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Strigolactone and Auxin Applications on Cotyledon Senescence in Sunflower Seedlings under Salt Stress

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ABSTRACT

Objective: Senescence is a programmed cell death process and is important in the growth, development and flowering process of the plant. Delaying senescence has a very important effect on agriculture in terms of product yield. Indole-3-acetic acid (IAA) and strigolactone (GR24) are growth regulators that affect plant development and senescence. Salt stress accelerates the senescence process. The aim of this study is to increase crop yield by delaying senescence by the application of auxin and GR24 under stress conditions, because the delay of senescence causes the prolongation of the vegetative process and the formation of more apical tips. In this case, the seedling produces more fruit.

Materials and Methods: In this study, senescent cotyledons of sunflower seedlings were used as experimental material. Half of the developing sunflower seedlings were irrigated with Hoagland solution, and the other half was irrigated with 150 mM sodium chloride (NaCl) solution. IAA and GR24 were applied by spraying on seedlings that are grown both in Hoagland solution and under salt stress.

Results: The degree of senescence of the cotyledons of the plants was determined in terms of the percentage of green area. When the green area percentage of cotyledons of seedlings grown in Hoagland solution was 50, all cotyledons were harvested. After that, fresh weight, pigment contents, total protein, malondialdehyde, and proline levels, peroxidase enzyme activities of cotyledons were determined. The application of IAA and GR24 to the cotyledons of seedlings grown in the salt medium significantly delayed the senescence.

Conclusion: This study was conducted in the plant growth chamber under controlled conditions. Results showed that the application of IAA and GR24 to leaves can ameliorate the adverse effects of salt stress and delay senescence due to the activation of chlorophyll components and modulation of photosynthesis as well as antioxidant defense capacity. The effect of IAA is more precise when all analyzes are considered. More importantly, showed that all findings (except MDA and Proline) IAA and GR24 promote senescence in Hoagland in this research. Delaying senescence contributes to basic science. It is desired to increase fruit and vegetable yield by delaying senescence. It is suggested that this information can be used practically in the field of agriculture. On the other hand in this study, we can say that IAA and relatively GR24 can play an important role in the protection of plants in agricultural areas in salt stress.

Keywords: Green area percentage, *Helianthus annuus* L., Hoagland, NaCl, Plant growth substance, Senescence

INTRODUCTION

Plants need optimum conditions for growth. When plants are suddenly exposed to an unexpected condition, their development and lifespan are affected (1). The factors that create all these conditions are defined as "stress". Stress conditions affecting plants include biotic conditions are created by living things such as animals, plants, and microorganisms, and abiotic conditions are created by environmental conditions such as water, minerals, gases, temperature, and radiation (2). Increasing land areas rich in salt (NaCl) poses a serious threat to plants and ecosystems, agriculture is endangered, and agricultural products are restricted with population increase.

Senescence is an important active process involving serious catabolic changes in gene expression and hor-

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monal signaling (3). Senescence is an indicator characterized by the yellowing of cotyledons and leaves, and multiple signals, such as various plant hormones can regulate this process. Senescence seen at the level of the whole organism causes the death of the plant in the last stage of plant ontogenetic development. Senescence, the last stage of plant development, has an important impact, especially in agriculture. The senescence process speeds up in seedlings exposed to salt stress. Delaying senescence causes the growth period to be extended in an organized manner, thus increasing the product yield (4).

Endogenous factors such as phytohormone levels, high-order epigenetic mechanisms, and expression of specific environment-dependent genes and environmental factors such as day length, drought, freezing, and insufficient light cause senescence. Plant hormones are metabolic regulators that control plant growth, development and many more processes. Hormones regulate plant growth and provide the necessary energy in plant life by affecting the metabolism.

Strigolactones (SLs) have recently been identified as new phytohormones that regulate plant growth and development by influencing plant metabolism (5). Strigolactones are derived from carotenoids and are involved in seed germination, photomorphogenesis, regulation of plant morphology (inhibition of bud growth and shoot branching) and physiological reactions to abiotic factors. SLs are synthesized in roots and stems and transported through the xylem (6). Although the effect of strigolactones in regulating leaf senescence is known (7), information on the molecular mechanism of the action is still insufficient. SL deficient or SL insensitive mutants show senescence delay (8). Some studies have shown that leaf senescence is accelerated by strigolactone (GR24). To investigate these effects, GR24 was applied to the leaves of *Arabidopsis* and rice (9,10). Exogenous application of GR24 increased leaf senescence in both *Arabidopsis* and SL-deficient mutants of rice (11,12). Auxin is an important hormone for plant growth and development. IAA can coordinate growth under stressful conditions by managing the plant's response to stress (13). The senescence delaying effect of auxin has been reported for many years in scientific studies (14). When the sources of auxins are examined, it is seen that the role of this phytohormone in regulating senescence by auxin is complex. Auxins and SLs link with each other in the feedback loop (15). Auxins play an important role in strigolactone biosynthesis through gene expression and are involved in developmental processes with strigolactone (16,17). Strigolactones promote auxin transport in seedling stems (18). It has been noted that SLs affect and regulate auxin pathways by facilitating auxin transport and stimulating transcription of the auxin receptor TIR1 (19).

In summary, the aim of this study was to investigate the effects of indole-3-acetic acid and strigolactone on cotyledon senescence in sunflower seedlings due to salt stress. At the end of this study, it was determined that the application of IAA and GR24 played a beneficial role in accelerating the salt-induced senescence process in the sunflower.

MATERIALS AND METHODS

Plant Material, Growth Conditions and Treatments

Seedlings of sunflower [*Helianthus annuus* L. (Tar-San 1018 TR.00.00.1024.0265)] were grown in a plant growth chamber (6000-lux light intensity, 16 h light, 8 h dark photoperiod and 25±2°C, 59% humidity). 10^{-5} M IAA (Sigma-Aldrich) and 10^{-8} M GR24 (rac-GR24 from Chiralix) were applied to the seedlings grown in Hoagland solution for Control groups and Hoagland+ 150 mM NaCl (NaCl-CAS No: 7647-14-5) solution for salt stress groups. The IAA concentration used in this study was obtained from the results of our previous studies. The GR24 concentration was determined after several applications based on the article information. The cotyledons of all plants were harvested on the day when the cotyledons of the seedlings in the salt group causing early senescence were 50%.

Determination of Senescence Degree

The degree of senescence in the cotyledons of plants was determined by a method developed by Lindoo and Noodén (20) for soybeans and then successfully used by modification. According to this method, the senescence degree of cotyledons of the sunflower plant was determined considering the green area percentage of cotyledons.

Determination of Total Chlorophyll and Carotenoid Amount

Chlorophyll and carotenoid amounts of the extracts obtained from fresh samples were determined according to Parsons and Strickland's method (21). The Elisa reader device was used in all experiments in which absorbance values were measured.

Determination of Soluble Total Protein Amount

The determination of soluble total protein amount was made by the Bradford method (22) in which homogenates were taken into Eppendorf tubes and centrifuged at +4 °C for 30 min at 13,000 x g. The samples were measured at 595 nm wavelength, and the protein amount calculations were made by comparing them with the previously prepared BSA standard.

Determination of Peroxidase (POD) Activity

In order to determine the differences in POD activity in sunflower cotyledons, Birecka et al.'s method (23) was used. The samples, which were homogenized with phosphate buffer (pH: 7), were centrifuged for 30 min at 13,000 x g. Phosphate buffer at pH: 5.8 was used for measurement. After all procedures were completed, it was placed in the device for measurement, and the kinetic absorbance was measured with a total of 13 measurements, 10 sec apart for 2 min.

Malondialdehyde (MDA) Determination

MDA measurement was made according to the Heath and Packer method (24). The prepared extracts were centrifuged at 10,000 x g for 15 min. The supernatants taken were incubated in a 96˚C water bath for 30 min, then taken into the refrigerator and cooled to terminate the reaction, then centrifuged at 10,000 x g for 10 min. After centrifugation, samples were measured at 532 nm and 600 nm.

Proline Determination

Proline measurement was made according to the Bates et al. method (25). The extracts were obtained at 12,000 x g for 7 min.

After centrifugation, necessary materials were added to the supernatant and left for 1 h to be incubated in a 98˚C water bath. Later, after the procedure was applied to the samples that were cooled in the refrigerator, 518 nm absorbance value was measured with an Elisa reader.

Statistical Analysis

Each treatment included six replicates and each experiment was carried out at least five times. The data obtained as a result of the analysis were analyzed by one-way analysis of variance (ANOVA) included in the Statistical Package for Social Sciences (SPSS for Windows 10.0) package program. The differences between the means were determined as significant with $p < 0.05$ according to Duncan's new multiple range test.

RESULTS

Change in Average Green Area (A.G.A.) Percentage of the Cotyledons

The cotyledons of the seedlings grown in Hoagland and salt solutions and treated with IAA and GR24 were observed. The A.G.A. percentages of cotyledons were determined at regular intervals for 30 days. While the cotyledons of all groups were 100% green, the senescence process started from the 19th day. The differences between the Hoagland and salt groups increased day by day. Most of the cotyledons of the seedlings treated with IAA completed the senescence process on the 30th day. IAA caused faster senescence than GR24. While cotyledons in the salt medium experienced rapid senescence, the application of GR24 slowed down the senescence process a little but accelerated it in the next process. On the other hand, IAA had a retarding effect on the senescence and healing effect on salt toxicity under NaCl stress.

Analyses of the harvested cotyledons were carried out when the average percentage of the green area of the Hoagland group was approximately 50% (Figure 1).

Changes in Total Chlorophyll and Carotenoid Amount

In the Hoagland solution, the chlorophyll content in cotyledons of the seedlings treated with IAA and GR24 decreased by 50% and 48%, and the carotenoid content decreased by 68% and 50%, respectively. On the other hand, the amount of chlorophyll in seedlings grown in the salt medium increased by 185% and 178%, and the carotenoid content by 99% and 36%, respectively, with the application of IAA and GR24 (Table 1). This indicates that IAA and GR24 in Hoagland's solution encourage senescence but delay senescence in salt stress.

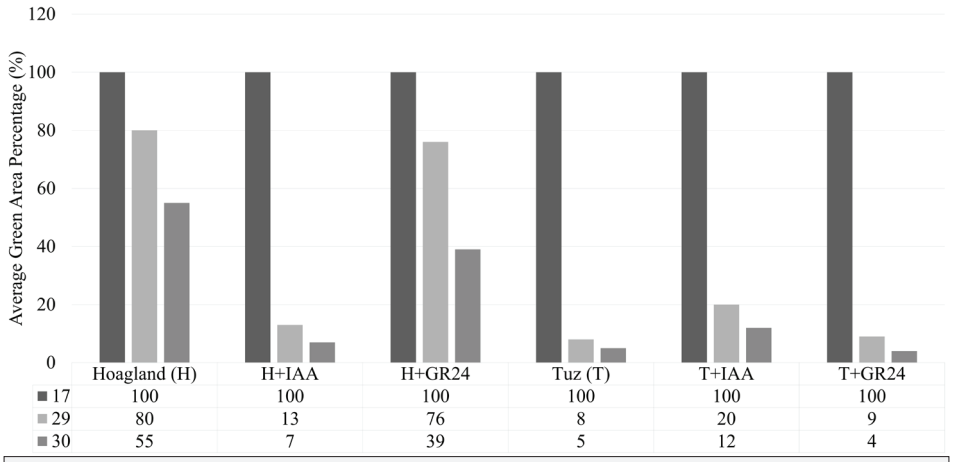

Figure 1. Average Green Area (A.G.A.) percentage of senescence occurring in cotyledons of *Helianthus annuus* L. (sunflower) seedlings grown in Hoagland solution and salt solution for 30 days.

Table 1. Comparison of total chlorophyll ($p < 0.05$) and carotenoid ($p < 0.05$) amount in cotyledons of *Helianthus annuus* L. seedlings grown in Hoagland and salt medium and treated with 10^{-5} M IAA and 10^{-8} M strigolactone (GR24) at approximately 50% senescence point of Hoagland group.

Treatment Groups	Total Chlorophyll Amount (µg/g fr wt)	Carotenoid Amount (µg/g fr wt)
Hoagland	126.2129±2.1157[a]	0.8570±0.0270[a]
Hoagland+IAA	64.1296±3.5607[b]	0.2746±0.0378[b]
Hoagland+GR24	66.4460±0.7634[b]	0.4282±0.0338[c]
Hoagland+NaCl	64.6257±1.6693[b]	0.5799±0.0429[d]
Hoagland+NaCl+IAA	120.0975±5.5115[c]	1.1546±0.0252[e]
Hoagland+NaCl+GR24	116.7482±5.3101[d]	0.7866±0.0485[f]

Data are means ± standard deviations (SD) of least five independent experiments with six replicates. Different letters indicate values that differ significantly from the control and strigolactone-indoleacetic acid treatments, respectively at $P<0.05$ according to Duncan's new multiple range test.

Changes in the Amount of Soluble Total Protein

While the total amount of protein in the cotyledons of the seedlings treated with 10^{-5} M IAA decreased by 41% compared to the cotyledons of the Hoagland group, it was determined that there was no difference in the 10^{-8} M GR24 application (Figure 2). As a result, it was found that IAA application decreased the amount of protein due to early senescence in cotyledons and increased salt stress with a healing effect.

Changes in POD Activity

POD activity in cotyledons of seedlings grown in Hoagland solution increased 2.1 times with IAA application and 1.4 times with GR24 application (Table 2). The POD activity in cotyledons of seedlings exposed to salt stress increased 2.2 times when compared to Hoagland group cotyledons. In addition, it was determined that IAA and GR24 application increased POD activity by 31% in cotyledons of seedlings grown in Hoagland solution compared to cotyledons of seedlings in salt.

Changes in the MDA Ratio

Compared to cotyledons harvested at 50% senescence of seedlings grown in Hoagland solution, MDA content in cotyledons of seedlings treated with IAA and GR24 decreased approximately 1.8 times and 1.16 times, respectively (Table 2). The amount of MDA in cotyledons of seedlings grown in salt solution decreased by 1.6 and 1.2 times, respectively, with IAA and GR24 application.

Changes in the Proline Rate

Proline in cotyledons of the seedlings treated with IAA and GR24 reduced by 70% and 10% in Hoagland solution (Table 2). In cotyledons of seedlings grown in the salt solution, the amount of proline decreased by 47% and 28% with IAA and GR24 application, respectively.

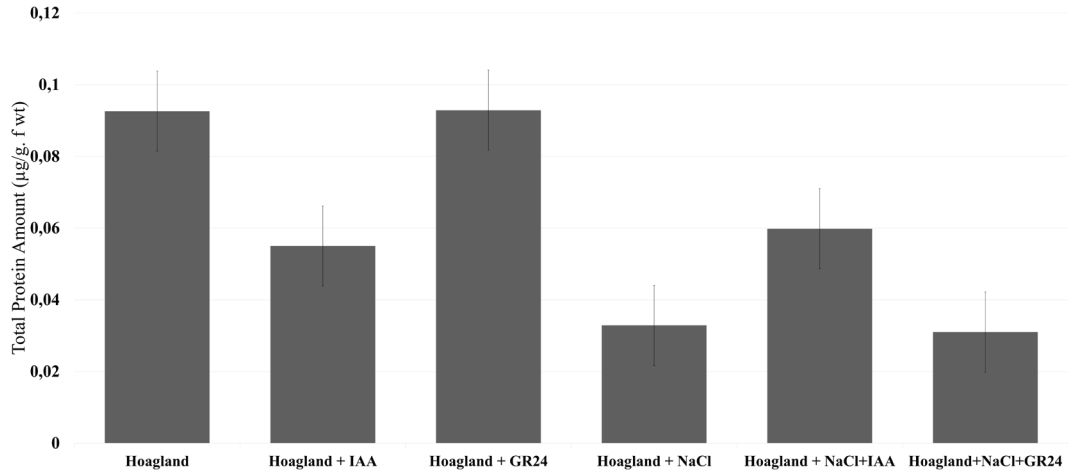

Figure 2. Comparison of the total protein amount in cotyledons of *Helianthus annuus* L. seedlings grown in Hoagland and salt medium and treated with 10^{-5} M IAA and 10^{-8} M GR24 at 50% senescence time ($p < 0.05$).

Table 2. Comparison of peroxidase (POD) enzyme activity, the amount of MDA and proline in cotyledons of *Helianthus annuus* L. seedlings grown in Hoagland and salt medium and treated with 10^{-5} M IAA and 10^{-8} M GR24 at 50% senescence time of Hoagland group ($p < 0.05$).

Treatment Groups	POD Activity (ΔA/g fr wt. min)	MDA Amount (µmol/g. f wt)	Proline Amount (µmol/g. f wt)
Hoagland	0.0547±0.0039[a]	12.9301±0.7366[a]	33.1518±4.6258[a]
Hoagland+IAA	0.1165±0.0059[b]	7.1640±0.8871[b]	9.9066±1.0937[b]
Hoagland+GR24	0.0773±0.0069[c]	11.1290±0.9140[c]	29.8773±2.3791[c]
Hoagland+NaCl	0.1227±0.0069[d]	39.7446±1.3817[d]	165.9861±7.0719[d]
Hoagland+NaCl+IAA	0.0806±0.0045[e]	24.9651±1.6667[e]	88.0193±6.3961[e]
Hoagland+NaCl+GR24	0.0629±0.0005[a]	34.2204±2.5806[f]	119.0541±3.1777[f]

Data are means ± standard deviations (SD) of least five independent experiment with six replicates. Different letters indicate values that differ significantly from the control and strigolactone-indoleacetic acid treatments, respectively at $P<0.05$ according to Duncan's new multiple range test.

DISCUSSION

The role of auxins in the senescence process has been discussed for a long time. While some researchers state that auxins delay senescence (26,27), some recent studies have shown that auxins accelerate senescence. Noh and Amasino (28) found a link between auxin gene expression and senescence. In this study, IAA and a synthetic strigolactone analog GR24 were sprayed to the foliage to evaluate the response of sunflower to the application of GR24 with IAA in optimum and salt conditions. According to the findings, the fact that it encourages senescence in the presence of IAA in the Hoagland medium is in line with the previous findings. Zn is known to provide IAA stabilization (29,30). Some researchers found that auxin was effective in the speed of the senescence process by establishing a link between Zn and IAA (31). In their study, Saglam-Cag and Okatan (31) applied C^{14} to the apical end, preventing IAA from reaching cotyledons, and found that senescence does not occur in these cotyledons. Technological advances in plant molecular biology will help unravel the mystery between auxin and senescence. In recent research on senescence and auxin-related genes, information about current molecular regulatory senescence patterns has been obtained (27).

In this study, parallel to the acceleration of the senescence observed in cotyledons of the seedlings grown in Hoagland solution and treated with IAA, a decrease in fresh weight, chlorophyll, carotenoid contents, total protein amount, MDA and proline, and an increase in peroxidase activity were detected. Our results show that IAA practice increases senescence in Hoagland solution. Similarly, Hou et al. (26) stated that auxin increases the expression of the SAUR 36 gene, which is a positive regulator of the senescence process. Moreover, some researchers stated that auxin measurements in senescing leaves have shown that the abundance of free, bioactive IAA increased two fold, which correlates with an increased expression of key enzymes involved in IAA biosynthesis during age-dependent leaf senescence (32). Looking at the average green area percentage, the increase in the senescence rate observed in the cotyledons of the seedlings grown in Hoagland solution and treated with GR24 also decreased in fresh weight, chlorophyll, and carotenoid contents similar to the IAA effect. In recent years, it has been reported that SL accelerates leaf senescence, which is also regulated by sugar signals and ethylene (3,33,34). On the other hand, there are studies that GR24 delays senescence (35). It was reported that sugar suppresses SL-induced leaf senescence in the dark. But there was no difference in the total protein amount compared to the control of the untreated seedlings. This difference may have affected the transcription stability of the enzyme proteins that controlled the anabolic and catabolic degradation processes in those days of the application of GR24 to the cotyledons of the seedlings grown in the Hoagland solution. Since senescence occurred earlier and faster during IAA application, a significant difference was observed in the amount of protein. The fact that IAA and GR24 applications also cause an increase in peroxidase activity indicates that the application of IAA and GR24 promotes senescence. Proline in cotyledons treated with IAA and GR24 was reduced by 70% and 10% in Hoagland solution (Table 2). According to this result, it is obvious that IAA and GR24 promote senescence. These results are parallel to some sources (32). Indeed, it is known that proline is also protective under normal conditions and is important in preventing cell death. It is known that chlorophyll and total soluble proteins are degraded and proteolytic activity is increased during the senescence process. Most findings in this study indicate that IAA promotes senescence**.** However, it has been stated that proline accumulation in barley plays no role in salinity tolerance, but rather represents a sensitivity symptom (36). On the other hand, in this research, MDA and proline contents were found to be high in rapidly senescent cotyledons under salt conditions (Table 2). Indeed, MDA activity reaches high levels in the NaCl environment, and this is a sign that the cell membrane is damaged by the effect of salinity (37). MDA, which increased as a result of the damage of salt, decreased with the application of IAA to the seedlings. This result suggests that IAA plays a curative role in salt stress by following a different path. In recent years, many researchers have stated that IAA applications to seedlings can be effective in developing resistance to salt stress (38,39). However, there is limited information about the ameliorating role of IAA in salt stress. Some researchers (40,41) have determined that synthetic GR24 treated plants showed higher MDA content by different stress when compared to the control.

In our study, GR24 application under salt stress conditions decreased the amount of MDA, proline and activity of POD (Table 2), whereas increased the content of chlorophyll and carotenoids (Table 1). This result may suggest that GR24 delays senescence and exerts a curative effect. The same researchers found higher chlorophyll content and photosynthesis rate in synthetic GR24 treated plants under drought stress. Lu et al. (42) found low MDA content and POD activity in low light stress.

To summarize, senescence occurs earlier in seedlings grown under abiotic stress conditions. It has been shown that in seedlings exposed to stress conditions, auxin may at least partially participate in the positive regulation of stress resistance by affecting the expression of abscisic acid response genes and reactive oxygen species metabolism (43). As a matter of fact, in this study, it was determined that the senescence of the cotyledons of sunflower seedlings grown in a salt environment accelerated significantly compared to the cotyledons of seedlings grown in the Hoagland environment. However, IAA application to grown seedlings in a salt environment had a delayed effect on senescence. The GR24 application, on the other hand, accelerated the senescence at the end of the process. Although the causes of proline accumulation in salt stress are not fully explained, it is accepted as an important indicator of salt tolerance (44). In many studies, it was stated that lipid peroxidation and consequently MDA increased with salt stress (45,46). In this study, a statistically significant increase was found in the proline and MDA content in the cotyledons of the seedlings grown in a salt environment compared to the proline content in the cotyledons of the seedlings grown in optimum conditions. These findings are in agreement with scientific sources (47-50). In our study, it was revealed that IAA and partially GR24 regulate salinity positively (51) and delay senescence. However, Mina et

al. (41) reported that GR24 application decreased the level of IAA in roots and leaves under stress. Briefly, when we consider the average percentage of green area, during the delay of the senescence observed in cotyledons of the seedlings grown in Hoagland+NaCl solution and treated with IAA, chlorophyll and carotenoid contents increased in total protein amount. A decrease in the peroxidase activity, MDA and proline amounts of cotyledons under the same conditions was detected. These results proved that IAA application reduced lipid peroxidation, preserved membrane structure, and delayed senescence, and the toxic effect created by salt stress had a healing effect. Considering the average green area percentages, the senescence rate observed in the cotyledons of the seedlings grown in a salty environment and treated with GR24 is not as strong as the IAA effect. GR24 application in salt stress caused an increase in chlorophyll, carotenoid and a decrease in POD enzyme activity, MDA and proline amount. It is thought that the GR24 application has a delaying effect on senescence. Little is known about the regulation of leaf senescence by strigolactone. Ueda and Kusaba (10) showed that SL promotes leaf senescence in a study they did. Moreover, their results showed that both ethylene synthesis and SL synthesis were induced during the effective progression of leaf senescence caused by darkness. Yamada and Umehara (11) stated that the production of strigolactones is stimulated in response to nitrogen and phosphorus deficiency and accelerates leaf senescence. Agusti et al. (52) showed that strigolactone signaling is necessary for promoting vascular cambium formation and strongly interacts with the auxin signaling pathway. This information supports our views put forward in our study that effectively the IAA and GR24 encourage senescence. If the receptors associated with the senescence process are not yet present in a cell, it is out of the question for that cell to perceive the senescence signal. Therefore, in order for IAA to function as a senescence signal, receptors that will perceive IAA as a senescence signal must be synthesized in the cells of target organs that will undergo senescence. In a study, two different receptor proteins that bind IAA and occur at a different time from the other were detected in the plasma membrane. The first of these proteins are related to growth, and the role of the second is still not determined (53,54). The possibility that the second protein that binds IAA is the receptor that detects the senescence signal can be considered.

As a result, this study was conducted in the plant growth chamber under controlled conditions. Results showed that the application of IAA and GR24 to leaves can ameliorate the adverse effects of salt stress and delay senescence due to the activation of chlorophyll components and modulation of photosynthesis as well as antioxidant defense capacity. The effect of IAA is more precise when all analyses are considered. More importantly, most of the findings showed that IAA and GR24 encourage senescence in Hoagland (control/without salt) in this research. Delaying senescence contributes to basic science. It is suggested that this information can be used practically in the field of agriculture. In this study, our aim is to delay senescence with various applications under stress condition, to extend the vegetative period and to increase the yield of the crop. IAA and relatively. GR24 can play an important role in the protection of plants in agricultural areas in salt stress.

CONCLUSION

This study was conducted in a plant growth chamber under controlled conditions. According to our results, while IAA and partially GR24 promote senescence under optimum conditions, these delay senescence under salt stress. The mechanism of the effect of auxin and GR24 on senescence has not been determined yet. Further studies are needed to obtain sufficient information on this subject. The results showed that IAA and GR24 delay senescence in salt stress. If senescence is delayed in salty conditions, fruit yield will increase. This research will support overcoming plant development problems related to global warming.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.S.; Data Acquisition- S.S.; Data Analysis/Interpretation- S.S., H.O.; Drafting Manuscript- S.S., H.O.; Critical Revision of Manuscript- S.S.; Final Approval and Accountability- S.S., H.O.

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