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Optimizing Sodium Azide (NaN_3) Mutagenesis for Cumin (*Cuminum Cyminum* L.) Improvement

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HIGHLIGHTS

- Optimizing mutagen application is crucial for balancing genetic variation and seed viability.
- Sodium azide application significantly affects cumin germination parameters.
- 3 mM x 3 h sodium azide combination enhances cumin breeding mutation.
- Decrease in germination parameters dependent on the dose with increasing sodium azide concentration

Abstract

Cumin (*Cuminum cyminum*), an anciently cultivated plant of the Apiaceae family, holds significance for its aromatic seeds widely used in culinary practices globally. Despite its culinary and medicinal value, cumin faces challenges in cultivation due to diseases, pests, and weed infestations, with Alternaria leaf blight and Fusarium wilt being notable threats. Mutation breeding, a favored technique among breeders, introduces genetic variation through chemical mutagens like sodium azide, enabling the development of cumin varieties resistant to herbicides and diseases while ensuring high yields. This study aims to optimize sodium azide application as a chemical mutagen to enhance cumin breeding programs, emphasizing the importance of dosage and treatment duration in achieving desired mutation efficiency. The experimental results demonstrate significant impacts of sodium azide on germination parameters, with an optimal treatment duration of 3 hours for 3 mM sodium azide. Further research is needed to determine the effects of other variables on mutagen action, as well as M_1 plant survival and reproduction.

Keywords: Cumin; Cuminum cyminum; mutation breeding; sodium azide; germination

1. Introduction

Cumin (*Cuminum cyminum*), belonging to the Apiaceae family, is one of the oldest cultivated plants in the ancient world. Native to the eastern Mediterranean region and parts of Asia, primarily cultivated for its aromatic seeds. Its seeds, with their distinctive slightly bitter flavor, are a fundamental ingredient in the culinary traditions of numerous cultures worldwide (Lal et al. 2014; Bharti et al. 2018). Beyond its culinary role, cumin is also valued for its medicinal properties in traditional healing systems (Lodha and Mawar 2014). Ancient texts from Ayurveda and traditional Chinese medicine its use for digestive ailments, respiratory issues, and even as a stimulant (Dhandapani et al. 2002).

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The chemical composition of cumin seeds has been extensively studied, revealing a rich array of bioactive compounds such as cuminaldehyde, cuminol, and various terpenoids. These compounds contribute not only to cumins flavor but also to its potential health benefits, including antimicrobial, antioxidant, and anti-inflammatory properties. (Kumar et al. 2015; Agarwal et al. 2017; Kanani et al. 2019).

Generally, cumin is sown in arid fields to reduce fallow areas in Central Anatolia/Türkiye. There are numerous factors that also limit cumin production. Diseases, pests, and weeds in cumin cultivation areas cause significant yield losses. Cumin is highly vulnerable to Alternaria leaf blight disease, with severe epidemics occurring during rainy and mild springs, while Fusarium wilt is another pathogen that can cause yield losses of up to 45% in cumin (Didvania 2019; Budak 2020). Moreover, weed control stands out as one of the most significant challenges in cumin cultivation fields. Therefore, it is crucial to develop cumin varieties that are resistant to herbicides and diseases, while being high yielding.

Mutation breeding, favored by breeders, induces genetic variation in plants through natural processes or mutagens. Chemical mutagens induce DNA mutations in plant cells, creating genetic variation. This variation is then used through selection and crossbreeding methods to develop plants with desired traits (Oladosu et al. 2016). The most commonly used chemical mutagens include ethyl methanesulfonate (EMS), sodium azide (NaN_3), nitrosomethylurea (NMU), and hydroxylamine. Chemical mutagens cause mismatches during DNA replication or breaks in the DNA strand. These changes can alter gene function or introduce new traits (Agrawal and Kumar, 2021)

Chemical mutagens are generally applied to seeds, seedlings, or tissue cultures. The duration and concentration of mutagen application are carefully controlled based on the plant species and the desired level of mutation (Wei et al. 2013). Mutagenized plants are grown and screened for specific traits. Plants with desired characteristics are selected for further breeding programs (Serrat et. 2014). The use of chemical mutagens in plant breeding has great potential to increase agricultural production and contribute to sustainable farming practices. This method helps increase genetic diversity, enabling plants to gain resistance to environmental stresses and diseases.

The principal objective of this investigation is to enhance the utilization of sodium azide (NaN_3) as a viable chemical mutagen, serving as the initial stage in the cultivation of cumin varieties suitable for genetic resource augmentation via classical breeding methodologies. This optimization endeavor involves the precise determination of both the appropriate dosage and duration of sodium azide treatment applied to seeds.

2. Materials and Methods

2.1. Plant Material

The cumin (Cuminum cyminum) seed sample used in the study was obtained from local farmer field in Kerpic Village (39°04'02" °N 32°34'20" °E 1007 m above sea level) of Haymana district of Ankara province, where cumin is intensively cultivated in Center Anatolia.

2.2. Sodium Azide (NaN3) Preparation and Seed Application

First, a batch of 300 grams of cumin seeds underwent a 24-hour soaking period in cold tap water. Subsequently, they were submerged in a solution containing 1.5% NaOCl (sodium hypochlorite) for 15 minutes to ensure sterilization. Following this, the seeds were rinsed three times with distilled water to complete the sterilization process. Considering the molecular mass of sodium azide (NaN3) as 65.01 g/mol, specific amounts of NaN3 were measured for different concentrations: 0.065 g for 1 mM, 0.130 g for 2 mM, 0.195 g for 3 mM, and 0.260 g for 4 mM. Each measured quantity of NaN3 for its respective concentration was dissolved in 250 ml of distilled water, the pH was adjusted to 3, and the solution volume was brought up to 1 L. Stock solutions were prepared following this procedure. These prepared stock solutions were then applied to the cumin seeds for durations of 1, 2, 3, and 4 hours, corresponding to the designated time intervals for variation.

The selected concentrations are at an optimal level to provide effective inhibition without causing excessive damage to the cells. This range is frequently tested in many cytotoxicity studies. Sodium azide (NaN_3) has

been used at these levels specifically to prevent unwanted contamination. Adjusting the pH of the solutions to 3 was done to ensure the stability of sodium azide or to achieve the optimal pH conditions for the reaction targeted by the experiment. The application duration is critical in determining the degree of mutagenic effect. In rice, mustard, and peas, it has been observed that prolonged exposure to NaN_3 increases mutation rates, but also leads to adverse outcomes such as seed mortality or failure to germinate. Therefore, application times ranging from 1 to 4 hours have been deemed appropriate to examine both the early and late effects of NaN_3 on cumin seeds (Zuo et al. 2019; Chaudhary et al. 2021; Turkoglu 2022).

After application, the seeds treated with sodium azide were placed in sealed tubes and subjected to magnetic stirring for the specified durations. Post-treatment, the sodium azide-treated seeds were removed from the tubes and air-dried at room temperature. From each treatment combination, 100 viable seeds were selected for assessment, while a control group treated with pure water was established to evaluate germination-related traits. Performance evaluation criteria included germination rate (%), germination rate coefficient, germination rate index, germination vigor index and germination time (day).

2.3. Statistical analysis

The study employed a randomized complete block design, incorporating five replicates, with one serving as the control group. To evaluate the significance of differences among the treatments, the Duncan multiple comparison test was utilized. All statistical analyses were executed using JMP Pro 16 software (SAS et al.).

3. Results and Discussion

Table 1 presents the variance analysis, mean values, and multiple comparison results for germination rate, germination rate coefficient, germination rate index, average germination time, and germination vigor index. These results are based on the different sodium azide concentrations and treatment durations applied to cumin seeds.

Table 1. Average germination parameters for cumin at different sodium azide concentrations and durations

Dose (mM)	Duration (h)	Germination rate (%)	Germination time (day)	Germination rate index	Germination rate coefficient	Germination vigor index
1	1	81,60	8,36	0,194	12,00	366,38
	2	76,00	8,61	0,180	11,62	405,40
	3	60,00	8,33	0,140	12,05	237,15
	4	59,20	8,45	0,138	10,05	162,99
Mean		**69,20 b**	**8,44 ab**	**0,163 b**	**11,43 bc**	**292,98 b**
2	1	79,20	8,56	0,188	11,68	394,76
	2	78,40	8,66	0,186	11,69	282,51
	3	51,20	9,23	0,120	11,37	285,76
	4	55,20	8,18	0,128	10,42	295,38
Mean		**66,00 bc**	**8,63 a**	**0,155 bc**	**11,29 c**	**314,60 b**
3	1	66,40	8,94	0,158	11,25	402,75
	2	52,80	7,95	0,122	12,58	278,72
	3	42,40	7,85	0,098	12,75	165,14
	4	60,00	8,39	0,140	10,52	350,23
Mean		**55,40 c**	**8,28 ab**	**0,129 c**	**11,78 b**	**299,21 b**
4	1	74,40	7,94	0,176	12,65	407,98
	2	36,00	7,96	0,086	12,59	97,62
	3	56,80	8,60	0,134	11,69	272,17
	4	49,60	8,20	0,116	10,02	197,71
Mean		**54,20 c**	**8,17 b**	**0,128 c**	**11,73 b**	**243,87 b**
Control		**87,20 a**	**8,10 b**	**0,208 a**	**12,34 a**	**471,24 a**
Mean (Time)	1	*77,76 a*	*8,38*	*0,184 a*	*11,89 ab*	*408,62 a*
	2	*66,08 b*	*8,24*	*0,156 b*	*12,12 a*	*307,10 b*
	3	*62,24 b*	*8,42*	*0,140 b*	*11,96 ab*	*286,29 b*
	4	*59,52 b*	*8,26*	*0,146 b*	*10,25 b*	*295,51 b*
General Mean		**70.70**	**8.14**	**0.170**	**12.04**	**357.56**
ANOVA		*p > F*				
	df					
Dose (D)	4	**	**	**	*	**
Time (T)	3	**	ns ‡	**	**	**
D x T	12	**	**	**	**	**

* P < 0.05; ** P < 0.01; ‡ ns, not significant. δ Means within a column not sharing a lowercased letter differ significantly at the P < 0.05 levels

It was determined that the dose and time application significantly affected all germination parameters. Also, duration x time interaction has been statistically significant in all parameters except germination time. As seen from Table 1, the lowest germination rate (54,20%) was obtained from 4 mM sodium azide doses. With each decreasing dose, the germination rate increased, and it was observed highest in the control group (87,20%). In other words, it means that as the concentration of sodium azide increased, the germination rate decreased, and the control group had a notably higher germination rate than the groups treated with sodium azide. As a result of the combination of treatments, the highest germination rate (81.60%) was achieved with the application of 1 mM x 1 hours. The lowest germination rate index (36,00%) was obtained by applying 4 mM x 2 hours (Figure 1). As shown in Table 1, the highest germination rate index (0.208) was observed in the control group. Following this, the germination rate index for the sodium azide doses were 1 mM (0.163), 2 mM (0.155), 3 mM (0.129), and 4 mM (0.128), respectively. The germination rate index decreased as the concentration of sodium azide increased, with the control group exhibiting a significantly higher germination rate compared to the sodium azide treatments.

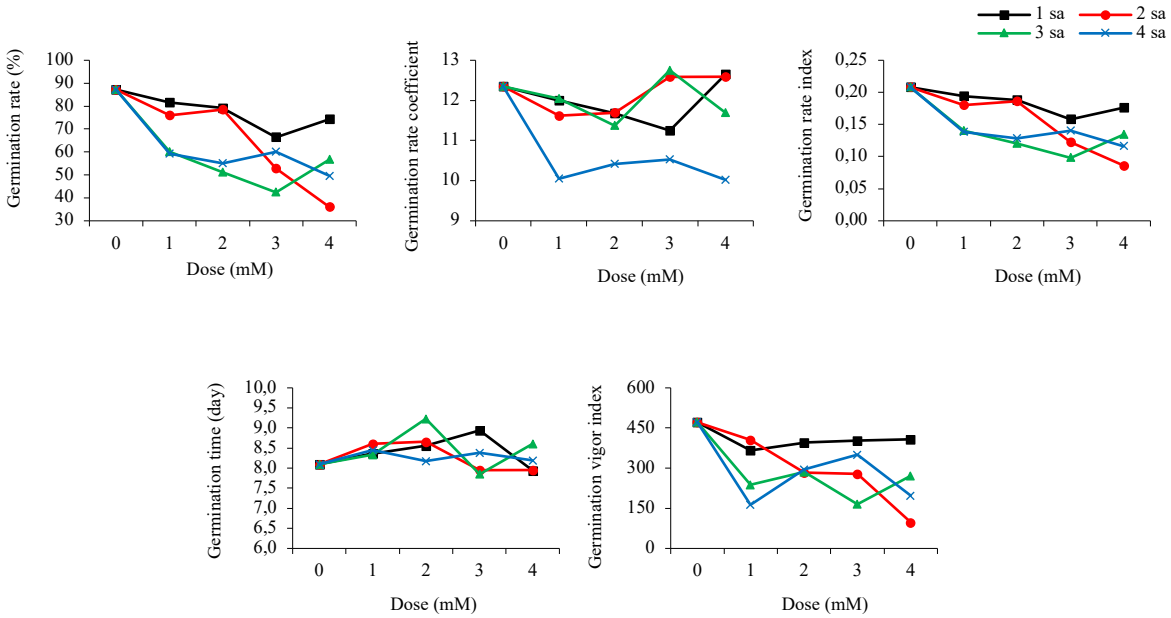

Figure 1. The effects of different sodium azide concentrations and durations on germination parameters

The average germination time varied depending on the sodium azide concentration, ranging from 8.10 to 8.63 days. The control group germinated the quickest, while the 2 mM sodium azide treatment resulted in the slowest germination. For the other sodium azide concentrations, the average germination times were 8.44 days for 1 mM, 8.28 days for 3 mM, and 8.17 days for 4 mM. Across all treatments, the overall average germination time was 8.14 days. Additionally, the interaction between time and dose was found to be insignificant for this parameter.

The control group exhibited the highest germination rate coefficient with an average value of 12.34. For the 1 mM sodium azide treatment, the average germination rate coefficient was 11.43, with values ranging from 10.05 to 12.05 across the four time points. The 2 mM sodium azide treatment showed an average germination rate coefficient of 11.29, with individual time point values between 10.42 and 11.69. The 3 mM sodium azide treatment had an average germination rate coefficient of 11.78, with the lowest value at 10.52 and the highest at 12.75. For the 4 mM sodium azide treatment, the average germination rate coefficient was 11.73, with values spanning from 10.02 to 12.65. Across all sodium azide treatments, the germination rate coefficients were lower than that of the control group.

The germination vigor index, a crucial indicator of seed viability and potential for successful germination, was meticulously assessed across various concentrations of sodium azide, along with a control group. Intriguingly, the control group displayed the most robust vigor index, with a mean value of 471.24, underscoring its superior seed quality and viability. However, as the concentration of sodium azide escalated, a noticeable decline in vigor index became evident. For instance, seeds treated with 1 mM sodium azide

exhibited a mean vigor index of 292.98, while those subjected to 4 mM sodium azide displayed a notably lower mean vigor index of 243.87. This dose-dependent reduction in vigor index implies a detrimental impact of sodium azide on seed viability and germination potential.

It is stressed that within the domain of plant breeding, there exist numerous alternative methodologies, which should ideally complement each other. Nonetheless, mutation breeding has emerged as a notably efficacious approach compared to its counterparts, chiefly because the outcomes of mutagenesis have often been swiftly integrated as new varieties. This method endows breeders with two pivotal advantages: firstly, it furnishes a pool of unselected genetic diversity, and secondly, it facilitates the augmentation of genetic variability, thereby facilitating selection and cross-breeding endeavors. Cumin emerges as an attractive candidate for mutation breeding owing to its diminutive floral structures and limited genetic variation available for conventional breeding efforts. This strategic application of mutagenesis has historically served as a viable avenue for enhancing quantitative traits, leveraging the innate potential for genetic enhancement in plant species. In the study conducted by Yadav and Krishna (2013) on cumin plants, three different doses of sodium azide (0.2, 0.4, and 0.6 mM) were applied to seeds for 6 hours to investigate their mutagenic effects. They observed a decrease in germination with increasing doses of sodium azide. The doses they used were relatively low compared to our study, while the duration was high. But, applying a higher dose is important to increase genetic damage and induce more variations. Emrani et al. (2011) reported that increasing doses of sodium azide reduced germination percentages and germination rate index in rapeseed plants, and the combination of 6 mM for 8 h reduced germination by 50% compared to the control group. The same result was reported by Ingle et al. (2018) in fenugreek, Prabha et al. (2010) in black cumin, and Raina et al. (2022) in cowpea, as well as by Srivastava et al. (2010) in wheat. Sodium azide can induce point mutations and disrupts the developmental, physiological, and metabolic processes of the treated plant species (Mensah and Obadoni 2007). The decrease in germination parameters observed following the application of sodium azide may be linked to point mutations or other forms of genetic damage (Vinithashri et al. 2020). In addition, in the study conducted by Patel et al. (2023) on cumin plants, it was found that high doses of gamma rays and EMS created a significant difference in the plant population in the M_1 generation, and these treatments increased the mutation frequency in the M_2 generation.

4. Conclusions

The significant effects of mutagen doses and application periods on germination rate, germination rate coefficient, germination rate index, germination vigor index, and germination time in cumin were observed. The study aimed to establish a balance between mutation efficiency and seed viability. Longer durations or higher concentrations can induce more mutations, but they may significantly reduce seed viability. Therefore, the selected duration and concentration represent a compromise that maximizes mutation efficiency while ensuring an acceptable level of seed viability. Hence, optimum treatment conditions were determined as 3 hours for 3 mM sodium azide combination. This study represents a step towards exploring the most suitable treatment conditions to enhance mutation efficiency in cumin breeding programs and genetic studies. Further research is needed to determine the effects of other variables on mutagen action, as well as M_1 plant survival and reproduction.

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