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Improving the Growth of Tobacco Plants (Prilep) Treated with Sodium Azide (NaN3) under Artificial Drought Stress Conditions PEG

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Author's contribution

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

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Original Research Article

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ABSTRACT

The research was conducted during the year 2023. The seeds were treated with three concentrations of the mutagen (1, 3 and 5%) and with a soaking time of (6) hours. In addition, to stimulate drought stress, polyethylene glycol (PEG) was used at concentrations (15, 30, and 45%). The experiment was carried out according to a randomized complete design (R.C.D.) in the village of Al-Jankeel, Latakia, Syria. Three replicates for each treatment were measured. Some germination indicators of treated seeds (germination percentage (%)), phenotypic indicators of plants (plant height (cm/plant)), phenotypic indicators (total leaf surface area (cm2), net photosynthesis rate (mg/cm2)/day) and specific gravity for leaves (g/cm2). Treatment with the chemical mutagen NAN3, especially at low concentration, increased the germination rate, plant height, total leaf surface area, net photosynthesis rate, and leaf specific gravity. Treating chemical mutagens under conditions of drought stress at low concentrations improved the values of the

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studied indicators compared to other treatments. The high concentration of drought led to negative effects on all indicators studied. Therefore, we recommend soaking the seeds with the chemical mutagen NAN3, due to its role in improving the germination and morphological characteristics of the Prilep tobacco variety.

Keywords: Soaking seeds; sodium azide; prilep; salt stress.

1. INTRODUCTION

Tobacco belongs to the Solanaceae family, and the genus Nicotiana, which includes about 76 plant species, of which only two species are of industrial importance [1].

Plants respond to drought stress by inducing changes in their various morphological traits and metabolic processes, such as photosynthesis, antioxidants, and phytohormone levels [2]. Plants can often tolerate drought conditions, but in return this will lead to lower plant productivity [3].

Plant response mechanisms to water shortage have been the focus of physiological and ecological research on water stress including drought stress and waterlogging stress, and are also very important for the breeding of drought-tolerant cultivars [4].

In the last decade, concern about the harmful effects of specific compounds found in tobacco smoke, including alkaloids, has increased. Most efforts have focused on nicotine, and work is currently underway to reduce it as much as possible by producing new varieties or improving cultivated varieties by causing mutations, whether using chemical or physical mutagens. Lai *et al*., [5].

Improving tobacco does not necessarily depend on long-term breeding programs, or on the production of genetically modified plants, but it is possible to benefit from the speed and simplicity of the method based on modifying certain highly beneficial traits through treatment with compounds that have a mutagenic effect [6]. Genetic improvement of tobacco generally leads to the production of varieties distinguished by their high production, good quality, desirable technological characteristics, adaptation to prevailing environmental conditions, and resistance to biotic (diseases and insects) and abiotic (drought, salinity, and frost) stress, and is characterized by the highest degree of originality and genetic similarity, and the lowest degree of Genetic mixing between plants occurs through continuous self-pollination over several successive generations [7].

The objectives of this study were:

(1). Effect of drought 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 NAN3 on the studied properties under and without drought stress conditions.

2. MATERIALS AND METHODS

The experiment was carried out during the 2023 season. The field experiment was conducted in the village of Al-Jankeel, within a greenhouse in Latakia.

2.1 Plant Material

Tobacco plant material, Praylep cultivar, was used as the plant material in this study. It was registered by the General Tobacco Corporation in Latakia. NAN3 Treatment In our study, the NAN3-induced pediatric trial of (Ren NAN3). Started, 25 seeds per LEVY technique replicates were soaked in 15 ml (0.6 ml/seed) in 0.05 M phosphate starting, pH 8.0 for 6 h, at 20°C at 100 rpm constant pumping.

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, 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).

Polyethylene glycol (PEG-6000) was used as a percentage (%) for novelty, artificial drought stress, and other osmotic stress [8] through irrigation. Diversity Retin provided 200 ml per plant at all times, and watering was done. To the bottom of the plant and with a difference between the popular pasture, during the growth period of the plants, which corresponds to the vegetative growth stage, after transplanting and after a month, where fatigue stress is applied in the first good generation, to achieve stress standards as follows:

- P0: Water fresh plants only.
- P1: The plants were irrigated with a 15% solution, which characterizes an osmotic pressure of 0.7 MPa.
- P2: The plants were irrigated with a solution of 30% Brazil osmotic pressure - 1.4 MPa.
- P3: The plants were irrigated with a solution of 45% Brazil osmotic pressure - 2.1 MPa.

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 Williams, [9].
  (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 [10].

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 NAN3 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 tobacco variety was not significantly affected by the soaking treatment with a low concentration (1)% of the mutagen NAN3, as it reached 90% in the N1 treatment, compared to the control, 92%. The germination rate decreased significantly ($P<0.05$) with the increase in the concentration of the mutagen NAN3 to be recorded. Its lowest value is 32% for N3 transactions.

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

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

Germination percentage(%)	Transactions
3[a] ± **9**2	N0
2.5[a] ± **9**0	N1
1.**5**[b] ± **6**4	N2
2 [C] ± 32	N3

The stimulating effect of mutagenesis at low concentrations on germination speed leads to RNA activation, or protein synthesis may occur during the early stage of germination after seed treatment [11].

Mutagenesis at high concentrations caused a delay in the start of metabolism after germination, which led to a delay in mitotic activity and rapid plant growth, and could be due to damage at the cellular level and even chromosomes [12].

3.2 Effect of Chemical Mutagens and Drought 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).

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

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 drought conditions at low concentration also led to an increase in plant height compared to the other treatments and the control.

The reason for the reduced height of tobacco plants when exposed to drought stress may be due to the lack of cell division of the stem and leaves and their small size as a result of the decrease in water potential in them due to the lack of soil water readiness, which leads to a decrease in the efficiency of converting solar energy into chemical energy and producing dry matter [13].

Mutagenesis treatments with NAN3 at low concentrations increased tolerance to drought stress applied to the plant and improved growth characteristics [14].

3.3 Effect of Chemical Mutagens and Drought 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.

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

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 drought 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.

The leaf area and the total leaf surface area of the plant are growth indicators that indicate the intensity and strength of the stress to which the plant is exposed under conditions of many biotic and abiotic stressors, including water stress, and this is consistent with the results of Potopová *et al*., [15].

The reason for the increase in leaf flatness at low concentration (0.1%) can be attributed to the increase in leaf length and width resulting from increased plant cell division, as indicated by Omosun *et al*., [16].

3.4 Effect of Chemical Mutagens and Drought 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).

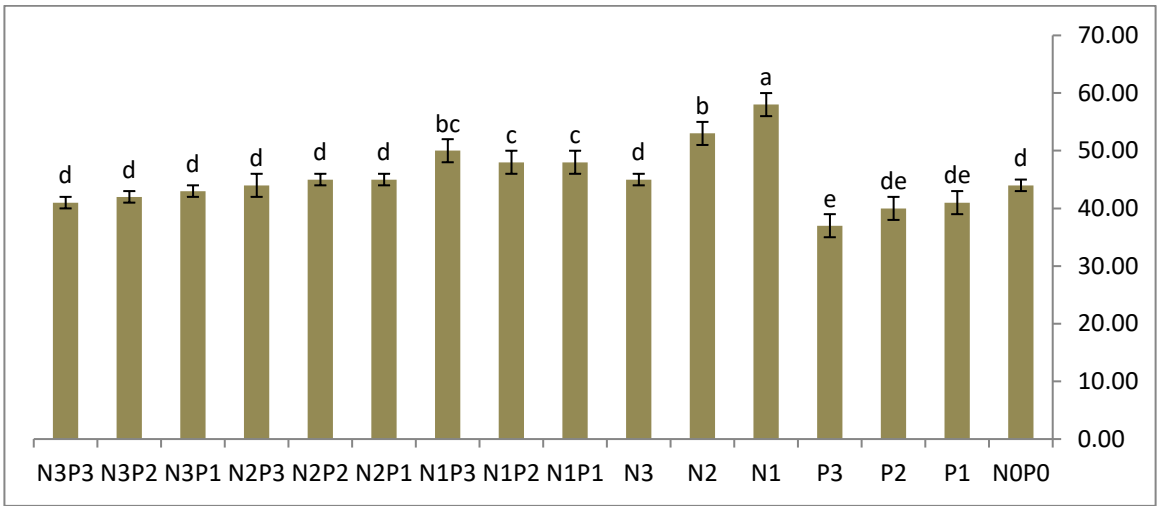

Fig. 1. Effect of NAN3 on the height of tobacco plants under drought stress

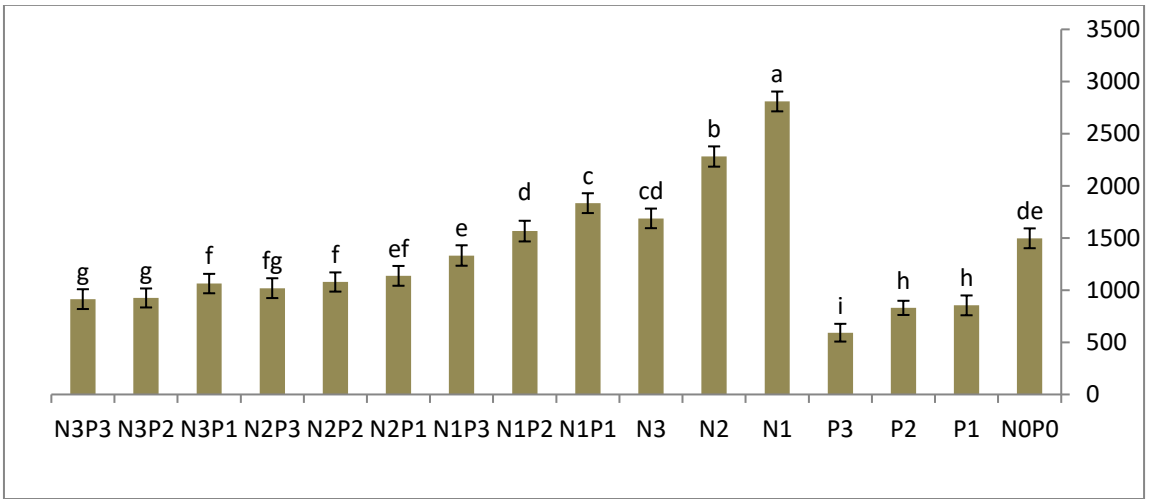

Fig. 2. Effect of NAN3 on the total leaf surface area of tobacco plants under drought stress

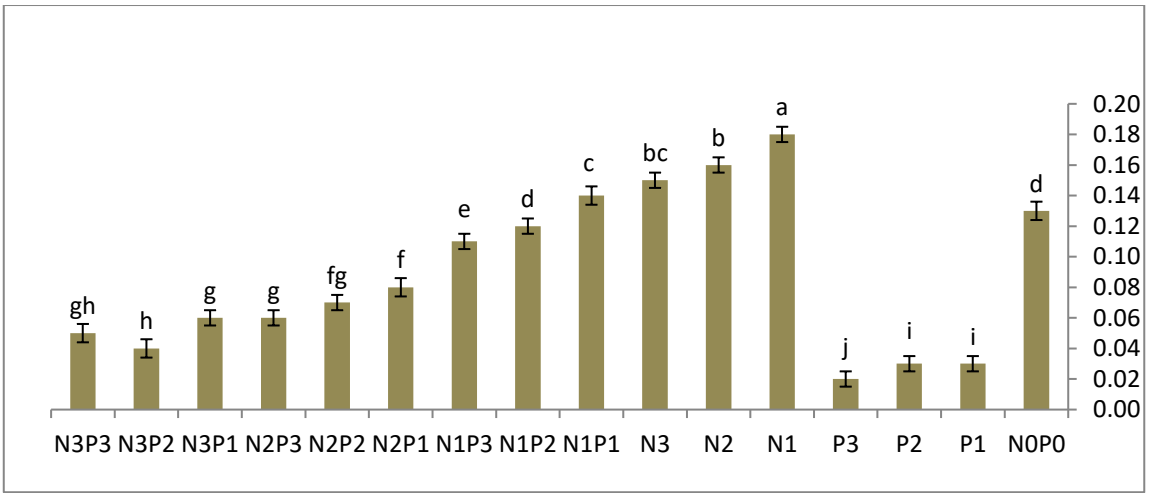

Fig. 3. Effect of NAN3 on the net photosynthesis rate of tobacco plants under drought stress

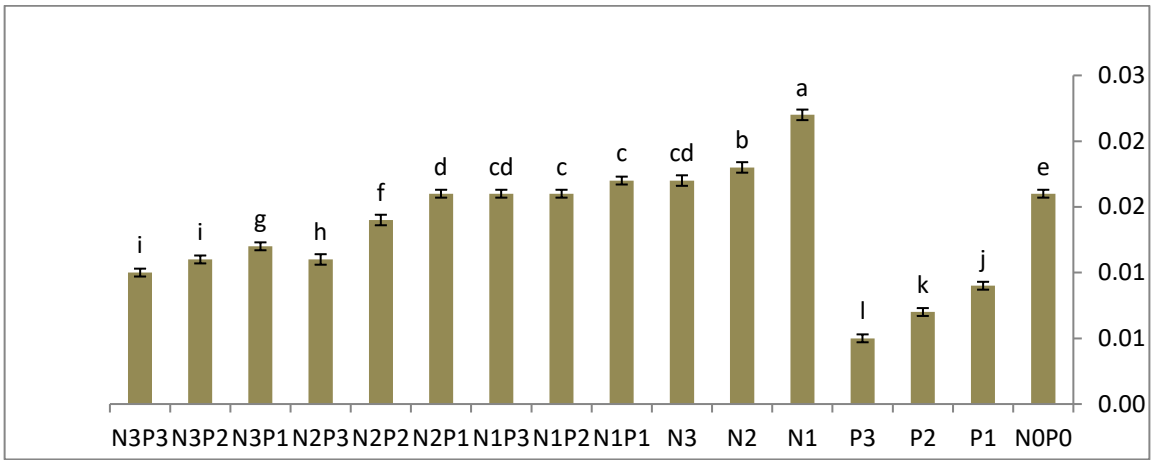

Fig. 4. Effect of NAN3 on the specific gravity of tobacco plants under drought stress

Drought stress led to a decrease in the net photosynthesis rate, and this decrease increased with an increase in the applied drought 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 drought conditions at low concentration also led to an increase in the net photosynthesis rate compared to the remaining treatments and the control.

The results showed by Chen *et al*. [17] reported on the effect of drought stress using polyethylene glycol (PEG) on two tobacco varieties, a decrease in plant growth rate (leaf surface area and plant wet weight), as well as a decrease in leaf chlorophyll content and net photosynthesis rate [18]. Other researchers attributed the noticeable decrease in the rate of photosynthesis under the influence of drought stress to the fact that water is an essential factor in all the photochemical-photochemical and biochemical reactions necessary to fix carbon in the process of photosynthesis. It has been noted that drought stress, over a short period, leads to a temporary cessation of growth followed by a decrease in the rate of photosynthesis. In the intensity of photosynthesis [19].

3.5 Effect of Chemical Mutagens and Drought 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).

Drought stress led to a decrease in the specific gravity, and this decrease increased with the increase in the applied drought concentration [20,21].

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 [22].

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

Drought inhibits growth due to a lack of cell filling pressure and causes a lack of water access to growing tissues due to the inability of the roots to grow and absorb water and mineral drought, and the lack of cell division and small size [23].

4. CONCLUSIONS

Increasing dehydration concentrations had a negative effect on the overall properties, but NAN3 doses administered reduced or reduced the negative effects of dehydration. According to the results of this research, the studied properties showed a lot of variation depending on the agents used, and a dose of 1% of NAN3 was usually selected to reduce the negative effect of dehydration. According to the study and literature search, it is necessary to conduct more research on developing drought-tolerant plants by taking advantage of mutations induced in laboratory 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.

REFERENCES

1. Knapp S, Chasem MW, Clarkson JJ. Nomenclatural changes and a new sectional classification in Nicotiana (Solanaceae). Taxon. 2004;53:73–82.
2. Zhu M, Chen G, Zhang J, Zhang Y, Xie Q, Zhao Z, Pan Y, Hu Z. The abiotic stress-responsive NAC-type transcription factor SlNAC4 regulates salt and drought tolerance and stress-related genes in tomato (Solanum lycopersicum). Plant Cell Reports. 2014;33:1851-1863.
3. Yahaya MA, Shimelis H. Drought Stress in Sorghum: Mitigation Strategies, Breeding Methods and Technologies—A Review. J. Agron. Crop. Sci. 2022;208:127–142.
4. Ren B, Zhang J, Dong S, Liu P, Zhao B. Responses of carbon metabolism and antioxidant system of summer maize to waterlogging at different stages. J. Agron. Crop Sci. 2018;204: 505–514.
5. Lai R, Ikram M, Li R, Xia Y, Yuan Q, Zhao W, Zhang Z, Siddique KHM, Guo P. Identification of novel quantitative trait nucleotides and candidate genes for bacterial wilt resistance in tobacco (*Nicotiana tabacum* L.) using genotyping-by-sequencing and multi-locus genome-wide association studies. Front. Plant Sci. 2021;12:744175.
6. Reddy TV, Dwivedi S, Sharma NK. Development of TILLING by sequencing platform towards enhanced leaf yield in tobacco. Ind. Crops Prod. 2012;40:324–335.
7. Sierro N, Battey JN, Ouadi S, Bakaher N, Bovet L, Willig A, Goepfert S, Peitsch MC, Ivanov N V. The tobacco genome sequence and its comparison with those of tomato and potato. Nature communications. 2014;5(1):3833.
8. Muscolo A, Sidari M, Anastasi U, Santonoceto C, Maggio A. Effect of PEG-induced drought stress on seed germination of four lentil genotypes. Journal of Plant Interactions. 2014;9(1): 354–363.
9. Williams RF. The physiology of plant growth with special reference to the concept of net assimilation rate. Annals of Botany. 1946;37:41-71.
10. Pearce RB, Brown RH, Blaser RE. Photosynthesis of alfalfa leaves as influenced by age and environment. Crop Science. 1968;8:677-680.
11. Bhat TM, Ansari MYK, Aslam R. Sodium azide (NaN3) induced genetic variation of Psoralea corylifolia L. and analysis of variants using RAPD markers. The Nucleus. 2012;55:149-154.
12. Borzouei, A., Kafi, M., Khazaei, H., Naseriyan, B. and Majdabadi, A. Effects of Gamma Radiation on Germination and Physiological Aspects of Wheat (*Triticum Aestivum* L.) Seedlings, Pakistan Journal of Botany. 2010;42(4):2281-2290.
13. Nezhadahmadi A, Prodhan ZH, Faruq G. Drought tolerance in wheat. Sci World J. 2013;17.
14. Mathew I, Shimelis H, Mutema M, Clulow A, Zengeni R, Mbava N. Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J. Agron. Crop Sci. 2019;205:385–400.
15. Potopová V, Boroneant C, Boincean B, Soukup J. Impact of agricultural drought on main crop yields in the republic of moldova. Int. J. Climatol. 2016;36:2063–2082.
16. Omosun G, Akanwa FE, Lazarus B. Effect of Ethyl Methanesulfonate (EMS) on the Germination, Growth and Yield of Two Okra (*Abelmoschus esculentus* (L.) Moench) Varieties. African Scientist. 2022; 22(2).
17. Chen Z, Xu J, Wang F, Wang L, Xu Z. Morpho-physiological and proteomic responses to water stress in two contrasting tobacco varieties. Scientific reports. 2019;9(1):18523.
18. Babitha KC, Vemanna RS, Nataraja KN, Udayakumar M. Overexpression of EcbHLH57 transcription factor from Eleusine coracana L. in tobacco confers tolerance to salt, oxidative and drought stress. PloS one. 2015;10(9): e0137098.
19. Alam SA, Huang JG, Stadt KJ, Comeau PG, Dawson A, Gea-Izquierdo G, Aakala

T, Holtta T, Vesala T, Makela A, Berninger F. Effects of competition, drought stress and photosynthetic productivity on the radial growth of white spruce in western Canada. Front Plant Sci. 2017;8:1915.

20. Ren XueLiang RX, Wang Yi WY, Yang ChunYuan YC, Shi YueWei SY, Wang MaoSheng W. M. Mutagenic effects of combined treatments of γ-rays and NaN3 on agronomic characteristics in tobacco.; 2008.
21. Purnima, and Pooja Singh. Plant Growth Promoting- Rhizobacteria (PGPR): Their Potential as Biofertilizer and Biopesticide Agents: A Review. Asian Journal of Advances in Agricultural Research. 2023; 22(1):25-37. Available:https://doi.org/10.9734/ajaar/2023/v22i1431.
22. Van Loon LC. Plant responses to plant growth-promoting rhizobacteria. New perspectives and approaches in plant growth-promoting Rhizobacteria research. 2007:243-54.
23. Li Z, Li Y, Zhang Y, Chen B., Peng Y, Zhang X, Ma X, Hung L, Yan Y. Indole-3-acetic acid modulates phytohormones and polyamines metabolism associated with the tolerance to water stress in white clover. Plant Physiology and Biochemistry. 2018;129: 251-263.

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