# Improvement of the Growth of Tobacco Plants Treated with Sodium Azide (NaN3) Under NaCl Stress

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Received: 4/06/2024 Accepted:13/07/2024

**Abstract**

The research was conducted during the year 2024. The seeds were treated with three concentrations of Sodium azide (1, 3, 5 mM ) and with a soaking time of (6) hours. In addition, to induce salinity stress, sodium chloride (NaCl) was used at concentrations (4, 8, 12 dS/m). The experiment was implemented according to a randomized complete design (R.C.D.) in the village of Burj Islam - 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)), Morphophysiological indicators (total leaf surface area (cm2), (Net Photosynthesis Rate (mg/cm2/day)), specific weight of leaves (g/cm2) and Leaf Area Index. High salinity concentration led to negative effects on all indicators studied. Treatment with the chemical mutagen NAN3 resulted in an increase in germination rate, plant height, total leaf surface area, net photosynthesis rate, leaf specific gravity and leaf area index. On the other side. Treatment with chemical mutagens under salinity stress conditions at low concentration (1 mM) outperformed all treatments and control. Therefore, we recommend soaking the seeds with a concentration of (1 mM) NAN3, due to its role in improving germination and the morphological and morphological characteristics of the local tobacco variety.

**Keywords**: soaking seeds, NAN3, tobacco plant, NaCl.

**Introduction:**

Tobacco (Nicotiana tabacum L.) is an economically important crop that is cultivated around the world, The genus Nicotiana, which is part of the family Solanaceae and comprises 76 naturally occurring species (Knapp et al., 2004), is one of the most extensively studied genera of flowering plants, in large part due to its economic and cultural importance (Lewis, 2011). Nicotiana species arose in the Americas and Australia, where they have been traditionally used by native peoples for recreational and therapeutic purposes (Goodman, 2004). After Columbus arrived in the Americas, tobacco smoking spread around the world due to the highly addictive nature of tobacco products, which has led to global health, economic, and social impacts ever since (Goodman, 2004).

Climate change and the consequent environmental stresses have been recognised as a severe danger to global food security (Azameti and Imoro, 2023). Soil salinity is one of the important environmental problems worldwide (Al-Turki *et al*., 2023; Xiao and Zhou, 2023). About 20% of the world's irrigated land is affected by salinity (Wang *et al*., 2023). Poor irrigation and industrial pollution continue to exacerbate soil salinization (Yu *et al*., 2020; Ge *et al*., 2023). Salt stress-induced phytotoxicity involves various plant physiological disorders (Nawaz *et al*., 2010). High salinity results in plant dehydration by inducing osmotic stress (Muñoz-Mayor *et al*., 2012). Plants activate osmotic adjustment to combat salt stress (van Zelm *et al*., 2020).

Mutation is the ultimate source of all genetic variation (Nei, 1988). Spontaneous mutations occur at very low frequency, meanwhile induced mutations facilitate the development of improved varieties (Zaky Zayed *et al*., 2014). Many studies used chemical mutagens such as sodium azide (NaN3) to produce plants resistant to biotic stress and abiotic stress such as salinity stress (El Kaaby *et al*., 2015). Sodium azide is functionally mutagenic chemical in various organisms, but not in Drosophila (Kamra and Gallopudi, 1979). Actually, mutagenicity in living systems is mediated through biosynthesis of organic metabolite of azide compound (Owais and Kleinhofs, 1988). This metabolite creates point mutation in DNA when entering into the nucleus (Gichner and Veleminsky, 1977). Aseptic plant regeneration has been an incremental tool for mutation induction, while totipotency of a single cell is a useful work for the establishment of pure form of species. It can facilitate the development of several numbers of its new genotypes (Mandal *et al*., 2000; Barakat *et al*., 2010).

Despite the wide range of concentrations at which sodium azide is used, the mutagen generally induces point mutation in the genome, impairing metabolic activity, growth and development, inhibiting protein synthesis and DNA replication (El Kaaby et al., 2015).

In this study, sodium azide (NaN3) was used. Such as seed treatment to produce salt-tolerant tobacco plants.

The aim of this work is to investigate the effect of the chemical mutagen NaN3 at graded concentrations on the growth of tobacco plants and to know the effect of salinity at graded concentrations on some different growth characteristics, in addition to knowing the response of tobacco plants to NaN3 treatment under salinity conditions.

**Material 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.

**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. NAN3 treatment in our study, the experimental procedure for NAN3-induced mutants of (Ren XueLiang *et al*., 2008) A triple technical iteration of three different dose ratios of NAN3 (sodium azide) (1 mM, 3 mM and 5 mM )

and a controlled dose (0 mM NAN3) was followed. To begin with, 25 seeds per technical replicates were pre-soaked in 15 ml (0.6 mL/seed) in 0.05 M phosphate buffer, pH 8.0 for 6 h, at 20 °C at 100 rpm static shaking. Treated seeds were rinsed under running tap water for 1 min to remove excess NAN3 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. Seedlings were transferred for planting in plastic bags with dimensions (15 x 30) cm and a capacity of (5-6) kg. the experiment was factorial with randomized complete design (R.C.D.), Soil was containing soil prepared as a mixture of sand and clay in a ratio of (2/1).

Irrigation with NaCl solutions (0, 4, 8, 12 dS/m) was carried out during active vegetative growth (before flowering), at a rate of three irrigations every week.

**Studied indicators:**

- Germination indicators:

- germination percentage %:

The germination percentage was calculated using the following equation (Kuswanto, 1996):

$$DK = (JK \div JC) \times 100$$

DK: percentage of germination, JK: number of germinated seeds, JC: total number of seeds.

- Morphological indicators:

- Plant Height (cm): 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) ($cm^2$):

The leaf area ($cm^2$) was calculated from the following equation:

Area of one leaf of a variety (cm2) = maximum length of the leaf (cm) x maximum width of the leaf (cm) x (0.6443) (Bozhinova, 2006).

- Net Photosynthesis Rate ($mg/cm^2/day$):

It is calculated from the following equation (Williams, 1946):

$$(NPR = \frac{(Log\ eL2\ - Log\ eL1\ )(W2 - W1)}{(T2 - T1)(L2 - L1)})$$

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 ($g/cm^2$):

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 (Pearce *et al*., 1968):

SLW = dry leaf weight (g/plant)/leaf area ($cm^2$/plant).

- Leaf Area Index:

The leaf area index was calculated after knowing the total leaf surface area and the area occupied by the plant on the soil according to the equation (Williams, 1946):

Leaf area index (LAI) = leaf area of the plant (cm2) / area of land occupied by the plant (cm2).

**Statistical Analysis:**

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

**Results and Discussion:**

**1. The distinct effect of the clear mutagen NAN3 on the germination rate (%) of tobacco plants:**

The results showed that the germination rate decreased with increasing NaN3 concentration (Table 1). NaN3 at a concentration of 1 mM showed a seed germination rate of 85% compared to the concentration of 3 mM, which achieved a germination rate of 76%, and the concentration of 5 mM, which achieved a germination rate of 58%.

**Table (1). Germination rate (%) of local tobacco plants under the influence of treatment with the chemical mutagen NAN3.**

| Transactions | Germination percentage(%) |
|---|---|
| N0 | $3^{a} \pm 90$ |
| N1 | $2^{a} \pm 85$ |
| N2 | $2.5^{b} \pm 76$ |
| N3 | $2.5^{C} \pm 58$ |

The symbols (N) indicate treatment with the chemical mutagen NAN3 (0, 1, 3 and 5)Mm 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.

The higher concentration of NaN3 affected some biological activities such as specific enzymes which are involved in seed germination processes and reduced germination percentages and other growth parameters (El Kaaby *et al*., 2015).

The survival percentage decreased progressively as the dosage increased (Mensah and Obadoni 2007). Mensah and Akomeah (1992) have reported that the higher the mutagenic dose, the lower the survival percentage.

**2. Effect of chemical mutagens and salt stress on plant height (cm):**

Data in Figure (1) indicate that there are significant differences at (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 increasing concentration of added salt.

While treatment with chemical mutagens increased plant height compared to the control group

Treatment with a chemical mutagen at a concentration of 1 mM under salinity conditions also outperformed all other parameters and the control.

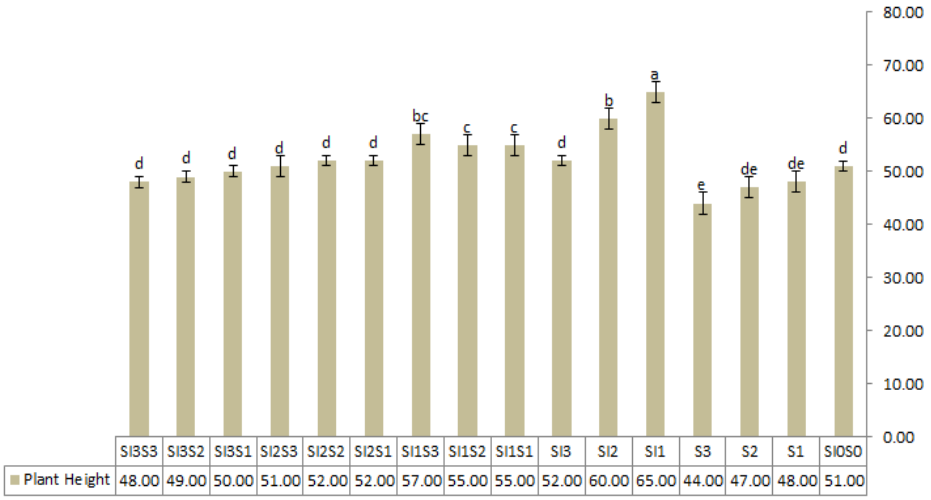


**Figure (1): Effect of NAN3 on the height of tobacco plants under salinity stress.**

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.

Azide ion plays an important role in causing mutation by interacting with enzymes and DNA in the cell. This is due to that NaN3 is a strong mutagen and growth of plant parts are strongly inhibited by increasing its concentration and treatment duration. Its impact has been observed on tobacoo and it was very effective in inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant and yield per plant (AlQurainy and Khan, 2009).

These results could be attributed to the effect of mutagens on the meristematic tissues of the seeds. These may be due to physiological and acute chromosomal damage, delay in the onset of mitosis, chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulting in reduced germination and survivability. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by SA leading to decrease in germination. Reduced growth due to higher doses was also explained differently by different workers. It may be attributed to one or more of the following reasons (i) the increase in growth promoters, (ii) the sudden increase in metabolic status of seeds at certain levels of dose, (iii) the increase in destruction of growth inhibitors, (iv) drop in the auxin level or inhibition of auxin synthesis and (v) decline of assimilation mechanism. Taking these as the preliminary consideration (Roychowdhury and Tah, 2011).

**3. Effect of chemical mutagens and salt stress on total leaf surface area (cm2/plant):**

Data in Figure 2 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the total surface area of tobacco plant leaves.

Salt stress decreased the total surface area of leaves, and this decrease increased with increasing salt concentration applied.

Treatment with chemical mutagens increased the total leaf surface area compared to the control group.

Treatment with a chemical mutagen at a concentration of 1 mM under salinity conditions also outperformed the rest of the treatments and the control.

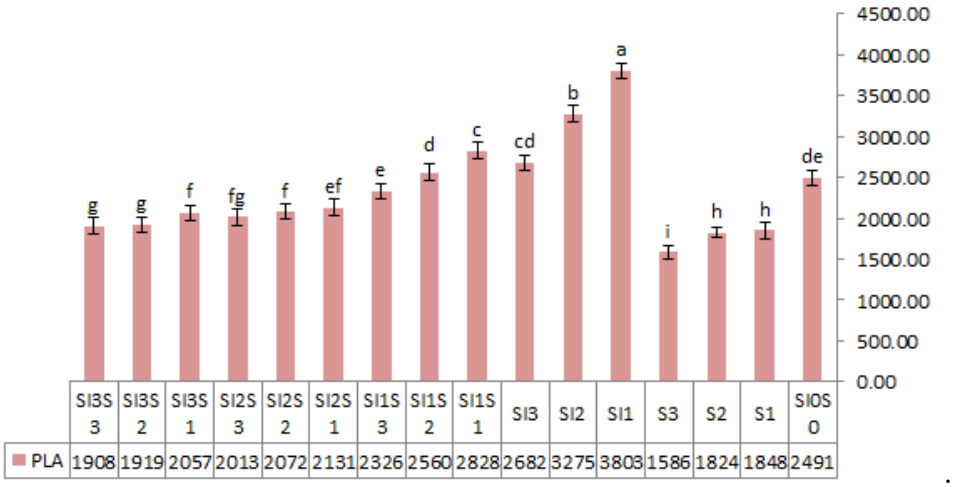


**Figure (2): Effect of NAN3 on the total leaf surface area of tobacco plants under salinity stress.**

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.

Salt stress affects growth, morphology, and anatomical structure of leaves and reduces their area (Delavari *et al*., 2014). Treatment with NAN3 at high concentrations caused significant changes in genetic balance and physiological functions, leading to a reduction in leaf surface area (Goyal and Khan, 2010).

**4. Effect of chemical mutagens and salt stress on the net photosynthesis rate (mg/cm2/day):**

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

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

Treatment with chemical mutagens leads to an increase in the rate of net photosynthesis compared to the control.

Treatment with a chemical mutagen under salinity conditions at a concentration of 1 mM also outperformed all treatments and the control.

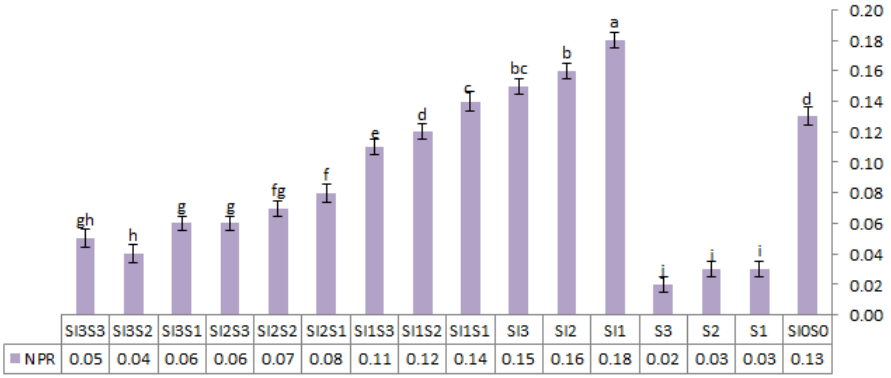


**Figure (3): Effect of NAN3 on the net photosynthesis rate of tobacco plants under salinity stress.**

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.

The expression of numerous genes and the activity of several enzymes related to photosynthesis are depressed under these conditions (Rodrigues *et al*., 2013).Ion cytotoxicity is caused by the

replacement of K+ by Na+ in biochemical reactions conformational changes and loss of function of proteins (Chaves *et al*., 2009).

Salt stress often induces ROS accumulation, leading top membrane lipid peroxidation and cell death (Zhao *et al*., 2021).

The decrease in photosynthetic capacity under salinity might be due to the stomatal closure, which limits stomatal conductance and photosynthetic CO2 assimilation (Yang and Lu, 2005).

**5. Effect of chemical mutagens and salt stress on specific gravity (g/cm2):**

The data in Figure (4) indicate that there are significant differences (P<0.05) between the studied treatments in terms of the specific gravity of the tobacco plant (cm).

Salt stress leads to a decrease in the specific gravity, and this decrease increases with increasing salt concentration.

Treatment with a chemical mutagen led to an increase in the specific gravity compared to the control

Treatment with a chemical mutagen under salinity conditions at a concentration of 1 mM also outperformed all other treatments and the control.

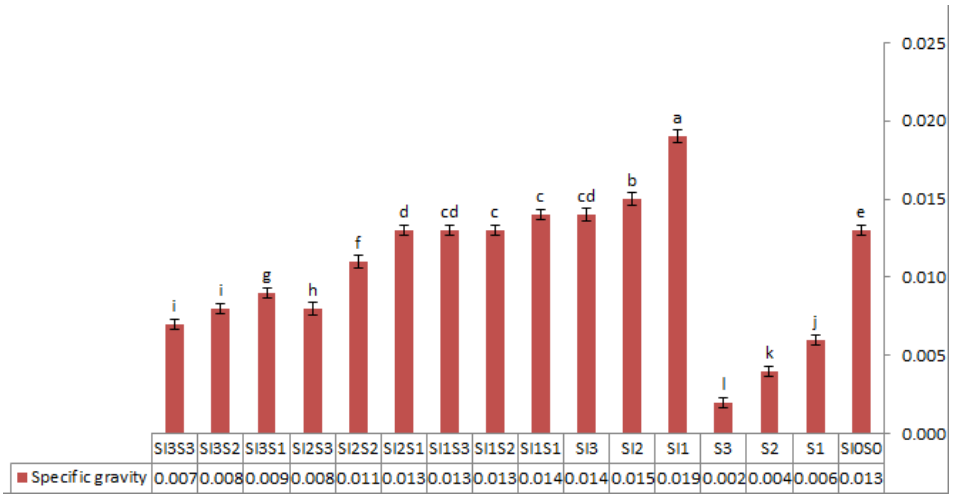


**Figure (4): Effect of NAN3 on the specific gravity of tobacco plants under salinity stress.**

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.

In the current study, during in vitro exposure to sodium azide at high concentrations, the specific gravity of leaves decreased due to the phenotype. The rapid effects of sodium azide on meristematic cells in the cultivated explant are the inhibition of cell proliferation due to its structure. The process of proliferation is in the S phase (S-phased) during... The cell cycle, in addition to some biochemical reasons, causes inhibition of the respiratory chain and the electron transport chain (Farhan *et al*., 2020).

The results of this study are consistent with those of Srivastava *et al*. (2019) Reduced vegetative growth when plants are exposed to chemical mutagens.

**6. Effect of chemical mutagens and salt stress on Leaf Area Index :**

Data in Figure (5) indicate that there are significant differences (P<0.05) between the studied treatments in terms of the leaf area index of the tobacco plant (cm). Salt stress leads to a decrease in the leaf area index, and this decrease increased with increasing salt concentration applied.

Treatment with chemical mutagens increased the leaf area index compared to the control. The treatment with chemical mutagen under salinity conditions at a concentration of 1 mM also outperformed all other treatments and the control.

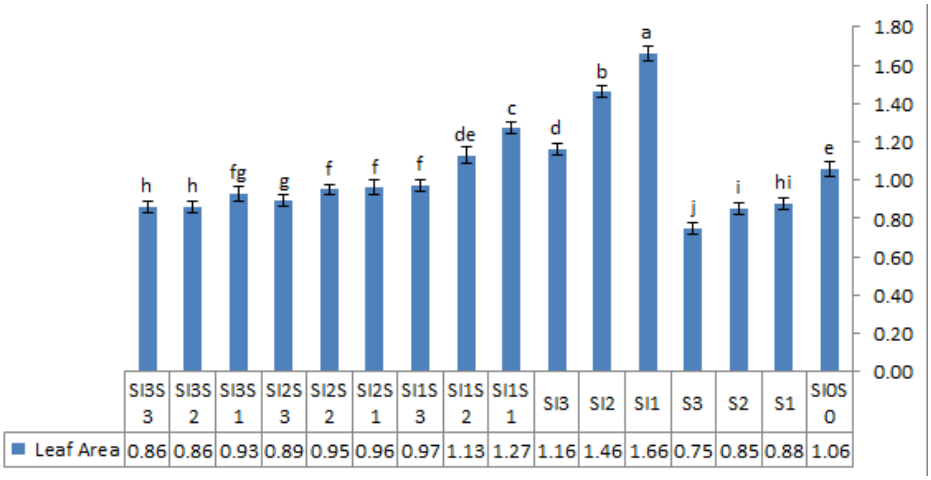


**Figure (5): Effect of NAN3 on Leaf Area Index of tobacco plants under salinity stress.**

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.

It indicated that leaves were the most susceptible plant part in tobacco and even low salt concentrations may depress the yield of leaves and thus, production of this non-food forage crop. The higher sensitivity of leaves was likely resulted in reduction of leaf expansion under lower water potentials. Positive turgor is necessary for the expansion growth of cells, thus, plants respond immediately to osmotic stress as reduction of the rate of leaf expansion (Munns *and* Tester, 2008).In agreement with this, leaf area and leaf expansion rate were the most sensitive parameters to salt in tobacco. Of the two components of salt stress, i.e. hyperosmotic effect and disturbance of ionic equilibrium, the former is the main reason for growth reduction under lower salt concentrations (Hasegawa *et al*., 2000).

**Conclusions:**

Increased NaCl concentrations harmed the properties in general, but the administered NAN3 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, a 1 mM NAN3 dose was 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.

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# تحسين نمو نباتات التبغ (*Nicotiana tabacum* L.) المعاملة بأزيد الصوديوم (NaN3) تحت إجهاد كلوريد الصوديوم

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تاريخ الاستلام: 4/ 06/ 2024 تاريخ القبول:2024/07/13

**الملخص**

أجري البحث خلال عام 2024. عوملت البذور بثلاثة تراكيز من المطفر الكيميائي أزيد الصوديوم (1، 3، 5 ميلي مول) وبزمن نقع قدره (6) ساعات. بالإضافة إلى ذلك، لتحفيز إجهاد الملوحة، تم استخدام كلوريد الصوديوم (NaCl) بتراكيز (4، 8، 12 ديسي مول/سم). نفذت التجربة وفق التصميم العشوائي الكامل (R.C.D.) في قرية برج إسلام – اللاذقية – سورية. ثلاث مكررات لكل معاملة. تم قياس بعض مؤشرات إنبات البذور المعالجة (نسبة الإنبات (%))، المؤشرات المظهرية للنباتات (ارتفاع النبات (سم/نبات))، المؤشرات المورفولوجية (مساحة المسطح الورق الكلي (سم2)، معدل التمثيل الضوئي الصافي (ملجم/سم2) /يوم)(،الوزن النوعي للأوراق (جم/سم2) ودليل مساحة الورقة) .أدى ارتفاع تركيز الملوحة إلى تأثيرات سلبية على جميع المؤشرات المدروسة. أدت المعاملة بالمطفر الكيميائي NAN3 إلى زيادة في معدل الإنبات وارتفاع النبات و مساحة مسطح الورقي الكلي ومعدل التمثيل الضوئي الصافي والوزن النوعي ودليل مساحة الورقة .على الجانب الآخر تفوقت المعاملة بالمطفرات الكيميائية تحت ظروف الإجهاد الملوحة بتركيز منخفض (1 ملم) على جميع المعاملات والشاهد. لذلك، ننصح بنقع البذور بتركيز (1 ملم) NAN3، نظراً لدورها في تحسين الإنبات والخصائص الشكلية والمورفيزيولوجية لصنف التبغ المحلي.

**الكلمات المفتاحية:** نقع البذور،NAN3 ، نبات التبغ، NaCl.