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Effects of Nitrogen Fertilizer Doses on Some Germination Parameters in Bread Wheat (*Triticum aestivum* L.) and Examination with Scanning Electron Microscopy

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Abstract

Wheat is a staple food that plays an important role in human nutrition. It is one of the most widely grown crops in the world and can be grown in a variety of climates and soil conditions. Wheat plants need nitrogen for their growth and yield. Nitrogen deficiency in wheat can lead to poor plant growth, yield reduction, and decreased protein content. Wheat plants also need to take up adequate nutrients to produce strong roots and seedlings, especially after germination. In this study, conducted in the Field Crops Department of the Faculty of Agriculture at Ordu University in 2023, the effects of different nitrogen fertilizer doses (1.5%, 2%, 3%, 4%, and 5%) on wheat seed germination were determined, and at the same time, the stoma structures located on the underside of the first leaves of the germinated seedlings were imaged with SEM microscopy. The root/shoot length ratio, seedling and root fresh weight, shoot/root, and root/shoot weight ratio were found to be significant ($p<0.05$). According to the results, the germination rate of various nitrogen treatments varied from 43.33 to 96.7%, while the control application was 33.33%. The application of 0.04 yielded the highest germination rate. The control group exhibited the lowest rate of germination. The LSD means for the majority of parameters, including seedling length, root length, shoot length, root/shoot length ratio, shoot/root length ratio, and seedling fresh weight, were highest for nitrogen administrations of 0.04 and 0.05. In contrast, the LSD means for these parameters were lowest for the 0.02 nitrogen application. A number of parameters, including seedling length, root length, shoot length, root-to-shoot and shoot-to-root length ratios, seedling and shoot fresh weights, shoot and root dry weights, and shoot water content, showed significant differences in germination characteristics under different nitrogen

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doses. The study's results revealed that the differences in stoma structures formed in young leaf tissues were striking when examined under SEM.

Keywords: Germination, Nitrogen, SEM, *Triticum aestivum* L.

Azotlu Gübre Dozlarının Ekmeklik Buğday (Triticum aestivum L.)'da Bazı Çimlenme Parametrelerine Etkileri ve SEM ile İncelenmesi

Özet

Buğday, insan beslenmesinde önemli bir yere sahip olan temel gıda ürünlerinden biridir. Dünya çapında en çok üretilen tarım mahsullerinden biri olan buğday, hemen hemen her türlü iklim ve toprak koşullarında yetiştirilebilmektedir. Buğday bitkisi, gelişimi ve verimi için azota ihtiyaç duyar. Buğdayda azotun yetersizliği, bitkilerin zayıf büyümesine, verim düşüklüğüne ve protein içeriğinin düşmesine neden olmaktadır. Buğday bitkisi, özellikle çimlendikten hemen sonra oluşturacağı kök ve fidenin güçlü olması için yeterli bitki besin maddesi alımına ihtiyaç duymaktadır. Ordu Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü'nde 2023 yılında gerçekleştirilen bu çalışmada, farklı azotlu gübre dozlarının (1.5%, 2%, 3%, 4%, and 5%) buğday tohumu çimlenmesi üzerine etkileri belirlenmiş ve aynı zamanda çimlenen fidelerin ilk yapraklarının alt kısmında yer alan stoma yapıları SEM mikroskobu ile görüntülenmiştir. Kök/sürgün uzunluk oranı, fide ve kök yaş ağırlığı, sürgün/kök ve kök/sürgün ağırlık oranı önemli bulunmuştur ($p<0.05$). Sonuçlara göre çeşitli azot uygulamalarının çimlenme oranı %43.33 ile %96.7 arasında değişirken, kontrol uygulamasının çimlenme oranı %33.33 olarak gerçekleşmiştir. 0.04 uygulaması en yüksek çimlenme oranını vermiştir. Kontrol grubu en düşük çimlenme oranını göstermiştir. Fide uzunluğu, kök uzunluğu, sürgün uzunluğu, kök/sürgün uzunluğu oranı, sürgün/kök uzunluğu oranı ve fide taze ağırlığı da dahil olmak üzere parametrelerin çoğunluğu için LSD değerleri, 0.04 ve 0.05 azot uygulamalarında en yüksek düzeyde tespit edilmiştir. Buna karşılık, bu parametreler için LSD değerleri 0.02 azot uygulaması için en düşük bulunmuştur. Farklı azot dozları altında, çimlenme özelliklerinde fide uzunluğu, kök uzunluğu, sürgün uzunluğu, kök/sürgün ve sürgün/kök uzunluk oranları, fide ve sürgün yaş ağırlıkları, sürgün ve kök kuru ağırlıkları ve sürgün su içeriği gibi birçok parametrede anlamlı farklılıklar gözlenmiştir. Çalışmanın sonuçları, SEM altında incelendiğinde genç yaprak dokularında oluşan stoma yapılarındaki farklılıkların dikkat çekici olduğunu ortaya koymaktadır.

Anahtar Kelimeler: *Azot, Çimlenme, SEM, Triticum aestivum* L.

1. Introduction

Wheat is a strategic crop in both the world and Turkey. The main reasons for this strategic importance are its wide adaptation ability, its importance in terms of planting area and production volume in field agriculture, its being a field crop from which the most basic food items in human nutrition are obtained, its being a basic raw material for the bread, bulgur, pasta and biscuit industries, and its being one of the basic cereals in solving the world hunger problem (Yıldırım and Yılmaz (2022); Yorulmaz and Akıncı (2022)).

Germination parameters in wheat are affected by various environmental conditions. In general, the germination time of wheat varies between 4 and 18 hours depending on the variety of the seed, temperature and humidity (Martínez et al., (2010); Yıldırım and Yılmaz (2023)). Fertilization is a cultural practice that is essential for the healthy and productive growth of plants. Proper fertilization helps to achieve higher yields, better product quality and healthier plants

(Aydemir vd., 2023; Akgül ve Aydemir 2022). Nitrogen is one of the main nutrient sources in plant production. Nitrogen supports plant growth and development. Nitrogen deficiency can lead to poor plant growth, reduced yields, and reduced protein content. Nitrogen fertilizers, as well as helping to support the development of the root system, also contribute to the formation of stronger seedlings (Aras and Uygun, 2017). In this study, the aim was to investigate the effects of different nitrogen dose applications on some germination characteristics in the Dimenit wheat variety and the stoma structures formed in young seedlings.

2. Material and Method

2.1. Material

This study was conducted in 2023 at the Faculty of Agriculture, Department of Field Crops, Ordu University. Five different doses of nitrogen fertilizer (1.5%, 2%, 3%, 4%, and 5%) were applied. Dimenit wheat variety was used as plant material in the study. The trial was planned as a randomized block design with three replications according to factorial design. MultiReflex brand EC fertilizer solution was used as nitrogen fertilizer (Table 1). The experiment was carried out in the laboratory under room temperature conditions.

Table 1. The composition of the nitrogen fertilizer solution used in the study

Content	% w/w
Total Nitrogen (N)	15
Ammonium Nitrogen (NH_4N)	2
Nitrate Nitrogen (NO_3N)	1
Urea Nitrogen (NH_2N)	12
Chlorine Content	0.06
pH	4-6
Biuret Reaction	Low

2.2. Method

The nitrogen doses were determined by the preliminary germination studies conducted. Five different nitrogen percentage concentrations (0.015, 0.02, 0.03, 0.04, 0.05) were used to generate the solutions. 2 ml of liquid solution was added to single-use plastic petri dishes to moisten the filter paper. It was routinely seeded with sterilized seeds using forceps. Following seed sowing, seed germination studies were started in the dark in petri dishes that had been covered with stretch film and put in an oven set at 20±5 °C. It was acknowledged that the radicle had emerged from the 2 mm testa for germination. Ten days in total were given before measurements were taken (Yıldırım and Yılmaz (2022); Yıldırım and Bilgen (2022)).

Germination rate (power) (%), seedling length (mm), shoot length (mm), root length (mm), seedling fresh weight (mg), stem fresh weight (mg), and root fresh weight (mg) were all taken into consideration in the study. The stem dry weight (mg), root dry weight (mg), shoot/root-root/shoot length ratio (%), shoot/root-root/shoot fresh weight ratio (%) and other parameters have been evaluated.

At the end of the tenth day of the experiment, researchers counted the seeds that had begun to germinate and used the formula (number of seeds germinated/total number of seeds) x 100 to determine the germination rate (Akıncı and Çalışkan, 2010). Seedling, shoot, and root lengths (cm) were measured using a millimetric ruler, and the stem length of 10 randomly selected plants was also measured (Yılmaz and Kısakürek 2021). The fresh weight of the green parts of the plants was measured in mg with a precision scale (Yıldız and Özgen, 2004), and the shoot and root dry weight was determined after drying in a 70°C air circulation oven (Yılmaz and Kısakürek 2021). The shoot/root and root/shoot wet weight ratio was found by weighing the shoot and root fresh weights of each plant and proportioning them to each other. The shoot/root and root/shoot length ratio was also measured using a millimetric ruler and proportioned to each other. The average was taken for all measurements on 10 plants. For taking captures of wheat leaf stomata were used a scanning electron microscope (SEM). Well dried material was processed for SEM. Before taking SEM photos, samples were coated with gold at 10 nm for 60 seconds by sputter. The photos were taken by SU1510 Hitachi SEM.

2.3. Statistical analysis

The XLSTAT program was used to do the statistical analyses. The ANOVA test was used for the analysis of variance at a 95% confidence interval, and the Tukey test was used for multiple comparisons.

3. Results and Discussion

Table 2 shows the results of the effects of 5 different nitrogen doses (1.5%, 2%, 3%, 4%, and 5%) on some germination parameters in the Dimenit bread wheat variety. Significant differences

($p<0.05$) were observed in the root/shoot length ratio, seedling and root fresh weight, shoot/root and root/shoot weight ratios.

Seed germination is a critical stage in plant life, seed dormancy is affected by external environmental conditions and affects individual development, seedling establishment and community stability (Chen et al., 2022; De Malach, Kigel and Sternberg, 2020; Jiang et al., 2016; Milla and Lopez, 2014; Zhang et al., 2020). In this study, the germination rate of different nitrogen applications ranged from 43.33% to 96.7%, while the control application was 33.33%. The highest germination rate was found in the 0.04 application. The lowest germination rate was in the control.

Table 2. Effects of different N applications on some parameters of bread wheat

Applications	Germination rate	Seedling lenght	Root lenght	Shoot lenght	Root/shoot lenght ratio	Shoot/root lenght ratio	Seedling fresh weight	Shoot fresh weight
control	33.33	13.73	3.23	10.5	0.307	3.308	0.163	0.062
0.015	46.67	13.03	3.43	9.6	0.355	3.154	0.495	0.253
0.02	43.33	6.93	3.23	3.7	0.875	1.143	0.103	0.029
0.03	46.67	12.52	3.74	8.77	0.422	2.429	0.09	0.056
0.04	96.67	18.75	3.98	14.77	0.265	3.83	0.146	0.068
0.05	73.33	19.79	7.24	12.54	0.601	1.741	0.129	0.084
p-value	0.101	0.539	0.820	0.699	**0.050***	0.718	**0.029**	0.122

Applications	Root fresh weight	Root/shoot weight ratio	Shoot/root weight ratio	Shoot dry weight	Root dry weight	Shoot water amount (mg)	Amount of shoot dry matter (mg)	Seedling dry matter ratio (%)
control	0.1	1.662	0.614	0.006	0.007	0.055	0.006	0.111
0.015	0.726	1.055	1.706	0.007	0.008	0.246	0.007	0.029
0.02	0.075	2.379	1.485	0.002	0.001	0.026	0.004	0.097
0.03	0.034	0.598	1.689	0.009	0.006	0.047	0.009	0.164
0.04	0.078	1.462	1.753	0.01	0.007	0.058	0.01	0.156
0.05	0.045	0.541	1.851	0.009	0.009	0.075	0.009	0.121
p-value	**0.007**	**0.015**	**0.041**	0.981	0.190	0.113	0.785	0.461

*Values in bold correspond to tests where the null hypothesis is not accepted with a significance level alpha=0.05

Table 3 shows the minimum and maximum values, mean (average), standard deviation (SD), and coefficient of variation (CV) for each characteristic. The characteristics investigated include seedling length, root length, shoot length, root/shoot length ratio, shoot/root length ratio, seedling fresh weight, shoot fresh weight, root fresh weight, root/shoot weight ratio, shoot/root weight ratio, shoot dry weight, root dry weight, shoot water content (mg), shoot dry matter amount (mg), and seedling dry matter ratio (%).

Seedling length varies between 6.067 cm (minimum) and 23.867 cm (maximum), with an average length of 14.128 cm. Root length varies between 1.867 cm and 8.033 cm, with an average length of 4.144 cm. Shoot length varies between 3.2 cm and 17.8 cm, with an average length of 9.983 cm. Root/shoot length ratio varies between 0.221 and 0.896, with an average ratio of 0.471. Plant root/shoot length ratio varies between 1.116 and 4.523, with an average ratio of 2.601. Chen Jing et al. (2020) found that a moderate nitrogen application of 240 kg per hectare significantly increased root length, surface area, and biomass in soil layers, increased cotton seed yield, and improved N efficiency.

Seedling fresh weight varies between 0.037 mg and 0.711 mg, with an average weight of 0.188 mg. Shoot fresh weight varies between 0.022 mg and 0.324 mg, with an average weight of 0.092 mg. Root fresh weight varies between 0.011 mg and 0.496 mg, with an average weight of 0.096 mg. Root fresh weight increased up to 300 mg l-1 or 450 mg l-1 nitrogen application in autumn/winter crops, but did not increase further in spring crops, high nitrogen reduced root weight (Akoumianakis et al., 2011).

The root-to-shoot weight ratio ranged from 0.347 to 6.082, with an average of 1.283. The shoot-to-root weight ratio ranged from 0.164 to 2.879, with an average of 1.425. The shoot dry weight ranged from 0.002 mg to 0.011 mg, with an average of 0.007 mg. The root dry weight ranged from 0.001 mg to 0.010 mg, with an average of 0.007 mg. A study found that appropriate nitrogen fertilization increased root growth in winter wheat, while excessive fertilization inhibited root growth, and yield and root-shoot ratio showed a significant correlation with irrigation level (Wu Rong, & Li YuanNong, 2013). The amount of water in shoots ranged from 0.019 mg to 0.320 mg, with an average of 0.085 mg. The amount of dry matter in shoots ranged from 0.002 mg to 0.011 mg, with an average of 0.008 mg. The seedling dry matter ratio ranged from 0.012% to 0.214%, with an average ratio of 0.113%.

Figure 1 shows the least square means (LS means) for various germination parameters under different nitrogen fertilizer applications. 0.04 and 0.05 nitrogen applications showed the highest LS means for most characteristics, such as seedling length, root length, shoot length, root/shoot length ratio, shoot/root length ratio, and seedling fresh weight. Conversely, the 0.02N treatment had the lowest LS means for these parameters. There were significant differences in various parameters, including seedling length, root length, shoot length, root/shoot, shoot/root length ratios, seedling and shoot fresh weights, shoot and root dry weights, and shoot water content. This also demonstrates the effect of varying nitrogen doses on these germination characteristics.

Table 3. An overview of all the dependent variables of the normality tests

Variable	Minimum	Maximum	Mean	Std. deviation	CV
seedling lenght	6.067	23.867	14.128	5.006	35.43344
root lenght	1.867	8.033	4.144	1.718	41.4611
shoot lenght	3.200	17.800	9.983	3.953	39.5926
root/shoot lenght ratio	0.221	0.896	0.471	0.234	49.60115
shoot/root lenght ratio	1.116	4.523	2.601	1.096	42.12845
seedling fresh weight	0.037	0.711	0.188	0.164	87.32862
shoot fresh weight	0.022	0.324	0.092	0.080	86.76992
root fresh weight	0.011	0.496	0.096	0.111	116.1939
root/shoot weight ratiı	0.347	6.082	1.283	1.427	111.1784
shoot/root weight ratio	0.164	2.879	1.425	0.798	56.00194
shoot dry weight	0.002	0.011	0.007	0.003	38.60962
root dry weight	0.001	0.010	0.007	0.003	48.08368
shoot water amount (mg)	0.019	0.320	0.085	0.080	94.54515
amount of shoot dry matter (mg)	0.002	0.011	0.008	0.003	35.02353
seedling dry matter ratio (%)	0.012	0.214	0.113	0.057	50.10116

Figure 2 shows the stoma structures in leaf samples taken from young shoots of the treatments. Stomata are small openings distributed over the leaf surface. Stomata allow plants to exchange gases, that is, to take in carbon dioxide and oxygen. The number and size of stomata affect the physiology of the plant and its adaptation to the environment. Stomata cells use changes in water potential to close and open the stoma aperture. Stomata were observed to be smaller and less numerous in the leaf of the control treatment. This may suggest that it would help reduce water loss in plants. Stomata were observed to be larger in the leaf treated with a 1.5% nitrogen solution. Observations taken in other applications are generally in the middle of the two, with respect to the control and 1.5% dose. Stomata opening or closing can also vary depending on many physiological characteristics of the plant. Therefore, the effectiveness of nitrogen can also change this.

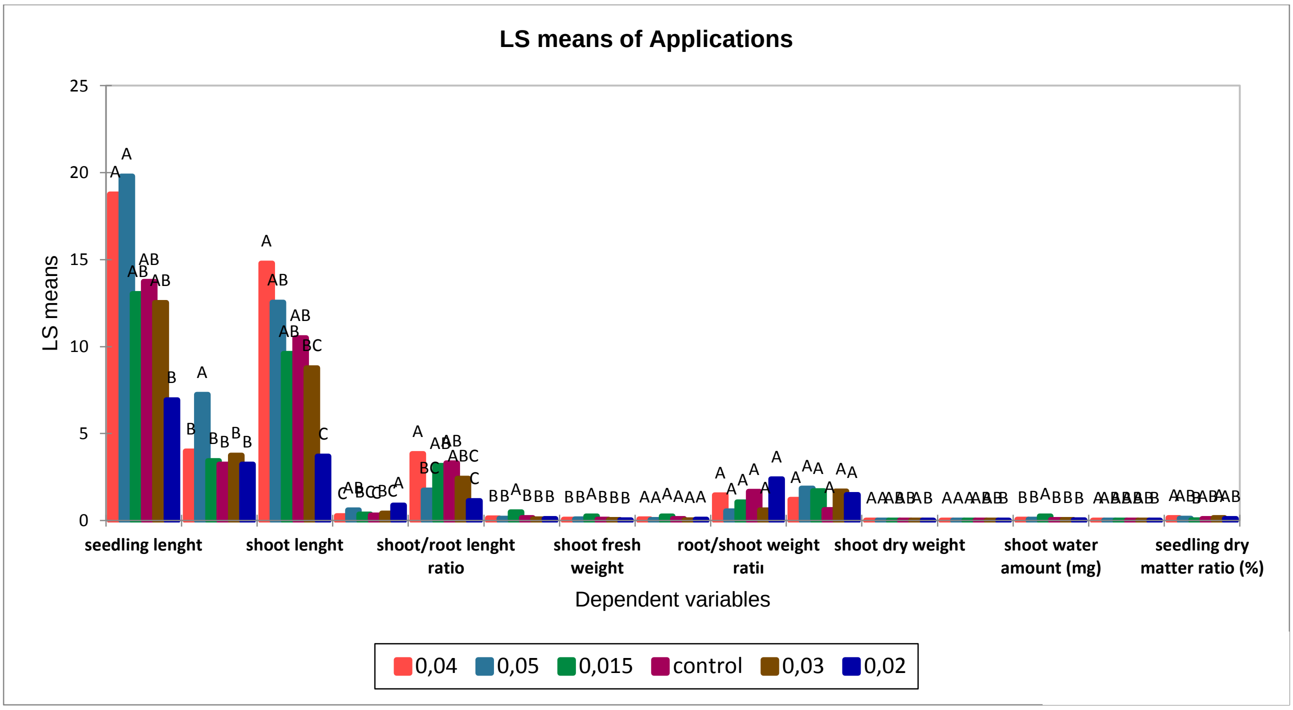

Figure 1. Summary of all pairwise comparisons for all characteristics (Tukey (HSD))

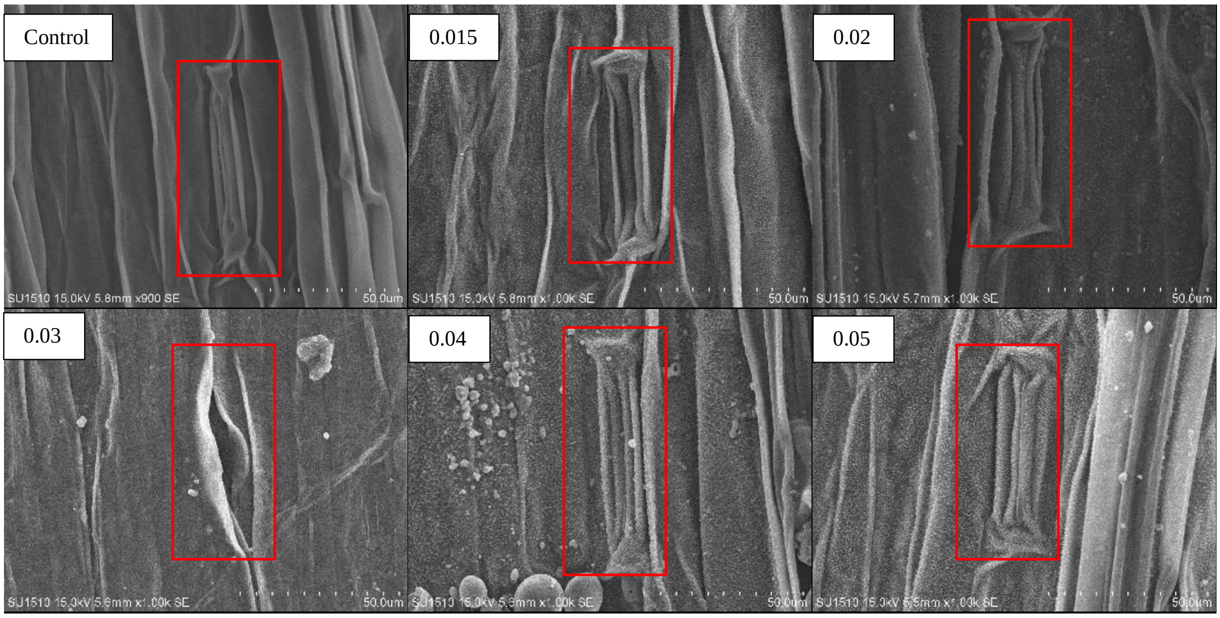

Figure 2. SEM images of wheat leaf stomata that belongs to six separate applications

4. Conclusion

The effect of various nitrogen fertilizer doses on a number of germination characteristics in Dimenit bread wheat was examined in this research. The results show the importance of nitrogen dosage by demonstrating significant changes in a number of important parameters. Notably, we saw a considerable improvement in the shoot/root and shoot/root weight ratios as well as a large rise in the root/shoot length ratio and seedling and root fresh weights. In contrast to the control group, germination rates varied greatly, with the greatest rate being attained at a nitrogen dose of 0.04. This study emphasizes the critical part nitrogen fertilization plays in controlling wheat germination and related characteristics, providing useful information for improving wheat cultivation practices. These results can be expanded upon by additional research to optimize nitrogen fertilizer applications for improved wheat crop yields.

Competing Interests

The authors declare that they have no competing interests.

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