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The effects of SNP and some plant hormones on sunflower (*Helianthus annuus L.*) seedling leaves exposed to salt stress

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Summary. In this study, the effects of salt stress, sodium nitoprusside (SNP) and hormones (Abscisic acid, Indol acetic acid and Gibberellic acid) applications on fatty acids concentrations of sunflower (*Helianthus annuus* L. cv. Tarsan-1018) plant leaf were investigated. *Helianthus annuss* L. cv. TARSAN - 1018 seeds were obtained through the Edirne Thrace Agricultural Research Institute, in Turkey. Following surface sterilization, the seeds were kept in water with aquarium pomp for 24 hours. The light intensity was 222 μ mol / m^2 s on the leaf surface. Seeds were grown in 16 hours light, 8 hours dark photoperiod. Seeds were irrigated with Hoagland culture solution for 5 weeks. At the end of the fifth week salt, SNP and hormone applications were performed with foliar application for 72 hour. At the end of 72 hours samples were taken. Fatty acid composition of leaf tissues of sunflower (*Helianthus annuus* L. cv. Tarsan-1018) were determined by using gas chromatography. 300 mM salt application caused decrease concentrations of C16:0, C17:0, C18:1 and C18:3. 100 μ M sodium nitoprusside has reduced the concentrations of C16:0, C17:0, C18:1. Hormone applications. adversely affected fatty acid concentrations. The results show that salt stress, sodium nitoprusside and hormone applications have negative effects on C16:0, C17:0, C18:1 and C18:3 concentrations in Tarsan-1018 sunflower leaf tissues.

Key words: fatty acid, hormone, salt stress, sodium nitoprusside, sunflower

Introduction

Stress can be defined as the destabilized physiological conditions of organisms. In the biological systems, a stress type may provide stress-producing for plants or it may provide optimum conditions for plants. The most practical definition of biological stress can be defined as negative conditions that inhibit normal functions and occurrences in plants (1-3). Salinity is a type of stress that has been increasing in the last few years worldwide. Salt stress causes decrease in precipitation and increase high humidity formation, and create difficulties in achieving water and nutrients for plants. As a result, water deficiency and drought cause stressful results (4-6). High salinity has negative effects on vegetation in many ways such as water stress, ion toxicity, nutrient deficiency, metabolic process changes, membrane disorders, regression in cell division and genotoxicity (7). The increase in NaCl stress causes negatively affected significant physiological steps such as protein synthesis, photosynthesis, energy and lipid metabolism (8). The osmotic effect of NaCl stress can be observed immediately. Cell growth and division are inhibited and stomata are closed (9, 10). Many of the plants have protective or countervailing mechanisms against adverse effects of salinity. In this way, plants can struggle with stress by controlling their stomata, improving osmotically, developing photoprotective effects, providing secondary metabolite. and phytohormone production (11-14).

Plant hormones are molecules that play an important role in the growth and development of plants. When plants are exposed to abiotic stress, some endogenous plant hormones play an important role in signal transduction and regulation of gene expression (15). Plant hormones regulate cell division, differentiation

and growth (16). In addition, gibberellins, ethylene, cytokinins and brassinosteroids can regulate seed germination and development (17). Abscisic acid (ABA) plays a role in the regulation of responses to stress in various stress conditions. It regulates stomatal activity, dormancy and other plant activity in abiotic and biotic stress conditions (18-20). ABA is a vital cellular signaling molecule that mediates the expression of some stress genes (20, 21). It has been determined that Nitric oxide (NO) involved in Indol acetic acid (IAA) signal. Thus some physiological processes are regulated (22). Gibberellic acids are a group of hormones that regulate seed germination, leaf expansion, root elongation and flow, and are related to growth and development (23, 24). It has been reported that GA regulates the amount of other plant hormones in the Glycine max plant and can correct the adverse effects of NaCl stress (25-27). Nitric oxide (NO) has important roles in many different physiological in plants stages such as seed germination, growth and development, senescence, stoma movements. Nitric oxide plays an important role in some physiological stages in plants, such as reduction of promoting or seed dormancy of seed development and various stress types (28-31), plant ripening and regeneration of senescence (32-34), prevention of flowering (35), provision of stoma movements (36-39). Fatty acids and lipids, which are important components of plant cells, not only provide structural integrity and energy for various metabolic processes, but also function as signal molecules (40). Researchers reported that drought stress could cause a change in fatty acid contents of different sunflower seeds (41).

Oilseed plants are located in vegetable production in Turkey and are defined as the basic necessities of vital importance in human nutrition (42). Sunflower, sesame, peanut, poppy, canola, aspir and cottonseed are cultivated in Turkey. Sunflower is in the first place in terms of production amount. Sunflower contains high vegetable oil (22–50%). For this reason it is an important plant in the production of vegetable oil.

Studies with oil plants have focused on the seeds of plants. In this research, which is a different study, the concentrations of fatty acids in sunflower plant leaves were investigated depending on salt (NaCl) stress, hormones (ABA, IAA and GA) and sodium nitoprusside (SNP) applications. The results indicate

that the concentrations of fatty acid in sunflower leaves vary depending on the applications.

Material and Methods

Plant material and experimental design

Sunflower plant (Helianthus annuss L. cv. TAR-SAN - 1018) seeds were obtained through the Edirne Thrace Agricultural Research Institute. Maximum salt tolerance of plant was determined. As a result, it was determined that the concentration of 300 mM NaCl was the maximum salinity concentration and it was used to create salt stress in the study. Then, seeds were sterilized using sodium hypochloride solution (1% v/v). Followed by washing with dH₂O. Following surface sterilization, the seeds were kept in the aquarium pump for 24 hours in water. After that, pot planting process was applied. The seeds were grown at 25 ± 2°C in plant growth chamber with 60-65% humidity. The light intensity was 222 µmol / m²s on the leaf surface. Seeds were grown in 16 hours light, 8 hours dark photoperiod. Hoagland culture solution was used as the main culture solution in the study. Seeds were irrigated with Hoagland solution for 5 weeks. At the end of the fifth week, salt and hormone applications were performed with foliar application for 72 hour and samples were taken at the 72nd hour. Each group has three aerated pots and 40 seeds germinated in each pot. SNP was prepared as 100 μM and the hormones were also applied as 100 μM that these applications were performed with foliar. Thus, 300 mM NaCl, 100 μM SNP, 100 μM ABA, 100 μM IAA and 100 µM GA applications and combinations were generated (43). The applications were made with the stated concentrations for 72 hours and the following 13 groups were formed:

Control, 300 mM NaCl, 100 μ M SNP, 300 mM NaCl + 100 μ M SNP, 100 μ M ABA, 100 μ M IAA,100 μ M GA, 300 mM NaCl + 100 μ M ABA, 300 mM NaCl + 100 μ M IAA, 300 mM NaCl + 100 μ M GA, 300 mM NaCl + 100 μ M SNP + 100 μ M ABA, 300 μ M NaCl + 100 μ M SNP + 100 μ M IAA and 300 mM NaCl + 100 μ M SNP + 100 μ M GA. The samples were frozen in liquid nitrogen and stored in deep freeze at -40°C until analysis.

Determination of Fatty acid

Fatty acids in the lipid extracts were converted into methyl esters including 2% sulphuric acid (v/v) in methanol (44). The fatty acidmethyl esters were extracted with 5 mL n-hexane. The analysis of fatty acid methyl ester was performed in a Shimadzu GC-17A instrument gas chromatograph, equipped with a flame ionization detector (FID) and a 25mm, 0.25 mm i.d.permabond fused-silica capillary column (Macherey- Nagel, Germany). The oven temperature was programmed between 145-215°C, 4°C / min. Injector and FID temperatures were 240 and 280°C, respectively. The rate of nitrogen carrier gas was at 1 mL/ min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 was used to process the data.

Statistical analysis

All experimental data were obtained repeatedly three times under some conditions. A comparative analysis of variance was performed between the control group and the experimental group. Stastical analyses of the data were performed by using SPSS 15.0 software program. All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences (set as P≤0.05). The data were shown as mean ± SD. Each group was compared with its own control group.

Results

Palmitic acid (C16:0) concentrations

Palmitic acid (C16:0) concentrations in leaf tissues of Tarsan-1018 sunflower plant were determined to be close to the control group in all treatment groups, except for 100 mM GA application (Table 1). When Table 1 is examined, it is understood that the many application groups result in a lower concentration of 16.0 than the control group. The lowest concentration of C16:0 was determined in 100 µM GA application (5,71±0,03 %). SNP treatment caused decrease concentration of C16:0. 100 µM IAA, 300 mM NaCl+ 100 μM GA and 300 mM NaCl+ 100 μM SNP+ 100 µM GA applications provided high concentration of C16:0 than the control group. NaCl application was almost ineffective. In the same way, 100 µM ABA application was found to be ineffective on the C16:0 concentration (Table 1).

Palmitoleic acid (C16:1) concentrations

Concentration of the Palmitoleic acid (C16:1) in leaf tissues of Tarsan-1018 sunflower plant were determined higher than control group in all applications, except for 100 mM GA and 300 mM NaCl+ 100 μ M SNP applications (Table 2). Especially 100 μ M ABA treatment provided the highest C16:1 concentrations (4,65±0,09%). NaCl stress and 100 μ M SNP caused increase con-

Treatment Groups	C16:0 Concentration (%) at 72nd Hour
Control	20,32±0,09
300 mM NaCl	19,97±0,02
100 μM SNP	15,44±0,09***
100 μM ABA	20,22±0,09
100 μM IAA	22,99±0,55"
100 μM GA	5,71±0,03***
300 mM NaCl+ 100 μM SNP	17,28±0,09**
300 mM NaCl+ 100 μM ABA	22,03±0,09**
300 mM NaCl+ 100 μM IAA	16,27±0,03***
300 mM NaCl+ 100 μM GA	22,96±0,42**
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	19,69±0,07
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	15,09±0,31***
300 mM NaCl+ 100 μM SNP+ 100 μM GA	21,91±0,06***

Treatment Groups	C16:1 Concentration (%) at 72nd Hou
Control	2,61±0,07
300 mM NaCl	3,69±0,06***
100 μM SNP	3,23±0,06***
100 μM ABA	4,65±0,09***
100 μM IAA	3,14±0,05***
100 μM GA	2,38±0,09***
300 mM NaCl+ 100 μM SNP	1,46±0,09***
300 mM NaCl+ 100 μM ABA	2,89±0,06°
300 mM NaCl+ 100 μM IAA	2,75±0,03°
300 mM NaCl+ 100 μM GA	2,75±0,04°
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	2,74±0,06°
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	3,03±0,01 ^{**}
300 mM NaCl+ 100 μM SNP+ 100 μM GA	3,46±0,06***

centraion of C16:1 than control. But 300 mM NaCl+ $100~\mu M$ SNP application reduced the concentration of C16:1 than control. Moreover, this value is the lowest value obtained from applications (1,46±0,09 %).

Heptadecanoic acid (C17:0) concentrations

Heptadecanoic acid (C17:0) concentrations were founded less than the control group in all treatment groups. These reductions were found to be serious. This reduction was about 7.5-fold in the 100 μ M IAA+ 100 μ M SNP+

300 mM NaCl treatment. 100 μ M IAA treatment resulted in 50% reduction compared to the control group. The concentration of C17:0 more severely decreased in 100 μ M GA and 100 μ M ABA applications (Table 3).

Strearic acid concentrations (C18:0)

Strearic acid concentrations (C18:0) of Tarsan-1018 leaf tissues were founded higher than the control group except for 300 mM NaCl+ 100 μ M IAA, 300 mM NaCl+ 100 μ M SNP+ 100 μ M IAA and 100 μ M GA appli-

Table 3. Heptadecanoic acid (C17:0) concentrations in leaf tissues of Tarsan-1018 sunflower plant	
Treatment Groups	C17:0 Concentration (%) at 72nd Hour
Control	6,63±0,08
300 mM NaCl	2,38±0,03***
100 μM SNP	2,48±0,07***
100 μM ABA	1,55±0,08***
100 μM IAA	3,25±0,05***
100 μM GA	1,76±0,03***
300 mM NaCl+ 100 μM SNP	2,01±0,05***
300 mM NaCl+ 100 μM ABA	2,09±0,07***
300 mM NaCl+ 100 μM IAA	3,15±0,01***
300 mM NaCl+ 100 μM GA	1,65±0,02***
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	1,98±0,06***
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	0,89±0,01***
300 mM NaCl+ 100 μM SNP+ 100 μM GA	1,02±0,08***
Significant differences between treatments at *** P≤0,001 level	indicated by different.

Treatment Groups	C18:0 Concentration (%) at 72nd Hour
Control	2,86±0,09
300 mM NaCl	3,94±0,06***
100 μM SNP	3,25±0,08***
100 μM ABA	4,09±0,06***
100 μM IAA	3,60±0,08***
100 μM GA	2,50±0,05**
300 mM NaCl+ 100 μM SNP	3,89±0,03 ^{***}
300 mM NaCl+ 100 μM ABA	4,40±0,06***
300 mM NaCl+ 100 μM IAA	2,14±0,06***
300 mM NaCl+ 100 μM GA	4,26±0,01***
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	3,36±0,04 ^{***}
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	1,82±0,06 ^{***}
300 mM NaCl+ 100 μM SNP+ 100 μM GA	3,21±0,01 ^{***}

cations. The highest concentration was detected in the 300 mM NaCl+ 100 μ M ABA application (4,40±0,06%). Only NaCl, SNP and IAA applications provided increase C18:0 concentration but their combinations caused decrease concentration of C18:0 and 100 μ M GA application made a negative impact on C18:0 (Table 4).

Significant differences between treatments at *** P≤0,001 and ** P≤0,001 level indicated by different.

Oleic acid (C18:1) concentation

All applications caused decrease oleic acid (C18:1) concentrations than the control except for 300 mM NaCl+

100 μ M SNP+ 100 μ M ABA. The lowest concentration of C18:1 was determined in 300 mM NaCl+ 100 μ M GA application (1,70±0,07%). The lowest concentration detrmined 2.22 fold less than the control group. Interestingly, application of 300 mM NaCl+ 100 μ M SNP+ 100 μ M ABA increased the concentration. However, only NaCl, SNP or ABA application did not raise the concentration. Only IAA treatment caused decrased C18:1 concentration. Combination of IAA, SNP and NaCl provided increase concentration of C18:1 (Table 5).

Treatment Groups	C18:1 Concentration (%) at 72nd Hour
Control	3,78±0,09
300 mM NaCl	3,05±0,03***
100 μM SNP	2,50±0,07***
100 μM ABA	2,97±0,08***
100 μM IAA	1,91±0,07***
100 μM GA	2,18±0,03***
300 mM NaCl+ 100 μM SNP	2,14±0,05***
300 mM NaCl+ 100 μM ABA	2,88±0,05***
300 mM NaCl+ 100 μM IAA	2,78±0,01***
300 mM NaCl+ 100 μM GA	1,70±0,07***
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	4,18±0,09***
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	1,84±0,01***
300 mM NaCl+ 100 μM SNP+ 100 μM GA	2,76±0,09***

Table 6. Linoleic acid concentration (C18:2) in leaf tissues of Tarsan-1018 sunflower plant	
Treatment Groups	C18:2 Concentration (%) at 72nd Hour
Control	17,44±0,08
300 mM NaCl	27,43±0,06***
100 μM SNP	23,12±0,05***
100 μM ABA	22,24±0,08***
100 μM IAA	22,88±0,04***
100 μM GA	19,01±0,04°
300 mM NaCl+ 100 μM SNP	31,47±0,06***
300 mM NaCl+ 100 μM ABA	19,88±0,07°
300 mM NaCl+ 100 μM IAA	16,45±0,01
300 mM NaCl+ 100 μM GA	23,44±0,02***
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	24,18±0,02***
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	27,93±0,06***
300 mM NaCl+ 100 μM SNP+ 100 μM GA	28,13±0,08***
Significant differences between treatments at *** P≤0,001 and *	F P≤0,05 level indicated by different.

Linoleic acid (C18:2) concentration

Linoleic acid concentration of Tarsan-1018 leaf tissues were founded higher than the control group except for 300 mM NaCl+ 100 µM IAA. Especially NaCl treatment provided an increase about 58% (Table 6). On the other hand, the combination of NaCl and SNP caused an increase approximately 80%. Only SNP treatment provided increased concentration of C18:2. The hormones have positive effects on C18:2 concentration. When the table is examined, it is understood that the IAA application increased the concentration of 18: 2, while the combination of NaCl and IAA caused a decrease.

α-Linolenic acid (C18:3) concentration

α-Linolenic acid (C18:3) concentration reached its highest value in 100 μM ABA treatment (54,66±0,04%) in Tarsan-1018 leaf tissues. NaCl, SNP, NaCl+SNP and NaCl+GA, NaCl+ GA applications caused decrease concentration of C18:3. ABA, IAA and GA provided high C18:3 concentration than control. The lowest concentration of C18:3 was determined in 300 mM NaCl+ 100 μM SNP application (16,79±0,06%). SNP treatment caused decrease concentration of C18:3 (Table 7).

Discussion

In the current study, we identified fatty acid concentrations (C16:0, C16:1; C17:0, C18:0, C18:1, C18:2 and C18:3) of *Helianthus annuus* L. cv Tarsan-1018 sunflower plant leaves.

As is known, plants produce a variety of responses to abiotic and biotic stresses. Diverse studies have shown that the fatty acid content of various industrial plants changes under stress conditions. The rate of oleic / linoleic acid increased during germination at high temperature, while decreased at low temperature (45). It has been reported that the sunflower plant's concentration of palmitic acid increased and the concentration of stearic acid decreased in drought stress. The same researchers reported that drought negatively affected the oleic acid concentration, while had an enhancing effect on linoleic acid concentration (46). In a different study it has been reported that water stress causes a decrease in oleic acid content (47). It was also concluded that water stress increased oleic acid concentration and decreased stearic acid concentration (48). Our results are consistent with the literature. According to our results, the concentration of oleic acid increased in the plant due to the stress effect, which is different from the literature. This may be due to genetic differences. Because genotype is the most important factor for identifies the fatty acid composition (49).

Treatment Groups	C18:3 Concentration (%) at 72nd Hou
Control	34,06±0,08
300 mM NaCl	28,60±0,03***
100 μM SNP	23,12±0,05***
100 μM ABA	54,66±0,04***
100 μM IAA	37,20±0,02°
100 μM GA	39,39±0,03**
300 mM NaCl+ 100 μM SNP	16,79±0,06 ^{***}
300 mM NaCl+ 100 μM ABA	37,74±0,07°
300 mM NaCl+ 100 μM IAA	39,75±0,01"
300 mM NaCl+ 100 μM GA	30,90±0,08**
300 mM NaCl+ 100 μM SNP+ 100 μM ABA	34,38±0,05
300 mM NaCl+ 100 μM SNP+ 100 μM IAA	34,79±0,02
300 mM NaCl+ 100 μM SNP+ 100 μM GA	31,34±0,08***

Literature knowledge on the effects of hormones on sunflower leaf fatty acid concentrations are limited. In a study conducted with salicylic acid (SA) application was shown that the concentration of some fatty acids (C16:0, C16:1, C18:0, C18:2 and C18:3) decreased and the concentration of C18:1 increased. In the same study it was also reported that SA treatment caused increase concentration of IAA and GA while reducing concentration of ABA (50). According to our results, ABA application increased concentrations of 16: 1, C18:0, C18:2 and C18:3. and it decreased concentrations of C17:0 and C18:1. There may be an increase or decrease in unsaturated fatty acids under different stress conditions. Due to these different reactions, the increase in unsaturated fatty acids does not provide endurance in all types of stress (51). For example, concentration of C18:3 increases in saline conditions (52) and decreases at heavy metal stress (53). However, in the present study it was determined that the concentration of C18:3 decreased under saline conditions. Of course, it is difficult to obtain same physiological responses in living organisms. There are very few studies on salinity and temperature stress that increase or no effect on fatty concentrations It is generally reported that there is a decrease of oil concentrations in saliniyt or temparature stress (54).

In our study, it was determined that concentrations of fatty acids (C16:0, C17:0, C18:1 and C18:3)

decreased with application of salt stress. In a different study (55), C16:0, C18:2 and C18:3 fatty acids were identified in sunflower leaves. We have also identified C17:0, C17:1, C18:0 and C18:1 fatty acids in addition to these. Researchers reported that leaf fatty acid composition in sunflower plants showed significant changes with Cd stress and this negative effect was alleviated by salicylic acid treatment (56). Peroxidation of unsaturated lipids in biological membranes is the most obvious indication of oxidative stress in animals and plants (57). According to results, it was revealed that concentration of C18:3 decreased with salt application while it increased with hormone applications. On the other hand concentration of C18:2 increased with salt treatment. Concentration of C18:1 decreased salt, ABA, IAA ve GA applications. It was determined that the concentration of C18:0 increased with salt application. In the same way, ABA and IAA applications increased concentration of C18:0. GA application decreased C18:0 concentration. These results suggest that GA is ineffective on desaturase activity. In addition, ABA and IAA improved desaturase activity and transformation of C18:0 to C18:2 and C18:3. Studies have shown that when the plants are exposed to drought stress, the fatty acid composition may change and the content of higher unsaturated fatty acids may increase (58, 59). In addition salt stress (250 mM) caused increase concentrations of C16:0, C18:0 and C18:2, and decrease concentration of C16:1 and C18:3 in Peanut (60). In the *Spartina patents* plant, salt sress increased contents of C16:0 and C18:0, and decreased concentrations of C16:1, C18:2 and C18:3 (61). We observed in the present study, salt stress decreased concentrations of C16:0, C17:0, C18:1 and C18:3 while increased C16:1, C18:0 and C18:2. Our results are partly consistent with studies. The reason for this may be genetic differences and different applications.

The data show that SNP application has effects on fatty acids. Researchers have reported that 10 μ L/L NO application provides a significant increase in C16:1, C18:1 and C18:3 content in peach (62). They stated that a decrease occurred in content of C18:2. In the same study it was determined that 5 µL/L and 10 μL/L NO applications caused decrease the content of C18:3 but 15 µL/L NO application provided increase it. Because of these results, it has been reported that the fatty acid content depends on NO concentration. In the present study, SNP has reduced the concentrations of C16:0, C17:0, C18:1 and C18:3 fatty acids and it increased concentration of C16:1, C18:0 and C18:2 fatty acids. Apparently, our results are in contrast with the literature. These different results may be due to plants. Because genotype is the most important factor for identifies the fatty acid composition (49).

Conclusion

Fatty acids are important substances in plant tissues and are affected by a variety of factors. Also, fatty acids play role in different metabolic events in all living organizms. Omega fatty acids are effective in brain development, strengthening of the immune system and prevention of heart diseases. Ratio of unsaturated / saturated fatty acids is important in nutrients and if this rate is low, it is effective for health. Present study showed that hormone applications provided increase concentraion of unsaturated fatty acids. This is a positive result for health. Hormones at the appropriate dose can make a positive effect. In our study, it was observed that concentrations of fatty acids (C16:0, C17:0, C18:1 and C18:3) decreased with application of salt stress. NaCl stress caused increase C18:0, C18:2 and C16:1. SNP has negative effect on the concentrations of C16:0, C17:0, C18:1 and C18:3 fatty acids and it increased concentration of C16:1, C18:0 and C18:2 fatty acids.SNP has reduced the concentrations of C16:0, C17:0, C18:1 and C18:3 fatty acids and it increased concentration of C16:1, C18:0 and C18:2 fatty acids. Although nitric oxide is a signaling molecule, it has not a positive effect on all unsaturated fatty acids. This conclusion shows that the adaptation mechanism of plants to stress conditions is complicated. Hormone applications have negative effects on C16:0, C17:0, C18:1 and C18.3 concentrations. Espacially GA has very restrictive effects on the fatty acids. In conclusion, signal molecules and hormones are important for plants. The mechanism of adaptation to stress conditions is influenced by many factors. Although sunflower leaf is not consumed by humans the results suggest that attention should be paid to the consumption of nutrients.

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