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Original Article

Optimization of Sodium Azide Application in Black Cumin (*Nigella sativa* L.) and Determination of Agronomic Characteristics in M_1 Generation

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ABSTRACT

This research represents the first step in enhancing the potential of black cumin seeds for developing new varieties through classical breeding methods. For this purpose, the application conditions of sodium azide, a chemical mutagen, were optimized to produce M_1 seeds. To achieve this optimization, we applied sodium azide mutagen to seeds at various concentrations (1, 2, 3, and 4 mM) and durations (1, 2, 3, and 4 hours) and assessed its impact on germination parameters. A field study involving single plant selections from the mutant and control groups was also conducted to evaluate the agricultural characteristics of plants in the M_1 generation. The results revealed that as the concentration of sodium azide increased, the germination rate, germination rate coefficient, germination rate index, and germination vigor index decreased, while the average germination time increased. The differences in dose significantly affected all variations in germination parameters. The germination rate decreased from 85.6% in the control group to 38.0% with increasing doses. In terms of field performance, significant differences in plant height, first branch height, first capsule height, capsule diameter, and 1000-seed weight were detected between the mutant and control groups of M_1 plants. However, there were no significant differences in the number of branches, capsules per plant, number of seeds in the capsule, or yield per plant. In conclusion, a 3 mM x 2 hour application of sodium azide to seeds effectively induced genetic variation in black cumin, demonstrating its potential for improving black cumin through mutagenesis.

Key words: Mutation breeding, Chemical mutagenesis, *Nigella sativa* L., sodium azide, black cumin

Çörek otu (*Nigella sativa* L.) bitkisinde sodyum azid uygulamasının optimizasyonu ve M_1 generasyonunda tarımsal özelliklerin belirlenmesi

ÖZ

Bu çalışma, çörek otu tohumlarının klasik ıslah yöntemleriyle yeni çeşitlerin geliştirilmesi potansiyelini artırmak için ilk adımı oluşturmaktadır. Bu amaçla, M_1 tohumlarını üretmek için bir kimyasal mutajen olan sodyum azidin uygulanma koşulları optimize edilmiştir. Bu optimizasyonu sağlamak için, tohumlara farklı konsantrasyonlarda (1, 2, 3 ve 4 mM) ve sürelerde (1, 2, 3 ve 4 saat) sodyum azid mutajeni uygulanmış ve çimlenme parametreleri üzerindeki etkisi değerlendirilmiştir. Mutant ve kontrol gruplarından tek bitki seçimlerini içeren bir tarla çalışması da, M_1 jenerasyonundaki bitkilerin tarımsal özelliklerini değerlendirmek için gerçekleştirilmiştir. Sonuçlar, sodyum azid konsantrasyonu arttıkça çimlenme oranı, çimlenme oranı katsayısı, çimlenme oranı indeksi ve çimlenme canlılık indeksinin azaldığını; ortalama çimlenme süresinin ise arttığını ortaya koymuştur. Dozdaki farklılıkların tüm çimlenme parametrelerinde önemli etkileri olduğu saptanmıştır. Çimlenme oranı kontrol grubunda %85.6 iken, artan dozlarla %38.0'a düşmüştür. Tarla performansı açısından M_1 bitkilerinin mutant ve kontrol grupları arasında bitki boyu, ilk dal yüksekliği, ilk kapsül yüksekliği, kapsül çapı ve 1000-tane ağırlığı açısından önemli farklılıklar tespit edilmiştir. Ancak, dal sayısı, bitki başına kapsül sayısı, kapsüldeki tohum sayısı veya bitki başına verim açısından önemli bir farklılık bulunmamıştır. Sonuç olarak, tohumlara 3 mM x 2 saatlik bir sodyum azid uygulaması, çörek otu bitkilerinde mutagenez yoluyla genetik varyasyonun indüklenmesinde etkili olmuş ve çörek otunun iyileştirilmesinde potansiyelini ortaya koymuştur.

Anahtar kelimeler: Mutasyon ıslahı, Kimyasal mutagenez, *Nigella sativa* L., sodyum azid, çörek otu

INTRODUCTION

Black cumin (*Nigella sativa* L.) is an herbaceous plant belonging to the Ranunculaceae family, with a height ranging from 30 to 70 cm. It has small, black, oval seeds measuring 2-3 mm, capsule-shaped fruits, light blue or white flowers, and a taproot. This plant is an annual herb. The origin of black cumin seeds is thought to be Egypt and the Mediterranean coast (Paarakh, 2010; Hossain et al., 2021). Black cumin has been utilized as a traditional complementary medicine in various civilizations, including Unani, Ayurveda, Arab, Indian, and Chinese traditions. For instance, in Ayurveda, an alternative medical system, black cumin balances Vata (movement energy) and Kapha (structure and lubrication energy) and increases Pitta (metabolism or digestion energy). In the Unani system of alternative medicine, the therapeutic uses of the seeds include relief from stomach issues, laxative effects, gas reduction, combatting coughs, and asthma, and the expulsion of kidney stones (Botnick et al., 2012; Datta et al., 2012; Melnyk et al., 2015; Srinivasan 2018; Hossain et al., 2021; Sommer et al., 2021). Furthermore, numerous studies have reported on the biological effects of black cumin. These studies have indicated that black cumin possesses antimicrobial (Iqbal et al., 2017), antibacterial (Gasong et al., 2017), antifungal (Benazzouz-Smail et al., 2023), antihistaminic (Gholamnezhad et al., 2019), antidiabetic (Rani et al., 2018), anti-inflammatory (Ahmad et al., 2021), anticancer (Butt et al., 2019), anticonvulsant (Noor et al., 2012), antihypertensive (Shahbazi et al., 2022), and antiviral (Basurra et al., 2021) properties.

Black cumin plays a significant role among medicinal and aromatic plants. However, research on yield, yield components, and quality aspects still needs to be completed. Producers commonly use seeds harvested from the previous year's population. Additionally, weed control is a significant challenge due to the lack of suitable herbicides, leading to a reliance on traditional methods such as hoeing and manual weeding. These weeds adversely affect the growth of black cumin plants, resulting in yield and quality losses. Therefore, the development of herbicide-resistant, high-yielding black cumin varieties is crucial.

Genetic variation refers to the differences in DNA sequences among individuals within a population, primarily stemming from mutations. Mutations are sudden heritable changes in the genetic makeup of plants. Mutations can occur spontaneously (naturally) or can be induced artificially. Identifying mutant types can be challenging for breeders, especially when recessive genes are involved and population frequencies are low. Therefore, researchers have attempted to induce artificial mutations through radiation or chemical mutagens (Genç & Özar, 1986). Inducing mutations is one of the methods employed in resistance breeding. The identification of resilient genotypes will facilitate weed control in black cumin farming. Establishing a large breeding population is a prerequisite. Chemical mutations have been effectively utilized as a source of variation in breeding programs, resulting in the development of numerous varieties (Oladosu et al., 2016). Mutation breeding plays a crucial role in plant breeding and assists in creating genetic variation to introduce innovations. Various researchers emphasize that artificial mutation induction through colchicine (Col), ethyl methane sulfonate (EMS), and sodium azide (SA) provides a tool to overcome limitations on variability that can lead to specific improvements in plants without detrimental effects. Sodium azide is a chemical commonly used in mutagenesis studies. An organic metabolite known as β-azidoalanine [N3–CH2–CH(–NH2)–COOH], an amino acid analog, mediates the mutagenicity of sodium azide. This organic metabolite interacts with DNA, inducing AT→GC base pair transitions and transversions (Khan et al., 2009) and chromosomal aberrations (Gruszka et al., 2012). Sodium azide alters the numerous developmental, physiological, and metabolic activities of applied plants. Chemical mutagens induce changes in gene sequences or chromosomes within the plant genome. Compared with chromosomal mutations, mutations in gene sequences have minor effects. Recessive gene mutations are detected in the M_2 generation. However, whether these changes are permanent or modifications can be detected in the M_3 generation through progeny control (Raina et al., 2022).

The primary objective of this research was to optimize the application of sodium azide as a suitable chemical mutagen, the initial step in developing black cumin lines that can be used as a genetic resource in breeding using classical breeding methods. This optimization included determining the appropriate dosage and duration of sodium azide treatment of seeds to obtain the M_1 generation.

MATERIALS AND METHODS

Preparation and Application of a Sodium Azide Solution to Seeds

In our study, we used the 'Çameli' variety of black cumin, which is the only registered variety in Türkiye. Initially, 300 grams of black cumin seeds were soaked in cold tap water for 24 hours. Subsequently, they were immersed in a 1.5% sodium hypochlorite (NaOCl) solution for 15 minutes for sterilization. In the next step, they were rinsed three times with distilled water for sterilization. Considering that the molecular mass of sodium azide is 65.01 g/mol, the following amounts of sodium azide were weighed: 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. The weighed sodium azide for each concentration was dissolved in 250 ml of distilled water, adjusted to pH 3, and adjusted to 1 L. Stock solutions were prepared in this manner. These prepared stock solutions were then applied to black cumin seeds for 1, 2, 3, and 4 hours, corresponding to the time intervals that constitute the source of variation. Subsequently, the seeds treated with sodium azide were placed in closed tubes and subjected to magnetic stirring for each duration. The sodium azide-treated seeds were removed from the tubes and air-dried at room temperature. One hundred healthy seeds were selected from each treatment combination, and a control group (pure water) was established to assess their germination-related traits. The performance was evaluated based on the germination rate, germination velocity coefficient, germination velocity index, average germination time, and germination vigor index. The most suitable dose and duration combination (3 mM x 2 hours) for determining germination-related traits was determined. On May 12, 2022, this combination was applied to black cumin seeds to obtain M_1 plants. The treated seeds and control seeds that had not undergone any mutagen treatment were set aside for planting in the field the following day.

Seeds Site and Experiment Description

This study was conducted during the 2022 growing season at the experimental station of the Ataturk University Research and Extension Center in Erzurum, Turkey (39.933° N, 41.237° E, altitude 1789 m above sea level). The experimental region falls under the Dsc climate type, according to the Köppen−Geiger classification (D: severe in winter, s: dry in summer, c: mild cool in summer) (Kottek et al., 2006). Table 1 presents data related to precipitation, temperature, and relative humidity for the experimental site during the vegetation period, including the long-term average (2000-2021) and the data for the year 2022. Climatic data for the study area were collected for specific months, considering the following planting and harvesting times: May (May 13-31), June, July, August, and September (September 01-06).

Table 1. The meteorological data of the experimental area during the long-term (2000–2021) growing periods of black cumin in 2022.

Year	Month					Total/ Average
	May#	June	July	August	September&	
			Precipitation (mm)			
Long-term	71.50	49.40	22.30	19.40	4.20	166.80
2022	44.00	65.20	3.10	8.10	0.30	120.70
			Temperature (°C)			
Long-term	9.60	13.70	17.70	15.30	12.80	13.82
2022	10.87	16.70	20.48	23.30	21.66	18.60
			Relative humidity (%)			
Long-term	60.80	55.70	41.50	40.60	47.70	49.26
2022	60.44	60.75	48.55	39.76	38.71	49.64

Calculated from data between 13-31 May;
& Calculated from data between 1-6 September

Based on these values, the total precipitation during the 2022 vegetation period was 120.70 mm, approximately 46.10 mm lower than the long-term average. Although sufficient rainfall was received during May and June, the region transitioned into a dry period starting in July before the black cumin emergence and flowering stages. The average temperature data for 2022 (18.60°C) indicated a significantly higher temperature than the long-term average (13.82°C). Black cumin were observed, and the experimental fields were irrigated during critical periods to mitigate any adverse effects on black cumin. Furthermore, the relative humidity values obtained for the long-term average (49.26%) and for 2022 (49.64%) showed parallel trends. The test soils had a clay-loam texture. The plants were deficient in available phosphorus (8.31 kg ha^{-1}) and total nitrogen (0.827%) but rich in available potassium (145.60 kg ha^{-1}). The experimental area had no salinity problems (0.54%) and exhibited a slightly alkaline (pH 7.38) structure. The organic matter content (1.31%) and lime content (0.71%) in the trial area were low (Klute, 1986).

The sowing was carried out on May 13, 2022, with a hand planter (Pocta, Model CP-1 SR-1) at a row spacing of 20 cm, using 20 kg of seeds per hectare. Considering the soil moisture content in the designated experimental area and the favorable climate conditions for sowing, a land area of 250 m^2 (25 × 20 m) was allocated for the mutant group, which underwent chemical mutagen treatment. In comparison, 50 m^2 (25 × 2 m) was designated for the control group, which did not receive mutagen treatment. Before sowing in the designated area, 60 kg per hectare of nitrogen (21% AS) and 60 kg per hectare of phosphorous (46% TSP) fertilizers were applied and incorporated into the soil using a disc harrow. Throughout the trial period, three irrigations (on July 1, 2022, July 14, 2022, and July 28, 2022) and three hoeing (on June 6, 2022, June 20, 2022, and July 5, 2022) were carried out.

Plant sampling and harvest

Harvesting began when the capsules started to turn brown and the seeds began to blacken. During harvesting, a 1-meter-wide strip along the edge of the mutant planting area and a 0.5-meter-wide edge of the control planting area were considered edge effects and were not included in the measurements. The remaining plants in the field were harvested together. The harvesting was conducted between September 2, 2022, and September 6, 2022. The capsules were dried with the seeds positioned on top. After the drying process, the plants were threshed to obtain the seeds. Since these seeds were the first generation grown after the mutagen treatment, they were symbolized as "M_1". All plant parameters, including plant height, number of branches, first branch height, capsule diameter, number of capsules per plant, number of seeds per capsule, thousand seed weight, and yield per plant, were calculated based on 100 plants in the randomly selected mutant group and 25 plants in the control group, ensuring that all measurements were taken from the same plant.

Statistical analysis

Following a randomized complete block experimental design, the laboratory study was conducted with five replicates, including the control group. The Duncan multiple comparison test was applied to determine the significance levels of differences between applications. Statistical analyses were performed using JMP Pro 16 software. In the field study, for the determination of agricultural traits in the M_1 generation, 100 plants were selected from the mutant group, and 25 plants were selected from the control group. The following measurements and observations were carried out. The data were subjected to t test analysis using JMP Pro 16 statistical software, and differences were determined using the Duncan multiple comparison test. All observational parameter measurements were conducted on the same plant from the 100 randomly selected plants in the field.

RESULTS AND DISCUSSION

The variance analysis, mean values and multiple comparison results and results for the germination rate, germination rate coefficient, germination rate index, average germination time, and germination vigor index, based on different sodium azide concentrations and durations applied to black cumin seeds, are presented in Table 2. The dose application and dose × duration interaction significantly affected all germination parameters. The effect of duration of application was statistically significant only for the germination vigor index.

As shown in Table 2, the highest germination rate (85.60%) was obtained for the control group. This was followed by 1 mM (60.20%), 2 mM (46.00%), 4 mM (43.80%), and 3 mM (38.00%) sodium azide, respectively. The germination rate decreased with increasing sodium azide concentration, and the control group exhibited a significantly greater germination rate than did the sodium azide treatment group. As a result of the combination of treatments, the highest germination rate (67.20%) was achieved with the application of 1 mM x 3 hours. The lowest germination rate (32.80%) was obtained when 3 mM was applied for 3 hours. The approximately 51.20% decrease in the germination rate between these two combinations indicated the significance of the dose × hour interaction. The germination rate coefficient increased with increasing sodium azide concentration (1, 2, 3, and 4 mM), with values of 11.30, 10.90, 10.75, and 10.99, respectively. In the control group, this value was 12.06. The highest germination rate coefficient was observed for the control group, while the lowest was for the 3 mM sodium azide treatment group. The germination rate indices, depending on increasing sodium azide dose, were determined to be 0.140 (1 mM), 0.109 (2 mM), 0.092 (3 mM), and 0.104 (4 mM), while in the control treatment, the germination rate index was 0.204. The data showed that the highest germination rate index was obtained in the control group, and the lowest was obtained in the 3 mM sodium azide treatment group. The average germination time was between 8.32 and 9.33 days for different sodium azide applications. The earliest germination occurred in the control group, while the latest germination was observed in the 3 mM sodium azide treatment group. For the other sodium azide treatments, the average germination time was 8.88 days for the 1 mM dose, 9.20 days for the 2 mM dose, and 9.11 days for the 4 mM dose. Considering all factors, the average germination time was 8.97 days. The germination vigor indices of *Nigella sativa* seeds in the 1, 2, 3, and 4 mM sodium azide treatment groups and the control group were 396.10, 286.48, 231.47, 245.99, and 828.96, respectively. The data showed that the highest germination vigor index was obtained in the control group, while the lowest was obtained in the 3 mM treatment group. As shown in Table 2, the germination vigor indices for the 1-, 2-, 3-, and 4-hour sodium azide treatments were 464.81, 420.53, 369.34, and 336.51, respectively, and these differences were found to be statistically significant (p<0.01). The germination vigor index decreased linearly with increasing hour applications. Consequently, the lowest germination vigor index was obtained from the 4-hour application, while the highest was detected in the 1-hour application. With increasing sodium azide concentration, the germination rate, germination rate coefficient, germination rate index, and germination vigor index decreased, and the mean germination time increased (Figure 1).

Table 2. Average Values of Germination Parameters in Black Cumin with Different Sodium Azide Concentrations and Durations

Dose (mM)	Duration (sa)	Germination rate	Germination time	Germination rate index	Germination rate coefficient	Germination vigor index
1	1	57.60	8.68	0.134	11.54	517.38
	2	55.20	8.87	0.128	11.32	412.55
	3	67.20	9.55	0.158	10.49	374.76
	4	60.80	8.44	0.142	11.85	279.73
Mean		60.20 b	8.88 b	0.140 b	11.30 b	396.10 b δ
2	1	49.60	9.17	0.116	10.92	426.21
	2	54.40	8.97	0.126	11.18	348.41
	3	40.00	8.91	0.098	11.23	200.75
	4	40.00	9.76	0.096	10.27	170.58
Mean		46.00 c	9.20 ab	0.109 c	10.90 bc	286.48 c
3	1	44.80	9.69	0.106	10.33	319.73
	2	36.80	9.38	0.090	10.71	268.77
	3	32.80	9.09	0.080	11.03	143.20
	4	37.60	9.16	0.090	10.96	194.17
Mean		38.00 d	9.33 a	0.092 d	10.75 c	231.47 c
4	1	43.20	9.31	0.102	10.76	231.79
	2	43.20	9.39	0.104	10.66	244.01
	3	44.80	8.88	0.106	11.26	299.05
	4	44.00	8.87	0.104	11.29	209.14
Mean		43.80 cd	9.11 ab	0.104 cd	10.99 bc	245.99 c
Control		85.60 a	8.32 c	0.204 a	12.06 a	828.96 a
Mean (Duration)	1	56.16	9.03	0.132	11.12	464.81 a
	2	55.04	8.98	0.130	11.19	420.53 ab
	3	54.08	8.95	0.129	11.21	369.34 bc
	4	53.60	8.90	0.127	11.28	336.51 c
General Mean		54.72	8.97	0.130	11.20	397.80
ANOVA		*p > F*				
	df					
Dose (D)	4	**	**	**	**	**
Duration (D)	3	ns ‡	ns	ns	ns	**
D x D	12	*	**	**	**	**

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

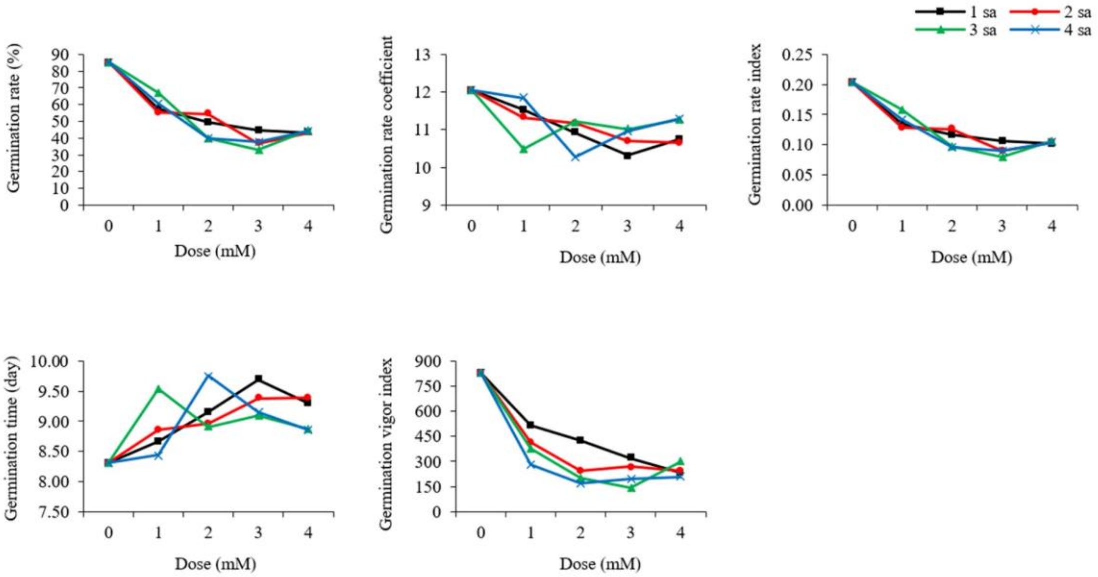

Figure 1. Effects of different sodium azide concentrations and durations on the germination rate, germination rate coefficient, germination rate index, germination vigor index and germination time

The average values and t test results of the yield and yield parameters observed for the M1 generation and control groups of *Nigella sativa* seeds subjected to different concentrations of sodium azide for different durations are presented in Figure 2. During the 2021 vegetative period, differences in plant height, first branch height, first capsule height, capsule diameter, and 1000-seed weight were found to be statistically significant between the control and mutant *Nigella sativa* plants, while the number of branches, number of capsules per plant, number of seeds per capsule, and yield per plant were not significantly different (Figure 2).

The average plant height was determined to be 49.98 cm in the control group and 58.13 cm in the mutant group. The shortest plant height in the control group was 39.5 cm, while in the mutant group, it was 42.5 cm. Conversely, the tallest plant height recorded was 64.5 cm in the control group and 79.5 cm in the mutant group. Overall, the plant heights obtained from the mutant group samples were notably greater than those obtained from the control group samples. As depicted in the scatterplot, clustering was more densely observed in the mutant group (Figure 2).

In the M_1 generation, the difference in the number of branches between the treatment and control plants was not statistically significant. Indeed, the average number of branches for the mutant and control groups was 5.14 and 5.25, respectively, with similar results. In the M_1 generation, the initial height of black cumin was greater than that in the control plants. According to the research results, the first capsule height of the control group varied between 26.2 cm and 47.2 cm, with an average first capsule height of 36.6 cm. The average capsule diameter for the control group was 9.9 mm, while in the mutant group, it was 7.6 mm. When examining the outliers, the lowest capsule diameter measured in the control group was 8.0 mm, whereas in the mutant group, it was 3.4 mm. The greatest capsule diameter was 11.8 mm in the control group and 11.7 mm in the mutant group (Figure 2).

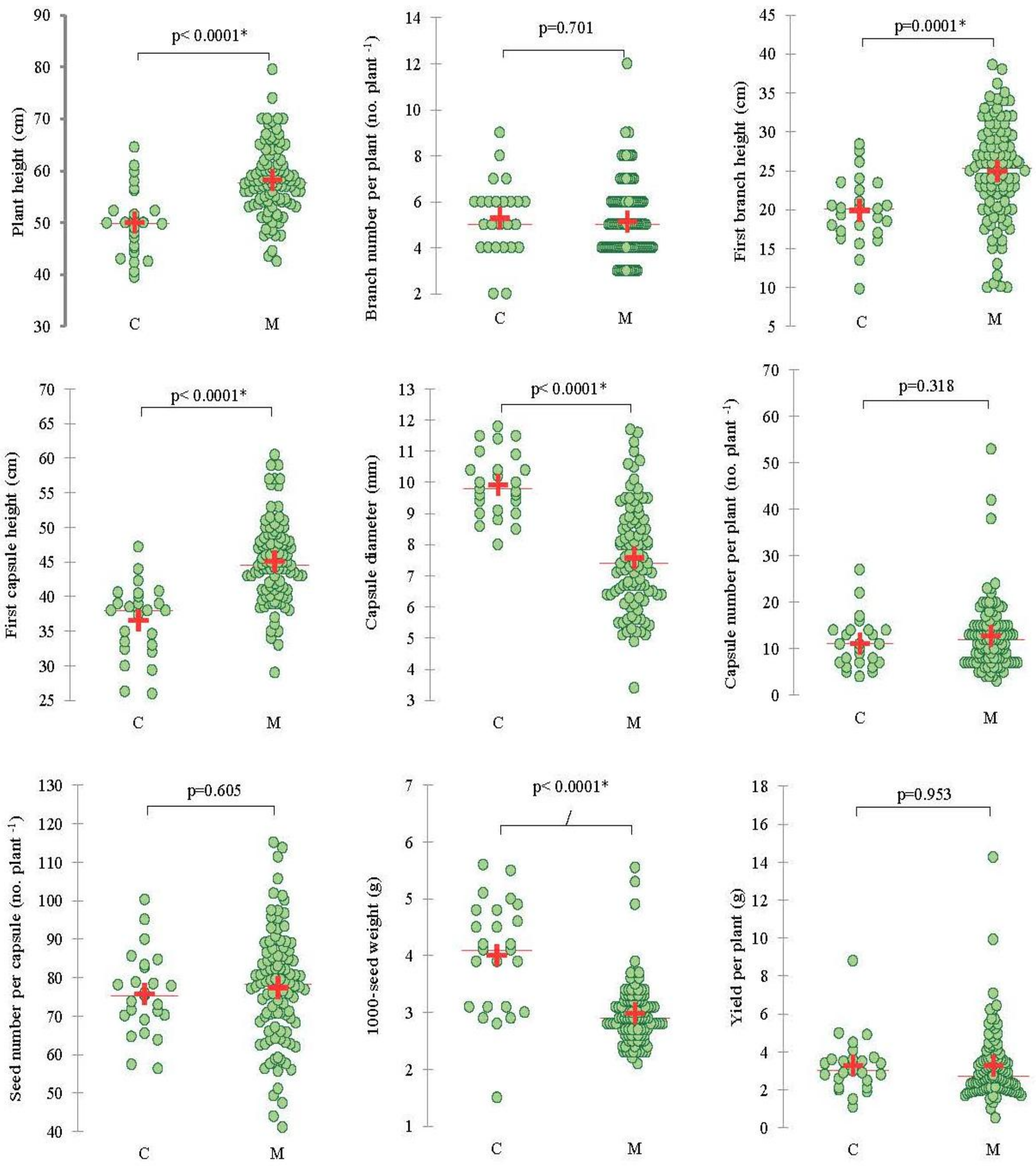

Figure 2. Scatter plot of the yield and yield parameters of the control and mutant black cumin populations.

The control group of plants exhibited larger capsule diameters than did the mutant group, indicating that mutagen treatment reduced the black cumin capsule diameter. The control plants had an average of 11.6 capsules per plant, whereas the mutants had an average of 12.8 capsules per plant. The greatest number of capsules per plant, 53.0, was obtained through mutagen application. The average number of seeds per capsule was 75.7 for the control plants and 77.4 for the mutants, with a wide variation observed in the mutant group, ranging from 42.4 to 115.1 seeds per capsule. As shown in Figure 2, the thousand-seed weight of the plants in the control group ranged from 1.5 g to 5.6 g, with an average of 4 g (Figure 2).

In contrast, the 1000-seed weight of the plants in the mutant group ranged from 2.1 g to 5.5 g, with an average of 2.9 g. Compared to those in the mutant group, the average 1000-seed weight of the plants in the control group significantly increased (Figure 2). The average yield per plant of the mutant plants (3.29 g) was similar to that of the control plants (3.27 g), with a wide range of variation observed within the mutant group. The highest yield per plant in the mutant group was 14.26 g, and the lowest was 0.48 g. An analysis of the scatterplot revealed clustering within the mutant group, ranging from 0.48 g to 7.09 g, whereas the control group clustered between 1.1 g and 5.0 g (Figure 2).

The mutagenic activity was greater at increased sodium azide doses, leading to more cell mutations, but beyond a certain threshold, germination did not occur. Considering both mutagenic activity and germination parameters, the most suitable sodium azide application dose x duration combination for black cumin plants was determined to be 3 mM x 2 hours. Increased concentrations of sodium azide significantly reduced germination rates. Prabha et al. (2010) reported that the application of sodium azide reduced germination rates in black cumin plants. Similar to our study, the researchers obtained the highest germination rate in the control group plants. Additionally, in a study investigating the effects of physical mutagens on black cumin, Datta et al. (1986) reported that while the germination rate in the control group was 94%, it decreased to as low as 58% with increasing doses, which supports the results obtained in our study. Similarly, in other cultivated plants, such as barley (Cheng & Gao, 1988; Ilbas et al., 2005), chickpea (Khan et al., 2004), sesame (Mensah et al., 2007), canola (Emrani et al., 2011), sunflower (Mostafa et al., 2011), rice (Aurabi et al., 2012), maize (Eze & Dambo, 2015), and cowpea (Raina et al., 2022), increased sodium azide doses and durations resulted in decreased germination rates. Sodium azide induces point mutations at high rates (Jander et al., 2003). Sodium azide, which can induce point mutations, also alters the developmental, physiological, and metabolic activities of plants (Mensah et al., 2007). The observed reductions in germination speed after sodium azide application may be due to point mutations or other genetic damage (Vinithashri et al., 2020). In our study, as the sodium azide dose increased, the average germination time increased, and the germination rate index, germination vigor index, and germination rate coefficient decreased. This phenomenon may result from factors such as hormonal imbalances (Ananthaswamy et al., 1971), the effects of azide anions, which are inhibitors of cytochrome oxidase that inhibit oxidative phosphorylation (Kleinhofs & Sander, 1975), the inhibition of ATP biosynthesis (Cheng & Gao, 1988), the alteration of mitochondrial membrane potential (Zhang, 2000), and changes in enzyme activities (Khan & Goyal, 2009).

The differences in plant height, first branch height, first capsule height, capsule diameter, and thousand-seed weight between the mutant and control groups were significant. In contrast, differences in the number of branches, capsules per plant, seeds per capsule, and yield per plant were not statistically significant. Based on the values obtained, the control group of plants had an average plant height of 58.13 cm, 5.14 branches, a first branch height of 24.88 cm, a first capsule height of 45.06 cm, a capsule diameter of 7.58 mm, 12.76 capsules per plant, 77.4 seeds per capsule, a thousand-seed weight of 2.98 g, and a yield per plant of 3.27 g. In contrast, the mutant group had an average plant height of 49.98 cm, 5.25 branches, a first branch height of 19.86 cm, a first capsule height of 36.58 cm, a capsule diameter of 9.92 mm, 12.16 capsules per plant, 75.7 seeds per capsule, a thousand-seed weight of 4.00 g, and a yield per plant of 3.29 g. The values obtained in the M_1 generation in our study are in line with the results obtained by Ürüşan (2016) and Yıldız (2021), who conducted studies on black cumin cultivation in the region. Moreover, Prabha et al., (2010) found that differences in plant height, number of branches, and thousand-seed weight in the M_1 generation of black cumin plants were statistically significant, indicating wide variation among parameters, similar to our study.

CONCLUSION

In terms of its pharmacological and phytochemical properties, black cumin is a medicinal and aromatic plant with significant importance. Considering its suitability for dry farming rotations, availability of agricultural tools and equipment commonly used for other crops, positive market supply and demand trends, and ease of processing, its inclusion in agricultural patterns is important and necessary. In the future, morphological mutants with superior characteristics will serve as genetic sources for breeding programs. Considering the results obtained, a combination of 3 mM sodium azide treatment for 2 hours can be applied to black cumin seeds to increase genetic diversity. Since full expression of land values is not expected in the M_1 generation, it is important to reevaluate the results with M_2 values. In the subsequent phases of the research, given that the genetic mutations for breeding purposes, such as herbicide resistance and high yield, are likely to be recessive, genetic inheritance studies are aimed at determining the M_2 generation. Additionally, whether these changes are permanent or modified will be assessed in the M_3 generation through progeny control.

Declaration of interests

The authors declare that there are no identifiable conflicts of interest, either financial or personal, that might have influenced the work presented in this paper. This research article was derived from the master dissertation of Hivrun Turanlı.

Author Contributions

Hivrun Turanlı : Conceptualization; data curation; formal analysis; investigation; methodology; software; writing— original draft.
Furkan Çoban : Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.
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