International Journal of Multidisciplinary Research and Growth Evaluation
ISSN: 2582-7138
Received: 14-09-2021; Accepted: 03-10-2021
www.allmultidisciplinaryjournal.com
Volume 2; Issue 5; September-October 2021; Page No. 426-433

The role of ascorbic acid in the tolerance of *Helianthus annuus* L. to salinity stress in tissue cultures and field

Shima Mohammed Sultan [1], Sajida Aziz Abood [2]
[1-2] Department of Biology, Science Coll, Mosul University of Mosul, Iraq
Corresponding Author: **Shima Mohammed Sultan**

Abstract
The current study involved callus induction from stem segments of *Helianthus annuus* L. seedlings on the medium MS supported by adding BA with a concentration of 1.0 mg.l^{-1} and NAA with a concentration of 0.5 mg.l^{-1}. Callus was used to identify the effect of sodium chloride (150mM), ascorbic acid (20 mg.l^{-1}) and the interaction between them on some cell contents sunflower callus. The addition of sodium chloride to the growth medium resulted in the increase in the proline concentration, soluble saccharides, the activity of catalase and peroxidase enzymes, a decrease in the concentrations of proteins and nuclear acids in the callus. From the other hand, the ascorbic acid and its interference with sodium chloride had a positive effects on increasing the contents of the callus tissues compared to the salinity treatment. The differentiation of the salinity treatment callus failed while the callus of the ascorbic acid treatment was superior in terms of its differentiation into vegetative branches and forming the plantlets compared to the rest of the treatments. Soaking the sunflower seeds in the solution of ascorbic acid improved the indicators of the plant growth and reduced the negative effects of sodium chloride and this is evidenced by the increase in the plant height, the increase in the fresh and dry weight, the leaf area, number of seeds.disc^{-1}, the weight of 100 seeds, chlorophyll concentration in the leaves and the content of oil and protein in the seeds.

Keywords: *Helianthus annuus*, sodium chloride, ascorbic acid

Introduction
Helianthus annuus L. belongs to Asteraceae and it is planted for the purpose of producing oil from its seeds. It is considered the third best vegetables oils in terms of consumption on the international level after the soy bean and peanut [1]. Salinity is regarded as one of the major problems that the farmers encounter all over the world which causes the saline stress, which is, in turn, has primary impacts that are osmotic and ionic toxicity on the cells and secondary complex effects that include oxidative stress that leads to the damage of the cell contents such as the membrane fats, proteins, nucleic acids and also result in a defect in photosynthesis, and thus influence the growth and yield of crops [2]. Ascorbic acid is regarded as an antioxidant that scavenge the free radicals [3] and it plays a major role in the plant cells by maintaining the osmosis potential and the ionic balance in the cells. Moreover, it preserves cellular components including the proteins, fats and the nucleic acids from damage [4]. Ascorbic acid regulates the division and growth of the cells and improves the vegetative growth, which inturn is refleced in increasing yield and improving its quality [5]. The action of antioxidants is represented by overcoming the harmful effect of salinity stress by creating an accumulation of the products of nutritional transformation such as the proline, amines, saccharides, ion and others and all this results in increasing the osmotic potential of the cellular sap to resist the external salt stress. Proline plays an important role in plant stress tolerance and protects the proteins, mitochondria, cell membranes and chloroplast from the harmful effects of the stresses [6]. The soluble sugars play an important role in regulating the osmosis of cells under the salinity stress conditions [7]. Study [8] found an increase in the quantity of proline in rice callus tissues by increasing the concentration of sodium chloride to 200 mM in the culture media. The addition of sodium chloride with a concentration of 200mM to the media of peas callus growth resulted in an increase of its content of proline and saccharides [9]. Moreover, salinity had a negative impact on the plant growth as it leads to a decrease in the concentrations of proteins, nucleic acids and lipids [10]. The study of [11] confirmed an increase in the activity of peroxidase and catalase enzymes when treating rice callus with sodium chloride with a concentration of 200mM, and the addition of ascorbic acid to the saline media also increased the activity of these enzymes. Salinity is considered the most problems that affecting seed germination and its development to whole plants, and the salt effect in this process is due to limiting the uptake of water and nutrients [12] or to the toxicty effect on the embryo due to the absorption of sodium and chloride ions [13].

There are evidences indicate that the role of ascorbic acid in regulating the transition from the vegetative stage to the production stage, meaning that ascorbic acid is not only an important antioxidant, but it appears to connect between flowering time and senescence and the cells programmed death, through a complicated network of signal transport [14]. The main goal of this research is increasing sunflower plants resistance to salinity by using ascorbic acid as an antioxidant by investigating some physiological and chemical changes that shown in the indicators of the growth and differentiation of callus by the effect of sodium chloride solution, ascorbic acid and the interaction between them, and the extent of the growth and development of the sunflower plants which are grown from seeds soaked in those solutions.

Materials and Methods

Callus cultures induction from the stem segments

After the sterilizing the sunflower seeds and planting them to form the seedlings, pieces of the stems of these seedlings were cultured on MS medium [15] supported by adding BA with a concentration of 1.0 mg.l^{-1} and NAA with a concentration of 0.5 mg.l^{-1} and kept bottles in the growth room at a temperature of 20±2 ℃ [16].

Effects of sodium chloride in callus growth

From the initiated calli with an age of 21 days are transferred to MS media to which the same combination of growth regulators used for the initiation with the addition of sodium chloride at a concentration of 150 Mm. Other media were provided with ascorbic acid with a concentration of 20 mg.l^{-1}, in addition to the interference treatment between sodium chloride and ascorbic acid as well as the control treatment. After 21 days of culture the indicators of callus growth were calculated.

Proline

The quantity of proline in the 21-day-old callus samples for all the treatments were estimated [17].

Protein

A test of [18] was used to estimate the amount of protein that extracted from the seeds and the callus samples for all the treatments.

Soluble saccharides

Soluble saccharides of callus tissues were extracted and estimated for all the treatments in the age of 21 days [19].

Nucleic acids

Cherry's method [20] was used for extracting and estimating the concentration of the total nucleic acids in the callus samples for the different treatments. DNA content was determined by using the Diphenylamine reagent for the different treatments [21].

Activity of the antioxidant enzymes

Catalase and peroxidase enzymes were extracted from the callus tissues for the different treatments [22].

Catalase activity

The activity of catalase was estimated depending on measuring the amount of increase of light absorption of the reaction solution at the 240nm [23].

Peroxidase activity

The activity of peroxidase measurement depended on measuring the change in absorption at wavelength 420nm [24].

Callus regeneration

Callus differentiation into vegetative branches

Approximately 0.5 g of callus samples with an age of 30 days, was transferred to MS media, which supplement with 1 mg.l^{-1} of BA + 1 mg. l^{-1} of NAA), sodium chloride (150 mM) and ascorbic acid (20 mg. l^{-1}) and the interaction between them. Then planting were transferred to the growing room under the same conditions mentioned before.

Rooting the differentiated vegetative branches and plantlets acclimatization.

After 30 days from the planting date, the branches formed from the differentiated callus of the different treatments were eradicated and each branch containing some leaves was transferred to an MS medium with half of its composition strength and without any growth regulators. The plantlets were kept in the growth room. The plantlets were transferred to plastic pots that contain a mixture of mixed soil and peat moss with a ratio of 1:1. The pots were covered with plastic perforated covers and left for 3 days in the growth room condition. After removing the covers they were transferred to the greenhouse.

Soaking sunflower seeds

A group of sunflower seeds were soaked in sodium chloride solution with a concentration of 150mM and another group of seeds was soaked in ascorbic acid with a concentration of 20 mg. l^{-1}, while a third group of seeds was soaked in a solution consisting of sodium chloride solution and ascorbic acid with the same concentrations above in addition to the control treatment in which its seeds were soaked in distilled water only. After 24 hours of soaking, the soaked seeds in the different solutions were planted in plastic pots consisting of a mixture of mixed soil and peat moss with a ratio of 1:1.

The measurements

All the growth indicators of sunflower plants with an age of 90 days were taken except for the weight and number of seeds after 120 days of planting for the different treatments and as follows:

Plant height (cm): The plant height was measured from the surface of the soil to the highest growing apex of the plant using tape measure.

Fresh and dry weight (g): The fresh weight of the adult plants was weighted by using a scale after cutting them into small pieces. The plants were dried in an electrical oven at a temperature degree ranged between 65-70 C° for 48 hours until the weight is stabilized.

Leaf area (cm^2): The leaf area for all the treatments were calculated (25)

Number of seeds (seed.$disc^{-1}$): The number of seeds was calculated for three plants and the mean after that was found.

The weight of 100 seeds (g): 100 seeds were taken randomly for each treatment and they were weighted by using a sensitive scale.

Total chlorophyll in the leaves (mg.cm^{-3}): Lichtenthaler's [26] method was used in extracting the chlorophyll and determining its quantity in sunflower leaves after a growth period of 90 days.

Protein percentage in the seeds (%): The percentage of the protein in seeds was determined depending on estimating the nitrogen percentage in the seeds of sunflower for the different treatments [27].

Oil percentage in the seeds (%): The oil was extracted from of sunflower seeds for the different treatments [28].

Results

The effect of sodium chloride and ascorbic acid and interaction between them in the callus tissue cellular components

Data in table (1) demonstrates the increase of the concentration of proline and soluble saccharides in the callus samples after 21 days of growth on MS medium which supplemented with sodium chloride at concentration 150mM and the values were 14.077 µg.g^{-1} and 0.999 µg.g^{-1} respectively compared to the control treatment that had values of 8.103 and 0.578 µg.g^{-1} respectively. From the other hand, adding sodium chloride to the callus growth media resulted in a decrease in its content of protein, DNA and RNA with values of 0.433 mg.g^{-1}, 25.1 µg.g^{-1}, 237. 5 µg.g^{-1} respectively compared to the control treatment that had the values of 1.865 mg.g^{-1}, 44.4 µg.g^{-1} and 441.0 µg.g^{-1} respectively. The same table indicates that adding ascorbic acid with a concentration of 20 mg.l^{-1} to the callus growth medium which is provided with sodium chloride with a concentration of 150Mm led to an increase in the content of the callus of the previously mentioned cellular components compared to the treatment of salinity only.

Table 1: The effect of sodiun chloride (150mM) and ascorbic acid (20 mg. l^{-1}) and the interaction between them in the callus cellular contents of 21-day-old sunflower.

Treatments	Concentrations				
	Protein (mg.g^{-1})	Proline (µg.g^{-1})	Soluble saccharides (µg.g^{-1})	DNA (µg.g^{-1})	RNA (µg.g^{-1})
Control treatment	1.856	8.103	0.578	44.4	441.0
Ascorbic acid	2.552	9.998	0.976	55.6	605.5
NaCl	0.433	14.077	0.999	25.1	237.5
Ascorbic acid+NaCl	1.1001	16.993	1.987	29.1	`290.0

Catalase and peroxidase activity

The results showed an increase in the activity of catalase and peroxidase enzymes extracted from the sunflower callus in the age of 21 days when adding sodium chloride with a concentration of 150mM to the growth media and the values were 49.672 and 59.907 unit.cm^{-3} respectively. This increase in the activity of the two enzymes kept on when adding ascorbic acid with a concentration of 20 mg.l^{-1} to these saline media to reach the values of 60.558 and 85.702 unit.cm^{-3} respectively compared to the other treatments (table 2).

Table 2: The activity of catalase and peroxidase enzymes extracted from the sunflower callus after 21 days of treatment with NaCl (150 mM) and ascorbic acid (20 mg.l$^{-1)}$ and interaction between them.

Treatments	Activity (unit.cm^{-3})	
	Catalase	Peroxidase
Control treatment	10.221	12.84
Ascorbic acid	20.760	13.449
NaCl	49.672	59.907
Ascorbic acid +NaCl	60.558	85.702

Callus regeneration

Results showed that the failure of the differentiation of the sunflower callus growing on MS media, which are provided with sodium chloride with a concentration of 150mM in addition to selected additions of growth regulators. It didn't show any response or behavior in this respect. Ascorbic acid played a stimulating role for callus differentiation to vegetative branches as their number was superior over the number of those differentiated from the callus of the control treatment using the differentiation medium MS to which BA(1 mg.l^{-1}) and NAA(0.1 mg.l^{-1}) was added. The callus growing on MS medium containing 150mM of sodium chloride restored its capability to differentiate into vegetative branches when ascorbic acid with a concentration of 20 mg/l^{-1} was added to that salt medium (Figure 1).

Fig 1: Patterns of vegetative branches of sunflower callus for the different treatments after 50 days of cultivation on MS media supported by adding (1mg.l^{-1} of BA + 0.1 mg/l^{-1} of NAA).

Rooting of the vegetative differentiated branches succeeded for the different treatments after 20 days from transferring them to MS media, which don't contain growth regulators. The treatment of ascorbic acid was characterized with more and longer branches of roots compared to the control treatment (Figure 2). Also, the process of acclimatization of plantlets resulting from the tissue culture succeeded for all the treatment that rooted after 30 days of acclimatization. The treatment with ascorbic acid was superior over the other treatments in forming and growth of the acclimatized plants (Figure3، A. B. C) with the plants emerging from callus differentiation treated with ascorbic acid with a concentration of 20 mg.l^{-1} being distinguished in terms of their capability in forming of sunflower heads(Figure 3, D).

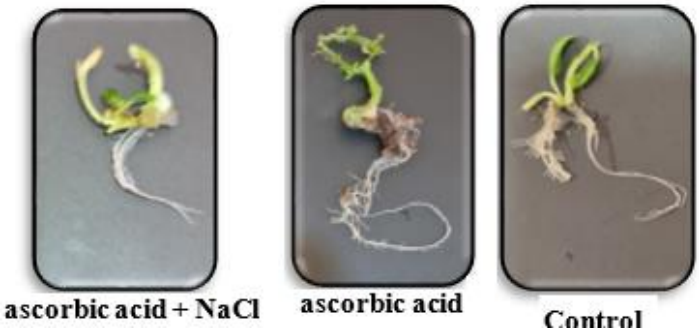

Fig 2: The variation between the characteristics of the roots emerging from the vegetative branches which differentiated from the sunflower callus for the different treatments after 20 days of their growth on MS media lacking growth regulators**.**

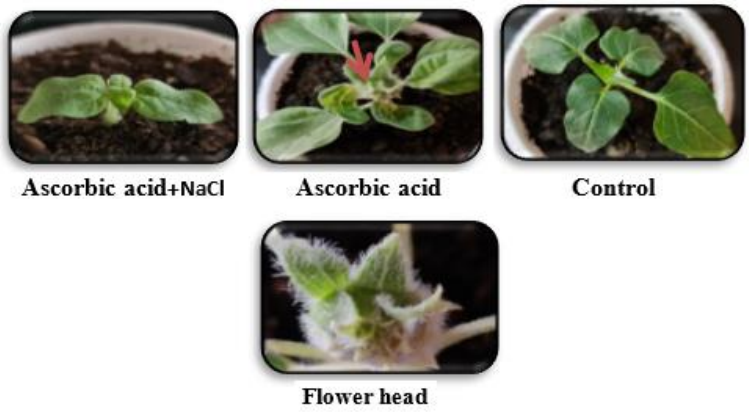

Fig 3: The effect of the interaction of ascorbic acid (20 mg.l^{-1}) and sodium chloride(150mM) in the growth of plantlets that resulting from the differentiated from the sunflower callus after 30 days of their acclimatization.

The effect of sodium chloride and ascorbic acid and interaction between them in the growth of sunflower plants

Physiological effects

All the sunflower plant characteristics studied (plant height, number of leaves, leaf area, the fresh and dry weights, number of seeds.$disc^{-1}$ and the weight of 100 seeds.$plant^{-1}$), were negatively affected by soaking the seeds in sodium chloride solution for 24 hours compared to the control treatment. From the other hand, the interaction that resulting from soaking the seeds in sodium chloride and ascorbic acid decreased that effect for all these indices after 90 days of planting (table3). Data in figure (4) demonstrates the clear differences in the plant growth with an age of 30 days according to the treatment. The plants kept on growing forming sunflower discs which differed by completing its growth and forming these seeds with different treatments (Figure 5, A, B, C, D). Moreover, results showed that the emergence of a unique case in the sunflower plants growing from seeds soaked in ascorbic acid and this case involved that the plant had more than one flower heads and the number of heads ranged between 3 to 6. $plant^{-1}$, compared to the other treatments in which each plant yielded one disc only (Figure 5, E).

Table 3: Growth indicators of *H. Annuus* plants resulting from soaking the seeds for 24 hours in NaCl (150mM) and ascorbic acid (20mg.l^{-1}) solution and the interaction between them.

Treatments	Plant height (cm)	No. of leaves. $plant^{-1}$	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	No. of Seeds ($seed.disc^{-1}$)	Weight of 100 seed. $plant^{-1}$ (g)
Control	182	22	175.554	378.638	75.728	250	12.245
Ascorbic acid	195	30	198.501	508.151	98.108	263	15.476
NaCl	67	12	98.795	142.712	35.678	95	8.330
Ascorbic acid +NaCl	150	16	140.152	201.757	53.810	181	11.251

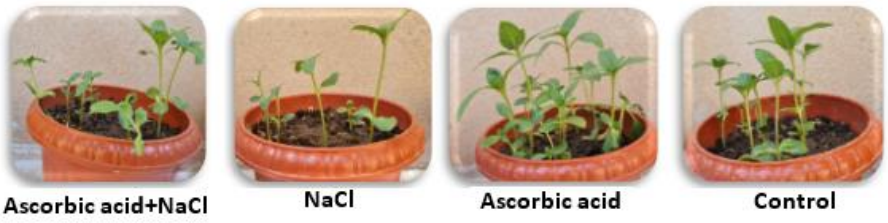

Fig 4: Soaking effect of sunflower seeds for 24 hours in NaCl ascorbic acid solution and the interaction between them i n the growth of the plants after 30 days of planting.

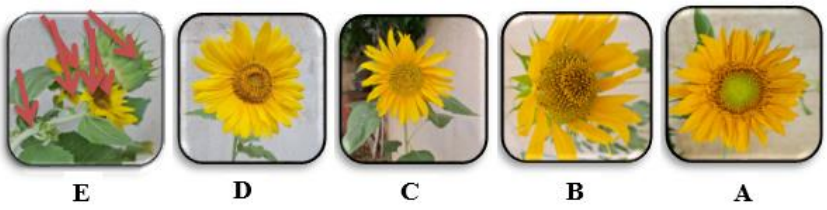

Fig 5: The effects of treating sunflower seeds with NaCl, ascorbic acid and the interaction between ascorbic acid and NaCl on forming the florets discs of the plant after 90 days of cultivation**.**
A- Control. **B**- Ascorbic acid (20 mg.l^{-1}). **C**- NaCl (150mM). **D**-Interaction of ascorbic acid and NaCl. E- A grown plant from seeds soaked in ascorbic acid (20 mg.l^{-1}).

Chemical effects

Total chlorophyll in the leaves

The leaf content of chlorophyll differed according to the different treatments after 90 days of planting. The highest value of chlorophyll was recorded in the leaves of the plants grown from seeds soaked in ascorbic acid as the value was 2.65 mg.g^{-1} compared to the rest of the treatments. The interaction between the ascorbic acid and salt achieved an increase in the leaves content of chlorophyll with a value of 1.12 mg.g^{-1} compared to the treatment with salt only, which was 0.98 mg.g^{-1} (table 4).

The percentages of oil and protein in the seeds

Data in table (4) indicates that there is a positive effect of soaking the seeds before planting them in ascorbic acid with a concentration of 20 mg.l^{-1} in increasing the percentages of oil and protein in the seeds of the plants growing from those treated seeds. The percentages were 47% for oil and 25% for protein and these percentages decreased significantly in the seeds produced from seeds treated with sodium chloride before planting them to be 30% for oil and 16% for protein compared to the control treatment that had the values of 40% and 21% respectively. The interaction between ascorbic acid and salt had an effect on the percentages of oil and protein as these percentages increased to be 35% for oil and 18% for protein compared to the salt treatment.

Table 4: The effect of soaking sunflower seeds in the solution of NaCl (150mM) and ascorbic acid (20 mg.l^{-1}) and the interaction between them in the chlorophyll concentration of the leaves and the percentages of oil and protein in seeds.

Treatments	Chlorophyll (mg.g^{-1}	Oil (%)	Protein (%)
Control	1.83	40	21
Ascorbic acid	2.65	47	25
NaCl	0.98	30	16
Ascorbicacid +NaCl	1.12	35	18

Discussion

Several studies demonstrated that the salinity stress results in the accumulation of some metabolites such as soluble saccharides, proline and other materials. The role of these materials is connected to the osmotic balance to enhance the plant ability for water uptake from the external medium in addition to other functions to provide the energy and nitrogen when the photosynthesis process declines due to the salinity stress [29, 30]. The increase in proline concentration in the sunflower callus tissues as an effect of the treatment with sodium chloride is due to a certain type of cell resistance to the salinity stress by stimulating the construction of proline synthesis from the glutamic acid and the decrease in catabolism and decreasing its participation in protein synthesis [31]. One of the studies showed an increase in corn callus content of proline when the concentration of sodium chloride increased [32]. Soluble unreduced saccharides are considered to play an important role in cell osmosis regulation under the salinity stress conditions [7]. The increase of unreduced saccharides concentration in the sunflower callus tissues was in agreement with what concluded in the pea's callus [9]. The decrease in protein concentration may be due to the reduction caused by the salinity stress in terms of the capability of the cells in involving the amino acids to synthesize the protein [33] or due to the decrease in the elements involved to synthesize the protein from the nitrogen and phosphorus and the decrease in the concentration of potassium that stimulates some enzymes to build the protein in addition to the increase of ROS concentration that breaks the peptide chains and thus causes protein decomposition [34]. The increase in the protein concentration in the sunflower callus tissues when adding the ascorbic is due to the role of this acid in stimulating Peptidylprolyl hydroxylase enzymes, which are responsible for building Glycoproteins in the cellular membranes, with a decrease in the activity of the protease enzymes [35]. Adding sodium chloride to the growth media of the sunflower callus led to a decrease its content of DNA and RNA and that was confirmed by a study conducted by [36] on the peas callus, as the researcher mentions that the reason behind that is the high salinity, which decreased the activity of three enzymes that participate in building the thymine nucleotide, which is the most important nucleotides in synthesizing the DNA. The increase in the quantities of DNA and RNA in the tissues of sunflower callus when adding ascorbic acid to the medium of growth might be due

to the role played by this acid in decreasing the harmful effects of ROS generated by adding sodium chloride to the nucleic acid molecules, which represents an important feature of enduring the salinity stress [37]. The interaction between sodium chloride and ascorbic acid caused a remarkable increase in the activity of peroxidase and catalase compared to the salinity treatment only and this is due to the role of ascorbic acid in stimulating these two enzymes to get rid of the toxicity of hydrogen peroxide and repress the free radicals under stress conditions [38, 39]. This result was in conformity with the results of [8] when treating rice callus with ascorbic acid and the addition of the ascorbic acid to the saline media increased the activity of those enzymes too.

The failure of the differentiation of the sunflower callus growing on media that include sodium chloride with a concentration of 150mM might be due to the direct and indirect impacts of sodium chloride salt at the molecular level of the tissue of the cells cultures leading to a dysfunction in the hormone balance and consequently to the decrease of the average division and expansion [40]. These findings are in conformity with what was concluded by [41] that adding sodium chloride to the media of rice callus growth caused a decreased in the number of the differentiated branched of the callus.

The results of the current study also showed the negative effects of sodium chloride on the morphological and physiological characteristics of sunflower during its different stages of growth beginning from the seed to the adult plant. Soaking the seeds of sunflower in sodium chloride caused a reduction in the plant height, its fresh and dry weights with a reduction in the leaf and the number of leaves. Soaking the seeds in the salt solution led to changes in the growth, morphology and roots physiology, which resulted in a lack of water uptake and nutrients from the soil. The reduction of the leaf area of the plant by salinity is due to the inhibition of elongation process of the cell division or both of them [42]. These results are in conformity with the effect of sodium chloride on the characteristics of the growth and development of wheat seedlings [43]. The decrease of chlorophyll in the leaf content with the increase of salinity stress might be due to the increase of salinity, which leads to the transfer and accumulation of some minerals in the leaves and a decrease in others such as ammonium ions that accumulate in the leaves and break the chlorophyll by means of breaking down of the plastids [44] or the lack of magnesium ions uptake that are components of chlorophyll synthesis and the iron ions that assists in chlorophyll formation [45], or may be because of the inhibition of the enzymes that contribute to building it[46]. The decrease of the vegetative growth and the long period required to reach flowering stage of sunflower growing from the seeds soaked in sodium chloride may be due to the lack of the main elements because of the replacement and competition of sodium and chloride ions and the increase of osmotic potential of soil solution in addition to the decrease in the level of the water available in the area of the roots will lead to a decrease in photosynthesis, with which the levels of transfer and accumulation of the dry material inside the seed decrease and this will consequently affects the number and weight of the seeds [15]. Soaking the sunflower seeds in ascorbic acid reduced the effect of salt on the plant growth as this acid has several functions in the chloroplastids, has a main role in scavenging ROS and the removal of hydrogen peroxide [47]. It was mentioned by[48] that the decrease of ascorbic acid in Arabidopsis mutant plant caused the emergence of senescence signs faster than the wild types. Ascorbic acid connects the time of flowering, aging and the programmed death of the cells through a complicated signal transferring network [14]. Which is achieved by various plant hormones. Ascorbic acid functions as an assisting agent to create these hormones such as the gibberelline and salicylic acid, which supports flowering and regulate the function of ABA and ethylene [49]. The disruption of the physiological processes resulting from stress effects on sunflower plants contributed to the small size of the flower disc, and this was reflected on the number of the seeds in the disc [50]. The number of seeds increased per disc by adding ascorbic acid only or adding it with sodium chloride and this is due to the role of this acid as an antioxidant, which causes an improvement in the vegetative growth characteristics of plant. I.e. increasing the materials produced in the leaves and their movement to the disc and this will, eventually, leads to the increase in the diameter of the disc and increasing the number of the seeds in the disc compared to the treatment using the salt. Soaking the seeds of sunflower in ascorbic acid before planting them resulted in the branching of the end of the stem with multiple flowers, which is similar to the wild species, and making the plant more tolerant to salinity. The study of [51] indicated that cultivated sunflower plants are less tolerant than their wild ancestors and this is manifested by the decreased biomass. These results might be interpreted as ascorbic acid made changes in the plant cultivated on the genetic level that made it more tolerant to salinity that it became similar to its wild type. The decrease of protein percentage in seeds when treating the plant with sodium chloride can be attributed to a disorder in nitrogen metabolism or to the inhibition in nitrate absorption [52], or to a reduction in the periods of protein and oil accumulation with the increase of salinity [53]. It was mentioned by [54] that the treatment with ascorbic acid prevents the degradation of the protein and decreases the oil peroxide in the germinated seeds of sunflower.

References

1. Byrareddy K, Uppar DS, Vyakaranahal BS, Hiremath SM, Hunje R, Nadaf HL. Effect of Integrated Nutrient Management on Sunflower Hybrid (KBSH-I) Seed Production. Karnataka J. Agric. Sci. 2008; 21:171-175.
2. Zhao C, Zhang H, Song C, Zhu JK, Shabala S. Mechanisms of Plant Responses and Adaptation to Soil Salinity, The Innovation. 2020; 1:1-41.
3. Siddiqui MH, Alamri SA, Al-Khaishany MYYA, Qutami MA, Ali HA. Ascorbic acid application improves salinity stress tolerance in wheat. Chiang Mai. J Sci. 2018; 45(10):1-11.
4. Nunes LR, Pinheiro PR. Silva JB Dutra AS. Effects of ascorbic acid on the germination and vigour of cowpea seeds under water stress, Rev. Ciênc. Agron. 2020; 51:2.
5. Paciolla C, Fortunato S, Dipierro N, Paradiso A, Leonardis SD, Mastropasqua L, *et al*. Vitamin C in Plants: From Functions to Bio-fortification. Antioxidants (Basel). 2019; 8:519.
6. Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Yoshiba Y. Effect of free Proline accumulation in petunias under drought stress. J. Exp. Bot. 2005; 56:1975-1981.
7. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Bio-chem. 2010; 48:909-930.

8. Alhasnawi AN, Cheradziah CMZ, Kadhimi AA, Isahak A, Mohamad A, Yusoff WMW. Enhancement of antioxidant enzyme activities in rice callus by ascorbic acid under salinity stress. Biologa Planturm. 2016; 60(4):783-787.
9. Yassin ET. Assessment of diode laser radiation and folic acid pretreatments on germination, seedlings and callous growth pf *Pisum sativium* L. plant under salinity stress. Thesis, Colloge of Science, University of Mosul, 2020.
10. Zhu JK. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology. 2002; 53:247-273.
11. Alhasnawi AN, Che Radziahi CMZ, Kadhimi AA, Isahaki A, Yusoff WMW, Mohammad A. Enhancement of antioxidant enzyme activities in rice callus by ascorbic acid under salinity stress, Biologia Plantarum. 2019; 60(4):783-787.
12. Cruz JL, Coelho EF, Fikho MAC, Santos AA. Salinity reduce nutrients absorption and efficiency of their utilization in cassava plants. Soil Sc. 2018; 48(11):1-12.
13. Moradi M, Saberali SF. Effect of salinity on germination and seedling growth of *Trigonella foenum-graecum*, *Dracocephalum moldavica*, *Satureja hortensis* and *Anethum graveolens*. J saudi Soc. of Agric. Sci. 2019; 18:316-323.
14. Barth C, De Tullio M, Conklin PL. The role of ascorbic acid in the control of flowering time and the onset of senescence. J Exp. Bot. 2006; 57:1657-1665.
15. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 1962; 15:473-477.
16. Mohammad AMS, Al-Barhawi RK, Abood SA. Effect of some growth regulators on the initiation and growth of sunflowers callus. J Univ. Kuwait (Sci.). 1986; 13(2):199-205.
17. Bates LS, Waldren RP, Teare, JD. Rapid determination of free proline for water stress studies. Plant Soil. 1973; 93:205-207.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall R.J. Protein measurement with the folin-phenol regents. J. Biol. Chem. 1951; 193:265-275.
19. Herbert D, Phipps PJ, Strange RE. Chemical Analysis of Microbial Cells. Method Microbial. 1971; 5:209-344.
20. Cherry JH. Nucleic acid determination in storage tissue of higher plants. Plant Physiol. 1962; 37:670-678.
21. Giles KW, Mayer A. Determination of DNA concentration with diphenylamine reagent. Meth. Enzymol. 1967; 12:163.
22. Pitotti A, Elizalde BE, Anese M. Effect of caramelizetion and mail lard reaction products on peroxidase activity. J Food Biochem. 1995; 18:445-457.
23. Aebi H. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.), 2nd Ed., Academic Press, New York. 1974.
24. Nezih M. The peroxidase enzyme activity of some vegetables and its resistance to heat. Food Agric. 1985; 36(9):877-880.
25. Baskaran L, Sundaramoorthy P, Chidambaram ALA, Ganesh KS. Growth and physiological activity of green gram (*Vigna radiata* L.) under effluent stress. Bot. Res. Int. 2009; 2(2):107-114.
26. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic bio-membranes. Method Enzymol. 1987; 148:350-382.
27. Vopyan VG. Agricultural Chemistry. English Translation, Mir publishers. 1st ed, 1984.
28. Bratf-Alean D, Cristea VM, Agachi PS, Irimie DF. Improvement of sunflower oil extraction by modeling and simulation. Revue Roumaine de Chimie. 2008; 53(9):881-888.
29. Smirnoff N, Cumbes Q. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989; 28:1057-1060.
30. Alhasnawi AN, Kadhimi AA, Mohamad A, Yusoff WW, Zain CM. Plant tissue culture and proline accumulation in abiotic stress: A review. J basic appl. Sci. Res. 2014c; 4:119-124.
31. Sukma KPW, Daryono BS, PurnomoI, Suprapt I. Callu response of hybrid and Madura local corn to salt stress. The 3rd International Conference on Biosciences IOP Conf. Series. Earth and Environmental Science. 2020; 457.
32. Ben-Zioni A, Itai C, Vaadia Y. Water and salt stresses, kinitine and protein synthesis in tobacco leaves, Plant Physiol. 1967; 42:361-365.
33. Ibrahim MM, Arafa NM, Aly UI. Antioxidant activity, phenol and flavonoid contents of plant and callus cultures of Plectranthus barbatus andrews. Egyptian Pharm. J. 2018; 17(1):32-39.
34. Hussein KA. The roles of glutathione and ascorbic acid in Na-detoxification in terms of rooting response of mungbean cuttings. J University of Karbala. 2018; 16(1):136-142.
35. Yassin ET. Assessment of diode laser radiation and folic acid pretreatments on germination, seedlings and callus growth of *Pisum sativum* L. plant under salinity stress, A Thesis, University of Mosul, Iraq.
36. Khan TA, Mazid M, Mohammed F. Ascorbic acid: an enigmatic molecule to developmental and environmental stress in plant. Int. J Appl. Biol. Pharm. Tech. 2011; 2(3):468-483.
37. Seidlitz M, Zabeau M, Vanmontagu D, Inze D, Vanbreusegem F. Catalase deficiency drastically affects gene expression includ- ed by high light in Arabidopsis thaliana. Plant J. 2004; 39(1):45-58.
38. Gang N, Manchanda G. ROS generation in plants: boon or bane. Plant Bio. Sys. 2009; 143:8-96.
39. Gupta B, Huang B. Mechanism of Salinity Tolerance in Plants. Physiological, Biochemical and Molecular Characterization. J Genomics. 2014, 1-18.
40. Dogan M. Effect of salt stress on *in vitro* organogenesis from nodal explant of Limnophila aromatica (Lamk.) Merr and Bacopa monnieri (L.)Wettst and their physio-morphological and biochemical responses, Physiology and Molecular Biology of Plants. 2020; 26:803-816.
41. Azmi, AR, Alam, SM. Effect of salt stress on germination, growth, leaf anatomy and mineral elements composition of wheat cultivars. Acta Physiol. Plant. 1990; 12(3): 215-224.
42. Kandil AA, Sharief AE, Alkhamsa KD. Effect of antioxidants and salinity stress on seedlings parameters of some wheat cultivars, Research Journal of Seed Science. 2018; 11:12-21.
43. Stevens R, Harvey G, Davies G. Separating the effects of folair and root salt uptake on growth and mineral composition of four grapevine cultivars on their own roots and on Ramsey rootstock, Journal of the American Society for Horticultral Science. 1996; 121(3): 569-575.

44. Khan DH, Frakland B. Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil profile and plant, Plant and Soil. 1983; 70(3):335-345.
45. Gomes MA, Pestana IA, Santa-Catarine C, Hauser- Davi RA, Suzuki MS. Salinity effects on photosynthetic pigments, nitric oxide in *Salvinia auriculata* Auble. Acta Limunologica Brasiliensia. 2017; 29:1-13.
46. Sajedi N, Shahbazi S, Jashni R. Effect of salinity levesl and seed weight on germination of wheat cultivars. Advances in Environmental Biology. 2019; 6(11):2917-2921.
47. Awad J, Stotz HU, Fekete A, Krischke M, Engert C, Havaux M, *et al*. 2-Cysteine peroxiredoxins and thylakoid ascorbat peroxidase create a water-water cycle that is essential to protect the photosynthetic apparatus under high light stress conditions. Plant Physiol. 2015; 167:1592-1603.
48. Barth C, Moeder W, Klessig DF, Conklin PL. The timing of senescence and response to pathogens is altered in the ascorbate-deficient Arabidopsis mutant vitamin c-1. Plant Physiol. 2004; 134:1784-1792.
49. Weaver LM, Gan S, Quirino B, Amasino RM. A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment, Plant Molecular Biology. 1998; 37:455-469.
50. Ahmad SAH. Effect of water stress and hill spacing on seed yield and some growth traits of sunflower. The Iraqi Journal of Agricultural Sci. 2012; 43(4):43-72.
51. Tran VH, Andries A, Temme and Lisa A. Donovan. Wild and Cultivated Sunflower (Helianthus annuus L.) Do Not Differ in Salinity Tolerance When Taking Vigor into Account, Agronomy. 2020; 10(7):1013.
52. Strogonov BP, Kabanov VV, Pakova MM. Feature of protein and nucleic acid metabolism during formative changes in plant under salinization conditions. Soviet Plant Physio. l970; 17:394-397.
53. Ghassemi-Golezani K, Taifeh-Noori M, Oustan S, Moghaddam M, Seyyed-Rahmani S. Oil and Protein Accumulation in Soybean Grains under Salinity Stress, Not Sci Biol. 2010; 2(2):64-67.
54. Dolatabadian A, Sanavy SAM. Effect of the Ascorbic Acid, Pyridoxine and Hydrogen Peroxide Treatments on Germination, Catalase Activity, Protein and Malondialdehyde Content of Three Oil Seeds, Not. Bot. Hort. Agrobot. Cluj. 2008; 36(2):61-66.