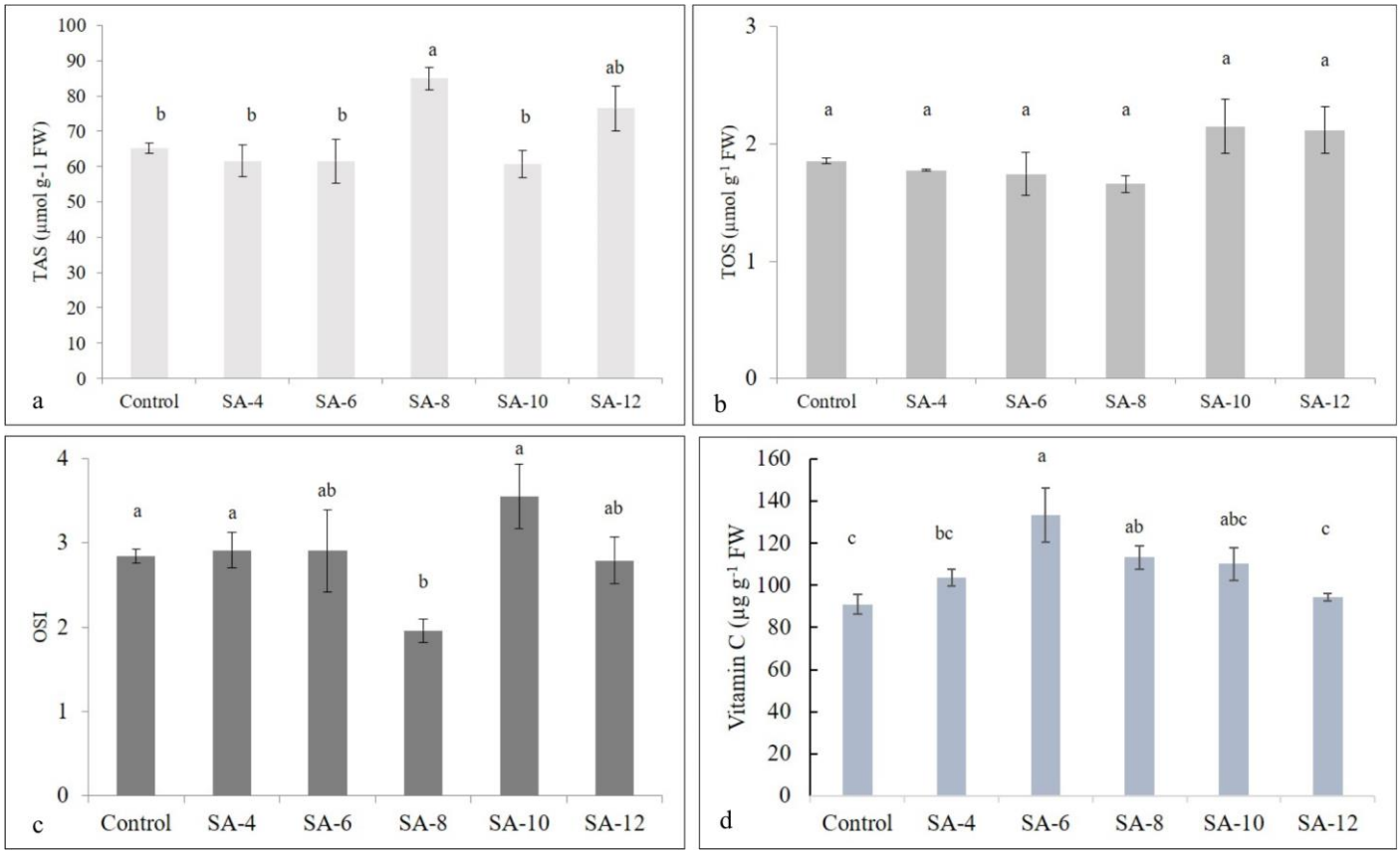

Figure 2. The effect of SA on TAS, TOS, OSI and vitamin C content. Different letters in the each column indicate significant difference (P<0.05).

Şekil 2. SAnın TAS, TOS, OSI ve C vitamini içeriğine etkisi. Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

The Effect of SA on Wheatgrass Vitamin C Content

We evaluated vitamin C content of shoot tissue of SA treated wheat seeds. According to results, the highest vitamin C content was measured at SA-6 treatment. SA-6 treated wheat shoot had significantly more (46.7 %) vitamin C content than control (Fig. 2d). Additionally, SA-8 treatment caused significantly increase (24.7 %) in vitamin C content compared to the control (Fig. 2d). While both of SA-6 and SA-8 applications had significantly more vitamin C content than SA-12, the vitamin C content of SA-6 treatment was higher than SA-4. Vitamin C (ascorbic acid) is one of the vitamins present in wheatgrass juice powder (Thakur et al., 2019). Drying methods affected ascorbic acid content of wheatgrass, especially oven drying method (Devi et al., 2019). Harvesting of wheatgrass in different days after sowing indoor and outdoor affected vitamin C content in wheatgrass, especially thirteenth day harvesting of indoor planted wheat seedlings was significantly higher than outdoor ones (Devi et al., 2020). Thakur et al., (2019) found high amount of vitamin C (9.3 mg/100 g) in wheatgrass juice powder. Spraying of SA caused increase in ascorbic acid content of wheat plants and protected plants from negative effects of fenoxaprop-p-ethyl herbicide (Yaman and Nalbantoğlu 2020). We found that SA caused increase in vitamin C content of the wheatgrass. Additionally, vitamin C content of SA-6 and SA-8 pretreated plants was significantly higher than control (Fig. 2d). The high level of vitamin C in SA-8 treated plants could be a reason for the highest TAS level in this application.

DNA Protective Role of Lyophilized Wheatgrass Extracts

Lyophilized ethanol extracts were prepared from the homogenized wheatgrass powder. The prepared extracts were dissolved at 10 mg/mL for DNA protection. The *pUC19* plasmid was used to investigate the DNA protective effect of lyophilized wheatgrass extract. After growing the bacteria with the plasmid overnight, plasmid isolation was made and its purity and concentration were determined (Fig. 3a-b). The purity of the isolated plasmid was measured as OD260/280 ratio between 1.8-2.1. The plasmid concentration was diluted to 62.5 ng/µL.

Plasmids are mostly in supercoil form and to a lesser extent in linear form. However, when exposed to stress conditions for a long time, the phosphodiester bonds in both chains are broken and converted to the nicked form. It was observed that DNA was in two different forms, namely supercoiled and linear form, in the well loaded only with plasmid. In order to determine the DNA protective effect, three different plasmid forms were observed in the wells containing plasmid DNA in which oxidative damage was induced in vitro by generating hydroxyl radical by Fenton reaction. In addition to the natural forms of the plasmid, it was observed that nicked plasmid forms were formed by the effect of hydroxyl radicals.

When the intensity of the bands was observed, it was observed that while the brightness of the linear form decreased in the well containing only the plasmid, especially in the wells containing only Fenton reaction (Well 2) and quercetin for control (Well 3), the nicked form was formed. In addition, although the nicked plasmid DNA form was formed in the wheatgrass extracts, it was observed that the nicked form band density was less than the well 2 and well 3 samples (Fig. 3c). However, no difference was observed in the band density of the wheatgrass extracts. Based on these observations, it appears that wheatgrass extracts have a DNA protective effect by reducing the nicked form formation of plasmid pUC19.

DNA protective effects of methanolic extracts of ten medicinal plants belonging to six families were investigated and it was reported that they had protective activity related with their phenolic and flavonoids content (Kumar et al., 2010). In a study investigating the antioxidant properties ethanolic extract of the stem of the *Opuntiaficus indica* var. Saboten, it was reported that the extract protected plasmid DNA from the harmful effects of hydroxyl radicals (Lee et al., 2002). It was investigated that the *Polyalthia longifolia* leaf extract strongly inhibited hydrogen peroxide induced DNA damage (Jothy et al., 2013). It was found that water-methanol extract of shade dried tuber of *Eulophia nuda* Lindl. had the highest protective role against DNA damage caused by free radicals (Kumar et al., 2013). It was found that lyophilized ethanol extracts (10 mg/mL) of the wheatgrass prevented damage on the pUC19 plasmid from the hydroxyl radicals formed by Fenton reaction because of its high content of antioxidants and vitamins.

Physiological Parameters

The effects of SA imbibition of bread seeds on shoot and root length and FW were evaluated. The highest shoot length was measured at SA-6 application (23.040 cm), however not significant than control (22.413 cm) (Table 1). SA-6 application caused significant increase in shoot length compared to SA-12 application (21.100 cm). SA application affected significantly root length of bread wheat. SA-10 application had significantly longer root length according to other applications. The lowest root length was measured at control. SA-4, SA-6 and SA-8 applications caused significantly longer roots than control (Table 1). There was no significant increase in

shoot FW. The highest root FW was weighted at SA-8 application. Additionally, SA-8 application caused significantly weightier at root tissue compared to control, SA-4, SA-6 and SA-10 applications (Table 1). Moreover, SA-10 and SA-12 root tissues had importantly root weight than control, SA-4 and SA-6 roots. Saheri et al., (2020) found that foliar spraying of SA of common purslane plants under drought stress resulted with increase in length, fresh weight and dry weight. Foliar spraying of SA caused increase in shoot and root length and shoot fresh and dry weight of wheat seedlings in greenhouse (Behnam et al., 2018). It was reported that shoot and root fresh mass of 15 days old wheat plants growth from seeds soaked with SA was weightier than control (Loutfy et al., 2020). According to our results, while SA application caused significant increase in root length compared to control, it did not affect significantly in shoot length according to control plants. SA-8, SA-10 and SA-12 treatments caused importantly increase in root fresh weight according to control (Table1).

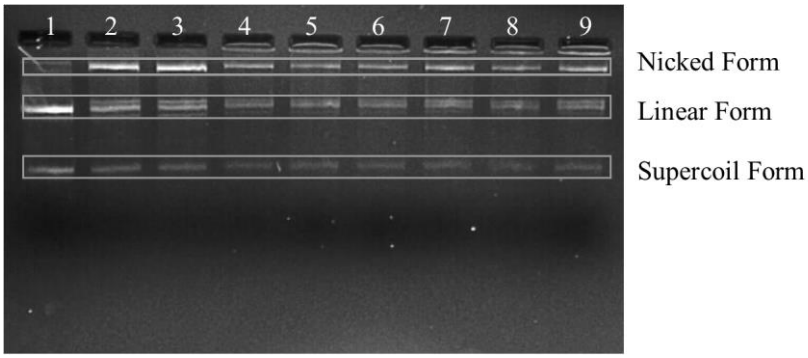

Figure 3. DNA protective activity of wheatgrass extracts. Well-1: Control (Plasmid), Well-2: Fenton solution (FS)+Plasmid, Well-3: FS+Plasmid+Quercetin (50 µg/mL), Well-4: FS+Plasmid+Control wheat grass extract (WGE), Well-5: FS+Plasmid+SA-4 WGE, Well-6: FS+Plasmid+SA-6 WGE, Well-7: FS+Plasmid+SA-8 WGE, Well-8: FS+Plasmid+SA-10 WGE, Well-9: FS+Plasmid+SA-12 WGE.

Şekil 3. Buğday çimi ekstrelerinin DNA koruyucu etkisi. Kuyucuk-1: Kontrol (Plazmit), Kuyucuk-2: Fenton solüsyonu (FS)+Plazmit, Kuyucuk-3: FS+Plazmit+Kersetin (50 µg/mL), Kuyucuk-4: FS+Plazmit+Kontrol buğday çimi ekstresi (BÇE), Kuyucuk-5: FS+Plazmit+SA-4 BÇE, Kuyucuk-6: FS+Plazmit+SA-6 BÇE, Kuyucuk-7: FS+Plazmit+SA-8 BÇE, Kuyucuk-8: FS+Plazmit+SA-10 BÇE, Kuyucuk-9: FS+Plazmit+SA-12 BÇE.

Table 1. The effects of SA pretreatment of bread wheat caryopses on some physiological parameters.
Tablo 1. Ekmeklik buğday karyopsis meyvelerinin SA ön uygulamasının bazı fizyolojik parametrelere etkileri.

	Shoot		Root	
Treatment	Length (cm)	FW (g)	Length (cm)	FW (g)
Control	22.413±0.336^{ab}	0.268±0.008^{a}	15.707±0.194^{d}	0.162±0.006^{c}
SA-4	21.827±0.531^{ab}	0.285±0.009^{a}	16.840±0.178^{b}	0.164±0.007^{c}
SA-6	23.040±0.546^{a}	0.280±0.006^{a}	16.387±0.165^{bc}	0.169±0.006^{c}
SA-8	22.040±0.545^{ab}	0.284±0.006^{a}	16.633±0.153^{b}	0.223±0.009^{a}
SA-10	21.767±0.836$_{ab}$	0.262±0.011^{a}	17.607±0.210^{a}	0.192±0.006^{b}
SA-12	21.100±0.750^{b}	0.264±0.011^{a}	15.993±0.225^{cd}	0.223±0.011^{ab}

Different letters in the each column indicate significant difference (P<0.05).
Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

Biochemical Parameters

The highest MDA content was determined at control. While we calculated decrease in MDA level of SA applications compared to control, there was no significant decrease. The highest proline content was at SA-8 treatment, however not significant (Table 2). The highest proline content of wheat shoot tissue was at SA-8 treatment however not significantly different from other treatments and control (Table 2). The highest hydrogen peroxide contents were at SA-12 and SA-8 applications and significantly higher than SA-4, SA-6 and SA-10. However, they were not significantly different compared to control and SA-12 (Table 2). According to results of Saheri et al., (2020), foliar spraying of SA caused increase in proline content and decrease in MDA level and hydrogen peroxide content of common purslane under drought stress. While pretreatment of *Artemisia aucheri* BOISS with SA did not affect proline content of plants under normal conditions, proline content of plants under osmotic stress was higher than control (Abbaspour and Ehsanpour, 2020). Loutfy et al., (2020) demonstrated that SA pretreatment of wheat seeds caused decrease in proline content of

wheatgrass. It was reported that different concentration of SA caused decrease in MDA level and fluctuations of hydrogen peroxide content in foliar sprayed wheat seedlings (Alsahli et al., 2019). In the same study, proline content was not affected from spraying of SA. In our study, SA pretreatment did not significantly affect the MDA, proline and H_2O_2 content compared to control. However, pretreatment of SA-8 and SA-12 caused increase in H_2O_2 content compared to other SA treatments (Table2). Results found in this study for MDA, proline and H_2O_2 content are similar to previous studies.

SA applications did not cause significantly change in total Chl content. Plants are affected environmental changes and media composition. It was reported that different photoperiod and media caused change in wheatgrass height (Virdi et al., 2021). In the same study, it was reported that indoor and outdoor growth of wheatgrass caused significantly change in chlorophyll content and outdoor growth wheat seedlings had significantly more chlorophyll than indoor ones. Additionally, different cultivation conditions affected Chl content of wheatgrass (Fortună et al., 2018).

Table 2. Some biochemical parameters and antioxidant enzyme activities of wheatgrass growth from bread wheat caryopses pretreated with different concentrations of SA.

Tablo 2. Farklı SA konsantrasyonları ile ön uygulama yapılmış ekmeklik buğday karyopsis meyvelerinden büyümüş buğday çiminin bazı biyokimyasal parametreleri ve antioksidan enzim aktiviteleri.

	Control	SA-4	SA-6	SA-8	SA-10	SA-12
Biochemical Parameters						
MDA	25.76±4.36^{a}	19.40±1.41^{a}	19.81±1.37^{a}	17.13±2.86^{a}	22.56±1.96^{a}	17.69±2.41^{a}
Pro	6.69±0.40^{a}	7.04±0.20^{a}	7.40±0.40^{a}	7.28±0.10^{a}	6.93±0.24^{a}	6.86±0.38^{a}
H_2O_2	157.20±5.89^{ab}	149.74±2.36^{b}	149.13±2.51^{b}	167.86±5.13^{a}	149.67±1.58^{b}	169.39±1.06^{a}
SPC	9.15±0.34^{c}	14.33±1.06^{ab}	13.03±0.47^{b}	13.88±0.57^{ab}	13.86±0.33^{b}	15.16±0.18^{a}
Chl	358.68±19.62^{a}	363.55±29.24^{a}	353.44±37.00	364.88±41.54^{a}	346.35±30.00^{a}	356.16±27.66^{a}
Antioxidant Enzyme Activities						
CAT	226.90±10.90^{b}	252.79±13.19^{ab}	271.07±11.74^{a}	263.20±28.77^{ab}	254.32±12.97^{a}	257.61±15.01^{ab}
APX	552.50±45.53^{ab}	642.86±37.64^{ab}	583.21±36.82^{ab}	638.21±11.02^{ab}	648.57±8.98^{a}	573.21±27.04^{b}
SOD	100.01±9.53^{a}	96.15±4.89^{a}	99.94±8.16^{a}	95.56±7.21^{a}	90.17±8.65^{a}	90.24±5.91^{a}

Different letters in the each row indicate significant difference (P<0.05).
Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

Seed imbibition with SA caused significantly increase in soluble protein content (SPC) of bread wheat shoot tissue. The highest soluble protein content was at SA-12 treatment (Table 2). Wheatgrass has high amount of some amino acids such as histidine, glutamic acid, threonine, arginine and leucine (Ghumman et al., 2017). Different growth conditions affected protein content of wheatgrass (Devi et al., 2020). Additionally, photoperiod, media composition and genotype affected protein content in wheatgrass shoot powder, especially photoperiod (Virdi et al., 2021).

We investigated that the effect of SA on some antioxidant enzymes (CAT, APX and SOD). While SA application did not affect SOD enzyme activity, SA caused significantly changes on APX and CAT enzyme activities (Table 2). According to Table 2, there was increase in CAT activities of SA treatments compared to control. However, SA-6 and SA-10 treatments had significantly more CAT activity than control. The highest APX activity was at SA-10 treatment however only importantly higher than SA-12 treatment. Additionally, APX activities of other SA treatments were higher than control but not significant (Table 2). Plant antioxidant defense enzymes CAT, APX and SOD are important enzymes to adapt environmental changes. In this study, we investigated that how SA pretreatment affected CAT, APX and SOD enzyme activities. SOD enzyme presence in wheatgrass reduces negative effects of radiations and toxins (Cao et al., 1996; Bar-Sela et al., 2007). According to Alsahli et al., (2019), foliar spraying of wheat seedling with different concentration of SA resulted with increase in CAT and APX enzyme activities while it did not affect SOD activity. We found similar results in our study. Seed imbibition with SA resulted with fluctuations in APX and CAT enzyme activities (Table2). However, we did not find increase in SOD activity.

CONCLUSION

We concluded that the highest concentration of SA prevented bread wheat germination. Imbibition of wheat seeds with SA resulted with increase in TAS level and vitamin C content of wheatgrass. Additionally, lyophilized wheatgrass extract had a protective role against DNA damage induced by hydroxyl radicals. Additionally, SA caused increase in root length and FW compared to control. Moreover, we determined increase in APX and CAT antioxidant enzyme activities according to control. SA pretreatment seeds before planting could be used to enrich antioxidant potential and vitamin C content of plants.

ACKNOWLEDGEMENTS

We would like to thank to Transitional Zone

Agricultural Research Center, Eskişehir, Turkey for seed providing. Additionally, we would like to Van Yüzüncü Yıl University Scientific Research Projects Department for its financial support (Grant number FYL-2019-8007).

Researchers Contribution Rate Declaration Summary

AB contributed to the study conception and design. Material preparation, data collection and analysis were performed by SD and AB. The first draft of the manuscript was written by SD and AB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest.

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