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EVALUATION OF THE EFFECTS OF SOME BENZIMIDAZOLE DERIVATIVES ON GERMINATION PARAMETERS OF WHEAT VARIETIES

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Abstract: This study aims to investigate the effect of some benzimidazole derivatives on the germination parameters of bread and durum wheat varieties. These derivatives contain both unsubstituted and substituted benzimidazole structures to determine the effect of the substituents on germination. Three different durum wheat varieties (Çeşit-1252, Eminbey, and Kızıltan-91) and three different bread wheat varieties (Demir 2000, Bayraktar 2000, and Tosunbey) were used in the study. First, 1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole (2) compounds were used to synthesize dinitro compounds (5,6-dnitro-1*H*-benzimidazole (3), 5,6-dinitro-2-methyl-1*H*-benzimidazole (4)) in order to investigate the effects of wheat on germination characteristics. Subsequently, these compounds were each reduced to nitroamine compounds (2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5), 5-nitro-1*H*-benzimidazole-6-amine (6)). Azo dyes (1-[(5-nitro-1*H*-benzimidazol-6-yl)diazenyl]naphthalene-2-ol (7), 1-[(2-methyl-5-nitro-1*H*-benzimidazol- 6-yl)diazenyl]naphthalene-2-ol (8)) and Schiff base compound (2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-yl)imino]methyl}phenol (9)) were synthesized from nitroamine compounds. Wheat samples whose germination parameters were to be investigated were treated with benzimidazole derivatives synthesized at a concentration of 10^{-6}M. Germination rates, coleoptile lengths, and root lengths of wheat varieties were measured on the 8^{th} day of germination. The obtained results demonstrated that the efficiency of the benzimidazole compounds varied depending on the wheat varieties and germination parameters. 5-nitro-1*H*-benzimidazole-6-amine (6) had the greatest effect on germination rate while 1*H*-benzimidazole (1) had the greatest effect on root and coleoptile lengths in all varieties.

Key words: benzimidazole, wheat germination, chemical stimuli.

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Introduction

Among the heterocyclic compounds, benzimidazoles have found a wide range of applications and their derivatives are employed in many different fields of research, particularly in agriculture and medicine. The structure of the benzimidazole compound is very similar to the structure of the purine bases, which are the cornerstones of the biological system. Due to their low toxicity, metal binding, and great stability, benzimidazole derivatives are one of the most significant study subjects (Reddy, 2010; Al-ebaisat, 2011; Tahlan et al., 2019).

From an agricultural point of view, there are many studies on the germination of wheat. In recent years, the inhibition or activation effects of chemicals on germination parameters and the enzymatic activity during germination have been investigated (Ansari & Lal, 2009; Ezzat, 2021; Gudasi et al., 2005; Tobina et al., 2014; Tot et al., 2018; Vasilache et al., 2014).

Enzymatic activity rises during germination. However, not every cereal experiences this in the same manner. The kind of enzyme, the grain, and the germination circumstances all play a role. In most cereals, the fourth day of germination marks the beginning of the peak activity of the enzymes (Guzmán-Ortiz et al., 2019). Phytic acid and other macromolecules and chemicals can be hydrolyzed thanks to germination. The ingredient in cereals with the most morphological alterations is starch. Some enzymes become more active and mobilized during germination. Factors influencing the activation time include the variety, temperature, and steeping period (Guzmán-Ortiz et al., 2019; Miransari and Smith, 2014). Typically, the addition of enzymes such as amylases, proteases, and xylanases modifies the rheology of the dough, the gas retention, and the softness of the crumb amylases (Guzmán-Ortiz et al., 2019). However, the timing of enzymatic activation varies depending on the type of cereal, growth circumstances, germination, and the availability of hormones such as gibberellins, which are produced in the grain embryos and stimulate the synthesis and production of enzymes, especially alpha-amylases (Guzmán-Ortiz et al., 2019).

The seed germination rate is one of the standard parameters for seed quality. There are many chemical stimuli whose effects on wheat germination have been investigated (Dhanda et al., 2004; Dyar & Shade, 1974; Gudasi et al., 2005; Risca et al., 2006; Vasilache et al., 2014). Some of the benzimidazole derivatives were investigated in the literature (Gudasi et al., 2005; Vasilache et al., 2014) in terms of germination parameters. However, to the best of our knowledge, the nitrobenzimidazole derivatives and the benzimidazole Schiff base compound used in this study have not yet been investigated for their effects on wheat germination.

The aim of this study is to evaluate the possible effects of nitrobenzimidazole derivatives and benzimidazole Schiff base compounds on wheat germination by examining the impact of benzimidazole derivatives on germination parameters including germination rate, coleoptile length, and root length of three bread and three durum wheat varieties.

Material and Methods

Wheat varieties

Three different bread wheat varieties (Demir 2000, Bayraktar 2000, and Tosunbey) and three different durum wheat varieties (Çeşit-1252, Eminbey, and Kızıltan-91) obtained from the Ikizce field of the Ankara Field Crops Central Research Institute were used in this study. The wheat varieties were grown in 2018–2019 under rainfed conditions. The wheat varieties were selected based on their different grain qualities and features. Tosunbey is a hard white winter bread wheat, Demir 2000 is a red hard winter bread wheat and Bayraktar 2000 is a white medium-hard bread wheat. Tosunbey has strong gluten properties, Demir 2000 has medium-strong gluten properties and Bayraktar 2000 has medium-strong gluten properties. Eminbey is a durum wheat with high gluten quality, Çeşit-1252 has medium gluten quality and Kızıltan-91 has low gluten quality.

Germination method

Germination analyses were performed according to the modified ISTA method (ISTA, 2012). For germination analysis, 50 wheat seeds, which were firm, large and insect-free, were selected and placed on the special humidity papers in the germination pot and pre-soaked with 17 mL of the benzimidazole solutions prepared as 10^{-6} M in dilute methanol (1:20).

A filter paper wetted with 5 mL of the same solution was placed on it and the germination pot was placed in a 20ºC incubator (Stik-BI-250A) in the dark, at constant humidity and in random order. Each day, an additional 5 mL of benzimidazole solution was added to the germination pots. On the 8th day of germination, the coleoptile and root lengths were measured on the randomly selected 10 germinated wheat grains from each germination pot, with three replications for each chemical application in each wheat variety.

Statistical analysis

The results obtained were analyzed using analysis of variance (ANOVA) using the JMP program, version 11.0.0 (SAS Institute Inc., 2013). When significant differences ($p<0.05$) were determined, the least significant difference (LSD) test was used to determine the differences among the means.

Synthesis of benzimidazole derivatives

1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole (2) were purchased from Sigma. The compounds (3-9) were synthesized by the authors and their characteristic data: 5,6-Dinitro-1*H*-benzimidazole (3) and 5,6-dinitro-2-methyl-1*H*-benzimidazole (4) were obtained using the conventional nitration method of benzimidazoles (Patel et al., 2022). The preferential reduction of dinitrobenzimidazole derivatives gave 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) and 5-nitro-1*H*-benzimidazole-6-amine (6) (Dincer, 2002; Ozbay, 2010; Tsymliakov et al., 2023). 1-[(5-Nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol (7) was obtained as a red solid according to the standard methods of diazotization and coupling reactions using HNO_2 (Külen, 2021; Shafeeq et al., 2022), b.p. > 300 ºC, yield: 65%, FT-IR, (cm^{-1}) 3644, 3100, 2980, 2890, 1640, 1530, 1438, 1350); UV-VIS (nm, EtOH): 297, 497. 1-[(2-Methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol (8) was obtained as a red solid according to the method of (7) using naphthalene-2-ol, b.p. > 300 ºC, yield: 78%, FT-IR, (cm^{-1}) 3088, 1620, 1521, 1487, 1404, 1321, 1298; UV-VIS (nm, EtOH): 226, 298, 416, 502. 2-[(2-Methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol (9) was obtained as a yellow solid by the condensation reaction of 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) with salicylaldehyde (Gönülalan, 2011; Külen, 2021), m.p.: 325 °C, yield: 81%, element analysis, (%) $C_{15}H_{12}N_4O_3$: Found: C:58.73, H:4.07, N:18:42, Calculated: C:60.81, H:4.08, N:18.91; FT-IR, (cm^{-1}) 3103 cm^{-1}, 3054 cm^{-1}, 2979 cm^{-1}, 1623 cm^{-1}, 1605 cm^{-1}, 1573 cm^{-1}, 1516 cm^{-1}, 1352 cm^{-1}, ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm) 2.6 (3H, s), 6.8-7.2 (2H, m), 7.6 (1H, s), 8.2 (1H, s), 9.0 (1H, s), 12.5 (1H, s), 12.9 (1H, s). The chemical structures of the compounds used in the germination applications are given in Figure 1.

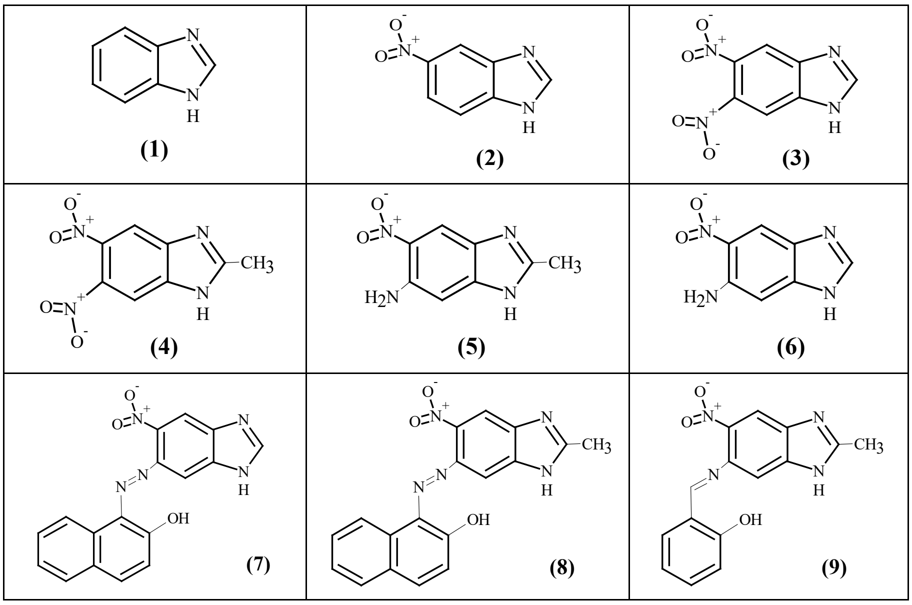

Figure 1. Compounds used in germination analysis.

Results and Discussion

Germination analysis

Coleoptile length (cm)

In monocotyledons, the coleoptile is a sheath that helps the shoot emerge through the soil crust. The maximum depth at which seeds can be sown depends on the length of the coleoptile. Therefore, genotypes with longer coleoptiles can be seeded deeper to avoid dry and hot conditions. Genotypes with shorter coleoptiles, on the other hand, may not sprout if sown too deeply, resulting in a weak stand and eventually causing production losses. Additionally, a rise in temperature has a negative impact on coleoptile length. Such variety-environmental interactions can therefore have a disastrous impact on crop productivity (Sidhu et al., 2020).

Table 1. Mean values of the coleoptile lengths measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan-91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	7.77 ı-q*	6.40 ou	7.98 h-p	9.41 d-ı	9.57 c-ı	8.16 h-o	8.22 BC
(1)	9.71 b-h	8.36 g-m	10.60 a-e	9.16 d-j	10.96 a-d	7.99 h-p	9.46 A
(2)	6.68 m-t	4.10 v	8.21 h-o	8.40 f-m	10.15 b-g	8.12 h-o	7.61 CDE
(3)	8.82 e-k	8.10 h-o	10.25 b-f	8.62 f-l	6.47 n-u	6.89 l-t	8.19 BCD
(4)	7.74 ı-q	7.00 k-s	8.29 g-n	8.87 e-k	7.43 j-r	5.33 s-v	7.44 DE
(5)	6.80 l-t	6.62 m-t	6.17 p-u	7.85 h-q	9.25 d-j	6.03 q-u	7.12 E
(6)	5.45 s-v	5.44 s-v	4.62 uv	11.58 ab	12.45 a	11.31 abc	8.48 B
(9)	5.10 tuv	5.57 r-v	7.15 k-s	7.98 h-p	8.80 e-k	7.11 k-s	6.95 E
Mean***	7.26 B	6.45 C	7.91 B	8.98 A	9.39 A	7.62 B	

*Different lowercase letters indicate statistical differences between application/variety interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The effects of the synthesized benzimidazole compounds on the coleoptile length of wheat varieties were evaluated and the results are given in Table 1. Better coleoptile length results were obtained by applying 5-nitro-1*H*-benzimidazole-6-amine (6) on bread wheat varieties. The mean values of the coleoptile length of Demir 2000, Bayraktar 2000, and Tosunbey varieties were 12.45, 11.58, and 11.31 cm, respectively. Besides, applying 1*H*-benzimidazole (1) had better results in

Demir 2000 and Kızıltan-91 varieties (10.96 and 10.60 cm) (Table 1). When comparing the effect of all chemical compounds on the mean coleoptile length of wheat varieties, Bayraktar 2000 and Demir 2000 obtained better results (8.98 and 9.39). Compounds (2), (4), (5), and (9) showed lower mean values of coleoptile length than the control sample, while the effects of compounds (3) and (6) on coleoptile length were statistically similar to the control sample (Table 1).

The effects of the compounds on the coleoptile length of wheat species were evaluated and it was observed that bread wheat varieties had a higher coleoptile length (8.66 cm) compared to durum wheat varieties (7.21 cm). Among the benzimidazole derivatives, the effect of 1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole-6-amine (6) on the coleoptile length (9.46 and 8.47 cm) was found to be better in the bread wheat varieties compared to durum wheat varieties (Table 2).

Table 2. Mean values of the coleoptile lengths measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	7.38 efg*	9.05 bc	8.22 BC
(1)	9.56 b	9.37 b	9.46 A
(2)	6.33 ghı	8.89 bcd	7.61 BCD
(3)	9.06 bc	7.33 efg	8.19 BC
(4)	7.67 def	7.21 e-h	7.44 CD
(5)	6.53 fgh	7.71 def	7.12 D
(6)	5.17 ı	11.78 a	8.47 B
(9)	5.94 hı	7.96 cde	6.95 D
Mean***	7.21 B	8.66 A	

*Different lowercase letters indicate statistical differences between application/species interactions, (p<0.05);**Different capital letters in the same column indicate statistical differences between applications (p<0.05);*** Capital letters in the same row indicate the statistical difference between varieties (p<0.05), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

Root length (cm)

The development of a plant root, an essential organ for absorbing nutrients, is one of the key indicators of a plant's ability to withstand drought. From a physiological perspective, having a healthy root-to-shoot ratio helps plants cope with drought stress (Liu et al., 2015). In our study, the effects of the synthesized benzimidazole derivatives on the root length of wheat varieties were evaluated. The results are given in Table 3. It was observed that the application of unsubstituted benzimidazole (1) on Eminbey (13.78 cm); 5-nitro-1*H*-benzimidazole (2) on

Tosunbey (13.11 cm); 5,6-dinitro-1H-benzimidazole (3) on Eminbey (12.36 cm); 5,6-dinitro-2-methyl-1*H*-benzimidazole (4) on Bayraktar 2000 (11.53 cm); 5-nitro-1*H*-benzimidazole-6-amine (6) on Demir 2000 (12.92 cm); 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) on Eminbey (12.05 cm); 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-yl)imino]methyl}phenol (9) on Tosunbey (11.48 cm) had the highest root length values (Table 3). Among the benzimidazole derivatives, the effect of 1*H*-benzimidazole (1) on the root length was better. When considering the mean value of root length after applying all chemicals, Bayraktar-2000 (10.54 cm), Eminbey (10.81 cm) and Tosunbey (11.00 cm) were the most prominent varieties (Table 3).

Table 3. Mean values of the root lengths measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan 91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	9.77 f-l*	11.44 a-h	10.42 e-k	11.83 a-g	10.45 e-k	10.43 e-k	10.72 BC
(1)	13.61 ab	13.78 a	11.41 b-ı	10.80 c-j	10.34 e-k	12.99 abc	12.15 A
(2)	8.39 k-l	9.79 f-l	12.28 a-e	11.51 a-h	11.13 c-j	13.11 abc	11.04 B
(3)	11.70 a-h	12.36 a-e	10.80 c-j	10.93 c-j	7.68 l-m	10.62 d-k	10.68 BC
(4)	9.45 h-l	10.58 d-k	9.88 f-l	11.53 a-h	9.73 f-l	9.69 g-l	10.15 BC
(5)	9.07 ı-l	12.05a-f	9.40 h-l	10.18 e-k	8.96 j-l	9.91f-l	9.93 C
(6)	5.62 mn	5.56 mn	4.71 n	7.62 lm	12.92 a-d	9.74 f-l	7.7 D
(9)	9.70 f-l	10.94 c-j	10.12 e-k	9.9 f-l	9.43 h-l	11.48a-h	10.26 BC
Mean***	9.67 D	10.81 AB	9.88 CD	10.54 AC	10.08 BCD	11.00 A	

*Different lowercase letters indicate statistical differences between application/variety interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole,; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The effects of the compounds on the root length value of wheat species were evaluated and the results are given in Table 4. There was no significant difference between the mean root lengths of bread wheat and durum wheat. Among the benzimidazole derivatives, the application of 1*H*-benzimidazole (1) had the highest mean root length value (12.15 cm) (Table 4). The application of 5-nitro-1*H*-benzimidazole (2) on bread wheat (11.91 cm) and 5,6-dinitro-1*H*-benzimidazole (3) on durum wheat (11.62 cm) had the better root length results. The application of unsubstituted benzimidazole also had positive effects on the root length of both bread wheat (11.38 cm) and durum wheat (12.93 cm) varieties.

Table 4. Mean values of the root lengths measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	10.54 b-e*	10.90 b-e	10.72 B
(1)	12.93 a	11.38 a-d	12.15 A
(2)	10.16 cde	11.91 ab	11.04 B
(3)	11.62 abc	9.75 e	10.68 B
(4)	9.97 de	10.32 cde	10.15 B
(5)	10.17 cde	9.68 e	9.93 B
(6)	5.30 f	10.10 cde	7.70 C
(9)	10.25 cde	10.27 cde	10.26 B
Mean^{ns}	10.12	10.54	

*Different lowercase letters indicate statistical differences between application/species interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);^{ns} There is no statistical difference between the species ($p<0.05$); (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino] methyl}phenol.

Germination rate (%)

In the germination analyses, germination was observed on the 8th day. The highest rate was generally noted in the control applications of Çeşit-1252 (97%), Eminbey (95%), Bayraktar 2000 (98%), Demir 2000 (97%) and Tosunbey (97%) (Table 5). The application of unsubstituted benzimidazole (1) to Bayraktar 2000 (97%), Demir 2000 (97%), Tosunbey (100%) cultivars, the application of 5-nitro-1*H*-benzimidazole (2) to Bayraktar 2000 (99%), Demir 2000 (98%), Tosunbey (99%) varieties, the application of 5,6-dinitro-1*H*-benzimidazole (3) to Eminbey (96%) Bayraktar 2000 (99%), Demir 2000 (95%), Tosunbey (97%) varieties, the application of 5-nitro-1*H*-benzimidazole-6-amine (6) to Çeşit-1252 (100%), Eminbey (98%), Kızıltan-91 (99%), Bayraktar 2000 (99%), Demir 2000 (100%) and Tosunbey (97%) varieties, the application of 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) to Bayraktar 2000 (99%) and Demir 2000 (97%) varieties, the application of 5,6-dinitro-2-methyl-1*H*-benzimidazole (3) to Eminbey (96%), Bayraktar 2000 (99%) and Demir 2000 (96%) varieties, the application of 2-[(2-methyl-5-nitro-1*H*-benzimidazole-6-yl)imino]methyl}phenol (9) to Bayraktar-2000 (99%), Demir 2000 (97%) and Tosunbey (97%) varieties had the highest germination rate on the 8th day (Table 5).

Table 5. Mean values of the germination percentage values measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan-91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	97 a-d*	95 a-f	93 c-h	98 abc	97 abc	97 a-d	96 B
(1)	89 h-k	87 ı-k	85 j-k	97 a-d	97 a-d	100 a	92 D
(2)	92 d-h	92 d-h	91 f-ı	99 a	98 abc	99 ab	95 BC
(3)	94 b-g	96 a-e	84 k	99 ab	95 a-f	97 a-d	94 CD
(4)	91 e-ı	96 a-e	89 hjk	99 ab	96 a-e	91 e-ı	94 CD
(5)	89 g-j	95 ae	91 e-ı	99 a	97 abc	93 c-h	94 BC
(6)	100 a	98 a-c	99 a	99 ab	100 a	97 abc	99 A
(9)	89 h-k	92 d-h	94 b-g	99 a	97 a-d	97 ad	95 BC
Mean***	93 C	94 C	91 D	99 A	97 AB	96 B	

*Different lowercase letters indicate statistical differences between application/variety interactions, (p<0.05);**Different capital letters in the same column indicate statistical differences between applications (p<0.05);*** Capital letters in the same row indicate the statistical difference between varieties (p<0.05), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The 5-nitro-1*H*-benzimidazole-6-amine (6) had the highest germination percentage (99%) among the mean germination rates of the applied compounds. The bread wheat varieties Bayraktar 2000 (99%) and Demir 2000 (97%) had the highest mean germination rate (Table 5). The effects of the compounds on the germination rate of wheat species on the 8th day were evaluated and it was observed that the bread wheat varieties had a higher germination rate (97%) compared to the durum wheat varieties (92%) (Table 6).

When the interaction of the applied chemicals with the species was examined, it was found that the effect of all chemical applications on the germination rate of bread wheat was better than that of durum wheat. Among the benzimidazole derivatives, only 5-nitro-1*H*-benzimidazole-6-amine (6) had a high germination rate value (99%) in the durum wheat varieties (Table 6).

Table 6. Mean values of the germination percentage values measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	95 bc*	97 ab	96 B
(1)	87 e	98 ab	92 D
(2)	92 d	99 a	95 BC
(3)	91 d	97 ab	94 BCD
(4)	92 cd	95 b	94 CD
(5)	92 cd	97 ab	94 BCD
(6)	99 a	99 a	99 A
(9)	92 d	98 ab	95 BCD
Mean***	92 B	97 A	

*Different lowercase letters indicate statistical differences between application/species interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

It was observed that bread wheat (97%) had a higher germination rate than durum wheat (92%) on the 8th day of germination. Another result obtained by evaluating the analysis data showed that Bayraktar 2000 (99%) and Demir 2000 (97%) were the bread wheat varieties with the best germination. Analyzing the impact of chemical applications on the coleoptile length of the varieties, it was concluded that the application of 5-nitro-1*H*-benzimidazole-6-amine (6) to Demir 2000 (12.45 cm), Bayraktar 2000 (11.58 cm) and Tosunbey (11.31 cm) varieties and the application of 1*H*-benzimidazole (1) to Demir 2000 (10.96 cm) and Kızıltan-91 (10.60 cm) varieties gave the highest coleoptile length values. While the highest result was obtained with 1*H*-benzimidazole (1) in the applied compounds, Bayraktar 2000 and Demir 2000 varieties were the most prominent among the varieties.

The viability of the grains for seeding, and the size and mass of the cereal grains can be used to explain the variance in germination rates between the various cereal grains (Kaur and Gill, 2020). On the other hand, the results obtained can be evaluated in terms of the chemical structures of the applied compounds (Dincer, 1992; Hameed et al., 2016, 2019). According to the study of Vasilache et al. (2014), the existence of methoxy or ethoxy radicals in the molecules is connected to the biological effects of imidazole derivatives, which are thought to have an auxin-like effect by stimulating cell elongation. Some authors have shown that imidazolium-based ionic liquids are toxic to barley and wheat seedlings during the early stages of growth. The most toxic ionic liquid is the one with the longest alkyl substituent on the imidazolium ring (Bubalo et al., 2014; Chen et al., 2016). Tot et al. (2018) studied how ionic liquids based on imidazolium affected the germination and growth of wheat and barley. The observed effects of the chemicals on wheat and barley germination strongly imply that the total toxicity of the ionic liquids was influenced by the addition of oxygen as a hydroxyl group in the alkyl substituent. These results are consistent with the results of the present study, according to which the Schiff base 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol (9), which has the -OH group, had no positive influence on the length of the coleoptile or the root. The chemical 5-nitro-1*H*-benzimidazole-6-amine (6), which contains a free -NH_2 group, was found to have a stronger impact on the germination rate in this study. Derivatives of benzimidazoles, which have a diazo group in their structure, were found to inhibit germination. Since the germination properties of these chemicals have never been investigated before, the results of the present study are significant.

Conclusion

The objective of this study was to investigate the effects of benzimidazole derivatives with different substituents and different structures on the germination parameters of wheat. These substituted benzimidazole derivatives have not yet been studied in the literature with regard to germination analysis.

The results indicated that the benzimidazole compounds had different effects on the germination parameters of the different species (bread wheat and durum wheat). Of all the benzimidazole derivatives, 5-nitro-1*H*-benzimidazole-6-amine (6) exhibited a high value for germination rate in durum wheat varieties. Besides that, the outcomes can also be assessed in terms of the chemical structures of the substances used. A molecule with a free -NH_2 group, 5-nitro-1*H*-benzimidazole-6-amine (6), had the highest germination percentage on the 8th day. However, at a concentration of 10^{-6} M, the azo benzimidazole derivatives, 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol (7) and 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol (8), with the -N=N- group in the structure, inhibited the wheat germination. If we consider the result that diazo compounds inhibit germination, the possibilities of using these compounds and their derivatives as herbicides should be investigated. The results of the present study suggest that some benzimidazole derivatives could be used to enhance the germination rate, coleoptile, and root lengths of wheat.

Further studies with a higher number of wheat genotypes containing different species are needed to confirm these findings.

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PROCENA UTICAJA NEKIH DERIVATA BENZIMIDAZOLA NA PARAMETRE KLIJANJA SEMENA PŠENICE

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R e z i m e

Ovo istraživanje ima za cilj da istraži uticaj nekih derivata benzimidazola na parametre klijanja semena hlebne i durum pšenice. Ovi derivati sadrže nesupstituisane i supstituisane benzimidazolske strukture kako bi se utvrdio uticaj supstituenata na klijanje semena. U istraživanju su korišćene tri različite sorte durum pšenice (Çeşit-1252, Eminbey i Kızıltan-91) i tri različite sorte hlebne pšenice (Demir 2000, Bayraktar 2000 i Tosunbey). Prvo su korišćena jedinjenja 1*H*-benzimidazol (1) i 5-nitro-1*H*-benzimidazol (2) za sintezu dinitro jedinjenja (5,6-dinitro-1*H*-benzimidazol (3), 5,6-dinitro-2-metil-1*H*-benzimidazol (4)) kako bi se utvrdila reakcija sorti na tretman. Nakon toga, svako od ovih jedninjena je redukovano na nitroaminska jedinjenja (2-metil-5-nitro-1*H*-benzimidazol-6-amin (5), 5-nitro-1*H*-benzimidazol-6-amin (6)). Azo boje (1-[(5-nitro-1*H*-benzimidazol-6-il)diazenil]naftalen-2-ol (7), 1-[(2-metil-5-nitro-1*H*-benzimidazol-6-il)diazenil]naftalen-2-ol (8)) i Šifovo bazno jedinjenje (2-[(2-metil-5-nitro-1*H*-benzimidazol-6-il)imino]metil)fenol (9)) sintetizovani su iz nitroaminskih jedninjenja. Seme svih sorti pšenice tretirano je derivatima benzimidazola sintetizovanim u koncentraciji 10^{-6} M. Stope klijanja, dužine koleoptila i dužine klijanca merene su osmog dana klijanja. Dobijeni rezultati su pokazali da efikasnost benzimidazolskih jedinjenja varira u zavisnosti od sorti pšenice i parametara klijanja. Najveći uticaj na stopu klijanja imao je 5-nitro-1*H*-benzimidazol-6-amin (6), dok je 1*H*-benzimidazol (1) imao najveći uticaj na dužinu korena i koleoptila kod svih sorti.

Ključne reči: benzimidazol, klijanje pšenice, hemijski stimulusi.

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