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Effect of Soaking Seeds with Graded Concentrations of Ethyl Methane Sulfonate (EMS) on Local Tobacco Plants under Salt Stress Conditions

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The research was conducted during the year 2024. The seeds were treated with three concentrations of the mutagen (0.1, 0.5, 1%) and with a soaking time of (8) hours. In addition, to induce salinity stress, sodium chloride (NaCl) was used at concentrations (4, 8, 12 dmol/cm2). The experiment was implemented according to a randomized complete design (R.C.D.) in the village of Salib - Latakia - Syria. Three replicates for each treatment. Some germination indicators of the treated seeds were measured (germination percentage (%)), morphological indicators of plants (plant height (cm/plant)), morphological indicators (total leaf surface area (cm2), (Net Photosynthesis Rate (mg/cm2/day)) and specific weight of leaves (g/cm2) .

Treatment with the chemical mutagen EMS at low concentration (0.1%) led to an increase in the germination rate, plant height, total leaf surface area, Net Photosynthesis Rate and leaf specific gravity.

On the other hand, increasing the EMS concentration by (0.5 and 1%) led to a decrease in the studied indicators.

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Treatment of chemical mutagens under salinity stress conditions at a low concentration (0.1%) improved the values of the studied indicators compared to other treatments. High salinity concentration led to negative effects on all indicators studied
Therefore, we recommend soaking the seeds with a concentration of (0.1%) EMS, due to its role in improving the germination, morphological and morphological characteristics of the local tobacco variety.

Keywords: Soaking seeds; ethyl methane sulfonate; tobacco plant; salt stress.

1. INTRODUCTION

"Tobacco is one of the most widely grown industrial crops worldwide, and is now grown in more than 125 countries" [1].

"Environmental stresses strongly influence plant growth and development. Salinity is one of the most important of these stresses and can limit crop yield" [2].

"The accurate selection techniques determines the success of induced variation and obtaining desired characteristics in breeding" [3]. "One way to induce mutation is through the use of chemical mutagens. EMS is a common, powerful, and one of the most effective chemical mutagen, especially recommended using when mutation is introduced to the seed materials, since the application and the monitoring of the outcome of mutations are relatively easy. In plants, EMS usually causes point mutations, on the other hand, loss of a chromosome segment or deletion can also occur in lesser extent" [4-7].

"Chemical mutagen to use base analogs is preferable for plant breeders to induce point mutations and have great capacity. A very high mutation rate invariably causes high sterility so plant breeders aim at the more mutagenic effect and the less physiological damage" [8].

In the study of Al-Korkhi et al. [9], "mutagenesis treatments with EMS at low concentration led to a significant increase in the traits (plant height, number of leaves, and root length) of chrysanthemum plants under salt stress conditions".

The objectives of this study were:

(1) Effect of salt stress on germination rate, plant height, total leaf surface area, leaf specific gravity and crop growth rate.
(2) The effect of treatment with graded concentrations of the chemical mutagen EMS on the studied properties under and without salt stress conditions

2. MATERIALS AND METHODS

The experiment was carried out during the 2024 season. The field experiment was conducted in the village of Salib, within a greenhouse in Latakia.

2.1 Plant Material

Tobacco plant material, Baladi tobacco cultivar, was used as plant material in this study. It was registered by the General Organization for Tobacco in Lattakia. EMS treatment in our study, the experimental procedure for EMS induced mutants of Soufi et al. [10] A triple technical iteration of three different dose ratios of EMS (ethylmethanesulfonate) (0.1%, 0.5%, 1%) and a control dose (0% EMS) was followed. To begin with, 25 seeds per technical replicates were pre-soaked in 15 ml (0.6 mL/seed) for 8 h in 0.05 M phosphate buffer, pH 8.0 for 8 h, at 20 °C at 100 rpm static shaking. Treated seeds were rinsed under running tap water for 1 min to remove excess EMS solution from seed surfaces, transferred to Petri dishes containing water-soaked filter paper and left to grow in the growth chamber at 20 °C in triplicate of 25 seeds per treatment dose. After the next day of treatments, the seeds were continuously assessed for germination and developmental stages daily.

The seeds were planted on agricultural medium in plastic dishes containing compost with a capacity of 2 kg for each treatment, and the seedlings were transferred for planting in a factorial experiment using a randomized complete design (R.C.D.), in plastic bags with dimensions (15 x 30) cm and a capacity of (5-6) kg. Soil containing soil prepared as a mixture of sand and clay in a ratio of (2/1).

Irrigation was carried out with salt water using solutions prepared from sodium chloride salt NaCl during the period of active vegetative growth before flowering, at a rate of three

irrigations every week after the next, so that the electrical conductivity corresponds according to the following parameters:

=S0 0 mm/cm2, S1 = 4 mm/cm2, S2= 8 mm/cm2, S3 = 12 mm/cm2

2.2 Studied Indicators

- Germination indicators:
  - germination percentage %:

The germination percentage was calculated using the following equation

DK = (JK÷JC) × 100

- Morphological indicators:
  - Plant Height (cm/plant): was measured for each experimental treatment, starting from the soil surface level to the growing top, before the plants entered the inflorescence formation stage, that is, about 6 weeks after transplanting.
- Morphophysiological indicators:
  - Total paper surface area (PLA) (cm2):

The leaf area (cm2) was calculated from the following equation:

Area of one sheet of a variety (cm2) = maximum length of the leaf (cm) x maximum width of the leaf (cm) x (0.6443).

- Net Photosynthesis Rate (mg/cm2/day):

It is calculated from the following equation [11]:

(NPR=(Log 〖eL2〗^( )-Log 〖eL1〗^)(W2-W1)/((T2-T1)(L2-L1)))

NPR: net photosynthetic production, mg/cm2/day, L2 and L1: leaf area (cm2) at the beginning and end of the measurement period, respectively, W2 and W1: plant dry weight at the beginning and end of the measurement period, respectively, T2 and T1: number of days between the two phases (At the beginning of the active vegetative growth phase and the end of this phase, i.e. at 30 and 60 days from transplanting).

Specific gravity of leaves (g/cm2):

The leaf specific weight (SLW) was determined after measuring the dry weight of the leaves at the beginning of the technical maturity of the leaves according to the researcher [12]:

SLW = dry leaf weight (g/plant)/leaf area (cm2/plant).

2.3 Statistical Analysis

Statistical analysis of the results from experiments with three or more mean values used a one-way analysis of variance (ANOVA) as dictated by the number of main effects, followed by Tukey's HSD post hoc test or Dunnett's HSD. The difference was considered to be statistically significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 The Distinct Effect of the Clear Mutagen EMS on the Germination Rate (%) of Tobacco Plants

The data in Table 1 indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of germination percentage (%) in tobacco plant seeds.

The germination rate of the local tobacco variety was not significantly affected by the soaking treatment with a low concentration (0.1)% of the mutagen EMS, as it reached 93% in the E1 treatment, compared to the control, 94%. The germination rate decreased significantly ($P<0.05$) with the increase in the concentration of the mutagen EMS to be recorded. Its lowest value is 29% for E3 transactions**.**

Table 1. Germination rate (%) of local tobacco plants under the influence of treatment with the chemical mutagen EMS

Germination percentage(%)	Transactions
$3^{a} \pm 94$	E0
$2^{a} \pm 93$	E1
$2.5^{b} \pm 68$	E2
$2.5^{C} \pm 29$	E3

The symbols (E) indicate treatment with the chemical mutagen EMS (0, 0.1, 0.5 and 1)% for the local tobacco variety. All data refer to averages plus standard error (means ± SE) n=3, and different letters (a, b, c... to show the significant differences between the averages for each indicator at each treatment (P<0.05**)**, ANOVA-Tukey test**.**

Many studies have indicated a decrease in the rate and speed of germination with an increase in the concentration of the chemical mutagen EMS used [13]. Chemical mutagen reduces germination, and seed germination slows down with an increase in the concentration of EMS according to the results of research conducted on the tobacco plant [14], and for results on Vigna radiata [15].

3.2 Effect of Chemical Mutagens and Salt Stress on Plant Height (cm)

Data from Fig. 1 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the height of tobacco plants (cm).

Salt stress led to a decrease in plant height, and this decrease increased with an increase in the concentration of applied salt.

While treatment with a chemical mutagen at a low concentration increased plant height compared to the other concentrations and the control

Treatment with a chemical mutagen under salinity conditions at low concentration also led to an increase in plant height compared to the other treatments and the control. The negative effect of salinity on plant height was pointed out by (Udovenko et al., 1970), who showed that high concentrations of salinity inhibit enzymatic activity and stop the elongation of growing shoot cells, which leads to shortening of the plant, in addition to not increasing the size of the meristematic cells. Preventing their transformation into adult parenchyma cells, which causes weakness in the overall growth of the plant and the formation of leaves of small size and area.

The negative effect with increasing the concentration of the EMS mutagen led to a decrease in plant height, especially at high concentration, and this is consistent with the results of Sudheeran et al. [16].

3.3 Effect of Chemical Mutagens and Salt Stress on Total Leaf Surface Area (cm2/Plant)

Data from Fig. 2 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the total leaf surface area of tobacco plants.

Salt stress led to a decrease in the total leaf surface area, and this decrease increased with an increase in the concentration of applied salt**.**

While treatment with a chemical mutagen at a low concentration increased the total leaf surface area compared to the other concentrations and the control.

Treatment with a chemical mutagen under salinity conditions at low concentration also led to an increase in the total leaf surface area compared to the rest of the treatments and the control**.**

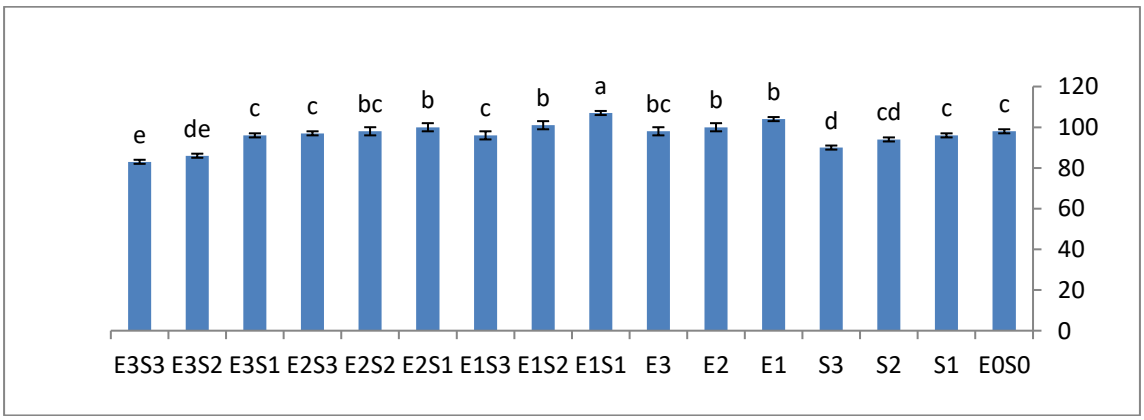

Fig. 1. Effect of EMS on the height of tobacco plants under salinity stress

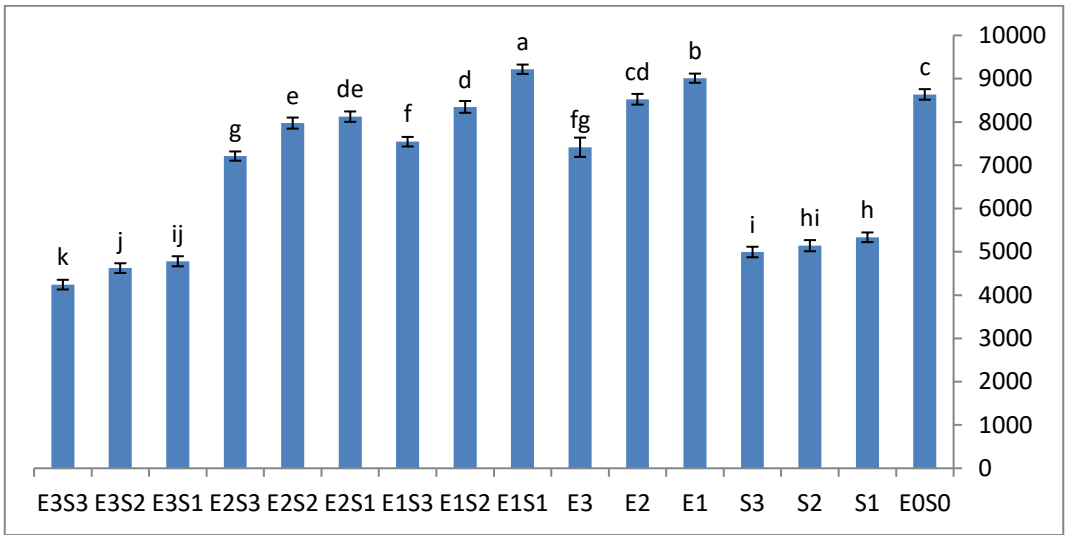

Fig. 2. Effect of EMS on the total leaf surface area of tobacco plants under salinity stress

Salt stress affects the growth, morphology, and anatomical structure of leaves and reduces their area. [17].

Treatment with EMS at high concentrations caused significant changes in genetic balance and physiological functions, including hormone synthesis, growth regulators, protein coding, and cell division, leading to a reduction in leaf surface area [18].

3.4 Effect of Chemical Mutagens and Salt Stress on the net Photosynthesis Rate (mg/cm2/Day)

Data from Fig. 3 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the net photosynthesis rate of tobacco plants (cm).

Salt stress led to a decrease in the net photosynthesis rate, and this decrease increased with an increase in the applied salt concentration.

While treatment with a chemical mutagen at a low concentration increased the net photosynthesis rate compared to the other concentrations and the control.

Treatment with a chemical mutagen under salinity conditions at low concentration also led to an increase in the net photosynthesis rate compared to the remaining treatments and the control.

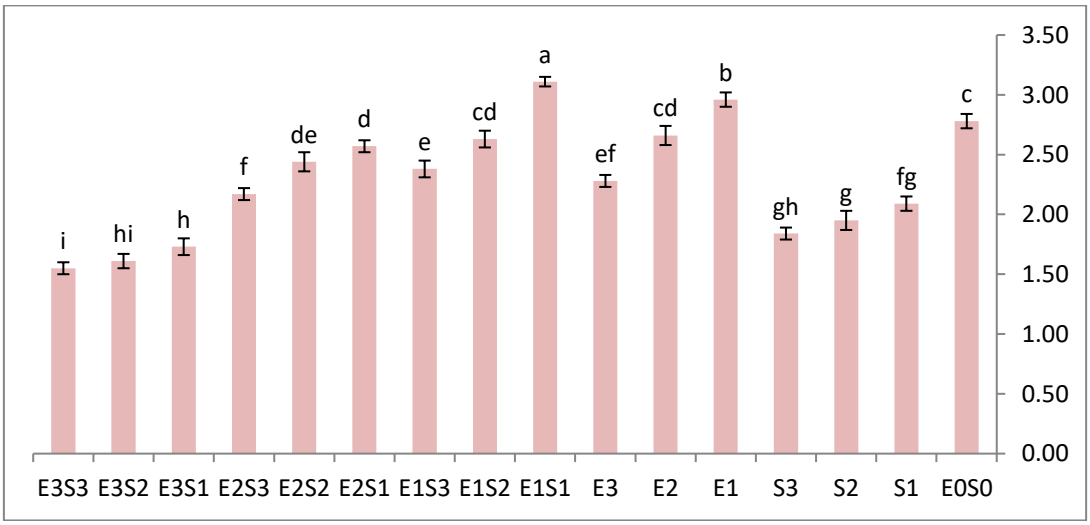

Fig. 3. Effect of EMS on the net photosynthesis rate of tobacco plants under salinity stress.

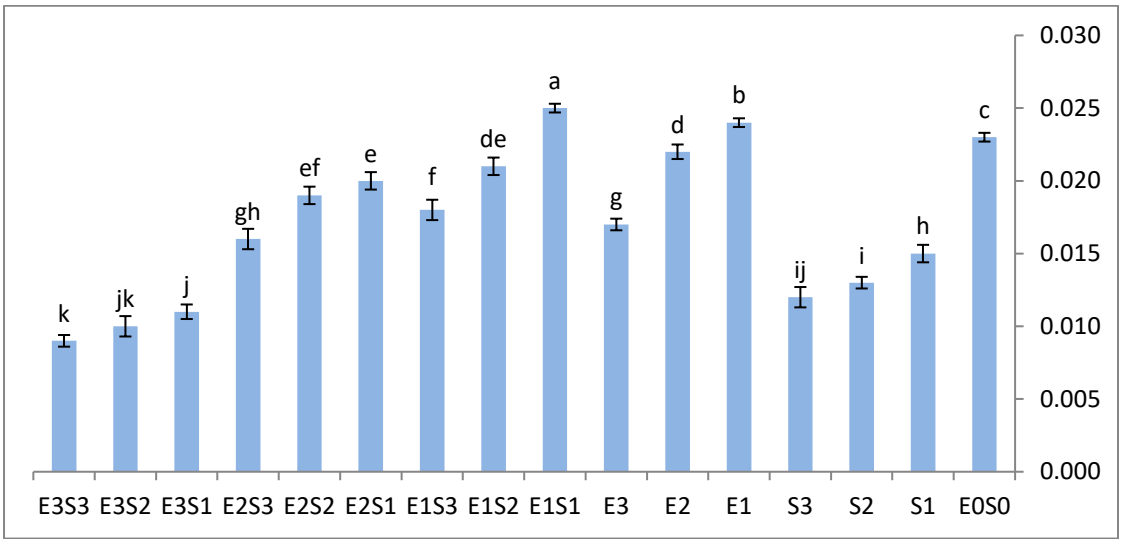

Fig. 4. Effect of EMS on the specific gravity of tobacco plants under salinity stress

In fact, [19] pointed out the negative effect of salt stress, especially at high concentrations of NaCl, on the rate of photosynthesis due to the defect caused by salts in the ability of plant roots to absorb all of the nitrogen, sulfur, and magnesium that enter into the synthesis of chlorophyll molecules. It causes a decrease in the content of photosynthetic pigments, which in turn negatively affects the rate of photosynthesis in the plant.

Low concentrations of the compound EMS stimulated the formation of solutes through the metabolic activity of mineral elements and water, thus increasing their accumulation, which acts as a regulator of osmotic pressure, retaining water, and preventing wilting, thus increasing the rate of plant photosynthesis. Mutations can occur at high concentrations of the chemical mutagen EMS. Chromosomes, auxin synthesis, impact on nutrient metabolism and accumulation of free amino acids leading to decreased plant photosynthesis rate [20].

3.5 Effect of Chemical Mutagens and Salt Stress on Specific Gravity (g/cm2)

Data from Fig. 4 indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of the specific gravity of tobacco plants (cm).

Salt stress led to a decrease in the specific gravity, and this decrease increased with the increase in the applied salt concentration.

While treatment with a chemical mutagen at a low concentration led to an increase in the specific gravity compared to the other concentrations and the control.

Treatment with a chemical mutagen under salinity conditions at low concentration also led to an increase in the specific gravity compared to the other treatments and the control.

High concentrations of the chemical compound EMS caused a decrease in the specific weight of the leaves, and the compound ethyl methane sulfonate EMS caused a decrease in most of the growth characteristics of the plant. This may be attributed to the occurrence of physiological disorders and genetic and cellular defects, causing the formation of free radicals in the cell and damage to the cellular membranes, thus affecting the effectiveness and activity. Enzymes, such as the prevention or absence of auxin formation, which helps in the division of meristematic tissues, thus influencing cell division and elongation, metabolic processes, and the various stages of plant growth, leading to a decrease in the specific gravity of leaves [21].

4. CONCLUSIONS

Increased NaCl concentrations had a negative effect on the properties in general, but the administered EMS doses reduced or diminished the negative effects of NaCl. According to the results of this research, the investigated features showed a lot of variability depending on the used factors, usually, 0.1% EMS dose were

determined to reduce the negative effect of NaCl. According to the study and literature search, it is necessary to conduct further research on the development of salt-tolerant plants by utilizing induced mutations in *In vitro* conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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