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Research Article

Effect of Sodium Azide Treatment at Different Duration and Concentration on germination and Seedling Growth Characters in Wheat (*Triticum aestivum* L.)

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Abstract

Wheat in the world and in Turkey to be essential nutrients to maintain the leading status in the still, as a result, it is widely cultivated. Therefore, it is important to increase the genetic and physiological diversity in wheat. Mutation breeding is an effective method for increasing genetic diversity in cultivated plants. The aim of this study is the identification of different duration [control (10 min), 1 h, 2 h and 3 h] effects and concentrations [0 (control), 0.5, 1, 1.5 and 2 mM] of sodium azide (NaN_3) mutagen in germination and seedling growth on wheat (Kırik cultivar) and determine the optimum doses of sodium azit which can be used in breeding programs. This study was conducted in 4 replicates according to the completely randomized factorial design. Some germination and seedling characters were investigated in this study. The results showed that except the germination percentage application of sodium azide in different concentrations and duration were significantly affected all considering traits. We believe that increase concentration and duration treatment of SA leads to decreases germination rate index and germination vigor index despite that means time to germination also was increased. Likewise, seedling growth characters significantly were affected by NaN_3 application concentration and duration. Also based on the value of LD_{50} 2 h treatment + 1 mM concentration was determined for the optimum dose of the applied root and shoot length.

Keywords: Wheat, Sodium azide, Germination, Seedling

Farklı Süre ve Dozlarda Uygulanan Sodyum Azitin Buğdayda (*Triticum aestivum* L.) Çimlenme ve Fide İle İlgili Bazı Karakterler Üzerine Etkileri

Öz

Buğday, Dünyada ve Türkiye'de en önde gelen temel besin maddesi olması durumunu halen korumakta, bunun sonucunda da yaygın olarak yetiştirilmektedir. Bu nedenle, buğdayda farklı özelliklere sahip yeni çeşitler geliştirebilmek için genetik ve fizyolojik çeşitliliğini artırmak önemlidir. Mutasyon, bitkilerde genetik çeşitliliğin artırılmasında etkili bir araçtır. Bu çalışma, ekmeklik buğdayın Kırik çeşidinde kimyasal bir mutagen olan sodyum azitin (NaN_3) çimlenme ve fide ile ilgili karakterler üzerine etkilerini ve ıslah amaçlı çalışmalarda uygulanabilecek optimum dozu belirlemek amacıyla yapılmıştır. Araştırmada sodyum azitin 5 farklı konsantrasyonu [0 (kontrol), 0.5 mM, 1 mM, 1.5 mM ve 2 mM'lık] ve 4 farklı uygulama süresi [kontrol (10 dk), 1 saat, 2 saat ve 3 saat] ele alınmış ve deneme şansa bağlı tam bloklar deneme deseninde faktöriyel düzenlemeye göre 4 tekerrürlü olarak yürütülmüştür. Denemede çimlenme ve fide ile ilgili bazı karakterler üzerinde durulmuştur. Sodyum azitin hem uygulama süresi hem de konsantrasyonu çimlenme oranı dışındaki çimlenme ve fide ile ilgili tüm karakterleri çok önemli derecede etkilemiştir. Uygulama süresi uzadıkça çimlenme hızı indeksi ve çimlenme gücü indeksinin azaldığı, buna karşın ortalama çimlenme zamanının uzadığı tespit edilmiştir. Kök ve sürgün uzunluğu esas alındığında LD_{50} değerine göre optimum dozun, 1 mM'lık konsantrasyon ve 2 saatlik uygulama süresi olduğu belirlenmiştir.

Anahtar Kelimeler: Buğday, Sodyum azit, Çimlenme, Fide

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1. Introduction

Turkey has a rich biodiversity and is the homeland and gene center of many plant species. The most important of these plant species is wheat. Wheat is a very important product for human nutrition, and it is stated that the agricultural industry based on both wheat and wheat products is one of the main sectors in the food sector and economy [1]. It is a cultivated plant that ranks first in the world in terms of cultivation area and production amount. Worldwide, product production is largely limited by the effect of environmental stress factors. Considering the climatic changes in recent years, stable, highly productive; developing high quality wheat varieties with high resistance to lodging, biotic and abiotic factors are the most important objectives of breeding studies. Therefore, in today's breeding studies; efficiency and quality elements are considered together, while studies and researches are carried out to increase the efficiency obtained from the unit area, on the other hand, it is aimed to improve the quality characteristics of different consumer segments [2]. The task of the plant breeder is to identify varieties with wide ecological adaptability, superior characteristics in terms of yield and quality, or to improve the negative aspects of existing varieties. For this purpose, breeders benefit from the variations existing in nature and the new techniques and methods they have developed.

Many varieties have been bred and put into the service of agriculture with traditional breeding methods, which is one of these methods and forms the basis of breeding. However, these traditional methods have many disadvantages. The most important disadvantages are the need for long time, labor and resources in cultivar development. Therefore, plant breeders are considering new, modern and advanced breeding approaches that will provide faster and easier variation. One of these breeding methods is mutation breeding [3]. Mutation is permanent changes that occur in the genetic structure of living things and can occur in both reproductive cells and somatic cells [4; 5]. The frequency of spontaneous occurrence of mutations with desired characteristics in plants is extremely low. Therefore, physical or chemical mutagens have been used to increase the mutation frequency in such breeding studies [6].

The number of cultivars bred worldwide through mutation breeding is 3218, and in terms of the number of mutant cultivars, wheat (284); It ranks third after paddy (814 units) and barley (309 units). Although the number of cultivars developed through mutation breeding is substantial throughout the world, the number of cultivars developed through mutation breeding is quite low in Turkey, and there is no wheat among them. As of 2012, the number of registered mutant varieties in Turkey is 7 in total, of which 2 belong to soybean, 2 to tobacco, 1 to barley, 1 to chickpea and 1 to sesame [7]. Gil et al. [8] stated that it is possible to introduce a new mutant variety after 4-6 years in annual plants after mutagen application.

With the use of mutation breeding, cultivars with high tolerance to biotic and abiotic stress factors can be developed in plants. The frequency of spontaneous occurrence of mutations with desired characteristics is very low. Therefore, physical or chemical mutagens have been used in many breeding studies to increase the mutation frequency [9]. Sodium azide is one of the strongest chemical mutagens in cultivated plants. The most critical factors in the induction of mutations are the mutagen concentration and the application time. As a general rule, as the mutagen concentration and duration increase at a given concentration and exposure time, more

mutations occur than normal. However, at very high concentrations and application times, undesirable conditions such as more seedling damage and death may occur [10].

In this study, it was aimed to determine the effect of sodium azide, which is a chemical mutagen, on germination and seedling-related characters in the Kirik variety of bread wheat (*Triticum aestivum* L.) and to determine the optimum dose and application time that can be applied in mutation breeding in wheat.

2. Material and Methods

In the research, seeds of the Kırik variety of bread wheat (*Triticum aestivum* L.) were used as material. 2000 seeds were counted and after washing these seeds in tap water, they were mixed in 70% ethyl alcohol (EtOH) for 3 minutes, washed 3 times with sterile distilled water in a sterile cabinet, and surface sterilization was performed by mixing them in 20% sodium hypochlorite containing a few drops of Tween for 25 minutes. Surface sterilized seeds were kept in aerated water for 24 hours after washing with sterile distilled water. Sodium azide was applied to the seeds at 5 different concentrations [0 (control), 0.5 mM, 1 mM, 1.5 mM and 2 mM] and 4 different times [control (10 min), 1 hour, 2 hours and 3 hours]. Accordingly, 5×4=20 combinations/applications were formed. After the application, the seeds were washed in tap water for 20 minutes to remove the mutagen. Chemically mutagen-treated seeds were divided into groups of 100 in order to determine some characteristics related to germination and seedling, and they were taken to the germination trial in petri dishes with four replications between paper. 14 ml of distilled water was placed in each petri dish. During germination, the temperature was adjusted to 25 ºC and 16 hours of light and 8 hours of dark periods were applied. After the seeds were placed in the germination medium, germination data were obtained by counting every day for 14 days (the ones with a root length of 1 mm and above were considered germinated).

a. Germination-related characters and characters related to seedlings developing on paper media;

At the end of the 14th day in seeds, germination rate (%) (GR), mean germination time (MGT), germination rate index (CHI), germination vigor index (GVI) was measured, in addition, 14 days after the seeds were placed in the germination medium, 10 seedlings were randomly taken from each petri dish and root length (cm) (RL), shoot length (cm) (SL), seedling length (cm) (SL) characters were calculated [5].

b_2. Characters related to seedling growing in peat medium

Seeds treated previously as described were used. 100 seeds from each of the 20 combinations were divided into four groups of 25 and each group was planted in a row. Thus, the experiment was carried out in four replications. 100×100×30 cm wooden crates containing peat were used in the greenhouse works. In M1 seedlings, at the end of twenty-eight days after sowing, germination rate (%) (GR), first leaf length (cm) (FLL), root length (cm) (RL), shoot length (cm) (SL) and seedling height (cm) (SL) characters was investigated [5].

3. Results and Discussion

a. Germination-related characters and characters related to seedlings developing on paper media

Germination rate (%) (GR):

The interaction of sodium azide application time and concentration with time × concentration on germination rate was insignificant (Table 1). The application time did not have a significant effect on the germination rate. As a matter of fact, the germination rate was 100% in 10 minutes of application, followed by 1 and 2 hours (99.40%) application times with a small difference, and 3 hours of application took place in the last place with 99.20% (Table 2). When evaluated according to the concentrations based on the averages, it was determined that the highest germination rate (99.80%) occurred at the control (0%) and 1.5 mM concentrations, and the lowest (99.30%) at the 1 and 2 mM concentrations. However, the differences between 0 and 1.5 mM concentrations and again between 0.5 and 2 mM concentrations were found to be insignificant (Table 2).

Mean germination time (MGT)

The interaction between the application time and concentration of sodium azide on the mean germination time and the interaction between these two factors was very significant ($P<0.01$) (Table 1). When evaluated according to the durations based on the averages, the longest average germination time was obtained from 3.79 days and 3 hours of application, followed by 3.14 days of 2 hours, 2.76 days of 1 hour and 1.56 days of 10 minutes. delayed (Table 2). The mean germination time of 1.48 days in the control was prolonged as the sodium azide concentration increased. As a matter of fact, the mean germination time was 2.29 days at 0.5 mM concentration, 2.61 days at 1 mM concentration, 3.55 days at 1.5 mM concentration, and 4.15 days at 2 mM concentration (Table 2). The effect of the application time on the mean germination time differed according to the concentration, therefore the interaction between the two factors was very significant ($P<0.01$) (Table 1). The highest mean germination time was determined at a concentration of 0.5 mM in 10 minutes of application, and at a concentration of 2 mM in applications of 1, 2 and 3 hours (Table 2).

Germination rate index (GRI)

The effect of the application time and concentration of mutagen and the interaction between these two factors on the germination rate index was very significant ($P<0.01$) (Table 1). According to the average of the application times, the germination rate index was found to be 32.55 in 10 minutes of application, followed by 1 hour (27.51), 2 hours (26.13) and 3 hours (25.51) application times in decreasing order. On the other hand, the differences between the 2 and 3 hour treatments were insignificant (Table 2). When evaluated according to the concentrations based on the averages, the highest germination rate index was found in the control group with 33.19, followed by concentrations of 0.5 mM (28.25), 1 mM (27.93), 1.5 mM (25.72) and 2 mM (24.55). Accordingly, as the concentration of sodium azide increased,

the germination rate index decreased (Table 2). The effect of the application time on the germination rate index differed according to the concentration. Therefore, the interaction between the application time and the concentration was very significant (P<0.01) (Table 1). The highest germination rate index was obtained from the control group at application times of 10 minutes, 1, 2 and 3 hours. The lowest germination rate index was found at a concentration of 0.5 mM in 10 minutes of application, and at a concentration of 2 mM in applications of 1, 2 and 3 hours (Table 2).

Germination vigor index (GVI)

The interaction between the application time and concentration of mutagen on the germination power index and the interaction between these two factors was very significant (P<0.01) (Table 1). When evaluated according to the durations based on the averages, the highest germination power index was obtained from the 10-minute application (2071.22), followed by the application times of 1896.13 for 1 hour, 1488.85 for 2 hours and 1386.54 for 3 hours. However, the differences between the 2 and 3 hour application times were statistically insignificant. As can be seen from the data presented here, the prolongation of the application time of the mutagen decreased the germination power index (Table 2). As with the application time, the germination power index decreased as the applied concentration increased. As a matter of fact, the highest germination power index was found in the control group with 2298.36, followed by concentrations of 0.5 mM (1893.39), 1 mM (1666.02), 1.5 mM (1492.37) and 2 mM (1203.27) (Table 2). On the other hand, when all combinations are considered separately, the highest potency index was obtained from the control concentration at 10 minutes, 1, 2 and 3 hours of application times. The lowest germination power index was recorded at a concentration of 0.5 mM in 10 minutes of application, and at a concentration of 2 mM in applications of 1, 2 and 3 hours (Table 2).

Root length (RL)

Both the application time and the concentration of the mutagen affected the root length very significantly (P<0.01) and the interaction between these two factors was very important (P<0.01) (Table 1). As the application time increased, the root length decreased. The maximum root length was obtained with 5.82 cm of application time of 10 minutes, followed by application times of 1 hour with 4.84 cm, 2 hours with 4.25 cm and 3 hours with 4.12 cm. However, the differences between the 1 and 2 hour treatments were statistically insignificant (Table 2). In terms of averages of concentrations, the longest roots were detected at 7.60 cm in the control group, followed by 4.89 cm at 1 mM, 4.80 cm with 0.5 mM, 3.38 cm with 1.5 mM, and 3.09 cm with 2 mM. However, the differences between the 0.5 and 1 mM concentrations were statistically insignificant. As can be understood from the information given here, the root length decreased as the mutagen concentration increased (Table 2). On the other hand, when all combinations are considered separately, the highest value in terms of root length was obtained from the control group at 10 minutes, 2 and 3 hours of application times, and from 0.5 mM concentration for 1 hour of application. The shortest root length at a concentration of 0.5 mM in 10 minutes of application; It was determined at a concentration of 2 mM in 1 and 3 hour applications, and at a concentration of 2 mM in 2 hours of application (Table 2).

Shoot length (SL)

The interaction between the application time and concentration of sodium azide on shoot length and these two factors was very important (P<0.01) (Table 1). According to the averages of the times, the longest shoots were obtained from the 10-minute application (15.60 cm), followed by 1 hour (13.64 cm), 2 hours (10.21 cm) and 3 hours (9.36 cm) applications in decreasing order, but 2 and 3-hour applications. the differences between them were insignificant (Table 2). When evaluated according to the concentrations considering the averages, the shoot length which was 17.20 cm in the control group increased to 13.66 cm at 0.5 mM concentration, 11.78 cm at 1 mM concentration, 10.35 cm at 1.5 mM concentration and 2 mM concentration. decreased to 8.02 cm. Accordingly, as the sodium azide concentration increased, the shoot length shortened (Table 2). The interaction between these two factors was very important (P<0.01), as the effect of the application time on the shoot length differed according to the concentration (Table 1). The highest shoot length was obtained from the control group at the application times of 10 minutes, 1, 2 and 3 hours. The lowest shoot length was at a concentration of 0.5 mM in 10 minutes of application; It was determined at a concentration of 2 mM in 1 and 3 hour applications, and at a concentration of 2 mM in 2 hours of application (Table 2).

Root dry weight (RDW)

The application time and concentration of mutagen on root dry weight and the interaction between these two factors were very important (P<0.01) (Table 1). Root dry weight, which was 0.022 grams in 10-minute application, decreased to 0.0013 grams in 2-hour application, 0.0012 grams in 1-hour application, and 0.0009 grams in 3-hour application, but the differences between 1- and 2-hour application times were insignificant (Table 2). When evaluated according to the concentrations considering the averages, the root dry weight, which was 0.0023 grams in the control group, decreased to 0.0017 grams at 0.5 mM concentration, 0.0013 grams at 1 mM concentration, and 0.0008 grams at 1.5 and 2 mM concentrations. However, the differences between the 1.5 and 2 mM concentrations were statistically insignificant (Table 1). On the other hand, when all combinations are considered separately, the highest value in terms of root dry weight was obtained from the control group at the application times of 10 minutes, 1, 2 and 3 hours. The lowest root dry weight was recorded at a concentration of 1.5 mM in 10 minutes of application, and at a concentration of 2 mM in applications of 1, 2 and 3 hours (Table 2).

Shoot dry weight (SDW)

Shoot dry weight was significantly (P<0.01) affected by both the application time and the concentration of mutagen, and the interaction between these two factors was very significant (P<0.01) (Table 1). As the application period of the mutagen increased, the shoot dry weight decreased. As a matter of fact, the shoot dry weight, which was 0.0028 grams in the 10-minute application period, decreased to 0.0024 grams in the 1-hour application, 0.0016 grams in the 2-hour application, and 0.0013 grams in the 3-hour application (Table 2). Considering the averages of the concentrations, the highest shoot dry weight was determined as 0.0034 g in the control group, followed by 0.0023 g with 0.5 mM, 0.0019 g with 1 mM, 0.0014 g with 1.5 mM,

and 0.0012 g with 2 mM. concentrations followed. Accordingly, as the mutagen concentration increased, shoot dry weight decreased. However, the differences between the 1.5 and 2 mM concentrations were statistically insignificant (Table 2). The effect of the application time on the shoot dry weight differed according to the concentration, so the application time × the concentration was very important ($P<0.01$) (Table 1). The highest shoot dry weight was obtained from the control group at the application times of 10 minutes, 1, 2 and 3 hours. The lowest shoot dry weight was determined at 2 mM concentration in 10 minutes, 1, 2 and 3 hours applications (Table 2).

Table 1. Variance analysis of some germination and seedling characters after sodium azide application at different times and concentration

		Mean of squares							
Variation Source	**SD**	**GR**	**MGT**	**GRI**	**GVI**	**RL**	**SL**	**RDW**	**SDW**
Duration (D) (hour)	3	2.40	17.58**	204.31**	2124314.12**	12.02**	170.85**	0.0000060**	0.000008**
Concentration (C) (mM)	4	1.00	17.63**	176.58**	2743490.61**	51.18**	192.93**	0.0000059**	0.000011**
D×C	12	2.06	2.10**	14.15**	324419.44**	8.38**	21.72**	0.0000006**	0.0000005**
Error	60	2.26	0.06	1.64	43627.18	1.91	3.25	0.00000007	0.0000001
Total	80								

[1]: **: significant at $p \leq 0.01$., ns: non-significant at $p \geq 0.05$

Table 2. Mean comparison of different NaN_3 concentration levels and duration of germination and seedling growth parameters of wheat

Duration (hour)	Concentration (mM)	GR (%)	MGT	GRI	GVI	RL (cm)	SL (cm)	RDW (gr)	SDW (gr)
10 minutes	0	100.00	1.38^{d1}	34.41^{a}	2453.10^{a}	8.06^{a}	19.14^{a}	0.0905^{a}	0.0046^{a}
	0.5	100.00	1.66^{c}	31.66^{b}	1870.50^{b}	4.31^{b}	13.96^{b}	0.0679^{b}	0.0034^{b}
	1	100.00	1.65^{c}	31.87^{b}	1979.50^{b}	6.16^{ab}	14.65^{b}	0.0700^{b}	0.0024^{c}
	1.5	100.00	1.52^{b}	33.08^{ab}	1971.50^{b}	4.38^{b}	14.47^{b}	0.0647^{b}	0.0018^{c}
	2	100.00	1.62^{a}	31.75^{b}	2081.50^{ab}	6.20^{ab}	15.78^{ab}	0.0675^{b}	0.0017^{c}
	Mean	**100.00**	$\mathbf{1.56^{D}}$	$\mathbf{32.55^{A}}$	$\mathbf{2071.22^{A}}$	$\mathbf{5.82^{A}}$	$\mathbf{15.60^{A}}$	$\mathbf{0.0721^{A}}$	$\mathbf{0.0028^{A}}$
1 hour	0	99.00	1.33^{d}	34.70^{a}	2488.36^{a}	5.67^{a}	18.93^{a}	0.0833^{a}	0.0036^{a}
	0.5	100.00	2.37^{c}	27.97^{b}	2265.00^{ab}	6.67^{a}	16.60^{ab}	0.0632^{b}	0.0024^{bc}
	1	100.00	2.25^{c}	28.47^{b}	2011.50^{bc}	5.60^{a}	14.65^{b}	0.0579^{b}	0.0026^{b}
	1.5	100.00	3.49^{b}	23.90^{c}	1768.50^{c}	4.10^{ab}	12.26^{c}	0.0515^{b}	0.0019^{cd}
	2	98.00	4.39^{a}	22.51	947.240^{d}	2.16^{b}	5.76^{d}	0.0387^{c}	0.0015^{d}
	Mean	**99.40**	$\mathbf{2.76^{C}}$	$\mathbf{27.51^{B}}$	$\mathbf{1896.12^{B}}$	$\mathbf{4.84^{B}}$	$\mathbf{13.64^{B}}$	$\mathbf{0.0589^{B}}$	$\mathbf{0.0024^{B}}$
2 hours	0	100.00	1.51^{d}	33.08^{a}	2218.00^{a}	7.32^{a}	16.12^{a}	0.0832^{a}	0.0023^{a}
	0.5	99.00	2.35^{c}	27.02^{b}	1530.78^{b}	3.78^{bc}	10.38^{b}	0.0509^{b}	0.0021^{ab}
	1	99.00	2.91^{b}	25.68^{b}	1480.50^{b}	4.58^{b}	10.23^{bc}	0.0560^{b}	0.0017^{b}
	1.5	99.00	4.38^{a}	22.76^{c}	1150.00^{c}	2.85^{c}	7.75^{cd}	0.0501^{b}	0.0011^{c}
	2	100.00	4.58^{a}	22.13^{c}	1065.00^{c}	2.75^{c}	6.60^{d}	0.0494^{b}	0.0011^{c}
	Mean	**99.40**	$\mathbf{3.14^{B}}$	$\mathbf{26.13^{C}}$	$\mathbf{1488.85^{C}}$	$\mathbf{4.25^{B}}$	$\mathbf{10.21^{C}}$	$\mathbf{0.0579^{B}}$	$\mathbf{0.0016^{C}}$
3 hours	0	100.00	1.72^{e}	30.58^{a}	2034.00^{a}	9.38^{a}	14.63^{a}	0.0628^{a}	0.0029^{a}
	0.5	98.00	2.80^{d}	26.35^{b}	1907.28^{a}	4.51^{b}	13.70^{a}	0.0492^{b}	0.0013^{b}
	1	99.00	3.64^{c}	25.68^{b}	1192.60^{b}	3.24^{c}	7.62^{b}	0.0468^{bc}	0.0011^{bc}
	1.5	100.00	4.81^{b}	23.13^{c}	1079.50^{b}	2.19^{cd}	6.93^{b}	0.0468^{bc}	0.0008^{cd}
	2	99.00	6.01^{a}	21.81^{c}	719.34^{c}	1.27^{d}	3.93^{c}	0.0382^{c}	0.0006^{d}
	Mean	**99.20**	$\mathbf{3.79^{A}}$	$\mathbf{25.51^{C}}$	$\mathbf{1386.54^{C}}$	$\mathbf{4.12^{B}}$	$\mathbf{9.36^{C}}$	$\mathbf{0.0487^{C}}$	$\mathbf{0.0013^{D}}$
Means Concentration (mM)	0	99.80	1.48^{E}	33.19^{A}	2298.36^{A}	7.60^{A}	17.20^{A}	0.0799^{A}	0.0034^{A}
	0.5	99.30	2.29^{D}	28.25^{B}	1893.39^{B}	4.82^{B}	13.66^{B}	0.0578^{B}	0.0023^{B}
	1	99.50	2.61^{C}	27.93^{B}	1666.02^{C}	4.89^{B}	11.78^{C}	0.0577^{B}	0.0019^{C}
	1.5	99.80	3.55^{B}	25.72^{C}	1492.37^{D}	3.38^{C}	10.35^{D}	0.0533^{BC}	0.0014^{D}
	2	99.30	4.15^{A}	24.55^{D}	1203.27^{E}	3.09^{C}	8.02^{E}	0.0484^{C}	0.0012^{D}

Differences between averages shown with the same lowercase letter in the same column for each treatment period are insignificant.

b_2. Characters related to seedling growing in peat medium

Germination rate (%) (GR)

While the effect of sodium azide concentrations on the exit rate was significant ($P<0.05$), the effect of application time and time × concentration interaction was insignificant (Table 3). The prolongation of the application period increased the emergence rate in general (except for 1 hour). For example, the exit rate, which was 89.40% in the 1-hour application, increased to 90.40% in the 10-minute application, 91.60% in the 2-hour application, and 93.60% in the 3-hour application, but the differences between all application times were insignificant (Table 4). When the averages of the concentrations were examined, it was seen that the application decreased the output rate in general (except for 2 mM). For example, the exit rate from 94.75% at the control and 0.5 mM concentration decreased to 91.00% at the 1 mM concentration, to 88.50% at the 2 mM concentration, and to 87.25 at the 1.5 mM concentration. However, the differences between the control and the 0.5 and 1 mM concentrations were insignificant (Table 4). On the other hand, when all combinations are taken into account separately, the highest shoot dry weight was found in the control group at the application times of 10 minutes, 1, 2 and 3 hours; the lowest shoot dry weight was obtained at a concentration of 2 mM in 10 minutes, 1, 2 and 3 hours applications (Table 4).

First leaf length (GFL)

The application time and concentration of mutagen and the interaction between these two factors had a very significant ($P<0.01$) effect on the first leaf length (Table 3). When evaluated according to application time on the basis of averages, the highest value in terms of first leaf length was obtained with 3.48 cm in 1 hour application, followed by applications with 3.43 cm for 2 hours, 3.30 cm for 3 hours and 2.95 cm for 10 minutes. the differences between hourly treatments were statistically insignificant (Table 4). As can be seen from Table 4, all concentrations of mutagen except the 2 mM concentration increased the first leaf length. The highest first leaf length occurred at 3.51 cm and 1 mM concentration, followed by 1.5 mM (3.40 cm), 0.5 mM (3.36 cm) and 0 mM (3.09 cm) applications, followed by 3.07 in the last row. cm to 2 mM concentration (Table 4). The interaction between the two factors was very important ($P<0.01$), as the effect of the application time on the first leaf length differed according to the concentration (Table 3). The highest first leaf length is 1 mM in 10 minutes of application, 1.5 mM in 1 hour of application; It was obtained from the control group at a concentration of 1 and 2 mM in 2-hour application and in 3-hour application. The shortest first leaf length at a concentration of 2 mM in 10 minutes of application; It was detected at a concentration of 0 mM in 1 and 2 hours of application, and at a concentration of 1.5 mM in 3 hours of application (Table 4).

Root length (RL)

The application time and concentration of mutagen were very important ($P<0.01$) effect on root length, and the interaction between these two factors was very important ($P<0.01$) (Table 3). The maximum root length was obtained with 18.64 cm in 1 hour application time, followed by 17.36 cm for 2 hours, 16.49 cm for 3 hours, and 15.63 cm for 10 minutes (Table 4). When the

averages of the concentrations were examined, the longest roots were detected at 18.81 cm and 0.5 mM concentration, followed by 1 mM (18.69 cm) and 0 mM (16.47 cm) and 2 mM (15.66 cm) concentrations in decreasing order. , the shortest roots were determined at a concentration of 1.5 mM (15.52 cm) (Table 4). On the other hand, when all combinations are considered separately, the highest root length is at a concentration of 1 mM in 10 minutes of application, at a concentration of 0.5 mM in 1 and 2 hours of application, and in the control group in 3 hours of application; the lowest root length at a concentration of 1.5 mM in 10-minute and 1-hour applications; It was obtained at a concentration of 0 mM in a 2-hour application period and at a concentration of 2 mM in a 3-hour application (Table 4).

Shoot length (SL)

The effect of mutagen application time concentration on shoot length was very significant ($P<0.01$), and the interaction between these two factors was significant ($P<0.05$) (Table 3). Based on the application time, the maximum shoot length was 29.60 cm in 2 hours of application, followed by 29.19 cm of 1 hour, 28.96 cm of 3 hours and 27.34 cm of 10 minutes of application. However, the differences between the 1, 2 and 3 hour treatments were statistically insignificant. Medicine, there was no parallel change between application time and root length (Table 4). When evaluated according to the concentrations based on the averages, it was determined that all concentrations of mutagen increased the shoot length. As a matter of fact, shoot length, which was 27.15 cm in the control, increased to 28.52 cm at 2 mM concentration, 29.06 cm at 0.5 mM concentration, 29.28 cm at 1.5 mM concentration, and 29.84 cm at 1 mM concentration. The differences between the control and all other concentrations were very significant (Table 4.26). The effect of application time on shoot length differed according to concentration, therefore the interaction between the two factors was very significant ($P<0.01$) (Table 3). The highest value in terms of shoot length was 0.5 mM concentration in 10 minutes of application; At a concentration of 1.5 mM in 1 and 3 hour applications; It was obtained at a concentration of 1 mM in 2 hours (Table 4).

Seedling height (SH)

The application time and concentration of sodium azide on seedling height and the interaction between these two factors were very important ($P<0.01$) (Table 3). When evaluated according to the application time based on the averages, the longest seedling length was obtained with 47.83 cm from 1-hour application, followed by 46.96 cm of 2-hour and 45.45-cm 3-hour applications. In terms of this character, the 10-minute application with 42.98 cm took the last place (Table 4). When evaluated by considering the averages of the concentrations, the longest seedling length was obtained at 47.97 cm and 1 mM concentration, followed by 0.5 mM (47.88 cm), 1.5 mM (45.36 cm) and 2 mM (44.18 cm) concentrations. The shortest seedling length was obtained from the control group with 43.63 cm, but the differences between this concentration and the 1.5 and 2 mM concentrations were insignificant. As can be understood from these data, sodium azide application caused an increase in seedling height (Table 4). On the other hand, when all combinations are considered separately, the longest seedling length is at a concentration of 1 mM in 10 minutes and 3 hours; It was obtained at a concentration of 0.5 mM in 1 and 2 hour applications. The shortest seedling length was determined at a concentration

of 0 mM in 10 minutes, 2 and 3 hours applications, and at 2 mM concentration in 1 hour application (Table 4).

Table 3. Variance analysis of some germination and seedling characters after sodium azide application at different times and concentration

Variation Source		Mean of squares					
		SD	GR	FLL	RL	SL	SH
Duration (D) (hour)	3	65.26	5.58**[1]	164.72**	98.11**	20653.52**	
Concentration (C) (mM)	4	192.50*	3.1**	207.41**	83.61**	11.13**	
D×C	12	66.1	4.08**	93.80**	23.42*	8.13**	
Error	380	63.53	0.69	25.93	11.85	1.54	
Total	400						

[1]: **: significant at $p \leq 0.01$., ns: non-significant at $p \geq 0.05$

Table 4. Mean comparison of different NaN_3 concentration levels and duration of germination and seedling growth parameters of wheat

Duration (hour)	Concentration (mM)	GR (%)	FLL (cm)	RL (cm)	SL (cm)	SH (cm)
10 minutes	0	93.00	3.11 ^{a1}	15.63 ^{ab}	25.38 ^{b}	41.01 ^{b}
	0.5	88.00	2.77 ^{ab}	15.20 ^{ab}	28.70 ^{a}	43.90 ^{ab}
	1	89.00	3.27 ^{a}	17.30 ^{a}	28.01 ^{a}	45.11 ^{a}
	1.5	89.00	3.25 ^{a}	13.63 ^{b}	27.81 ^{a}	41.64 ^{ab}
	2	93.00	2.37 ^{a}	16.42 ^{ab}	26.80 ^{ab}	43.22 ^{ab}
	Mean	**90.40**	**2.95 ^{BI}**	**15.63 ^{C}**	**27.34 ^{B}**	**42.98 ^{C}**
1 hour	0	91.00	2.65 ^{c}	17.09 ^{b}	29.18	46.26 ^{b}
	0.5	94.00	3.57 ^{ab}	23.41 ^{a}	29.08	52.49 ^{a}
	1	86.00	3.70 ^{ab}	22.97 ^{a}	30.48	50.97 ^{a}
	1.5	87.00	4.07 ^{a}	14.86 ^{b}	28.00	45.33 ^{b}
	2	89.00	3.40 ^{b}	14.87 ^{b}	29.23	44.10 ^{b}
	Mean	**89.40**	**3.48 ^{A}**	**18.64 ^{A}**	**29.19 ^{A}**	**47.83 ^{A}**
2 hours	0	95.00	2.87 ^{b}	15.03 ^{b}	28.03 ^{a}	43.06 c
	0.5	98.00	3.40 ^{a}	19.58 ^{a}	29.88 ^{abc}	49.47 a
	1	96.00	3.65 ^{a}	18.00 ^{ab}	31.29 ^{a}	48.50 ab
	1.5	87.00	3.60 ^{a}	17.72 ^{ab}	30.50 ^{ab}	49.01 ab
	2	82.00	3.65 ^{a}	16.49 ^{ab}	28.31 ^{bc}	44.80 bc
	Mean	**91.60**	**3.43 ^{A}**	**17.36 ^{B}**	**29.60 ^{A}**	**46.96 ^{AB}**
3 hours	0	100.00	3.75 ^{a}	18.16	26.03 ^{b}	44.20
	0.5	99.00	3.72 ^{a}	17.05	28.60 ^{a}	45.66
	1	93.00	3.45 ^{a}	16.49	29.61 ^{a}	47.31
	1.5	86.00	2.70 ^{b}	15.88	30.81 ^{a}	45.49
	2	90.00	2.87 ^{b}	14.88	29.74 ^{a}	44.62
	Mean	**93.60**	**3.30 ^{A}**	**16.49 ^{BC}**	**28.96 ^{A}**	**45.45 ^{B}**
Means Concentration (mM)	0	94.75 ^{A}	3.09 ^{B}	16.47 ^{B}	27.15 ^{C}	43.63 ^{B}
	0.5	94.75 ^{A}	3.36 ^{A}	18.81 ^{A}	29.06 ^{AB}	47.88 ^{A}
	1	91.00 ^{AB}	3.51 ^{A}	18.69 ^{A}	29.84 ^{A}	47.97 ^{A}
	1.5	87.25 ^{B}	3.40 ^{A}	15.52 ^{B}	29.28 ^{AB}	45.36 ^{B}
	2	88.50 ^{B}	3.07 ^{B}	15.66 ^{B}	28.52 ^{B}	44.18 ^{B}

[1]Differences between averages shown with the same lowercase letter in the same column for each treatment period are insignificant.

4. Conclusion

Both the application time and the concentration of sodium azide significantly affected all germination (except germination rate) and seedling-related characteristics. It was determined that as the application time increased, the germination rate, germination rate index and germination power index decreased, while the average germination time was prolonged. Likewise, as the application time increased and the concentration increased, all the characters related to the seedling developed in the paper medium decreased. Kleinhofs et al. [11] noted that high concentrations may cause the death of cells by causing dysregulation in genetic and physiological activity. Cheng and Gao [12] applied sodium azide to barley seeds and found that the germination rate was significantly reduced. Irregularities in the formation of enzymes involved during germination may be one of the physiological effects caused by chemical mutagenic applications [13]. Delay in germination in seeds treated with sodium azide can cause delay or inhibition of physiological and biological processes required for seed germination, including enzyme activity [14], hormonal imbalance [15], and inhibition of mitotic processes [16] has been reported. Azide ion plays an important role in the formation of mutation by interacting with enzymes and DNA in the cell.

All of the characters related to the seedling grown in peat medium were significantly affected by both the application time and the concentration of sodium azide. Generally, the first leaf length, coleoptile length, shoot length and seedling length were higher in the 1-hour application period compared to the other periods, but the germination rate was lower. When evaluated in terms of the concentration averages, it was determined that the concentrations of 0.5, 1.0 and 1.5 mM generally caused an increase in other characters, except for the exit rate. In contrast, almost all concentrations of sodium azide caused a decrease in the egress rate. The reduction in seedling height in treated populations may be due to variation in auxin level [17], change in the specific activity of several enzymes, and induced physiological damage to the seed or seedling [18]. Sparrow and Evans [19] noted that the main reason for the decrease in seedling growth was chromosomal damage and inhibition of cell division. Likewise, Yasmin and Arulbalachandran [20] reported that the inhibition of seedling growth may occur due to major damage in genes controlling biochemical processes or chromosomal abnormalities or both.

Both the application time and the concentration of sodium azide applied to the seeds significantly affected all the germination and seedling characteristics except the germination rate. It was determined that as the application time increased, the germination rate index and germination power index decreased, while the average germination time was prolonged. Based on the root and shoot length, it was determined that the optimum dose according to the LD50 value was 1 mM concentration + 2 hours of application time.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Haliloğlu, K., concept; Tosun, M. and Haliloğlu, K. design; Tosun, M. and Haliloğlu, K. resources; Tosun, M. materials; Haliloğlu, K. and Türkoğlu, A. data collection and/or processing; Türkoğlu, A. data validation; Türkoğlu, A. analysis and/or interpretation; Türkoğlu, A. literature search; Türkoğlu, A. writing; Türkoğlu, A. and Türkoğlu, A. critical reviews; Türkoğlu, A. project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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