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Effect of Sodium Azide Mutagen on Lavender Plant Salinity Tolerance

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Abstract

This study aimed to induce mutation in *Lavandula angustifolia* plants to enhance salinity tolerance using sodium azide as a chemical mutagen. Lavender seeds were treated with various concentrations of sodium azide (0.0%, 0.5%, 1.0%, 2.0%, and 3.0%). The mutagenized seedlings were subsequently subjected to different salinity levels (0.0, 500, 1000, 2000, and 3000 ppm NaCl). Results indicated that a 0.5 and 1.0% sodium azide concentration provided optimal mutagenesis, enhancing salinity tolerance at 500 and 1000 ppm NaCl.

Treated plants showed improved germination rates (77.1 and 75.5%), increased growth parameters, and enhanced biochemical evaluation, including higher chlorophyll content and proline accumulation. Proline content decreased to 5.6 mg/g fresh weight at 500 ppm but increased to 8.6 and 9.5 mg/g at higher NaCl concentrations. The highest carbohydrate levels were recorded at 500 and 1000 ppm NaCl. Sodium ion concentration in plants increased to 13.1 and 15.4 g/kg at 2000 and 3000 ppm NaCl, respectively. Potassium content was higher in plants treated with 500 and 1000 ppm NaCl. Iron ion content significantly increased with higher NaCl concentrations, peaking at 3000 ppm, while a decrease was observed at 500 ppm. Zinc ion content was highest at 3000 ppm NaCl (5.7 mg/kg) and decreased to 4.5 mg/kg at 500 ppm. Moderate salinity levels (500 and 1000 ppm) led to significant improvements in pigment concentrations and ion balance, maintaining higher potassium and lower sodium levels. Genetic variability induced by sodium azide was confirmed via ISSR-PCR analysis, revealing unique polymorphic banding patterns in treated plants.

Keywords: lavender plants, seeds germination, salinity stress, sodium azide, mutation, plant growth parameters, ISSR-PCR.

1. Introduction

Global warming and declining water quality due to salinization pose significant challenges for agriculture and food security. Excessive groundwater extraction, seawater intrusion, and water reuse have exacerbated salinity levels, impacting plant growth and productivity **(Alfarrah and Walraevens, 2018; Cai *et al*., 2014)**. The growth and development of various plant species can be influenced by saline irrigation, even when applied at low concentrations **(Pessarakli and Szabolcs, 2011)**. The impact of salt on plants in different environments, including the wilderness, fields, and gardens, is influenced by a range of factors. These factors encompass ion concentration, soil composition, closeness to the sea, height, evaporation rate, temperature, and frequency of rainfall **(Lambers, 2003)**. The impact of salinity on agricultural productivity is a significant concern in several regions globally, with projections indicating a future increase in salinity levels (**Batool *et al*., 2014**).

Lavender (*Lavandula angustifolia*), a perennial aromatic plant widely used in the fragrance, medicinal, and food industries, as well as in fragrant garden design, is particularly susceptible to saline conditions. It belongs to the Nepetoideae subfamily within the Lamiaceae family **(Hassanpouraghdam *et al*., 2011)**

and commonly found in the Mediterranean region. Its historical usage may be traced back to ancient times, as shown by **Pohrib and Nistor (2012)**. The pharmaceutical application of the lavender plant and its compounds has been historically employed for several purposes, including its anticonvulsant properties, treatment of cold and flu symptoms, diuretic effects, digestive aid, calming qualities, stimulation of perspiration, and management of anxiety and grief **(Afsharypuor and Azarbayejany, 2006)**. Many varieties of lavender are valued for their cosmetic and medicinal properties, including antimicrobial, analgesic, antifungal, soothing, relaxing, anti-inflammatory, rejuvenating, healing, antiseptic, and tonic properties (**Hanamanthagouda *et al*., 2010; Cantor *et al*., 2018).** The Lavandula genus includes about 45 species and approximately 400 subspecies **(Koulivand *et al*., 2013)**. According to **Mokhtarzadeh *et al*. (2013)**, the genus is native to the Mediterranean region, extending from southern Europe to northern and eastern Africa, and includes areas from the Middle East to southwestern Asia and southeastern India.

Mutations are of significant importance in enhancing the agronomic characteristics of plants and conferring resilience to both biotic and abiotic stressors. Seeds serve as a viable means to investigate chemical mutagens that induce alterations in the genetic material of a cell. Following exposure to chemical mutagens, seeds exhibit the effects of mutagens in the form of altered morphological characteristics resulting from disrupted physiological processes. Germination refers to the biological process via which a seed initiates growth subsequent to a period of quiescence. Therefore, the process of germination concludes upon the emergence of the root. The process of imbibition, which refers to the uptake of water by seeds, is characterized by concurrent phenomena such as cellular growth, cell wall synthesis, and metabolic stimulation. According to **Barroco *et al*., (2005),** there is evidence indicating that cell division typically takes place subsequent to the process of germination. The augmentation in cellular proliferation required for the process of germination can be attributed to the elongation of cells. Within a brief timeframe, a restricted quantity of cells experience elongation and engage in differentiation mechanisms driven by swift metabolic alterations that precede cellular division.

Chemical mutagenicity is posited as the sole factor responsible for inducing mutations in organisms. These mutagens have an impact on the process of seed germination. The rate of seed germination is contingent upon the characteristics of the mutagen and the dosage of its application. According to **Yuan and Zhang (1993)**, several mutagens have been found to have a chemical influence that results in damage to the chromosomes of plants. Chemical mutagens typically elicit induced mutations, causing alterations in the base pairs, particularly GCAT, which then lead to modifications in amino acids. These changes ultimately affect the functionality of proteins **(Van der Veen, 1966)**. The chemical mutagen induced significant morphological alterations and structural modifications in plants, resulting in a notable deviation from their typical characteristics.

Sodium azide (NaN_3) is a chemical mutagen that has demonstrated significant mutagenic properties in crop plants, rendering it one of the most potent mutagens in this context. Mutations arise as a result of the synthesis of the organic metabolite derived from the azide molecule. The aforementioned metabolite is capable of trans-locating into the nucleus, where it engages in interactions with DNA molecules, ultimately resulting in the induction of a point mutation within the genome. Various parameters, including mutagen characteristics, treatment time, pH levels before and after treatment, temperature, and oxygen concentrations, might influence the impact of mutagens. The dosage of mutagenesis employed is a crucial factor to be taken into account in any mutagenesis initiative. It has been widely documented that elevated quantities of mutagens have a propensity to amplify biological harm. According to **Khan *et al*. (2009),** using sodium azide is regarded as a mutagenesis method that has relatively low risks and high efficiency. This is due to the fact that sodium azide yields a substantial amount of mutagenesis with modest sterility rates, but its physiological effects are thought to be minimal.

Chemical mutagenesis is a widely recognized and valuable technique utilized for enhancing the yield and characteristics of agricultural crops. Sodium azide has demonstrated its efficacy as a chemical mutagen in the induction of genetic diversity. Therefore, this chemical mutagen has emerged as a significant instrument for augmenting the agronomic characteristics of cultivated plant species. Scientists have proved the significance of mutant breeding in enhancing genetic diversity for quantitative features in several crop plants **(Khan and Goyal, 2009; Mostafa, 2011).**

Soil salinity is widely recognized as a significant environmental stressor that poses detrimental effects on plant species **(Min and Su, 2016)**. According to **Rao *et al*. (2019)**, the presence of salt stress induces oxidative stress, which in turn has a detrimental impact on crop growth, development, and yield. Additionally, the ion toxicity component of salt stress further exacerbates these unfavorable effects. In response to stressful conditions, plants typically exhibit a reduction or total inhibition of growth **(Mittal *et al*., 2018),** a suppression of photosynthesis **(Sun *et al*., 2016)**, and the activation of diverse tolerance mechanisms, such as the accumulation of various osmolytes.

The growth of plants and crops is negatively impacted by salinity mostly due to the production of highly reactive oxygen species (ROS) (**Gohari *et al*., 2020)**. The accumulation of sodium ions (Na^+) in conditions of high salinity can have detrimental effects on various metabolic processes. Specifically, the function of the Calvin enzyme, as well as the phenylpropanoid pathway and glycolysis, may be compromised. The proper maintenance of ionic homeostasis and the stability of cell membranes are closely associated with sufficient plant nutrition in the presence of salt, as highlighted by **Zhao *et al*. (2020)**. The impact of salinity on ion homeostasis is mediated by a series of interconnected processes, leading to alterations in gene expression and metabolic profiles that enable organisms to cope with the stress induced by high salt levels. The presence of sufficient quantities of crucial minerals, such as iron (Fe) and zinc (Zn), greatly enhances the ability of plants to withstand the adverse effects of salinity-induced stress. Zinc has been identified as having both structural and functional significance in maintaining the integrity of cell membranes **(Marschner, 1995)**. Additionally, zinc plays a crucial part in the process of detoxification and regulation of free radicals, toxic fatty acids, and sulfhydryl residues inside cell membranes. Iron also has a significant impact on the functioning and behavior of several enzymes involved in the process of chlorophyll production, photosynthesis, and transpiration.

Salinity is widely recognized as a significant stressor in plant growth and yield, with the potential to cause plant mortality when exposed to chronic saline conditions **(Parida and Das, 2005).** The tolerance of plants to salinity stress is contingent upon their capacity to either prevent the entry of salt into buds or endure elevated levels of salt in leaves **(James *et al*., 2002)**. The following conditions have the potential to induce genetic variability in reproductive plants, as identified by **Werner *et al*. (2015)**. The presence of genetic variation necessitates the utilization of a technique to identify and discern morphological distinctions in grown plants **(Saha *et al*., 2016)**.

The present study employed ISSR-PCR as a molecular marker to evaluate the genetic variability of lavender plants. This study investigates the use of sodium azide to induce mutations in lavender plants, aiming to enhance their salinity tolerance. The objectives include evaluating the physiological, biochemical, and genetic responses of lavender to salinity stress and exploring the potential of sodium azide in breeding programs.

2. Materials and Methods

This study was conducted during 2022 to 2024 at Breeding Research department of fruit trees, ornamental and woody plants, Horticultural Research Institute, Agricultural Research Center, Egypt.

2.1. Plant Materials and Sodium azide Treatment

Lavender seeds **(Fig. 1)** imported from the Netherlands were stored at 4°C for two weeks. Seeds were divided into five groups (50 seeds per group) and treated with sodium azide concentrations (0.0%, 0.5%, 1.0%, 2.0% and 3.0%). After immersion in phosphate-buffered sodium azide solutions (pH_3) for two hours at 30°C with agitation (20 rpm). The control treatment consisted of untreated seeds.

Fig.1. The seeds of Lavender

Following the treatment, the excess chemical residue was removed by washing the seeds with continuously flowing tap water for at least 2 hours. The seeds were then air-dried on blotting paper at room temperature (24°C). Both the control and treated seeds were subsequently germinated in carefully prepared beds within a greenhouse. Five beds were used, one for each treatment. The clay soil used for the experiment was collected from the top layer (0–15 cm) of a cultivated field. The obtained soil was air-dried, crushed, sieved through a 2 mm sieve and mixed well before being treated with different treatments. fifty seeds for each treatment were sown, then seeds were covered with a

light layer of soil and the beds were watered. Afterwards, beds were covered with a transparent plastic cover to keep the required temperature and moisture, providing that the plastic cover remains away from the soil's surface. Lavender seeds began to germinate one month after planting. Once the lavender seeds grow, the plastic cover may be removed. When the seedlings start to sprout leaves, they would be transplanted into separate pots, one plant per pot and watered regularly. After one to three months new plants are grown enough to be transplanted outside, which eliminates any frost danger and prevents harm.

Seed germination was assessed by calculating the number of seeds that sprouted on the eighth day following sowing, with results presented as a percentage. On the 40th day after sowing, data was collected on the seeds germination percentage.

2.2. Salinity Treatment

a. First Experiment

At the start of the salinity experiment (Day 1), all lavender seedling mutant were irrigated monthly with the respective NaCl concentrations until the pots reached saturation, with one liter of solution applied to each pot. To prevent the accumulation of salts, the pots were washed with tap water once a week. Lavender seedlings were exposed to NaCl at five different concentrations (0.0, 500, 1000, 2000 and 3000 ppm), with a control group receiving only water, for a duration of two months. The pH of the nutrient solution was maintained at an optimal level of 5.8, measured every two days and adjusted using H_2SO_4 (5% v/v).

The measurements including plants height, seven plants were randomly selected from each treatment. The average value for each treatment was calculated from 21 plants.

b. Second Experiment

The lavender plants mutant by Sodium azide at 0.5 and 1.0% were irrigated bimonthly with the different concentrations of NaCl (0.0, 500, 1000, 2000 and 3000 ppm) until the pots reached saturation, with one liter of solution applied to each pot for a duration of three months. The measurements including physiological evaluating as plants height, Number of branches/plant, Root length (cm), Number of roots/plant, Fresh weight of herbs/individual plant (g), Herb dry weight/plant (g) and Index of Salt Tolerance Traits (%). The biochemical evaluating as Chlorophyll content, Proline content, Total carbohydrates percentage and Mineral Nutrient Content. The seven plants were randomly selected from each treatment. The average value for each treatment was calculated from 21 plants.

2.3. Physiological Evaluating

- **Yield Components**

At the start of the treatments, initial measurements were recorded for plant height (cm), number of branches, root length (cm), number of roots per plant, herb fresh and dry weight per plant (g), and salt tolerance trait index (STTI %). These parameters were reassessed at the end of the treatments after 90 days when the treated plants were harvested. To determine the water content percentage (WC%) of the fresh material in leaves, stems, and roots, the respective parts were initially weighed (fresh weight, FW) and then dried in an oven at 60°C for 72 hours to obtain the dry weight (DW). The water content percentage was calculated using the formula:

$$WC\% = [(FW - DW)/FW] \times 100$$

The salt tolerance trait index (STTI %) was calculated as a reliable indicator of salinity tolerance based on the method described by **Chen *et al.* (2007)** for asparagus bean. The index was determined using the equation:

STTI% = (Mean fresh herb yield of salt-treated plants / Mean fresh herb yield of control plants) × 100

2.4. Biochemical Evaluating

2.4.1. Chlorophyll Content

The measurement of chlorophyll a, chlorophyll b, and total carotenoids was conducted using the methodology given by **Lichtenthaler and Wellburn (1993)**. The fresh leaf material, weighing between 50-100 mg, was pulverized using a mortar while being exposed to liquid nitrogen. Subsequently, the pulverized material was subjected to extraction using 1.0 ml of ice-cold acetone solution with an 80% concentration (v/v). The extraction process involved shaking the mixture overnight in the absence of light at a temperature of 4 °C. The samples underwent centrifugation at a speed of 12,000 revolutions per minute (rpm) and a temperature of 4°C. Subsequently, the absorbance of the resulting supernatant was measured at wavelengths of 470 nm, 645 nm, and 663 nm. The concentrations of chlorophylls and carotenoids were determined through the utilization of the following mathematical equations:

Chl a (µg m/l) = 12.21 (A663) − 2.81 (A646);

Chl b (µg m/l) = 20.13 (A646) − 5.03 (A663);

Caro (µg m/l) = (1000 A470 − 3.27 [chl a] − 104 [chl b])/227.

The concentrations of photosynthetic pigments were ultimately reported in mg/g of dry weight (DW).

2.4.2. Proline content

The proline content was determined using the methodology outlined by **Fedina *et al*., (2006).**

2.4.3. Total carbohydrates percentage

Total carbohydrates content, were determined by the phenolsulfuric method described by **(Balbaa *et al*., 1986).**

2.4.4. Minerals Nutrient Content

The minerals present in the leaves were subjected to analysis using the methodology outlined extensively in the study conducted by **Chrysargyris *et al*., (2018)**. The flame photometric technique was employed to quantify the concentrations of sodium ions (Na^+) and potassium ions (K^+). The quantification of zinc (Zn) and iron (Fe) concentrations in plant tissues was performed using atomic absorption spectroscopy, following the methodology described by **Honarjoo *et al*., (2013)**.

2.5. ISSR-PCR Analysis

To evaluate genetic variability, DNA was extracted from treated and control plants. ISSR-PCR was conducted using primers to amplify genetic regions, with polymorphic band patterns analyzed for mutagenic effects.

Leaf samples from 21 plants per treatment (7 pots per replicate) were collected in triplicate, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent DNA extraction.

The inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) was used to assess genetic variability in treated and control lavender plants. DNA was extracted from fresh foliage of greenhouse-grown seedlings, with 0.5 g of leaves ground in 600 µl of cetyltrimethylammonium bromide (CTAB) extraction buffer. Samples were incubated at 55°C for 15 minutes, followed by the addition of 600 µl of $CHCl_3$ and centrifugation at 1400 rpm for 2 minutes to recover the supernatant. DNA particles were separated by adding 50 µl of sodium acetate and 500 µl of isopropanol, followed by centrifugation at 1400 rpm for 1 minute. The resulting pellet was washed with 500 µl of 70% ethanol, centrifuged for 1 minute, and resuspended in 50 µl of nuclease-free water. DNA samples were stored at −20°C.

DNA concentration and purity were assessed using spectrophotometry and gel electrophoresis. Amplification was conducted with a thermal cycler (GenPro model TC-E-96G) using 22 primers **(Table 1)**. Each reaction mixture contained 12.5 µl of Taq DNA polymerase mixture, $MgCl_2$, buffers, deoxyribonucleotide triphosphates, 1.5 µl of primer, 2 µl of DNA, and 25 µl of nuclease-free water. Thermal cycling conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at primer-specific temperatures for 1 minute, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes, and the reaction products were stored at 4°C. Agarose gel electrophoresis (1.5%, 100 V) was conducted using Tris-ethylenediaminetetraacetic acid (1X) as the running buffer, and bands were visualized under UV light **(Godwin *et al*., 1997**).

Table 1. Primers used in ISSR amplification

Primer	Sequence	Annealing temp. (°C)
UBC 808	(AG) 8T	54.3
UBC 812	(AC) 7 C	50.0
UBC 822	(TG) 7 A	67.2
UBC 828	(CA) 8 T	48.5
UBC 833	(CA) 7RG	54.3
UBC 839	(ACC) 6 T	63.4
UBC 844	(ACA) 4 A	49.0
UBC 848	(GT) 3 C	52.0

2.6- Statistical Analysis

The experiment followed a completely randomized block design as described by **Gomez and Gomez (1984).** Data were analyzed using the MSTAT computer program **(MSTAT Development Team, 1989).** Treatment means were compared using Duncan's Multiple Range Test **(Duncan, 1955)** to identify significant differences.

3. Results and Discussion

3.1. Sodium azide treatments and seeds germination

Mutation represents a practical alternative to conventional breeding methods in crop improvement programs. Genetic diversity resulting from induced mutations, facilitated by various mutagenic agents, has significantly advanced plant breeding initiatives. This approach, along with recombinant and genetically modified breeding techniques, has been instrumental in producing superior plant varieties with economic benefits, confirming the value of mutation breeding technology. The term "mutation" refers to the process of inducing changes in genetic material through the use of chemical, physical, or biological agents **(Oladosu *et al*., 2016)**. Chemically induced mutations have proven effective not only in promoting early maturity **(Gadakh *et al*., 2015)**, but also in enhancing salinity tolerance in rice **(Revathi and Arumugam Pillai, 2015)**, improving low pH and aluminum tolerance in sugarcane **(Purnamaningsih and Hutami, 2016)**, and herbicide resistance in sugarcane **(Koch *et al*., 2012)**.

Sodium azide has been widely used to enhance crop resistance to harmful pathogens **(Devi *et al*., 2008)** and to induce desirable variations in tolerance to salinity and other abiotic stresses. In our research, the application of sodium azide at different concentrations resulted in both negative and positive responses, depending on whether the concentration was high or low. **He *et al*. (2009)** reported that sodium azide has served as a mutagen across various plant species, including *Triticum aestivum, Triticum durum, Zea mays, Oryza sativa, Lactuca sativa, Musa spp and Helianthus annuus*. This application aims to create mutant varieties that exhibit resistance or tolerance to biotic and abiotic stresses, as well as to enhance quality.

Data collected on seed germination three months after sowing revealed that the 0.5% concentration of sodium azide had the highest germination rate of 77.1%. This was followed by germination rates of 75.5% at 1.0%, 65.3% at 2.0%, and 53.2% at 3.0%, compared to the control treatment, which had a germination rate of 80.5% **(Table 2)**. These results indicate that increasing concentrations of sodium azide reduced germination percentages in lavender seeds.

Table. 2. Impact of Sodium azide on seeds germination percentage of Lavender after three months

Sodium azide Treatment (%)	Seeds germination (%)
Control	80.5 **a**
0.5	77.1 **b**
1.0	75.5 **b**
2.0	65.3 **c**
3.0	53.2 **d**

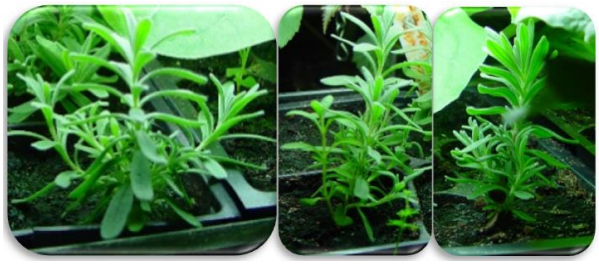

Fig. 2. Effect of different doses of Sodium azide on seeds germination of Lavender after two months

The application of sodium azide demonstrated its potential to induce variability in quantitative traits. This was evident from alterations in means, variances, and frequency distributions in treated populations. Similar findings have been reported by **Veleminsky and Angelis (1987)** in wheat. Sodium azide is a cost-effective and straightforward mutagenic agent widely used to enhance crop yield, improve quality traits, and confer resistance against biotic and abiotic stresses. However, a negative correlation was observed between increasing sodium azide concentrations and germination rates, consistent with findings in black gram by **Lal *et al*. (2009).** Elevated concentrations adversely impacted seed germination and seedling survival due to inhibitory effects.

The reduction in germination following mutagenic treatments can be attributed to disruptions in physiological and biological processes, including enzyme activity (**Khan and Al-Qurainy, 2009**), hormonal imbalances (**Ananthaswamy *et al*., 1971**), and mitotic inhibition **(Sato and Goel, 1967)**. Sodium azide's mutagenic activity involves interactions with cellular enzymes and DNA. According to **Zhang (2000),** azide anions inhibit cytochrome c oxidase and oxidative phosphorylation, leading to ATP depletion and reduced germination rates. This may also reflect adaptive mechanisms developed by seeds to tolerate sodium

azide's inhibitory effects, improving physiological conditions for germination. **Cheng and Gao (1988)** reported similar declines in barley germination rates under sodium azide treatment. Reduced ATP levels impair cellular energy supply, decelerating biological reactions and overall germination success.

Among the various mutagenic agents, sodium azide has recently been employed as a chemical mutagen to enhance agronomic characteristics. It has been applied to susceptible plant species to enhance their resistance to pests and diseases **(Khan *et al*., 2009)**. Sodium azide can result in the deletion of one or more nucleotides, a phenomenon referred to as point mutation **(Słoczyńska *et al*., 2014)**. Consequently, alterations in the amino acid sequence will take place, leading to variations in the synthesized protein and/or enzyme **(Al-Qurainy and Khan, 2009)**. Reaching the concentrations of SA from 3 to 10 µM markedly decreased both the rate of seed germination and the frequency of callus induction **(Kannan *et al*., 2015)**.

3.2. Salinity Treatment

Salt solutions are an important abiotic factor affecting the growth and health of various crops. The negative effects of salinity not only reduce horticultural crop yields and increase production costs, but also contribute to soil erosion and disruption of ecological balance **(Piwowarczyk *et al*., 2016)**. Elevated salt concentrations in the environment can lead to plant stress through two main mechanisms: elevated root zone osmotic potential due to elevated levels of solutes and the toxic effect of excessive ion concentrations **(Demir and Kocaçalişkan, 2002)**. Salinity poses a major challenge to crop development and agricultural production in many regions of the world. It is estimated that approximately 20% of the world's irrigated land is affected by salinity **(Wang *et al*., 2003)**. Soil salinity is known to be a critical abiotic stressor affecting agricultural productivity, especially in arid and semi-arid regions. In these challenging environments, water scarcity is a critical factor that severely limits crop productivity **(Tounekti *et al*., 2011).**

A. First Experiment

At the start of the salinity experiment, all lavender seedling mutant by different levels of sodium azide at (0.0, 0.5, 1.0, 2.0 and 3.0%) were irrigated bimonthly with the respective NaCl concentrations at five different concentrations (0.0, 500, 1000, 2000 and 3000 ppm), with a control group receiving only water, for a duration of three months.

The combination among different concentrations of sodium azide and NaCl were affected significantly on seedling height of Lavender. The highest seedling height resulted in the treatment of 500 ppm of NaCl with 0.5 and 1.0% of Sodium azide which recorded 28.0 and 33.0 cm/seedling compared with the control treatment 21.33 cm, following by the treatment of 1000 ppm of NaCl with 0.5 and 1.0% of Sodium azide which recorded 26.7 and 30.4 cm. However, with increasing the level of Sodium azide with high concentrations of NaCl at 2000 and 3000 ppm decreased the seeding height. The less seedling height were observed with treated the seedling by NaCl at 3000 ppm and different levels of Sodium azide, as shown in **Table 3.**

Table 3: Effect of salinity levels and Sodium azide concentrations as their combinations on seedling height of Lavender

NaCl Concentrations (ppm)	Sodium Azide Treatments (%)				
	Control	0.5	1.0	2.0	3.0
	Seedling Height (cm)				
Control	23.00 **a**	26.0 **b**	28.0 **b**	30.0 **a**	25.0 **b**
500	21.33 **ab**	28.0 **a**	33.0 **a**	30.8 **a**	28.3 **a**
1000	23.76 **a**	26.7 **b**	30.4 **ab**	26.0 **b**	22.6 **c**
2000	15.51 **c**	20.5 **c**	22.7 **c**	20.0 **c**	13.1 **d**
3000	10.62 **d**	15.2 **d**	16.8 **d**	10.2 **d**	6.0 **e**

B. Second Experiment

The lavender plants mutant by Sodium azide at 0.5 and 1.0% were irrigated bimonthly with the different concentrations of NaCl (0.0, 500, 1000, 2000 and 3000 ppm) until the pots reached saturation, with one liter of solution applied to each pot for a duration of three months. The physiological evaluating including plants height, Number of branches/plant, Root length (cm), Number of roots/plant, fresh weight of herbs/individual plant (g), herb dry weight/plant (g) and Index of Salt Tolerance Traits (%). The biochemical evaluating including Chlorophyll content, Proline content, Total carbohydrates percentage and Minerals Nutrient Content.

3.2.1. Physiological Evaluating

3.2.1.1. Plant Height (cm)

The findings of the study conducted by **Rodríguez *et al.* (2005)** provide confirmation that salinity stress mostly manifests in the growth factor of aboveground plants.

The findings shown in **Table (4)** indicated that the combination among 0.5 and 1.0% Sodium azide and different levels of NaCl at 500 and 1000 ppm resulted in the longest plant height 34.4, 35.2 cm and 30.5 and 31.1 cm, respectively. The application of salinity treatments at elevated concentrations (2000 and 3000 ppm) with two levels of Sodium azide resulted in a significant reduction in the height of lavender plants, as compared to both the control treatment. In general, the height of lavender plants exhibited a decline as the salinity level increased, reaching its lowest plant height at a salinity level of 3000 ppm with two doses of Sodium azide.

Fig. 3. Effect of various concentration of NaCl (ppm) on growth parameters of Lavender plants produced from seed treated with Sodium azide at o.5 and 1.0%.

Table 4. Effect of different NaCl concentrations (ppm) with Sodium azide at 0.5 and 1.0% on plant height (cm) of Lavender plants after three months

NaCl Concentrations (ppm)	Plant Height (cm)	
	Sodium azide (%)	
	0.5 %	1.0%
Control	36.2 **a**	37.9 **a**
500	34.4 **ab**	35.2 **ab**
1000	30.5 **c**	31.1 **c**
2000	18.3 **d**	18.5 **d**
3000	10.6 **e**	10.7 **e**

The phenomenon of growth inhibition in plants occurs as a prompt reaction to various environmental stressors, such as salinity, which leads to the imposition of osmotic stress on plants **(Munns and Tester, 2008)**. Nevertheless, the suppression of growth during periods of stress is linked to the redistribution of plant resources that are typically allocated towards primary metabolism and overall growth **(Hand *et al.*, 2017)**. The acquired results unequivocally demonstrated that the application of salt treatments at elevated concentrations resulted in a notable decrease in growth. It is important to acknowledge that all plant specimens demonstrated resilience in withstanding the adverse conditions of our experimental trials when subjected to low levels of watering using a sodium chloride solution, specifically up to 1000 (ppm). The data presented in the study by **Morosan *et al*. (2017)** suggest that Lavender has a higher degree of resistance to water and salt stress compared to conventional crop cultivars like Phaseolus. According to a study conducted by **García-Caparrós *et al*. (2019)**, previous research has indicated a decrease in the growth rate of Lavender and other species belonging to the same genus when exposed to circumstances of salt stress.

The findings of our study align with prior research conducted by **Navarro *et al*. (2007)**, which showed that the presence of salinity led to a reduction in the height of potted Arbutus unedo plants cultivated in substrates composed of peat, sand, and clay. These plants were subjected to irrigation with NaCl solutions of 52 µM and 105 µM over a period of 16 weeks.

Furthermore, **Shehata and Naseer (2019)** conducted a study that revealed that sweet basil plants exhibited diminished shoot length, plant height and number of

branches/plant when subjected to elevated saline levels of 2,000 and 4,000 ppm NaCl, in comparison to the control group. Similarly, the utilization of salinity in the rosemary plant was demonstrated by **Abdelkader *et al*. (2019)**. The experimental conditions including different levels of concentration (2000, 3000, 4000 ppm) resulted in a notable reduction in various growth parameters, including number of branches / plant, plant height, salt resistance index, dry weight of plant / plant and and overall total.

According to the study conducted by **Ibrahim *et al*. (2019)** on sweet basil, there was a notable reduction observed in various growth parameters such as number of branches / plant, plant height, salt resistance index, dry weight of both herbs and roots, as well as photosynthetic pigments (specifically chlorophyll a and chlorophyll b) as salinity levels increased.

3.2.1.2. Number of Branches / Plant

The findings presented in **Table (5)** demonstrate that the treatment 500 and 1000 ppm of NaCl combined with 0.5 and 1.0% of Sodium azide exhibited the maximum number of branches per plant, which was significantly different from the others doses of NaCl with two levels of Sodium azide. Overall, the application of salinity treatments at concentrations of 2000 and 3000 ppm with two levels of Sodium azide resulted in a decrease in the number of branches per plant when compared to the control treatment. Furthermore, there was a drop in the number of branches per lavender plant as the salinity levels increased with two levels of Sodium azide, reaching the lowest value at 3000 ppm.

Table 5. Effect of various NaCl concentrations (ppm) on number of branches per plant of Lavender plants

NaCl Concentrations (ppm)	Number of Branches / Plant	
	Sodium azide (%)	
	0.5%	1.0%
Control	23.6 **a**	24.2 **a**
500	21.5 **b**	22.0 **b**
1000	19.2 **b**	20.1 **bc**
2000	12.1 **c**	12.9 **d**
3000	7.6 **d**	8.2 **e**

Salinity represents a significant abiotic stress factor that exerts a profound impact on plant growth through the induction of osmotic stress and disturbances in ionic and nutritional equilibrium. The presence of these imbalances has been shown to have a detrimental impact on a range of physiological and biochemical processes associated with the growth and development of plants **(Zhang *et al*., 2013)**. Plants subjected to saline stresses have several metabolic alterations aimed at diminishing their water absorption capacity, resulting in significant reductions in their growth rate. The decline in growth can be ascribed to a reduction in osmotic capacity within the soil, resulting in diminished water uptake, lower transpiration, and the subsequent closing of stomata **(Ben-Asher *et al*., 2006)**. The decrease in plant growth may be attributed to the inhibitory effects of salinity on plant differentiation and meristematic activity, leading to reduced plant height, fewer branches per plant, and lower root weights per lavender plant **(Sanchesconde and Azura, 1979)**. The obtained outcomes were consistent with the findings reported by **Nishant and Dheeraj (2014)** regarding *Citronella java* and **Sumand and Varshney (2018)** regarding *Cassia angustifolia* plants. The findings of **Kumar *et al*. (2021)** demonstrated a significant decrease in plant growth, including total height, stem and root lengths, as well as the number of branches and leaves, with an increasing concentration of NaCl in all water drop cultivars. These results suggest that the presence of salt stress hampers the growth of these plants. The cultivar V11E0135 showed a significant decline in growth compared to other cultivars, however V11E0022 demonstrated superior adaptability in comparison to its counterparts. The outcomes of this study are consistent with previous research conducted on other plant species **(Menezes *et al*., 2017; Rahneshan *et al*., 2018)**.

3.2.1.3. Root Length (cm)

The results shown in **Table (6)** indicate that the application of salinity treatments with two levels of Sodium azide significantly reduced lavender root length, with the exception of the 500 and 1000 ppm treatments combined with Sodium azide at 0.5 and 1.0%, which showed the longest root lengths compared to the control treatment. Conversely, the 2000 and 3000 ppm salinity levels significantly reduced root length, resulting in the lowest number of observed roots.

Table 6. Effect of different NaCl concentrations (ppm) with Sodium azide at 0.5 and 1.0% on roots length of Lavender plants

NaCl Concentrations (ppm)	Roots Length (cm)	
	Sodium azide	
	0.5%	1.0%
Control	18.0 **a**	18.9 **a**
500	16.2 **ab**	16.7 **b**
1000	17.4 **b**	17.6 **b**
2000	10.3 **c**	10.4 **c**
3000	6.4 **c**	6.8 **d**

It is noteworthy that there was a substantial increase in root length observed in plants subjected to water stress. This behavior exhibits similarities to the natural response of plants, wherein roots are prompted to proliferate in conditions of low humidity in order to access deeper soil layers with higher moisture content. The impact of salinity on plant growth is intricately linked to several key factors. Firstly, salinity exerts an influence on the water flow resistance of roots and stomata. Secondly, under saline conditions, there is a notable alteration in the equilibrium between root and shoot hormones. Thirdly, salinity induces modifications in the structure of chloroplasts and mitochondria, potentially disrupting normal metabolic processes and growth. Lastly, salinity leads to an elevation in respiration while concurrently diminishing the products of photosynthesis **(Said-Al Ahl and Mahmoud, 2010)**.

This result is similar to the results of **Khorasaninejad *et al*. (2016)** exposure to salt stress causes a decrease in the level of photosynthesis. Also, with increasing salinity, the root length first increased and then decreased. **Safarnejad *et al*. (2007)** announced that with increasing salinity stress, the germination rate and seed germination strength index, root length, stem length, root dry weight, and stem dry weight decrease. This decrease in plant growth under salt stress is due to a decrease in Plant energy reserves are the result of disturbances occurring in biological activities and metabolism in plants. Also, the decrease in root length might be caused by ionic toxicity, followed by an increase since the root is one of the organs that begin extending to the surface to maximize plant absorption. As a result, the root length grows. It has been discovered that the intensity of water absorption rises as the effect of salinity stress diminishes.

3.2.1.4. Number of Roots / Plant

The data shown in **Table (7)** demonstrates a clear relationship among salinity treatments, Sodium azide and the number of roots per plant. Specifically, the highest levels of salinity treatments with Sodium azide at 0.5 and 1.0% were found to have a substantial negative impact on the number of roots / plant. Furthermore, the number of roots per lavender plant exhibited a significant rise to 17.4 and 19.0 root / plant, 18.0 and 19.9 root/plant, respectively when subjected to saline levels of 500 and 1000 ppm with Sodium azide at 0.5 and 1.0%, respectively, in comparison to the control treatment.

Table 7. Effect of various NaCl concentrations (ppm) with Sodium azide at 0.5 and 1.0% on number of roots/plant of Lavender plants

NaCl Concentrations (ppm)	Number of Roots / Plant	
	Sodium azide	
	0.5%	1.0%
Control	15.1 **c**	15.9 **c**
500	17.4 **b**	18.0 **b**
1000	19.0 **a**	19.9 **a**
2000	11.5 **d**	12.5 **d**
3000	8.0 **e**	8.7 **e**

The salinity mechanisms that influence plant growth are intricately connected to the following factors: According to **Said-Al Ahl and Mahmoud (2010)**, the presence of salinity has an impact on the resistance of roots and stomata to water flow. Additionally, under saline conditions, there is a notable alteration in the equilibrium of hormones between the root and shoot systems.

The present investigation observed a decline in stem thickness, plant height and root biomass as a result of elevated saline levels exceeding 1,000 ppm of NaCl, aligning with findings from other research. The present study examines the findings of **Cordovilla *et al*. (2014)** about the effects of lavender and thyme. Additionally, the study by **Nasreen *et al*. (2012)** is reviewed to explore the outcomes associated with chamomile. In a study conducted by **Hegazy Mahrezi *et al*. (2012)**, it was observed that the introduction of a 50 µM NaCl concentration in the nutrient solution resulted in a mere 7% reduction in the production of dry matter in both the shoots and roots of rosemary plants.

Conversely, lavender biomass experienced a more significant decline, with a 50 µM NaCl concentration leading to a 21% loss in dry matter. According to the findings of **Garcia-Caparros *et al*. (2017)**, it was observed that *Lavandula multifida* exhibits superior adaptability to salinity in comparison to other species belonging to the Lamiaceae family.

3.2.1.5. Fresh Weight of Herbs / Individual Plant (g)

The findings shown in **Table (8)** suggest that the application of salinity treatments at 2000 and 3000 ppm with two levels of Sodium azide resulted in a reduction in the fresh weight of lavender herb per plant, as compared to the control treatment. In the treatments Sodium azide (0.5 and 1.0%), with salt levels at 500 and 1000 ppm resulted in increased fresh weight of approximately 83.5 and 76.2 g/plant, and 84.0 and 77.7 g/plant, respectively, as compared to the control treatment.

Table 8. Effect of different levels of NaCl (ppm) with Sodium azide on fresh weight of herbs/individual plant (g) of Lavender plants

NaCl Concentrations (ppm)	Fresh weight of herbs/individual plant (g)	
	Sodium azide	
	0.5%	1.0%
Control	88.4 **a**	89.3 **a**
500	83.5 **b**	84.0 **b**
1000	76.2 **c**	77.7 **c**
2000	50.3 **d**	51.2 **d**
3000	32.4 **e**	33.3 **e**

3.2.1.6. Herb Dry Weight / Plant (g)

Based on the findings reported in **Table (9)**, it is evident that the application of salinity treatments (except the 500 and 1000 ppm levels) with two levels of Sodium azide resulted in a reduction in the herb dry weight per plant of lavender, as compared to the control treatment. The highest herb dry weight/plant (30.5 and 28.3 g) and (31.0 and 28.8 g), respectively were recorded with treatments NaCl at 500 and 1000 ppm combined with Sodium azide at 0.5 and 1.0%.The observed decrease was statistically significant at the levels of 2000 and 3000 (ppm).

Table 9. Effect of different levels of NaCl (ppm) with Sodium azide at 0.5 and 1.0% on herb dry weight/plant (g) of Lavender plants.

NaCl Concentrations (ppm)	Herb Dry Weight / Plant (g)	
	Sodium azide	
	0.5%	1.0%
Control	35.2 **a**	35.9 **a**
500	30.5 **b**	31.0 **ab**
1000	28.3 **b**	28.8 **b**
2000	15.8 **c**	16.0 **c**
3000	12.3 **c**	12.8 **d**

In their study, **Razmjou *et al*. (2008)** observed a negative correlation between heightened salt stress and various growth indices in *Matricaria chamomile*. The process of plant growth is dependent on the proliferation and elongation of cells. Consequently, when growth is hindered by salinity stress, it can be ascribed to many factors such as osmotic stress, ion imbalance, and ion toxicity. These factors ultimately lead to a decrease in turgor and hinder DNA synthesis, thereby impeding cell growth **(Yazici *et al*., 2007)**. The findings of the study indicated that exposure to salt stress resulted in a notable reduction in both fresh and dry weights. According to **Munns (2003)**, the limitation of plant growth in saline environments might be attributed to either a decrease in water accessibility or the harmful effects of NaCl. The reduction in dry weight observed in response to salinity stress can be linked to the suppression of nutrient hydrolysis and subsequent transfer to growing shoots. **Taher *et al*. (2010)** also documented comparable findings in their study on wheat, while **Dolatabadian *et al*. (2011)** discovered similar results in their investigation on soybeans. Similarly, the study conducted by **Hassanuzzaman *et al*. (2013)** revealed that the initial phase of growth decline occurs at a rapid rate as a result of osmotic stress. This is subsequently followed by a significantly slower process attributed to the accumulation of salt in the leaves, ultimately resulting in salt-induced toxicity in plants. **Khorasaninejad *et al*. (2016)** as salinity stress increased stem and root wet weight decreased. As reported by **Salami *et al*. (2006**), a decrease in germination rate, root length, fresh and dry weight of roots, and fresh and dry weight of stem due to salinity stress was found in herbaceous plants. This weight loss is the result of an effect on ionic balance and osmotic balance due to salinity stress.

A decrease in the fresh weight of the plant under salt pressure may occur due to the accumulation of harmful ions such as Cl, which are either harmful by themselves or cause a disturbance in the metabolism of other nutrients and reduce their competition with important nutrients due to disturbances in the absorption of the elements. In addition, as the salinity level increases, the number of leaves decreases due to decreased cell division and differentiation **(Zafari *et al*., 2015)**. **Khorasaninejad *et al*. (2016)** also, with increased salinity, root dehydration occurs. This result also agreed with the results of the experiments of **Salami *et al*. (2006)** on the effect of salt stress on cumin and sycamore, and **Azturk *et al*. (2004)** studied the effect of salinity on lemongrass. Because the toxicity of the ions is the result of their accumulation, the harmful elements cause disturbance in all the biological activities of the plant and finally cause the plant to lose weight. Salt stress, especially at high concentrations of sodium chloride, also causes loss of osmotic balance as a result of tissue dehydration becoming cellular and eventually leads to wilting.

In the study conducted by **Kumar *et al*. (2021)**, it was observed that there was a considerable decrease in the fresh biomass and dry weight of both shoots and roots in both water droplet selections. All sodium chloride (NaCl) treatments resulted in a decrease, with V11E0135 exhibiting a more significant reduction compared to V11E0022. In a study conducted by **Kapoor and Pande (2015)**, it was shown that many plant species exhibited a reduction in both fresh and dry weights of their roots and shoots when subjected to NaCl-induced stress. Significant variations in biomass decrease were detected between sensitive and tolerant coriander cultivars in response to various NaCl treatments. The observed reduction in height and biomass of waterdrop plants may be attributed to the adverse impact of NaCl treatment. Osmotic stress is a condition that hinders the absorption and movement of water, resulting in a cascade of hormone-induced reactions that can decrease the aperture of stomata, the uptake of carbon dioxide, and the rate of photosynthesis **(Menezes *et al*., 2017).** An additional factor contributing to the decrease in growth could perhaps be the allocation of energy towards maintaining a balance between salt stress and reduced carbon assimilation **(Sarker and Oba, 2020).**

3.2.2. Index of Salt Tolerance Traits (%)

The data presented in **Table (10)** indicate that the percentage of salt tolerance trait index was considerably reduced when subjected to salinity treatments of 2000 and 3000 ppm with two levels of Sodium azide, as compared to the control group. To clarify, the observed enhancements in the salt tolerance trait index were around 94.5, 86.7% and 97.3, 84.6%, respectively for the salinity level at 500 and 1000 ppm combined with Sodium azide at 0.5 and 1.0%. It is important to note that there was a statistically significant disparity between these treatments and the control treatments.

Table 10. Effect of various NaCl concentrations (ppm) with Sodium azide at 0.5 and 1.0% on Index of Salt Tolerance Traits (%) of Lavender plants.

NaCl Concentrations (ppm)	Index of Salt Tolerance Traits (%)	
	Sodium azide	
	0.5%	1.0%
Control	100.0 **a**	100.0 **a**
500	94.5 **ab**	97.3 **a**
1000	86.7 **c**	84.6 **b**
2000	67.9 **d**	60.3 **c**
3000	48.3 **e**	42.7 **d**

The global population is experiencing a significant growth rate, leading to an escalating need for plants utilized in both food production and therapeutic applications. Due to the aforementioned rationale, there exists a pressing imperative to enhance the productivity of plants cultivated in regions with high salinity levels and devise a resolution to mitigate the adverse effects of salinity stress **(Shahid *et al*., 2021)**. The assessment of salinity tolerance at the germination and emergence stages holds significance as it serves as a key determinant of salinity tolerance in subsequent growth stages **(Feghhenabi *et al*., 2021)**. According to **Abdelsadek *et al*. (2022)**, the findings revealed a significant decline in the salinity resistance index across all salinity levels with two levels of Sodium azide, as compared to the control treatment. Specifically, the decrease amounted to roughly 29.34% and 34.00% for the salinity level at 1000 ppm, in comparison to 300 ppm. In their study, **Ibrahim *et al*. (2019)** observed a notable decline in the salt resistance index of sweet basil plants as the salinity levels increased.

The tolerance and response mechanisms to salinity vary based on several characteristics, including the duration of salt exposure, the concentration of salt, the genotypes of plants, and environmental conditions. Certain plant species and varieties exhibit varying degrees of damage in response to stress stimuli, with some experiencing significant harm while others demonstrate a lower amount of damage **(Tlahig *et al*., 2021; Tokarz *et al*., 2021)**. Due to the inherent sensitivity of the majority of crops to salinity, an elevation in salt concentration leads to a reduction in crop production. The extent of yield reduction is subject to variation ranging from 10% to 50%, contingent upon the concentration of salt present in the surrounding environment **(Godoy *et al*., 2021)**. The presence of salinity in soil induces alterations in both the physicochemical and biological characteristics. The detrimental impact on plants is observed as a result of both osmotic stress leading to water scarcity and the ionic effect arising from the accumulation of ions **(Karabay *et al*., 2021)**. According to **Ergin *et al*. (2021)**, the germination stress tolerance index exhibited a substantial drop when the dose of NaCl was increased. The study conducted by **Kusvuran *et al*. (2015)** examined the impact of salinity on the germination of *Lolium perenne* L. It was noted that the salinity tolerance index exhibited a decline as the salinity levels increased. **Marium *et al*. (2019)** showed in a separate investigation that the stress tolerance index during germination exhibited a decline as the concentration of NaCl increased.

B. Biochemical Evaluating:

3.2.3. Chlorophyll Content (mg/g fresh weight)

The results presented in **Table (11)** indicate a reduction in the levels of chlorophyll a, chlorophyll b, and carotenoid when subjected the plants to salinity treatments at 2000 and 3000 ppm with two levels of Sodium azide. Conversely, an increase in chlorophyll content was observed when the treatment levels were set at 500 and 1000 ppm with Sodium azide at 0.5 and 1.0%. These differences were found to be statistically significant when compared to the control treatment.

Table 11. Effect of different levels of NaCl (ppm) with Sodium azide at 0.5 and 1.0% on Chlorophyll content (mg/g fresh weight) of Lavender plants.

NaCl concentrations (ppm)	Sodium azide		
	0.5%		
	Chlorophyll a	Chlorophyll b	Carotenoid
Control	1.70 **a**	0.66 **a**	0.26 **ab**
500	1.42 **ab**	0.51 **b**	0.30 **a**
1000	1.10 **b**	0.32 **c**	0.24 **b**
2000	0.63 **c**	0.22 **d**	0.13 **c**
3000	0.31 **d**	0.90 **e**	0.10 **c**
NaCl concentrations (ppm)	Sodium azide		
	1.0%		
	Chlorophyll a	Chlorophyll b	Carotenoid
Control	1.90 **a**	0.93 **a**	0.48 **b**
500	1.72 **b**	0.81 **a**	0.53 **a**
1000	1.36 **c**	0.56 **b**	0.32 **c**
2000	0.82 **d**	0.45 **bc**	0.18 **d**
3000	0.40 **e**	0.23 **d**	0.15 **d**

Numerous cultivated species have been found to exhibit significant alterations in their photosynthetic pigments, namely chlorophyll and carotenoids, under the influence of salt stress, as supported by compelling data from studies conducted by **Al Hassan *et al*. (2015)** and **Iqbal *et al*. (2019)**. Conversely, naturally tolerant

wild species, including certain plant varieties, do not appear to have comparable effects on their photosynthetic pigments under similar conditions. The study conducted by **González-Orenga *et al*. (2020)** examined the properties and characteristics of saltwater. Hence, the reduction in chlorophyll content is regarded as a biomarker indicating stress in plants. Previous studies conducted by **Said-Al Ahl and Omer (2011)** and **Chrysargyris *et al*. (2018)** have examined the impact of salt stress treatments on different species of aromatic plants, including *L. angustifolia*. These studies have consistently observed a decrease in photosynthetic pigments in response to the salt stress treatments. The aforementioned data have been validated under our experimental settings, wherein we provide a noteworthy decrease in the levels of chlorophyll a and b as a result of exposure to salt-induced stress. Significant reductions in carotenoid concentrations were seen under conditions of elevated salt.

The reduction in chlorophyll content in the leaves of lavender plants subjected to salt, in comparison to the control plants, exhibits a similar trend as seen in numerous other species **(Koocheki *et al*., 2008; El-Danasoury *et al*., 2010)**. According to **Yang *et al*. (2009)**, the reduction in chlorophyll levels observed in response to salt stress can be attributed to a decline in pigment production or enzymatic degradation of chlorophyll.

The growth of plants is influenced by the rate of photosynthesis, which exhibits a direct correlation with the levels of chlorophyll present in the leaves. Based on the findings, it was observed that salinity had a detrimental effect on the chlorophyll content. The results obtained in this study are consistent with the findings reported by **Amirjani (2011)**, which indicated a decrease in the levels of chlorophyll a and b in dill leaves (*Anethum graveolens*) under conditions of salinity-induced stress.

The decline in leaf chlorophyll concentration in response to salinity could be attributed to either an elevation in chlorophyll breakdown or a reduction in chlorophyll production, potentially caused by oxidative stress **(Blokhina *et al*., 2003)**. The phenomena of decreased chlorophyll content during salt stress has been widely documented and is extensively utilized as a sensitive indicator of the cellular metabolic condition **(Chutipaijit *et al*., 2011)**. According to **Abdelsadek *et al*. (2022)**, the presence of high salinity levels in soil or water can have a significant impact on carbon photosynthesis, photosynthesis efficiency, and leaf chlorophyll content. In comparison to the control plants, the presence of elevated salinity levels resulted in a notable reduction in the concentration of pigment components, specifically chlorophyll a and b. The findings presented align with the outcomes documented by **Helaly *et al*. (2018)** in their study on rosemary, as well as with the research conducted by **Shehata and Naseer (2019)** on sweet basil. The impact of increased NaCl concentration on photosynthesis has been observed, and prolonged exposure to salt stress has been found to result in a reduction in lipoprotein-chlorophyll biosynthesis **(Akbari Ghogdi *et al*., 2012)**. Various viewpoints have been reported on the influence of salinity on chlorophyll content, among which studies revealed a considerable decrease in chlorophyll content under salt stress **(Meriem *et al*., 2014; Sharif *et al*., 2017)**. Research findings suggest that the augmentation of chlorophyll levels in response to salt-induced stress may be attributed to an elevation in the quantity of chloroplasts. According to **Ekinci *et al*. (2012)**, previous findings on lettuce have shown comparable outcomes, suggesting that the augmented chlorophyll levels may be attributed to the buildup of NaCl within the chloroplasts. According to **Kumar *et al*. (2021)**, the findings suggest that the augmentation of chlorophyll levels in response to salt-induced stress may have advantageous effects on the growth of waterdrop plants in saline soil conditions.

Carotenoids are a class of antioxidants that play a crucial role in facilitating the acquisition of salt stress tolerance in plants through the mitigation of free oxygen radicals **(Ali *et al*., 2017)**. In the present investigation conducted by **Kumar *et al*. (2021)**, it was shown that the carotenoid concentration exhibited a modest increase under NaCl-induced stress conditions in both the chosen cultivars, as compared to the control group. Notably, V11E0022 demonstrated a considerably elevated concentration of carotenoids. According to a prior investigation conducted by **Çelik and Atac (2012)**, it was observed that the levels of carotenoids exhibited an increase in response to salt-induced stress. Carotenoids serve as antioxidants by mitigating the harmful effects of singlet oxygen, so preventing oxidative damage.

3.2.3. Proline Content

The data presented in the study demonstrate a notable impact of salt stress on the proline content in leaves, as

influenced by varying concentrations of NaCl combined with two levels of Sodium azide. An elevation in NaCl concentrations with Sodium azide at 0.5 and 1.0% resulted in a notable augmentation in proline content, reaching 8.6, 9.5 and 9.0, 9.8 mg/g fw with NaCl concentrations of 2000 and 3000 ppm, respectively. This increase was observed in comparison to the control treatment, which exhibited a proline content of 5.0 mg/g fw. In contrast, the application of a low concentration of salt stress at 500 ppm with two levels of Sodium azide resulted in a lower proline content of 5.6 and 5.8 mg/g fw, respectively. This difference was found to be statistically significant when compared to the control treatment and all other treatments, as indicated in **Table (12)**.

Table 12. Effect of different levels of NaCl (ppm) with Sodium azide at 0.5 and 1.0% on Proline content of Lavender plants.

NaCl Concentrations (ppm)	Proline Content (mg/g fw)	
	Sodium azide	
	0.5%	1.0%
Control	5.0 **d**	5.3 **de**
500	5.6 **c**	5.8 **d**
1000	6.2 **c**	7.9 **c**
2000	8.6 **b**	9.0 **b**
3000	9.5 **a**	9.8 **a**

Under stressful conditions, cells protect themselves by accumulating proline and soluble carbohydrates. This accumulation serves to maintain the osmotic strength of the cytosol in relation to the vacuole and the external environment. Proline has a dual purpose in cellular function, acting as both an osmotic protectant and a defense against reactive oxygen species. Furthermore, it plays a crucial role in safeguarding enzymes and maintaining their structural stability **(Rahneshan *et al*., 2018; Alzahrani *et al*., 2019)**. According to **Al-Amier and Craker (2007)**, when plants are subjected to salt stress, there are metabolic changes that result in an elevation in the synthesis of proline and a reduction in its breakdown, ultimately resulting to elevated levels of proline. The high buildup of proline in leaves serves as a significant adaptive strategy for enhancing salt tolerance. This is due to the fact that proline acts as a valuable source of energy, carbon, and nitrogen, which are essential for the recovery of plant tissues. According to **Hare *et al*. (1998)**, proline functions as an osmolyte, thereby decreasing the osmotic potential and thereby diminishing the uptake of harmful ions. Consistent with the results of **Annunziata *et al*. (2017)**, the proline content exhibited an increase in sage plants cultivated in saline environments. The findings align with the outcomes reported by **Woodrow *et al*. (2017)**, as they demonstrated that proline plays a role in osmotic regulation and the removal of reactive oxygen species (ROS). Rosemary plants have the ability to collect sodium in order to maintain leaf turgidity under saline circumstances, despite the need to generate liquid organic matter, particularly proline. The buildup of proline and sodium serves as a method employed to sustain cellular swelling and mitigate the adverse consequences of salt stress, as described by **Bandeh-Hagh *et al*. (2008)**. According to **Hong *et al*. (2000)**, it has been observed that proline has the potential to function as a radical scavenger, hence offering cellular protection against oxidative stress generated by salt. It can be inferred that proline plays a significant role in the alleviation of stress in plants. The findings presented here align with the conclusions drawn by **Mansour (2000)** and **Abraham *et al*. (2003)**, who observed an increase in proline accumulation in plants exposed to salt-induced stress. In their study, **Nasim *et al*. (2013)** demonstrated that an elevation in salinity levels resulted in a corresponding augmentation in the proline content of peppermint (*Mentha piperita* L.). The increase in proline levels is a commonly seen response to water and salt stress in plants. In the presence of saline circumstances, numerous plant species exhibit the capacity to amass proline as an osmolyte that serves a dual purpose of being non-toxic and protective, so enabling the maintenance of osmotic equilibrium **(Ashraf and Foolad, 2007)**. According to **Khosravi *et al*. (2011)**, their findings indicate that the elevation of proline levels in plants, as a result of osmotic stress, contributes to enhanced tolerance against leaf dehydration and promotes plant growth under stressful conditions. According to **Munns and Tester (2008)**, proline and sucrose are the predominant solutes that tend to concentrate under saline conditions. The study conducted by **Abdelsadek *et al*. (2022)** observed the proline concentration at elevated salinity levels of 3000 and 4500 ppm, revealing the highest reported values. The proline content exhibits an upward trend in both the roots and leaves of the tolerant cultivar as the NaCl stress level increases. Conversely, in the sensitive cultivar, the proline concentration first rises but subsequently declines after

reaching a threshold of 100 µM NaCl. The observed decline in response to 200 µM NaCl stress in V11E0135 may be attributed to a potential decrease in enzymatic activity within the proline biosynthesis pathway, as suggested by **Chun *et al*. (2018)**. Another potential factor contributing to this phenomenon could be the enzyme proline dehydrogenase (ProDH), which plays a significant role in the regulation of proline accumulation. Hence, it is plausible that the downregulation of ProDH genes occurred in V11E0135 under elevated NaCl concentrations, as suggested by **Funck *et al*. (2010)**.

3.2.4. Total Carbohydrates Percentage

The findings shown in **Table (13)** demonstrate a significant drop in the concentration of total carbohydrates percentage when the salt level is increased through the addition of NaCl concentration. This trend was observed consistently across both levels of Sodium azide. The greatest levels of total carbohydrates were observed at NaCl concentrations of 500 and 1000 ppm with Sodium azide at 0.5 and 1.0%, in comparison to the control treatment. Moreover, there was a notable reduction in the overall carbohydrate content observed in plants subjected to a concentration of 3000 ppm of NaCl, in comparison to the control plants, with two levels of Sodium azide.

Table 13. Effect of different levels of NaCl (ppm) with two levels of Sodium azide on total carbohydrates percentage of Lavender plants.

NaCl Concentrations (ppm)	Total Carbohydrates Percentage	
	Sodium azide	
	0.5%	1.0%
Control	15.57 **b**	16.02 **b**
500	16.8 **a**	16.9 **a**
1000	16.9 **a**	17.0 **a**
2000	12.2 **c**	12.4 **c**
3000	9.7 **d**	9.9 **d**

In order to maintain osmotic potential, plants accumulate several suitable solutes, including proline, soluble carbohydrates, proteins, and GSH. The elevated concentration of these chemicals facilitates the identification of a cultivar that exhibits tolerance to stress circumstances **(Sarker and Oba, 2019)**. The present findings are in line with previous research conducted by **Meriem *et al*. (2014)** and **Sharif *et al*. (2017)**, which showed that canola, coriander, and tobacco cultivars that are tolerant to salinity exhibited higher levels of proline and soluble sugars. According to **Ibrahim *et al*. (2019)**, the elevation in soluble sugars can be attributed to heightened enzymatic activities, which facilitate the regulation of cellular structures and functions by interacting with macromolecules. Tolerant cultivars exhibit enhanced water retention capabilities as a result of the presence of proline and carbohydrates. In the study conducted by **Abdelhamid *et al*. (2013)**, it was shown that the increment in salt levels, ranging from 0.0 to 50 to 100 mmol NaCl, resulted in notable enhancements in sucrose and total soluble solids (TSS) in bean sprouts. Conversely, a steady and considerable decline was observed in the concentrations of total sugars and carbs. In contrast, the application of foliar treatment resulted in a notable augmentation of overall sugars and carbs in the whole of bean plants when compared to both the untreated control group and the salinity levels that corresponded to them.

The findings obtained are in line with the results reported by **Hassanein *et al*. (2009)** and **Sadak *et al*. (2012)**. According to **Bartels and Sonkar (2005)**, the elevation in dissolved solids observed in response to salinity stress can be attributed to the protective and adaptive mechanisms of soluble carbohydrates. The findings of **Hasanin *et al*. (2009)** indicated that salinity-induced stress in fava bean plants resulted in a reduction in overall carbohydrate levels, as well as a decrease in leaf photosynthetic pigments. These observations suggest that salinity may have an inhibitory effect on photosynthetic activity and/or promote the utilization of carbohydrates in alternative metabolic pathways. **De Ridder and Salvucci (2007)** observed that the reduction in overall carbohydrate levels in response to salinity stress can likely be attributed to heightened sensitivity of photosynthetic system II, reduced concentration of CO in the intercellular spaces of stomata, diminished photochemical efficiency in the uptake of CO, and decreased oxygen levels. According to **Srivastava *et al*. (1995)**, the buildup of carbohydrates is a significant factor in mitigating the effects of salt stress on plant cells. This can occur by osmotic adjustment or by providing drought tolerance.

3.2.5. Mineral Nutrient Content

The growth of plants and the accumulation of Fe^+, Na^+, Zn^+ and K^+ were identified as the key factors influencing the specific effects of ions in the context of salt stress, particularly in the mineral analysis of Lavender leaves are shown in **Tables (14, 15, 16 and 17)**.

3.2.5.1. Na (g/kg)

The presence of salinity stress disrupts the process of nutrient intake and accumulation. The concentration of sodium ions had a positive correlation with the levels of salt with two levels of Sodium azide seen on the leaves of Lavender. The concentration of Na^+ in the plant increased to 13.1, 15.4 and 13.2, 15.6 (g/kg) when treated with 2000 and 3000 (ppm) of NaCl with Sodium azide at 0.5 and 1.0%, respectively. In comparison, the control treatment exhibited a reported Na^+ concentration of 0.8 and 1.0 g/kg with two levels of Sodium azide, as depicted in Table (14). The treatment plants that were exposed to a concentration of 500 ppm of NaCl show the lowest outcomes with Sodium azide at 0.5 and 1.0%.

Table 14: Effect of salinity with two levels of Sodium azide on Na ion concentration (g/kg) of Lavender plants.

NaCl concentrations (ppm)	Na (g/kg)	
	Sodium azide	
	0.5%	1.0%
Control	0.8 **e**	1.0 **e**
500	4.7 **d**	4.9 **d**
1000	8.2 **c**	8.5 **c**
2000	13.1 **b**	13.2 **b**
3000	15.4 **a**	15.6 **a**

3.2.5.2. K (g/kg)

In **Table (15)** displays the variations in potassium (K^+) levels observed in Lavender plants. The results indicate that the plants subjected to NaCl concentrations of 500 and 1000 ppm with two levels of Sodium azide exhibited the highest potassium percentage in comparison to the control treatment. The average potassium % exhibited a decline as the salinity concentration climbed to 2000 and 3000 ppm, reaching values of 5.3, 3.2 and 5.48, 3.26 (g/kg) respectively, in comparison to the unstressed control which had a potassium content of 11.0 and 11.30 g/kg.

Table 15: Effect of salinity with two levels of Sodium azide on K ion concentration (g/kg) of Lavender plants

NaCl Concentrations (ppm)	K (g/kg)	
	Sodium azide	
	0.5%	1.0%
Control	11.0 **a**	11.30 **a**
500	8.1 **b**	8.40 **b**
1000	7.5 **b**	7.70 **b**
2000	5.3 **c**	5.48 **c**
3000	3.2 **d**	3.26 **d**

3.2.5.3. Fe (mg/kg)

The results recorded in **Table (16)** indicated that, the Fe ion content in Lavender plants show a notable increase as the concentration of NaCl rise, particularly at a concentration of 3000 ppm NaCl, where it reached to 42.0 and 43.0 mg/kg, respectively with two levels of Sodium azide. This value is significantly higher compared to the control treatment, which had a Fe ion content of 33.0 and 36.0 mg/kg, respectively. The concentration of Fe^+ exhibited a decrease when the plants were subjected to a modest degree of salt stress at 500 ppm NaCl with two levels of Sodium azide. This resulted in a concentration of 36.0 and 40.0 mg/kg, as compared to the control treatment.

Table 16: Effect of salinity with Sodium azide at 0.5 and 1.0% on Fe ion concentration (g/kg) of Lavender plants.

NaCl Concentrations (ppm)	Fe (g/kg)	
	Sodium azide	
	0.5%	1.0%
Control	33.0 **c**	36.0 **c**
500	36.0 **b**	40.0 **ab**
1000	38.0 **b**	41.0 **b**
2000	40.0 **a**	42.0 **a**
3000	42.0 **a**	43.0 **a**

3.2.5.4. Zn (mg/kg)

The findings presented in **Table (17)** demonstrate that the concentration of zinc ions in lavender leaves was dramatically influenced by the application of salt treatments with two levels of Sodium azide. The stressed plants treated with 3000 ppm NaCl with Sodium azide at 0.5 and 1.0% had the greatest estimated zinc ion content, reaching 5.7 and 5.8 mg/kg,

respectively compared to control plants. The zinc ion level was lowered to 4.5 and 5.0 mg/kg, respectively when exposed to a salinity of 500 ppm NaCl with Sodium azide at 0.5 and 1.0%. When the salinity exceeds a certain threshold, there is a modest rise in the concentration of zinc ions.

Table 17: Effect of salinity on Zn with two levels of Sodium azide ion concentration (g/kg) of Lavender plants.

NaCl Concentrations (ppm)	Zn (g/kg)	
	Sodium azide	
	0.5%	1.0%
Control	4.2 **c**	4.8 **d**
500	4.5 **c**	5.0 **c**
1000	5.3 **ab**	5.6 **a**
2000	5.2 **ab**	5.4 **b**
3000	5.7 **a**	5.8 **a**

The saccharomyces species employs a broad approach to mitigate the effects of elevated soil salinity. This approach primarily involves minimizing the buildup of harmful ions, particularly sodium ions (Na^+), in the plant's leaves. This is achieved either by lowering the intake of these ions at the root level or by impeding their transportation to the aboveground portions of the plant. Nevertheless, the efficacy of this mechanism is limited to salt concentrations that are relatively modest, contingent upon the tolerance level of the specific genotype **(Gu *et al*., 2016)**. In the present study, it was observed that under the given circumstances, there was a notable elevation in the concentration of Na^+ ions within the leaves when exposed to high salinity.

The buildup of sodium ions (Na^+) is typically accompanied by a reduction in potassium ion (K^+) levels, as these two ions engage in competition for the same transport networks **(Rodríguez-Navarro, 2000)**. Potassium (K^+) is a crucial element in the metabolic processes of plants, as it participates in the activation of enzymes, adjustment of osmotic pressure, formation of swelling, modulation of membrane potential, and maintenance of cytoplasmic pH **(Almeida *et al*., 2017)**. A notable reduction in potassium (K^+) content was seen in the roots of lavender plants subjected to salt treatment, as well as in the stems when exposed to 100 and 200 µM sodium chloride (NaCl). Nevertheless, it was observed that the K^+ contents in the stems of plants subjected to a concentration of 300 µM NaCl, as well as in the leaves under all levels of external salinity, exhibited similarity to the control group. This similarity suggests that there was an activation of K^+ transport to the leaves, resulting in an increase in the K^+/Na^+ ratio. This increase in ratio partially counteracted the negative impacts caused by the presence of K^+. The presence of elevated levels of sodium ions (Na^+) in a solution. The aforementioned process has been documented in other species **(Koźmińska *et al*., 2019)** and is highly probable to play a role in the salt tolerance of lavender cultivars. The application of salinity resulted in a reduction in potassium (K^+) levels in lavender plants. Nevertheless, the sodium (Na^+) content exhibited an increase across all concentrations of salt. In the presence of salinity stress, there is a competitive relationship between the intake of sodium ions (Na^+) and potassium ions (K^+), resulting in a reduction of sodium toxicity. According to the findings of **Othman *et al*. (2006)**, it was observed that the concentration of potassium (K) reduced as salt levels increased. The study conducted by **Zahu *et al*. (2007)** found that plants subjected to salt stress saw a reduction in water potential due to the accumulation of Na^+ ions. This accumulation also led to changes in ion uptake and a decrease in leaf expansion, photosynthetic rate, and overall plant development.

The growth of plants is negatively impacted by nutrient imbalances in saline environments, as these imbalances disrupt the availability, transport, and distribution of essential nutrients. Salinity can lead to deficiencies or imbalances in nutrients, as sodium (Na^+) and chloride (Cl^-) ions compete with essential nutrients like potassium (K^+). Multiple studies have demonstrated that exposure to salinity leads to elevated concentrations of salt and chlorine, while concurrently resulting in reduced levels of phosphorus, nitrogen, potassium, calcium and magnesium in fennel **(Abdel-Wahab, 2006)**. The growth of roots under salt stress conditions is limited due to the combined effects of osmotic stress and ion toxicity. These effects result in a decrease in nutrient intake and hinder the movement of nutrients, particularly potassium (K^+). Due to their shared physicochemical features, sodium ions (Na^+) have the ability to compete with potassium ions (K^+) for crucial binding sites involved in essential metabolic processes. Consequently, this competition can disrupt the normal functioning of plant metabolism **(Marschner, 2012)**. The exposure of plants to salt stress results in the accumulation of sodium ions (Na^+) within

their cellular compartments. Excessive accumulation of Na^+ in the cytosol accompanies salt-induced K^+ efflux **(Sarker and Oba, 2020)**. The study conducted by **Kumar *et al*. (2021)** revealed a notable augmentation in sodium (Na^+) absorption and a corresponding reduction in potassium (K^+) absorption in both the leaves and roots of water drop cultivars when the concentration of NaCl increased. Furthermore, it was revealed that the V11E0135 cultivar exhibited a greater uptake of Na^+ ions in comparison to the V11E0022 cultivar.

The findings of the present study are consistent with previous research conducted by **Rahneshan *et al*. (2018)** and **Yassin *et al*. (2019)**, which also observed increased sodium (Na^+) uptake in susceptible cultivars of pistachio, mulberry, and wheat. The research conducted by **Inal *et al*. (2009)** and **Menezes *et al*. (2017)** demonstrated that the roots and shoots of carrots and amaranth exhibit a significant increase in sodium (Na^+) uptake and a decrease in potassium (K^+) absorption when subjected to salt stress. The progressive elevation of salt levels resulted in a gradual decline in the concentrations of Fe^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} within the *Faba bean* sprouts. According to **Abdel Hamid *et al*. (2010)**, plants cultivated in soils subjected to salinity stress frequently exhibit a deficit of micronutrients (Cu^{2+}, Mn^{2+}, Fe^{2+}, and Zn^{2+}) due to their reduced solubility. The aforementioned findings were corroborated by **Taie *et al*. (2013)**.

3.3. ISSR-PCR Extraction

Analysis Utilizing Inter-Simple Sequence Repeat Polymerase Chain Reaction (ISSR-PCR)

Our study reveals that the genetic stability of Lavender plants remains high over repeated *in vivo*. Furthermore, the observed banding patterns strongly suggest a close genetic link between the mother plants and their *in vivo* reproduced counterparts. Similarly, **Lata *et al*. (2010)** employed ISSR banding patterns to validate the genetic stability of propagated cannabis Pond plants and their respective mother plants.

The findings from our ISSR analysis indicated that chromosomal rearrangements occurred during intraspecific differentiation. The data reveal that utilizing sodium azide as a mutagen in conjunction with various NaCl treatments presents a promising strategy for developing new salt-tolerant Lavender cultivars. The salt-tolerant mutants generated can be directly employed as breeding stock to create new varieties with enhanced salt resistance.

Molecular analyses using ISSR markers **(Fig. 4)** showed significant differences between the transformed lavender plants under salt stress and their parental counterparts. The variations observed between the parent plants and those produced through mutagenesis with sodium azide suggest an enhancement in the expression of certain genes associated with salt stress tolerance at elevated levels. Consequently, the inability of the plants to thrive in saline conditions may stem from the loss or acquisition of functional mutations. This approach serves as an effective method for inducing new mutations that could improve desirable traits and enhance resilience to both biotic and abiotic stresses.

Numerous DNA markers have been employed to detect mutations and clonal somatic variants, including amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), and Intersimple sequence repeats (ISSR) **(Bedabadi *et al*., 2012)**. The ISSR technique utilizes primers that are complementary to simple sequence repeats, which bind to and amplify adjacent DNA sequences in opposite orientations. These ISSR markers are distributed randomly across the genome, facilitating the simultaneous identification of multiple loci without the need for prior sequence information **(Reddy *et al*., 2002)**. Their high level of polymorphism, along with their simplicity, rapidity, and cost-effectiveness, renders ISSR particularly advantageous for detecting randomly occurring mutations. In ISSR analysis, the patterns of the amplified DNA fragments are influenced by both the primer sequence and the genomic DNA being examined. Mutations caused by deletions, duplications, or single nucleotide alterations at the primer binding sites can be detected. In the context of orchids, ISSR assays have been utilized to evaluate genetic diversity in the cultivated spring orchid, assess the genetic stability of the micro-propagated jewel orchid, and explore the evolutionary relationships among *Dendrobium species* **(Zhang *et al*., 2010)**. This study aimed to assess the efficacy of ISSR assays in identifying Lavender mutations induced by varying concentrations of NaN_3, as well as to analyze the genetic variability associated with these mutations.

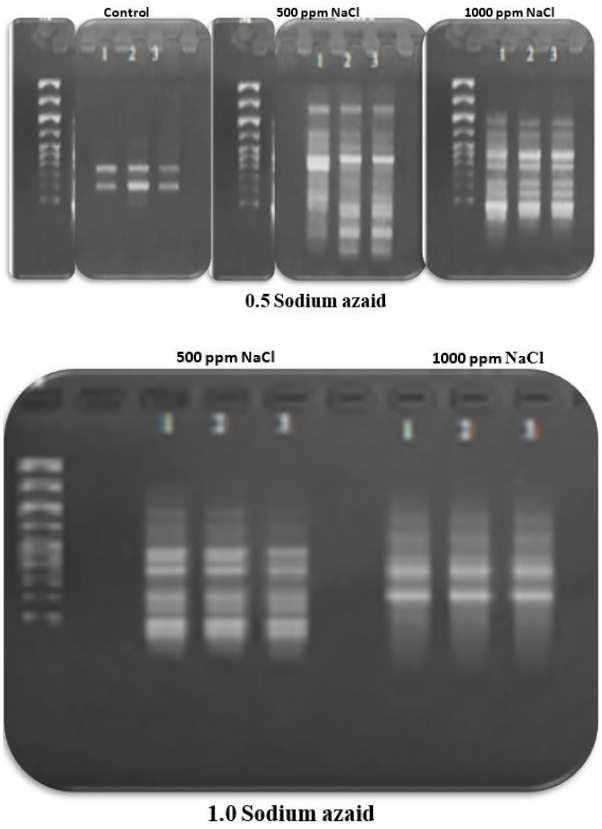

Fig. 5. The banding patterns of Lavender primers, as assessed through inter-simple sequence repeat polymerase chain reaction (ISSR-PCR), were analyzed following multiple subcultures.

Advances in genetic modification and mutagenesis methodologies have greatly enhanced our understanding of the genetic and molecular basis of plant salinity tolerance, facilitating the development of highly tolerant crops **(Apse and Blumwald, 2002)**. Plant responses to biotic and abiotic stressors exhibit considerable variability among different species. Mutational hybridization is a widely used technique for producing cultivars that exhibit resistance or tolerance. Breeding programs often incorporate different mutagenic agents to promote genetic diversity in plant responses to stressors **(Kozgar and Kozgar, 2014)**. Understanding the mechanisms that confer salt stress tolerance, at both the physiological and molecular levels, is important for enhancing the salinity tolerance of many crops **(Piowarczyk *et al.*, 2016)**. Mutations have the potential to produce new individuals exhibiting abnormal traits, expanding the range of genetic resources available for breeding and selection **(Liu and Zhen, 2004)**. NaN_3 is a commonly used mutagen due to its ability to induce a high frequency of mutations in random genomic regions while maintaining a low rate of chromosomal abnormalities. Typically, increasing mutagen concentrations increases the incidence of mutations; however, very high concentrations can lead to increased damage and mortality in seedlings **(Foolad, 2007)**. Mutagenesis facilitates the emergence of new genetic variations, particularly through somatic variation. Salinity tolerance screening is an effective breeding strategy for identifying genotypes capable of tolerating saline conditions **(Suprasana *et al.*, 2012)**. Mutagenes can induce genetic changes in organisms, disrupting unwanted genetic linkages, thereby revealing many promising traits, such as resistance to abiotic stress, resistance to dormancy, and dwarfing, which are useful for crop plant improvement **(Shah *et al.*, 2008)**. **Hussein *et al.* (2006)** used mutagenesis techniques to produce a salt tolerant chrysanthemum strain. A stable chrysanthemum strain exhibiting salt tolerance was developed through mutagenesis with 0.025% ethyl methanesulfonate (EMS). Salinity tolerance was assessed based on the plant's ability to maintain flower quality and productivity under salt-stress conditions.

Mutage was performed using EMS, NaN_3, and gamma rays to produce barley mutants with enhanced aluminum tolerance **(Zhu and Zhang, 2003)**. Twelve aluminum-resistant barley cell lines were generated through these mutagenesis methods, and four of these were selected for further characterization of their aluminum tolerance mechanisms. **Ganesan *et al.* (2005)** showed that somatic embryo germination rates in cotton improved from 44.6% to 50.9% when treated with 10 mM NaN_3. Similarly, **Ikramul Haq *et al.* (2011)** used a 0.5% NaN_3 concentration to induce mutations in sugarcane calli, with the goal of developing salinity-tolerant mutants. **Ahmed *et al.* (2010)** used concentrations of 0.0–0.5 mM NaN_3 to induce mutations in potato calli. In our research, we identified 0.5% and 1.0% NaN_3 concentrations as optimal mutagen concentrations for producing mutant populations, as these levels enhanced seed germination rates.

Traditional methods for selecting mutant plants based on their morphological and biochemical traits are often less reliable due to their sensitivity to environmental influences. Therefore, mutation detection using PCR and non-PCR methods is considered more reliable and stable and has been used to identify various mutations in crops. The main advantage of PCR in mutation analysis is its ability to detect the presence or absence of specific DNA sequences **(Azzam *et al.*, 2022)**.

4. Conclusion

The findings indicate that employing mutagens is advisable for enhancing the survival rates of mutant lavender plants exposed to varying concentrations of sodium chloride.

This research further substantiates the role of mutagens in creating new variants that exhibit better performance under stress conditions. The data revealed that salinity led to an increase in sodium ion levels while simultaneously decreasing potassium ion concentrations. This phenomenon likely occurs because the elevated sodium uptake from the nutrient medium hampers the absorption of other essential nutrients due to competition and interference with plant uptake. The application of different mutagens, including sodium azide, was shown to mitigate these adverse effects and promote the absorption of potassium and calcium ions within plant tissues.

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