Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Effects of sodium azide (NaN3) treatment on *in vitro* growth and genetic variation in the Garnem rootstock

Sarah Ali Muhammad Al- Hayany
* Corresponding author. Horticulture Department, College of Agriculture, University of Diyala, Iraq. E-mail: saraali@uodiyala.edu.iq

Mohammed D. Abdulhadi
Horticulture Department, College of Agriculture, University of Diyala, Iraq. E-mail: mohammed.daher@uodiyala.edu.iq

Ayad Assi Obaid
Horticulture Department, College of Agriculture, University of Diyala, Iraq. E-mail: ayadassi@uodiyala.edu.iq

Abstract

Objective

Sodium azide is a mutagenic chemical used to create genetic diversity in plants. The aim of this study was to investigate the effect of different concentrations of sodium azide on the in vitro growth of Garnem rootstock and to identify possible changes in the sequence of the chloroplast rbcL gene after treatment.

Materials and methods

This study was conducted on Garnem rootstock under in vitro conditions from August 2021 to June 2022. Homogenized explants were treated with sodium azide at concentrations of 13 and 26 mg/L for 60 minutes, and untreated samples were considered as controls. Each treatment consisted of 10 independent replicates. After treatment, the explants were cultured in proliferation medium containing 6 mg/L kinetin (Kin) and 1 mg/L 6-benzylaminopurine (BA). Mortality percentage, number of branches, branch length and number of leaves were evaluated. Also, a fragment of the rbcL gene was amplified by PCR and sequenced in the control and treated samples. Sequence similarity was examined using BLAST.

Results

Sodium azide affected the survival and growth of branches. The highest mortality of explants (70%) was observed at a concentration of 26 mg/L, while no mortality was recorded in the control treatment. The highest average number of branches and leaves was obtained in the control. The length of branches also differed between the treatments; the highest value was observed at a concentration of 26 and the lowest value at a concentration of 13 mg/L. Sequence comparison showed that in the studied region of the rbcL gene of the treated samples, there were possible

changes in some nucleotides. The studied sequences had a high similarity with the reference sequence of Prunus persica rbcL with accession number JN114836.1 in GenBank.

Conclusion

Sodium azide affected the viability and in vitro growth of Garnemo basal explants in a concentration-dependent manner. Also, sequence changes in the studied region of the chloroplast rbcL gene were observed after treatment. However, to confirm the mutational nature, heritability, and extent of these changes, further biological replicates and sequencing of additional genomic regions are necessary.

Keywords: garnem, mutagens, peach rootstock, sequencing, sodium azide

Paper Type: Research Paper.

Citation: Al- Hayany, S. A. M., Abdulhadi, M. D., & Obaid, A. A. (2026). Effects of sodium azide (NaN3) treatment on *in vitro* growth and genetic variation in the Garnem rootstock. *Agricultural Biotechnology Journal,* 18(4), 615-628.

Agricultural Biotechnology Journal, 18(4), 615-628. DOI: 10.22103/jab.2026.27271.1922

Received: May 30, 2026. Received in revised form: August 08, 2026.

Accepted: August 09, 2026. Published online: August 30, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

Introduction

Induced mutagenesis is one of the effective methods for creating genetic diversity in plants and has been widely used in plant breeding and improving agronomic traits. Mutations may occur naturally and are an important source of genetic diversity. However, when desirable traits are not found in existing genetic resources, new genetic diversity can be created using artificial mutagenesis. Physical and chemical mutagens are used to induce mutations in seeds, embryos, buds, and other plant tissues. The success rate of mutagenesis depends on factors such as the plant genotype, type and concentration of the mutagen, duration of treatment, and physiological status of the tissue used (Oladosu et al., 2016; Kharkwal, 2023). Chemical mutagens include alkylating agents, nitroso compounds, nucleotide base analogues, sodium azide, and acridine dyes. Among them, sodium azide (NaN_3) is one of the chemical mutagens of interest in plant studies due to its ability to induce genetic variation at relatively low concentrations (Kharkwal, 2023; Gruszka et al., 2012). The mutagenic activity of sodium azide is dependent on the treatment conditions, especially pH. This compound can be converted to azidoalanine in plants, which is known as a mutagenic metabolite in biological systems (LaVelle and Mangold, 1987). Also, previous studies have shown the role of acidic conditions in increasing the mutagenic activity of sodium azide (Gruszka et al., 2012). Therefore, appropriate determination of sodium azide concentration and treatment duration is essential to induce genetic variation and maintain tissue viability. Sodium

azide has been used to induce mutations in various plant species and can affect growth, physiological characteristics, and agronomic traits. Various studies have shown that this compound is capable of generating useful genetic diversity and can be used in mutation-based breeding programs to improve crop traits (Owais et al., 1983; Owais and Kleinhofs, 1988; Liang and Fournier, 1995; Khan et al., 2009; Gruszka et al., 2012). However, the plant response to sodium azide depends on the species, genotype, tissue type, concentration, and duration of treatment. Therefore, a given concentration may have a favorable effect in one species or tissue, but cause severe toxicity in another species or tissue. Recent studies have also provided further evidence for the mutagenic potential of sodium azide. Liu et al. (2025) showed using genome-wide analysis that sodium azide treatment can induce a large number of single nucleotide and other changes in DNA sequence. Also, the spectrum of changes induced can be influenced by genetic background and genomic location. These results confirm the capacity of sodium azide to induce genetic diversity and at the same time, indicate the necessity of molecular investigation of treated plant materials (Al-Hayany & Obaid, 2024; Liu et al., 2025). The effect of sodium azide under in vitro conditions has also been investigated in several plant species. Ramadan et al. (2016) investigated the effect of this substance on embryos, shoot tips and nodal explants of bitter and sweet almonds. The results showed that the survival of explants and vegetative growth depended on the concentration of sodium azide and the duration of treatment. The 60-min treatment caused changes in the number of branches, number of leaves and length of branches, indicating a concentration-dependent response of almond tissues to sodium azide (Ramadan et al., 2016). Also, Gómez et al. (2019) investigated the effect of sodium azide on micropropagated pineapple seedlings in temporary submerged bioreactors. With increasing sodium azide concentration, branch proliferation decreased significantly. At the highest concentration tested, the proliferation rate was reduced by about 87% compared to the control (Gómez et al., 2019). The physiological effect of sodium azide has also been reported in other horticultural crops. With increasing sodium azide concentration and increasing treatment time, the survival of the shoots decreases. These findings indicate that the appropriate dose for mutation induction depends on both concentration and duration of treatment. Yousif et al. (2025) also reported that 0.25 and 0.5 mM sodium azide concentrations reduced shoot proliferation in strawberry plants grown in vitro. Overall, the results of the available studies indicate that a good balance should be struck in the design of sodium azide treatments between inducing genetic changes and maintaining tissue viability. Garnem (G×N15) is an interspecific clonal rootstock obtained from a cross between almond and peach (Prunus amygdalus × Prunus persica). This rootstock is used for a number of stone fruit crops and is important due to its vigorous growth, good adaptability, and some desirable traits related to resistance. On the other hand, the ability of garnem to clonal proliferation makes it a suitable material for in vitro culture studies. Despite the importance of this foundation, information on the effect of chemical mutagens, especially sodium azide, on in vitro growth and molecular changes in garnemo is still limited. Molecular examination of treated plant material could provide further information on possible DNA changes after mutagenesis. The chloroplast gene rbcL encodes the large subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco is one of the key enzymes in the photosynthetic carbon fixation process. The rbcL gene

is located in the chloroplast genome, while the small subunit of Rubisco is encoded by the nuclear rbcS gene. Together, these two subunits constitute the complete Rubisco enzyme; therefore, coordination between the chloroplast and nuclear components is essential for the formation and healthy function of the plant.

Materials and methods

Plant material and in vitro culture: This study was conducted from August 2021 to June 2022 at the tissue culture laboratory located in Al-Fahma district, Baghdad, Iraq. Garnum rootstock was studied as the plant material. For mutagenicity testing, homogenous and healthy explants from in vitro culture were selected. The explants were cultured in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 6 mg/L kinetin (Kin) and 1 mg/L 6-benzylaminopurine (BA). 30 mL of the medium was distributed into each 300 mL glass vessel. The cultures were maintained under standard conditions used for micropropagation of Garnum rootstock.

Sodium azide treatment: Homogeneous explants were selected for sodium azide treatment. The samples were immersed in sodium azide (NaN_3) solutions at concentrations of 13 and 26 mg/L for 60 minutes. Untreated samples with sodium azide (0 mg/L) were considered as controls. Sodium azide solutions were prepared with double-distilled water and their pH was adjusted to 3.0. The explants were immersed in the solutions under sterile conditions for 60 minutes. After treatment, the explants were washed three times with sterile distilled water and then transferred to the propagation medium.

Experimental design and statistical analysis: The experiment was conducted in a completely randomized design (CRD). Each treatment consisted of 10 independent replications and one explant was considered as an experimental unit. The traits studied included explant mortality, number of branches per explant, branch length, number of leaves and seedling length. Data were analyzed using analysis of variance (ANOVA). Comparison of treatment means was performed with Duncan's multiple range test at a probability level of $P < 0.05$. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

DNA extraction: For molecular examination, three samples were selected: untreated control sample, sample treated with 13 mg/L sodium azide and sample treated with 26 mg/L sodium azide. Genomic DNA was extracted using a DNA extraction kit according to the manufacturer's instructions. The use of the commercial plant DNA extraction kit from Zymo Research in molecular studies of plants has been previously reported (Isuosuo et al., 2024).

Amplification of the chloroplast rbcL region by PCR: The rbcL region of the chloroplast genome was selected for molecular investigation and identification of sequence variations. This gene encodes the large subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Amplification of this region was performed using the rbcLa primer pair introduced by Maloukh et al. (2017). The forward primer sequence was 5′-ATGTCACCACAAACAGAGACTAAGC-3′. Its melting temperature (Tm) was 57.2°C and its GC percentage was 41%. The reverse primer sequence was 5′-GTAAAATCAAGTCCACCCAG-3′, melting temperature 52.0°C and GC percentage was 47%. The expected size of the PCR

product was determined to be approximately 650 base pairs (bp) (Maloukh et al., 2017). PCR reactions were performed according to the protocols reported by Abdulhadi et al. (2019) and Din et al. (2023).

Agarose gel electrophoresis and purification of PCR products: PCR products were separated using 2% agarose gel electrophoresis. After staining, DNA bands were visualized at 302 nm under ultraviolet (UV) light. PCR products of the expected size were purified before sequencing.

DNA sequencing and sequence analysis: The purified PCR products were sequenced by Sanger duplex sequencing. Sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting nucleotide sequences were edited and assembled. To identify similar sequences, a search was performed using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) database (NCBI Resource Coordinators, 2016). Then, the resulting sequences were compared with the reference sequences available in GenBank and multiple alignment of the sequences was performed. To identify possible changes, the sequence of the control sample was compared with the sequence of the samples treated with sodium azide. The changes examined included single nucleotide substitutions, insertions, and deletions of nucleotides. Sequence differences compared to the control sample and the corresponding reference sequence were evaluated.

Reference sequence: The reference sequence of Prunus persica rbcL was obtained from GenBank with accession number JN114836.1 for sequence comparison. Based on the sample identification system in the laboratory, the control sample and the samples treated with sodium azide were identified with the numbers 1, 2, and 3, respectively.

Results and discussion

Explant mortality: Figure 1 shows the effect of sodium azide concentrations on explant mortality; it shows that the highest explant mortality reached 70% at a concentration of 26 mg/L, while no explant mortalities were recorded in the control treatment.

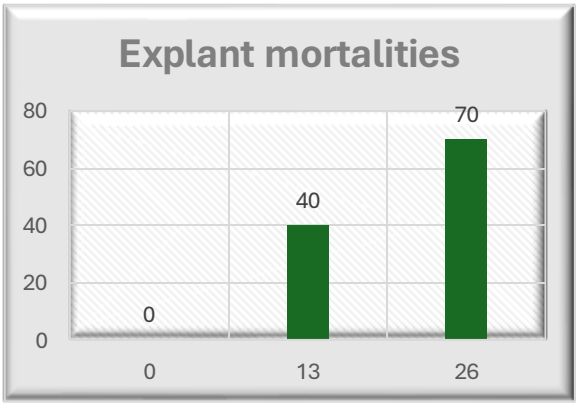

Figure 1. Effect of sodium azide concentrations on explant mortalities

Number of shoots: The results show significant differences between the concentrations of sodium azide used. The largest number of shoots was obtained with the control treatment, reaching 1.889 shoot expant shoots. It is noted that their numbers decrease with increasing concentration, reaching their lowest 1.331 shoot explants^{-1} at a concentration of 26 mg/L (Figure 2).

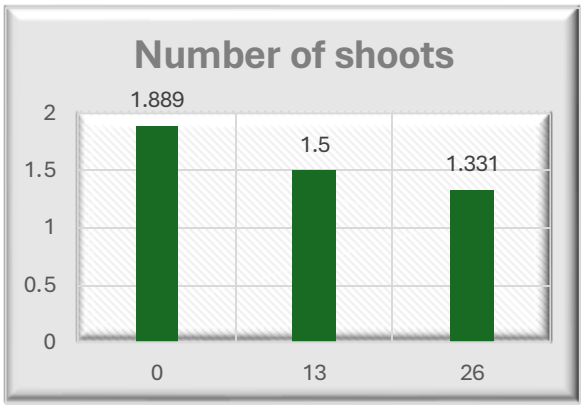

Figure 2. Effect of sodium azide concentrations on number of shoots

Shoot length: The results indicate that the treatment with sodium azide at 26 mg/L was significantly superior, giving the longest shoot length (12.639 mm), whereas the shortest shoot length was 8.341 mm when treated with a concentration of 13 mg/L (Figure 3).

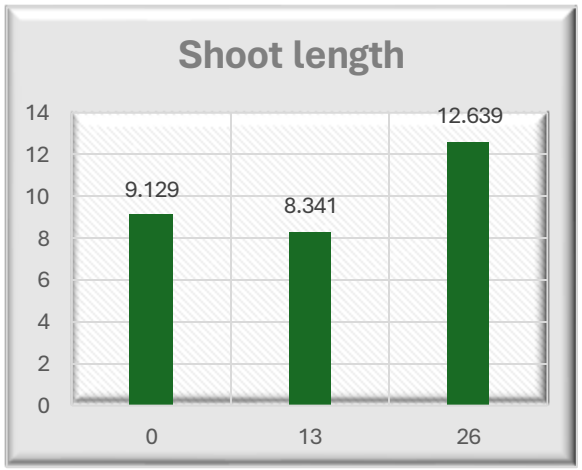

Figure 3. Effect of sodium azide concentrations on shoot lenght

Number of leaves: The results in showed that the control treatment was significantly superior, recording the largest number of leaves (17,670 leaf plant^{-1}), whereas the smallest number of leaves was obtained as a result of the treatment with sodium azide at 13 mg/L, (9,000 leaf explant^{-1}) (Figure 4).

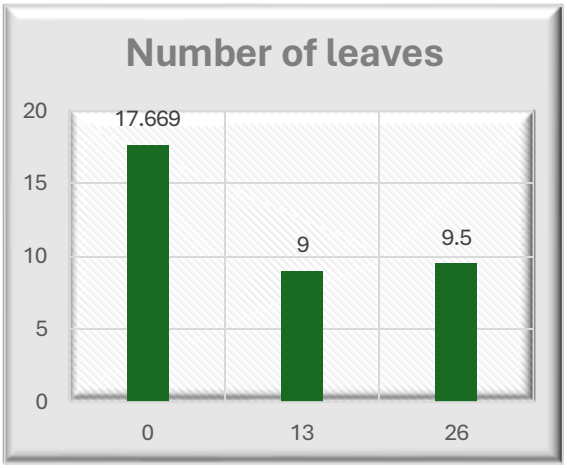

Figure 4. Effect of sodium azide concentrations on number of leaves

Shoot length (mm): From the results in Table 1, it is evident that the control treatment was superior, recording the longest seedling length (12.639 mm), thus significantly surpassing the other treatments. It is noted that the longest seedlings decreased with increasing sodium azide concentration, recording the lowest length of 14.770 mm at a concentration of 26 mg/L.

Table 1. The information on the mutant samples compared to samples from the database. NCBI

NO ID	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
Mutant 1	Transversion GAP Transversion Transition	18 57	A/T T/A	JN114836.1	Prunus persica voucher SBB-1089 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	99.64%
Mutant 2	GAP Transversion Transition	57	T/A	JN114836.1	Prunus persica voucher SBB-1089 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	99.82%

Plantlet length (mm): From the results in Table 1, it is evident that the control treatment was superior, recording the longest seedling length of 23.566 mm, thus significantly surpassing the other treatments. It is noted that the longest seedlings decreased with increasing so dium azide concentration, recording the lowest length of 14.770 mm at a concentration of 26 mg/L (Figure 5).

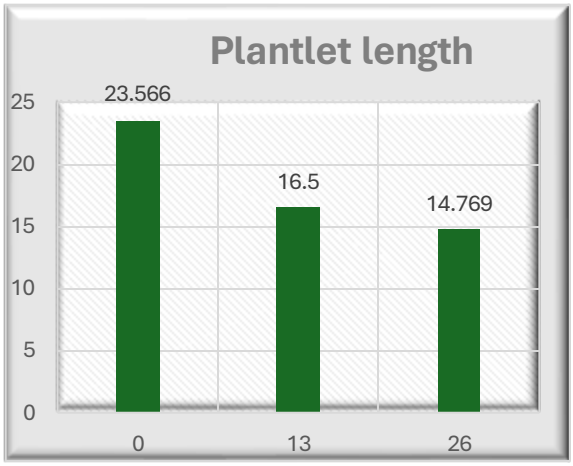

Figure 5 Effect of sodium azide concentrations on plantlet length

The results of the nucleotide sequence analysis of the gene showed rbcL is responsible for encoding the RuBisCO enzyme in chloroplasts by 99.64% and 99.82% for the first and second mutant samples with the control sample. The first sample showed two-point mutations at nucleotides 5 and 39 with respect to the control treatment (C/T and A/T in succession) (Figure 6).

Score	Expect	Identities	Gaps	Strand
1071 bits(548)	0.0	554/556(99%)	0/556(0%)	Plus/Plus

```
Query  2    CGACGGATTCAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAA  61
Sbjct  1    ...T.....................................T...................  60

Query  62   ACCAAAGATACTGATATCTTGGCAGCATTTCGAGTAACTCCTCAACCTGGAGTTCCACCT  121
Sbjct  61   ............................................................  120

Query  122  GAAGAAGCAGGGGCAGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGG  181
Sbjct  121  ............................................................  180

Query  182  ACTGACGGGCTTACTAGTCTTGATCGTTACAAAGGTCGATGCTACCACATCGAGCCCGTT  241
Sbjct  181  ............................................................  240

Query  242  GCTGGAGAAGAAAGTCAATTTATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAG  301
Sbjct  241  ............................................................  300

Query  302  GGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTGTTTGGGTTCAAGGCCCTG  361
Sbjct  301  ............................................................  360

Query  362  CGCGCTCTACGTCTGGAGGATTTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGC  421
Sbjct  361  ............................................................  420

Query  422  CCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGCCGCCCCCTATTG  481
Sbjct  421  ............................................................  480

Query  482  GGATGTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTAT  541
Sbjct  481  ............................................................  540

Query  542  GAATGTCTCCGYGGTG  557
Sbjct  541  ................  556
```

Figure 6. The location of point mutations in the first mutant sample compared to the control sample

554 nucleotides are identical as well as a single point mutation exists between the second mutant sample and the control treatment at nucleotide 39 relative to the control treatment A/T. The results show 551 nucleotide similarity, and the studied sequence contains only a point mutation without any gaps, insertions, or deletions of nucleotides compared to the standard sample. and since the plastid genome Plastome appeared stable comparing the sequence results, the first mutant sample showed the same two-point mutations recorded with the comparison sample above and with the sample recorded in the sequence. JN114836.1 In the database NCBI the second sample with the recorded mutation also showed similarity to the control sample when compared to the sample recorded in the sequence. JN114836.1 In the database NCBI (Figure 7).

Score	Expect	Identities	Gaps	Strand
1069 bits(547)	0.0	551/552(99%)	0/552(0%)	Plus/Plus

```
Query  6    GGATTCAAGCTGGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACCA  65
Sbjct  6    .....................................T......................  65

Query  66   AAGATACTGATATCTTGGCAGCATTTCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAG  125
Sbjct  66   ............................................................  125

Query  126  AAGCAGGGGCAGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTATGGACTG  185
Sbjct  126  ............................................................  185

Query  186  ACGGGCTTACTAGTCTTGATCGTTACAAAGGTCGATGCTACCACATCGAGCCCGTTGCTG  245
Sbjct  186  ............................................................  245

Query  246  GAGAAGAAAGTCAATTTATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAGGGTT  305
Sbjct  246  ............................................................  305

Query  306  CTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTGTTTGGGTTCAAGGCCCTGCGCG  365
Sbjct  306  ............................................................  365

Query  366  CTCTACGTCTGGAGGATTTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCGC  425
Sbjct  366  ............................................................  425

Query  426  CTCATGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGCCGCCCCCTATTGGGAT  485
Sbjct  426  ............................................................  485

Query  486  GTACTATTAAACCTAAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAAT  545
Sbjct  486  ............................................................  545

Query  546  GTCTCCGYGGTG  557
Sbjct  546  ............  557
```

Figure 7. The location of the point mutations in the second mutant sample compared to the control sample

Sodium azide is used as a potent mutagen to induce genetic changes in agricultural crops after its metabolism in the plant. This compound disrupts normal cellular activities by affecting intracellular calcium levels, thus reducing levels of Calmodulin production of adenine triphosphate (ATP) ATP low levels ATP in addition to disrupting spindle fiber organization and chromosome movement during mitosis, sodium azide also affects cellular respiration processes by inhibiting several enzymatic activities, including Catalase Peroxidase in addition to Cytochrome Oxidase Next to the proton pump Proton Pump it inhibits the cell cycle, and several studies have indicated that sodium azide acts as a mutagenic agent, transforming into an intermediate product that affects numerous cellular processes in the host plant, contributing to changes DNA for the host plant, all these metabolic disturbances ultimately contribute to the appearance of chromosomal abnormalities known as point mutations, which affect one or more

desirable traits in agricultural crops, ultimately improving production, quality, and resistance to biotic and abiotic stresses (Khan et al., 2009; Singh & Olejniczak, 1983; Jalil et al., 2023).

Conclusions: Sodium azide treatment affected the survival and in vitro growth of basal explants of Garnemo. The highest mortality of explants was observed at a concentration of 26 mg/L. Compared with sodium azide treatments; control samples generally produced more branches and leaves. Sequence analysis of the rbcL chloroplast region showed that the control and treated samples had high sequence similarity. However, a limited number of putative nucleotide changes were identified in the treated samples. These findings indicate that sodium azide treatment may cause changes in the sequence of the studied region of the rbcL gene. However, the data obtained from this study are insufficient to determine the functional effects, heritability, and extent of these changes at the genome level. To confirm the biological significance of these changes, it is necessary to conduct further studies using independent biological replicates, more markers from the nuclear and chloroplast genomes, and examining indices related to photosynthetic performance.

Author contributions

Sarah Ali Muhammad Al- Hayany; Methodology and Writing-Original Draft Preparation. Mohammed D. Abdulhadi; Software, Data Curation and Supervision. Ayad Assi Obaid; Investigation, Writing-Review & Editing.

Data Availability Statement

Sample recorded in the sequence JN114836.1 In the database NCBI the second sample with the recorded mutation also showed similarity to the control sample when compared to the sample recorded in the sequence JN114836.1 In the database NCBI.

Acknowledgments

The authors would like to express their sincere gratitude to Horticulture Department, College of Agriculture, University of Diyala for their support and provide the equipment

Ethical considerations

Not applicable. This study focused on agricultural pests and did not involve human subjects or animal experiments that require ethical approval.

Funding

This research received no external funding.

Conflict of interests

The authors declare no conflict of interest.

References

Abdulhadi, B. A., Kot, P., Hashim, K. S., Shaw, A., & Al Khaddar, R. (2019). Influence of current density and electrodes spacing on reactive red 120 dye removal from dyed water using electrocoagulation/electroflotation (EC/EF) process. *IOP Conference Series: Materials Science and Engineering, 584*(1), Article 012035. https://doi.org/10.1088/1757-899X/584/1/012035

Al-Hayany, S. A. M., & Obaid, A. A. (2024). Processing browning and increasing multiplication and rotting of peach rootstock cv. Garnem by using antioxidants and plant growth regulators in vitro. *Kufa Journal for Agricultural Sciences, 16*(2). https://doi.org/10.36077/kjas/2024/v16i2.10942

Din, A., Qadri, Z. A., Wani, M. A., Iqbal, S., Malik, S. A., Zargar, S. M., Banday, N., & Nazki, I. T. (2023). Comparative analysis of physical and chemical mutagenesis in chrysanthemum cv. 'Candid': Assessing genetic variation and breeding potential. *ACS Omega, 8*(46), 43836–43849. https://doi.org/10.1021/acsomega.3c05723

Gómez, D., Hernández, L., Martínez, J., Escalante, D., Zevallos, B. E., Yabor, L., Trethowan, R., Beemster, G. T. S., Sershen, & Lorenzo, J. C. (2019). Sodium azide mutagenesis within temporary immersion bioreactors modifies sugarcane in vitro micropropagation rates and aldehyde, chlorophyll, carotenoid, and phenolic profiles. *Acta Physiologiae Plantarum, 41*, Article 114. https://doi.org/10.1007/s11738-019-2911-0

Gruszka, D., Szarejko, I., & Maluszynski, M. (2012). Sodium azide as a mutagen. In *Plant mutation breeding and biotechnology* (pp. 159–166). CABI. https://doi.org/10.1079/9781780640853.0159

Isuosuo, C. C., Akaneme, F. I., & Urom, U. N. (2024). The yield and purity of DNA extracts from seeds of eight six accessions of *Treculia* species using Zymo Research mini-prep DNA extraction kit. *Bio-Research, 22*(2), 2325–2335. https://doi.org/10.4314/br.v22i2.1

Jalil, A. T., Abdulhadi, M. A., Al-Ameer, L. R., Abbas, H. A., Merza, M. S., Zabibah, R. S., & Fadhil, A. A. (2023). The emerging role of microRNA-126 as a potential therapeutic target in cancer: A comprehensive review. *Pathology - Research and Practice, 248*, Article 154631. https://doi.org/10.1016/j.prp.2023.154631

Khan, S., Al-Qurainy, F., & Anwar, F. (2009). Sodium azide: A chemical mutagen for enhancement of agronomic traits of crop plants. *Environment We: International Journal of Science and Technology, 4*, 1–21. https://api.semanticscholar.org/CorpusID:261051703

Kharkwal, M. C. (2023). Role of mutation breeding in crop improvement with special reference to Indian subcontinent. In S. Penna & S. M. Jain (Eds.), *Mutation breeding for sustainable food production and climate resilience*. Springer. https://doi.org/10.1007/978-981-16-9720-3_13

LaVelle, J., & Mangold, J. (1987). Structure-activity relationships of the azide metabolite, azidoalanine, in *S. typhimurium*. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 177*(1), 27–33. https://doi.org/10.1016/0027-5107(87)90018-2

Liang, W.-Q., & Fournier, M. J. (1995). U14 base-pairs with 18S rRNA: A novel snoRNA interaction required for rRNA processing. *Genes & Development, 9*(19), 2433–2443. https://doi.org/10.1101/gad.9.19.2433

Liu, C., Frascarelli, G., Stec, A. O., Heinen, S., Lei, L., Wyant, S. R., Legg, E., Spiller, M., Muehlbauer, G. J., Smith, K. P., Fay, J. C., & Morrell, P. L. (2025). Sodium azide mutagenesis induces a unique pattern of mutations. *PLoS Genetics, 21*(6), Article e1011634. https://doi.org/10.1371/journal.pgen.1011634

Maloukh, L., Kumarappan, A., Jarrar, M., Salehi, J., El-Wakil, H., & Rajya Lakshmi, T. (2017). Discriminatory power of *rbcL* barcode locus for authentication of some of United Arab Emirates (UAE) native plants. *3 Biotech, 7*, Article 144. https://doi.org/10.1007/s13205-017-0746-1

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

NCBI Resource Coordinators. (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research, 44*(D1), D7–D19. https://doi.org/10.1093/nar/gkv1290

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., Miah, G., & Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnology & Biotechnological Equipment, 30*(1), 1–16. https://doi.org/10.1080/13102818.2015.1087333

Owais, W., & Kleinhofs, A. (1988). Metabolic activation of the mutagen azide in biological systems. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 197*(2), 313–323. https://doi.org/10.1016/0027-5107(88)90101-7

Owais, W., Rosichan, J., Ronald, R., Kleinhofs, A., & Nilan, R. (1983). A mutagenic metabolite synthesized by *Salmonella typhimurium* grown in the presence of azide is azidoalanine. *Mutation Research/Genetic Toxicology, 118*(4), 229–239. https://doi.org/10.1016/0165-1218(83)90207-0

Ramadan, G. Y., ElDeep, M. D., & Ibrahim, I. M. (2016). Response of bitter and sweet almond to sodium azide as chemical mutagene. *Sinai Journal of Applied Sciences, 5*(1), 53–62. https://doi.org/10.21608/sinjas.2016.78630

Singh, C., & Olejniczak, J. (1983). Modification of mutagenic efficiency of sodium azide. *Cytologia, 48*(3), 437–444. https://doi.org/10.1508/cytologia.48.437

Yousif, S. A., Juameer, R. A. A., & Obaid, A. A. (2025). Effect of NaCl and sodium azide on physiological and enzyme traits of strawberry in vitro. *Alnakhla Journal of Science, 14*(2), 83–89. https://doi.org/10.63799/AJOS/14.2.5

مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

اثر تیمار آزید سدیم (NaN_3) بر رشد درون‌شیشه‌ای و تنوع ژنتیکی پایه Garnem

سارا علی محمد الحیانی

* نویسنده مسئول. گروه باغبانی، دانشکده کشاورزی، دانشگاه دیالی، عراق. ایمیل: saraali@uodiyala.edu.iq

محمد د. عبدلهادی

گروه باغبانی، دانشکده کشاورزی، دانشگاه دیالی، عراق. ایمیل: mohammed.daher@uodiyala.edu.iq

ایاد عاصی عبید

گروه باغبانی، دانشکده کشاورزی، دانشگاه دیالی، عراق. ایمیل: ayadassi@uodiyala.edu.iq

تاریخ دریافت: ۱۴۰۵/۰۳/۰۹ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۵/۰۵/۱۶ تاریخ پذیرش: ۱۴۰۵/۰۵/۱۷

چکیده

هدف: آزید سدیم یک ماده شیمیایی جهش‌زا است که برای ایجاد تنوع ژنتیکی در گیاهان مورد استفاده قرار می‌گیرد. هدف از این مطالعه، بررسی اثر غلظت‌های مختلف آزید سدیم بر رشد درون‌شیشه‌ای پایه Garnem و شناسایی تغییرات احتمالی در توالی ژن کلروپلاستی rbcL پس از تیمار بود.

مواد و روش‌ها: این مطالعه بر روی پایه Garnem تحت شرایط درون‌شیشه‌ای، از اوت ۲۰۲۱ تا ژوئن ۲۰۲۲ انجام شد. ریزنمونه‌های همگن با آزید سدیم در غلظت‌های ۱۳ و ۲۶ میلی‌گرم در لیتر به مدت ۶۰ دقیقه تیمار شدند و نمونه‌های تیمارنشده به‌عنوان شاهد در نظر گرفته شدند. هر تیمار شامل ۱۰ تکرار مستقل بود. پس از تیمار، ریزنمونه‌ها در محیط تکثیر حاوی ۶ میلی‌گرم در لیتر کینتین (Kin) و ۱ میلی‌گرم در لیتر ۶-بنزیل‌آمینوپورین (BA) کشت شدند. درصد مرگ‌ومیر، تعداد شاخه‌ها، طول شاخه‌ها و تعداد برگ‌ها ارزیابی شد. همچنین، قطعه‌ای از ژن rbcL با استفاده از روش PCR تکثیر و در نمونه‌های شاهد و تیمارشده تعیین توالی شد. میزان شباهت توالی‌ها با استفاده از BLAST بررسی گردید.

نتایج: آزید سدیم بر زنده‌مانی و رشد شاخه‌ها تأثیر گذاشت. بیشترین میزان مرگ‌ومیر ریزنمونه‌ها (۷۰ درصد) در غلظت ۲۶ میلی‌گرم در لیتر مشاهده شد، در حالی‌که در تیمار شاهد هیچ مرگ‌ومیری ثبت نشد. بیشترین میانگین تعداد شاخه‌ها و برگ‌ها در تیمار شاهد به دست آمد. طول شاخه‌ها نیز بین تیمارها متفاوت بود؛ به‌طوری‌که بیشترین مقدار در غلظت ۲۶ میلی‌گرم در لیتر و کمترین مقدار در غلظت ۱۳ میلی‌گرم در لیتر مشاهده شد. مقایسه توالی‌ها نشان داد که در ناحیه مورد مطالعه از ژن rbcL در نمونه‌های تیمارشده، تغییرات احتمالی در برخی نوکلئوتیدها وجود دارد. توالی‌های مورد مطالعه شباهت بالایی با توالی مرجع ژن rbcL در گونه Prunus persica با شماره دسترسی JN114836.1 در بانک ژنی GenBank داشتند.

نتیجه‌گیری: آزید سدیم به‌صورت وابسته به غلظت بر زنده‌مانی و رشد درون‌شیشه‌ای ریزنمونه‌های پایه Garnem تأثیر گذاشت. همچنین، پس از تیمار، تغییراتی در توالی ناحیه مورد مطالعه از ژن کلروپلاستی rbcL مشاهده شد. بااین‌حال، برای تأیید ماهیت جهشی این تغییرات، قابلیت وراثت آن‌ها و میزان گسترش این تغییرات، انجام مطالعات بیشتر با تعداد تکرارهای زیستی بیشتر و تعیین توالی نواحی ژنومی اضافی ضروری است.

کلمات کلیدی: آزید سدیم، پایه هلو، تعیین توالی، مواد جهش‌زا، Garnem

نوع مقاله: پژوهشی

استناد: سارا علی محمد الحیانی، محمد د. عبدلهادی، ایاد عاصی عبید (۱۴۰۵). اثر تیمار آزید سدیم (NaN_3) بر رشد درون‌شیشه‌ای و تنوع ژنتیکی پایه Garnem. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۴)، ۶۱۵-۶۲۸.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.