A COMPREHENSIVE STUDY ON THE IMPACT OF SALT ON SUNFLOWER GROWTH: BIOCHEMICAL ALTERATIONS IN SEEDING AND EARLY GROWTH STAGES

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ABSTRACT

Sunflower plants are semi-tolerant to salt stress. Calcium modulates the expression of oubain-sensitive ATPases, responsible for sodium fluxes in cells. Salt stress delays degradation of oil body (OB) membrane proteins. Serotonin and melatonin contents are elevated in response to salt stress. NO negatively regulates the total glutathione homeostasis and regulates polyamine and glycine betaine homeostasis in response to salt stress. An intricate biochemical crosstalk is thus observed to control salt tolerance mechanisms in sunflower. Climate change is causing soil salinization, resulting in crop losses throughout the world. The ability of plants to tolerate salt stress is determined by multiple biochemical and molecular pathways. Here we discuss physiological, biochemical, and cellular modulations in plants in response to salt stress. Accelerated antioxidant activities and osmotic adjustment by the formation of organic and inorganic osmolytes are significant and effective salinity tolerance mechanisms for crop plants. In addition, polyamines improve salt tolerance by regulating various physiological mechanisms, including rhizogenesis, somatic embryogenesis, maintenance of cell pH, and ionic homeostasis.

Keywords: *Sunflower plants, salt tolerance, organic and inorganic osmolytes, multiple bio-chemical, Salt stress, Sunflower oil, maintenance of cell pH.*

INTRODUCTION

Several crucial agronomic factors have impeded the further expansion of sunflowers. Because sunflowers are so susceptible to disease, two of its genetic potentials—improving oil quality and seed yield—are severely limited. Genetic diversity in wild sunflowers is far higher than in cultivated sunflowers, according to experts in population genetics. There is little optimism for the future development of this economically important crop, according to DNA data on the origins and growth of cultivated sunflower. Nevertheless, genomic research has shown that local populations and wild sunflower species exhibit a great deal of variability, sometimes called "primitive epolymorphism." "Interspecific hybridization" refers to matings between various species that belong to the same class. Two distinct varieties are cultivated. Obtaining oilseed comprises the first stage. Because of its high oil content, the little black seed is ideal for processing into sunflower meal and oil. This seed is also often used in bird feeders. The confectionary sunflower, often called the non-oilseed sunflower, comes next. Many breads and snacks include this bigger seed, which has black and white stripes. One may get high-oleic, linoleic, and mid-oleic sunflower oils. In order to create each one, conventional breeding methods were used. Different concentrations of oleic acid are present in these special oils. There are three varieties of sunflower oil, and they're all great for humans and the planet since they're safe, effective, and not transgenic.

The kernels or seeds of sunflowers, when candied, often have white stripes on a black backdrop. A seed is considered "in-shell" if it has retained all of its "meat" inside its shell and has not crumbled. Roasted and spicy variants are among the most popular. The presence of salts in both water and soil is a major concern for farmers worldwide.

LITERATURE REVIEW

Uttam Kumar Kanp [2021] the vigorousness of Helianthus annuus L. cv. Morden sunflower seeds was markedly reduced when exposed to circumstances that hastened ageing. Alternately, you might subject the seeds to the mist of eucalyptus oil for 60 days under the same settings to speed up the ageing process. Another alternative is to use them in an accelerated ageing process for 0 to 60 days at 99.5% relative humidity and 32±2°C. The seeds were treated for 8 hours with ascorbic acid and sodium-dikegulac (Na-DK; 2,3:4-6- di-O-isopropylidine-α-L-xylo-2-hexalofuranosate) before the ageing process was accelerated. Compared to the control group, plants grown from chemically treated seeds performed better. Some of the metrics used to measure this are root and shoot length, fresh and dried weight, field emergence capacity, and weight. Catalase and peroxidase enzyme activity, together with increases in chlorophyll, protein, DNA, and RNA, proved that pretreatments greatly improved the plants' potential, independent of storage conditions.

Muhammad Naveed [2021] claims that in semi-arid and dry places, agricultural productivity is significantly affected by soil salt deposition. In regions with little precipitation or high evaporation rates, salt may collect. The end effect is that excess salts are unable to reach crop root zones quickly enough. To lessen the impact of salts on plants, inorganic and biological amendments may be used. In naturally saline soils, the sunflower plants (Helianthus annuus) were studied for their growth, seed production, chemical and physiological traits, and beneficial impacts as a result of gypsum (GP), composted cow dung (CCD), and a mix of the two. As a result of adding salts like $CaCl_2$, $MgSO_4$, and NaCl, soil with saline-sodic components was created. At 15, 6, and 12 dS m∐1, the electrical conductivity (EC) was determined by using the sodium adsorption ratio (SAR). Overstimulation with salt had a deleterious effect on sunflower development, production, physiological processes, and chemical components.

Gebremedhn Yohannes [2020] Camelina sativa shows promise as a low-input biodiesel crop for marginal sites due to its high yields and abundance of omega-3 fatty acids. Because of its ionic and osmotic properties, salt has the ability to negatively impact germination, growth, and yield, which might be a problem in agriculture. Here, Camelina sativa seedlings were subjected to varying salt concentrations (KCl and NaCl). The agricultural research department of Wachemo University provided us with seedlings of Camelina sativa. We varied the concentrations of sodium chloride and potassium chloride in four separate controlled trials. A range of 0 to 5 g/L was seen in the results. Several variables related to C. sativa seedlings were studied, including germination %, root-shoot ratio, relative injury rates, relative germination index, relative germination speed, gunning index, and relative injury rates of NaCl and

KCl. These traits differed considerably ($p<0.05$), according to the research. At the 5% level of significance, only the decrease in shoot height and shoot length, not the salinity level or salt type, were shown to be important factors. We couldn't find anything comparable when we looked through the other criteria.

Haimeid, El-Hamaa R. [2020] Using presoaked sunflower seeds treated with different concentrations of glutathione, investigated the effects of salt stress on growth, metabolic markers, and yield characteristics in 2020. The experiment was conducted in a greenhouse over the course of two growth seasons. Progressive release of seawater, specific minerals, photosynthetic pigments, and salts occurred when sunflower plants were exposed to concentrations of 0, 3, and 6000 mg L^{-1}. When sunflower seeds were exposed to different levels of salt, their oil percentage, protein percentage, and yield metrics were significantly lowered. On the other hand, total soluble carbs, proline, free amino acids, hydrogen peroxide, and lipid peroxidation were all increased upon exposure to salt. The salt content and levels of some antioxidant enzymes were both elevated when salt stress was present.

Abdullah Ahmed Bjaili [2019] was released from prison. King Abdulaziz University's Agricultural Research Station in Hada Al-Sham studied three sunflower varieties—Cv. Sakha-53 of Egypt, Cv. Argentina-11 of Argentina, and Cv. May Hybrid of Turkey—during the 2017 and 2018 harvest seasons. Defoliation (0, 2, and 4 leaves/plant) and nitrogen fertilizer concentrations (100, 200, and 300 kg N/ha) were varied for each cultivar. Nitrogen, defoliation, cultivars, and interactions were shown to have a substantial effect on all of the characteristics that were studied. Seed protein content, total chlorophyll levels, and days to flowering were all improved by 300 kg N/ha of nitrogen fertilizer, however seed oil content was reduced. Defoliation, the act of removing all four leaves from a sunflower plant at once, causes the plant to grow taller and produces more oil, protein, and chlorophyll in its seeds. Turkish variants 'May Hybrid,' 'Sakha 53,' and 'Argentina-11' have much increased protein content. The variety 'May Hybrid' yielded the most protein with only two chopped leaves, while 'Sakha 53' generated the most oil.

Seed germination

After sterilising the sunflower (Helianthus annuus) seeds with 0.005% $HgCl_2$, they were placed in plastic trays on top of germination paper. While the seedlings were being grown in darkness at a temperature of 25˚C, we watered them with a half-strength Hoagland solution. In order to make a salt treatment solution, 120 mM NaCl was added to the Hoagland solution. For our future studies, we harvested cotyledons and roots from seedlings that grew normally. At equimolar doses (100, 250, 500, and 1000 µM, produced in half-strength Hoagland solution), several foreign NO-related therapies, including sodium nitroprusside (SNP), aminoguanidine, peroxynitrite, and cPTIO, were administered after the radical had erupted from the seeds.

Sunflower seeds

The fact that sunflower seeds contain oil is common knowledge across the globe. Oilseed plants do best in colder climates with less precipitation since they are hardy and can endure dry conditions. It is traditional to display mature sunflower seeds with their heads erect. The epicarp and mesocarp are the two layers that encase the seeds. In this context, the "endocarp" refers to the plant's underside. In an achene, the kernel accounts for about 80% of its weight, with cellulolytic components and lignin of the shell accounting for the remaining 20%. Perhaps the seeds are concealed inside this husk. Through painstaking breeding, sunflowers' seed oil content has been increased from 25% to 40%. The oil from seeds may be extracted primarily by either heating the seeds or pressing them cold. Margarine, salad dressing, and, of all things, cold-pressed oil are all possible byproducts of this multipurpose oil. But hot-press oil is used in many household products, including paints, soaps, detergents, and insecticides. The fatty and protein-rich sunflower seeds constitute a staple diet for birds that live in the open air. Statistical analysis shows that the size of a farm is significantly connected to the possible effect of a certain bird population on sunflower seed output at any given time.

Sunflower oil (Sunfoil)

Pure sunflower oil is made by refining and distilling the nonvolatile oil that is taken from sunflower seeds. To make frying pans and other home appliances, emollients are an essential ingredient. Russia and Ukraine, the top two sunflower oil producers in the world, gathered over 16 million metric tonnes in 2017. The countries that produced sunflower oil, with a total of 15.8 million metric tonnes in 2014, are included in this list. With 0.9 MMt, Argentina, Russia, and Turkey are the top three producers globally. The world's leading manufacturers in this field are located in Russia and Ukraine. Sunflower oil is mostly composed of oleic and linoleic acids. Unsaturated linoleic acid stands in contrast to saturated oleic acid, another kind of lipid. Processing methods and plant type are two variables that could impact the exact fatty acid ratio of sunflower oil. The oil has a distinct aroma and a somewhat amber hue.

Substance and nutritional constituent of sunflower seeds

The importance of a well-rounded diet in warding off diseases like obesity, cardiovascular disease, and neurological problems is being acknowledged by food scientists, nutritionists, and dietitians on a growing scale. Vegetable oils, including sunfoil, are hence in high demand. Sunfoil is delightful for humans to eat because of its unique flavor and texture. Edible oils often include phytonutrients, minerals, vitamins (especially vitamin E), antioxidants, and dietary fiber; nonetheless, their biological origin mostly determines their exact content. Diabetes, cancer, and obesity are all heightened when saturated fats are abundant in the diet. Using chemical and biological methods, meals might have their useful properties enhanced. The higher concentration of polyunsaturated fatty acids in plant-based lipids makes them healthier than animal-based fats. These acids improve organ function and lower the risk of fatal diseases like cardiovascular disease. Soybean, sunflower, rapeseed, and olive oils are the most common types of cooking oils.

Benefits of Sunflower

The field of phytomedicine has recently seen a boom in the study and use of plant extracts. Sunflower oil is an example of nutraceutical functional oil. Vit E, chlorophylls, phytosterols, phenolics, carotenoids, and antioxidants like tocopherols and tocotrienols are just a few of the beneficial compounds found in abundance in this food. Based on the research that is currently available, common oilseed crops might potentially provide nutritional advantages. A growing numbers of people are also of the belief that phytomedicines—which use functional oils derived from plants rather than manufactured pharmaceuticals—could one day help find treatments for a wide range of human illnesses. The potential for bioactive food extracts derived from medicinal plants to control and avoid illnesses has generated considerable interest.

RESEARCH METHODOLOGY

The sunflower seeds, classified as HelianthusannuusL. cv. KBSH44, were kindly provided by the Agricultural Sciences department of Bangalore University. Every one of us feels their gratitude. We cleaned the seeds with distilled water after sterilising them with 0.005% $HgCl_2$. Then, to encourage germination, we immersed them in water for four hours. The seeds were placed in plastic trays and covered with germination sheets after soaking in distilled water. The sheets were irrigated using Hoagland feeding solution, which was mixed at half strength. The Hoagland solution was supplemented with 120 mM NaCl after some minor laboratory changes. Before placing the seeds in the growing chamber, they were fanned out on a tray. During the allotted period, which might range from 2 to 6 days, they were maintained at a temperature of 25±2°C. No sodium chloride was added to the watering solution that was used on Hoagl and the control plants. When watering seedlings that had been treated with either nothing or NaCl, an extra solution containing 30µM ouabain was sometimes added to the Hoagl mixture. After that, 5-µM 1-napthylphthalamic acid (NPA), exogenous serotonin, and melatonin were mixed with the Hoagland solution in equal amounts. The concentrations ranged from 5 to 40 µM. Supplements containing 15µ M serotonin and melatonin was administered to seedlings treated with NaCl at regular intervals. It was noted how long the main roots were and how wide the hypocotyl was. All of the seedlings used in these tests exhibited typical hypocotyl and root length development.

RESULTS AND DISCUSSION

NaCl Stress Slows Down Sunflower Seedling Growth

When exposed to salt stress, several sunflower varieties react in unique ways. Previous work by researchers at Delhi University used seedling responses to NaCl stress to establish optimal NaCl concentrations for sunflowers. Sunflower seedlings exhibit concentration-dependent patterns of hypocotyl expansion and root proliferation when exposed to 40, 80 and 120 mM NaCl, respectively.

Table 1: as a defence mechanism against salt stress, seedlings secretes serotonin and melatonin along separate branches of their maternal lineage

Days of seedling growth	Serotonin (µg.gm FW) Melatonin (µg.gm FW)			
	(-)NaCl	120mM NaCl	(-)NaCl	120mM NaCl
2	9.12 ±0.32	$15 \pm 0.57^{*}$	4.6 ± 0.49	6.3± 0.32
4	27.8 ±1.2	$32 \pm 0.50^{**}$	7.1 ±0.4	$14.3 \pm 0.32^{*}$

			4	
6	10.8 ±0.92	12.3 ±0.32*	7.6 ±1.3	5.1 ±0.57
Values indicate mean ± SD (n=3) and analysis by independent t-test (*P<0.03.**P<0.01				

To do this, we used antibodies that specifically target the chemical localization of melatonin and serotonin to probe a three-centimeter area around the main root differentiation zone. This study made use of both longitudinal radial and transverse sections. The pericycle, endodermis, and vascular bundle of the root were already damaged by the stress of NaCl while germination was underway.

Table 2: in reaction to sodium stress, melatonin and serotonin build in the cells of sunflower cotyledons that contain the oil body

Days of seedling growth	Serotonin (µg.gm FW) (-)NaCl	Serotonin (µg.gm FW) 120mM NaCl	Melatonin (µg.gm FW) (-)NaCl	Melatonin (µg.gm FW) 120mM NaCl
2	23.4 ±2.7	37.4±2.4	6.1±1.0	35±1.8
4	22.3 ±1.5	28.9 ±2.02	13.4 ±0.66	18.7 ±1.41
6	18.9 ±1.7	30.2 ±0.63	7.8 ±1.02	8.5±0.90
Values indicate mean ± SD (n=3) and analysis by independent t-test (*P<0.03.**P<0.01				

Serotonin and melatonin accumulation in cotyledon cells, induced by NaCl stress, was seen at 2, 4, and 6 days into seedling growth. The accumulation of both the control and salt-treated cotyledons increased 2 days after germination, but then decreased as the plants aged. More serotonin was found in oil bodies of cotyledon cells that were treated with salt. Melatonin, like serotonin, accumulates in response to salt stress; two days after germination, cotyledons display the greatest amounts. The accumulation of serotonin and melatonin, which are produced by NaCl stress, was shown by the increased retention of oil bodies in seedling cotyledons that were treated with salt, as demonstrated by Nile red stained sections.

There is a direct correlation between salt stress and the release of IAA, serotonin, and melatonin in sunflower seedlings

Animals and plants both contain the indoleamines melatonin and serotonin. All of these processes—organogenesis, adaptation to biotic stress, ageing, and root and shoot growth—are regulated by these components. Some intriguing in vitro studies on root and shoot development has shown that melatonin and serotonin behave similarly to auxin. There has been a lot of recent focus on enzymes and their function in regulating plant serotonin synthesis. Our findings indicate that the application of exogenous melatonin and serotonin significantly impacts the root growth of seedlings. Serotonin has the potential to extend roots and increase terminal branch number, according to previous research. The available evidence suggests that melatonin promotes the development of bigger main roots and more intricate lateral root systems in sunflower seedlings.

Possible Involvement of Auxin Efflux Proteins (Pin) and Actin Distribution in the Sensing of Nacl Stress Through Endogenous Seroton In Distribution

The process of root elongation and lateral root initiation is aided by lesions in auxin outflow and associated acropetal transfer. The SOS system regulates auxin levels and lateral root growth in response to mild NaCl stress. One of the several routes via which salt stress regulates root development in Arabidopsis is ABA signaling.it was noted that stress with 150 mM NaCl retarded Arabidopsis lateral root growth and decreased active basipetal auxin transport both simultaneously. The findings showed that both primary root development and lateral root elongation were reduced when Arabidopsis was treated with 20µM NPA.

CONCLUSION

Even though sunflower seeds are immune to sodium chloride stress when they sprout, etiolated seedlings may experience this stress as early as 48 hours (or 2 days) after germination. Shorter main roots and reduced hypocotyl elongation are two of them. Plants respond to salinity stress at physiological, cellular, genetic, and metabolic levels. Previous research demonstrates that among plant responses to salinity, mechanisms that control ion uptake, transport, and balance, as well as water potential, photosynthesis, cell division, osmotic adjustment, enzymatic activities, polyamine regulation, stress signaling, and regulation of root apoplastic barriers play critical roles in plant tolerance to salinity. The last phases of seedling growth, which begin around four or six days after seeds germinate, are characterized by an absence of cotyledon development and a very infrequent lateral root branching. Sunflower seedlings of various stages respond differently to NaCl stress, and this is because the root and cotyledon concentrations of melatonin and serotonin vary. Applying synthetic melatonin and serotonin to seedlings may protect them from the negative effects of salt stress. As compared to seedlings grown in a control environment and treated with NaCl, those exposed to 15 µM serotonin showed a 20% increase in main root length and a 14% increase in hypocotyl elongation at 2 days of age.

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