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Numerical and statistical analysis of nitric oxide effects on bread wheat species under drought stress

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ABSTRACT

In study, three cultivars of bread wheat *Triticum aestivum* L. and *Triticum monococcum* L. were used. Plants were grown in growth room. Effect of nitric oxide treatments on drought stress of wheat genotypes were examined. The paraffin method was used for preparing a cross-section of leaves. Transverse sections were made using a sliding microtome and stained with safranin-fast green. Results were presented by photographs and tables. Photographs were taken with Leica DM 3000 microscopy. Anatomical numerical measurements were made on sections of leaf tissues of the plant samples help of micrometric ocular. This numerical results were evaluated statistically by ANOVA test.

Keywords: Numerical analysis, Drought stress, Statistical analysis, Nitric oxide

Kuraklık Stresi altındaki ekmeklik buğday türlerinde nitrik oksit etkisinin istatiksel ve nümerik analizi

ÖZ

Bu çalışmada ekmeklik buğdaylardan *Triticum aestivum* L. ve *Triticum monococcum* L. türlerine ait üç çeşit kullanılmıştır. Bitkiler çimlendirme odasında yetiştirilmiştir. Kuraklık stresi altındaki buğday genotiplerininde nitrik oksit uygulamasının etkileri çalışılmıştır. Anatomik çalışmalarda bitki örnekleri %70 alkolde fikse edilmiştir. Yaprak enine kesitlerinin hazırlanmasında parafin metodu kullanılmıştır. Sonuçlar tablo ve fotoğraflarda sunulmuştur. Fotograflar Leica DM 3000 mikroskop ile çekilmiştir. Bitki örneklerinin yaprak dokularından alınan kesitlerde mikrometrik oküler yardımıyla anatomik ölçümler yapılmıştır. Bu sayısal sonuçlar istatistiksel olarak ANOVA testi ile değerlendirilmiştir.

Anahtar kelimeler: Nümerik analiz, Kuraklık stresi, İstatiksel analiz, Nitrik oksit

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1. INTRODUCTION

In the twenty-first century, world will be faced with threats, including mainly drought caused by global climate change. Drought stress, affecting the growth and development of plants is one of the most widespread environmental stress. Nitric oxide is known to take place in the plant defense mechanism against some stress conditions. In this study, impacts of the nitric oxide application on leaf anatomy of *Triticum* L. genotypes under drought stress conditions were investigated and evaluated statistically [1, 2].

Drought stress has the highest percentage (26%) when the usable areas on the earth are classified in view of stress factors. Nitric oxide (NO) is a free radical that had been known for many years simply as a molecular gas. NO release in plants and the effects on plant growth, was first described in the 1970s [3,4,5]. Nitric oxide growth and development processes in the plant, Plant growth and development processes in Nitric oxide has been found that the signaling molecule in the formation of biotic and abiotic stress response [6, 7, 8, 9]. Nitric oxide (NO) is a important free radical as biologically. In case of threat caused by environmental stress created by abiotic and biotic factors nitric oxide can be produced in different plant species and organs. Nitric oxide (NO) is a very active molecule involved in many and diverse biological pathways where it has proved to be protective against damages provoked by oxidative stress conditions [10].

In recent years, many studies have mentioned the effects of nitric oxide on the plants physiology [6, 11, 12, 13], but there are few articles in which anatomical variations have been quantified [14, 15] .

The aim of this research is to statistically and numerical evaluate the variations that are observed in plant tissues growing in drought stress and NO concentrations that affect of this crop.

2. MATERIAL AND METHOD

In study, three cultivars of bread wheat *Triticum aestivum* L. (1.variety: Improved for aqueous conditions Göksu 99, 2. variety: Improved for dry conditions Karahan 99) and *Triticum monococcum* L. (3. variety: Kaplıca) were used. Plants were grown in growth room. Nitric oxide treatment on drought tolerance of wheat genotypes were examined. Applications were made as follows: Control (Hoagland) groups, Nitric oxide control (Hoagland+100 µm Nitric oxide) groups, drought (%15 PEG 6000) groups, Drought (%15 PEG 6000+100 µm nitric oxide) groups. For anatomical studies plant samples were fixed in 70 % alcohol. The paraffin method and hand cut method was used for preparing a cross-section of leaves [16,17]. Transverse sections were made using a sliding microtome and stained with safranin-fast green. Anatomical measurements were made with the help of micrometric ocular using sections from the different parts of the plant samples. Results were presented by photographs and tables (Figures 1-3, Tables 1-3). Photographs were taken with Leica DM 3000 microscopy. The results were evaluated statistically by ANOVA test (Tables 4-6). For the numerical analysis, 5 characters of the leaf which belong to three different *Triticum* genotypes were selected. The applications were coded as 1-5 and the genotypes were coded as A,B,C (Tables 4-6). Significance of the differences between the applications and characters for the three genotypes were evaluated by Pearson correlation.

3. RESULTS AND DISCUSSION

Nitric oxide applications of leaf tissue in vascular bundle caused by an increase in the diameter of the trachea and reduced mesophyll cell diameters in Peg 6000 groups. It has also been found to prevent the thickening of the upper and lower epidermis.The leaf anatomical measurements of the investigated plants were shown in Tables 1-3. Significance of the differences between two bread wheat (*T. aestivum*) genotypes was evaluated by Pearson correlation. The statistical analysis of the results were given in table 4-5. According to the tables, there are important correlations between the applications: PEG (4), PEG+NO (5) and leaf anatomical characters for the three genotypes at levels of 0.01 and 0.05.By the analysis of investigated three Triticum genotypes with different the applications responses, from five leaf anatomy related characters, it has been determined that trachea diameter and mesophyll are the best characters pairs. It has been also found that the results from numerical analysis of the leaf anatomy characters can provide distinct evidences, which are corresponding to the anatomy for recognition of the taxa.

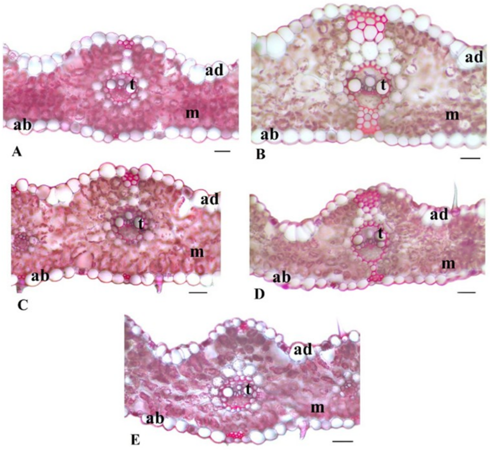

Figure 1. Cross section of leaf of Karahan 99 genotype (scale bars 50 mikrometer), ad: adaxial epidermis, t:trachea, m:mesophyll , ab:abaxial epidermis, A: 0.day, B: Control Group, C: Control+NO, D: PEG 6000, E: PEG 6000+NO

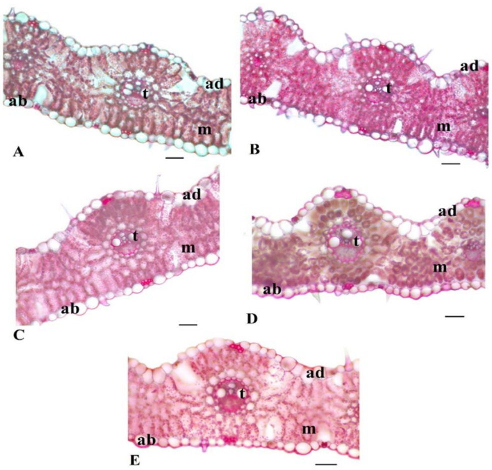

Figure 2. Cross section of leaf of Kaplıca 99 genotype (scale bars 50µm). ad: adaxial epidermis, t: trachea, m: mesophyll, ab: abaxial epidermis, A: 0.day , B: Control Group, C: Control + NO, D: PEG 6000, E: PEG 6000+ NO.

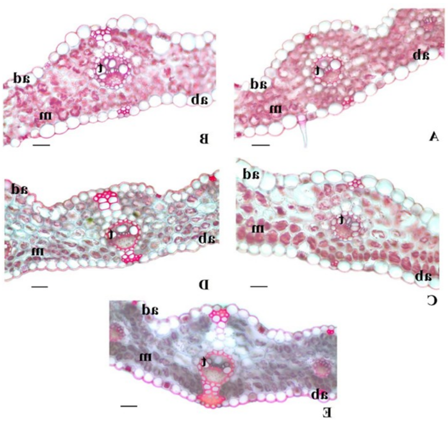

Figure 3. Cross section of leaf of Göksu 99 genotype (scale bars 50µm), ad: adaxial epidermis, t: trachea, m: mesophyll, ab: abaxial epidermis, A: 0.day , B: Control Group, C: Control + NO, D: PEG 6000, E: PEG 6000+ NO

Table 1. Measurements of leaf of Karahan 99 genotypes

	Width (µm)		Lenght (µm)	
	Min.–Max.	**Mean ± S.D.**	**Min.–Max.**	**Mean ± S.D.**
0.day				
Adaxial Ep.	22.51 - 47.71	32.43 ± 06.84	19.29 - 40.93	29.64 ± 05.60
Trachea	19.02 - 28.94	24.07 ± 02.93		
Mesophyll	25.18 - 38.30	31.72 ± 03.26		
Abaxial Ep.	22.22 - 43.56	30.41 ± 06.52	17.83 - 41.52	29.00 ± 07.00
Control				
Adaxial Ep.	23.97 - 44.15	30.49 ± 05.74	21.92 - 40.05	28.39 ± 05.24
Trachea	23.05 - 28.65	25.81 ± 02.14		
Mesophyll	24.35 - 41.22	29.94 ± 04.86		
Abaxial Ep.	25.73 - 40.93	33.20 ± 05.47	18.71 - 38.86	31.55 ± 06.16
C+NO				
Adaxial Ep.	28.65 - 43.56	33.80 ± 04.60	23.99 - 40.01	30.17 ± 05.41
Trachea	20.17 - 27.48	25.32 ± 02.53		
Mesophyll	21.40 - 42.69	34.98 ± 05.96		
Abaxial Ep.	30.99 - 44.73	38.12 ± 04.38	27.77 - 42.69	37.08 ± 05.43
PEG				
Adaxial Ep.	21.34 - 31.57	26.56 ± 02.52	16.50 - 28.36	24.50 ± 02.86
Trachea	19.05 - 28.94	25.08 ± 03.71		
Mesophyll	16.37 - 44.75	29.72 ± 07.39		
Abaxial Ep.	16.95 - 36.84	24.74 ± 05.60	17.54 - 31.74	23.91 ± 04.97
PEG+NO				
Adaxial Ep.	18.42 - 28.65	22.21 ± 02.96	18.12 - 26.02	21.24 ± 02.14
Trachea	14.40 - 22.24	17.86 ± 02.79		
Mesophyll	17.25 - 35.67	27.16 ± 05.75		
Abaxial Ep.	21.26 - 32.45	27.36 ± 03.36	20.15 - 28.07	23.94 ± 02.36

Table 2. Measurements of leaf of Kaplıca genotypes

	Width (µm)		Lenght (µm)	
	Min.–Max.	**Mean ± S.D.**	**Min.–Max.**	**Mean ± S.D**
0.day				
Adaxial Ep.	19.88 - 30.41	22.63 ± 03.06	17.83 - 26.61	21.11 ± 03.05
Trachea	19.66 - 24.57	22.00 ± 01.92		
Mesophyll	19.29 - 38.88	28.10 ± 05.63		
Abaxial Ep.	19.00 - 31.29	23.82 ± 04.53	17.84 - 31.58	23.43 ± 04.12
Control				
Adaxial Ep.	15.78 - 30.12	23.07 ± 04.10	15.78 - 36.26	24.57 ± 05.76
Trachea	19.59 - 23.45	21.62 ± 01.88		
Mesophyll	15.49 - 35.67	23.01 ± 07.66		
Abaxial Ep.	18.13 - 38.30	25.99 ± 05.41	16.08 - 40.06	24.85 ± 06.93
C+NO				
Adaxial Ep.	22.22 - 33.62	27.25 ± 04.20	20.47 - 47.95	30.53 ± 07.67
Trachea	21.64 - 25.85	23.15 ± 01.64		
Mesophyll	17.83 - 40.05	26.98 ± 06.40		
Abaxial Ep.	17.25 - 37.14	26.08 ± 06.80	16.08 - 35.67	25.16 ± 06.39
PEG				
Adaxial Ep.	16.08 - 30.11	21.94 ± 04.69	16.08 - 31.87	22.35 ± 04.90
Trachea	16.95 - 22.51	20.02 ± 02.30		
Mesophyll	24.56 - 40.05	32.60 ± 05.02		
Abaxial Ep.	15.20 - 31.87	25.36 ± 04.82	14.91 - 29.23	23.97 ± 03.64
PEG+NO				
Adaxial Ep.	17.54 - 23.39	20.78 ± 01.75	16.65 - 26.02	21.14 ± 02.61
Trachea	16.08 - 26.02	20.98 ± 02.85		
Mesophyll	19.20 - 39.14	30.63 ± 06.01		
Abaxial Ep.	16.66 - 40.64	23.63 ± 05.53	14.91 - 28.36	20.63 ± 03.16

Table 3. Measurements of leaf of Göksu 99 genotypes

	Width (µm)		Length (µm)	
	Min.–Max.	**Mean ± S.D.**	**Min.–Max.**	**Mean ± S.D.**
0.day				
Adaxial Ep.	23.25 - 31.66	27.46 ± 03.09	18.89 - 30.84	25.30 ± 03.22
Trachea	23.83 - 32.26	27.86 ± 03.60		
Mesophyll	23.25 - 41.56	31.55 ± 04.60		
Abaxial Ep.	25.00 - 39.82	32.49 ± 04.85	23.83 - 34.88	29.07 ± 03.44
Control				
Adaxial Ep.	15.11 - 37.20	29.73 ± 06.50	18.60 - 36.04	25.53 ± 06.52
Trachea	13.08 - 28.48	21.14 ± 04.64		
Mesophyll	17.73 - 30.52	24.30 ± 03.85		
Abaxial Ep.	22.09 - 40.98	30.37 ± 05.63	18.02 - 37.50	28.53 ± 05.43
C+NO				
Adaxial Ep.	27.32 - 46.22	35.46 ± 06.06	27.90 - 41.86	34.03 ± 03.97
Trachea	19.47 - 34.88	28.34 ± 04.76		
Mesophyll	21.22 - 40.40	30.99 ± 05.70		
Abaxial Ep.	20.94 - 41.27	32.69 ± 05.98	23.22 - 36.62	29.77 ± 04.43
PEG				
Adaxial Ep.	16.26 - 29.65	21.70 ± 02.99	14.53 - 22.08	19.14 ± 02.74
Trachea	21.93 - 27.61	24.02 ± 01.74		
Mesophyll	16.56 - 32.26	23.24 ± 04.29		
Abaxial Ep.	19.30 - 31.39	25.20 ± 04.40	13.37 - 25.58	18.99 ± 03.95
PEG+NO				
Adaxial Ep.	21.22 - 36.33	30.27 ± 05.20	17.73 - 29.94	24.98 ± 04.13
Trachea	15.98 - 25.29	20.05 ± 03.28		
Mesophyll	15.98 - 29.65	21.83 ± 03.93		
Abaxial Ep.	18.31 - 33.43	23.59 ± 04.90	10.46 - 27.03	19.06 ± 04.48

Table 4. Pearson correlation based on leaf anatomical characters of the genotypes (A)

	1	2	3	4
2	0,384			
	0,616			
3	0,145	0,476		
	0,855	0,524		
4	0,424	0,880	0,171	
	0,576	0,048*	0,829	
5	0,439	0,856	0,911	0,223
	0,561	0,050*	0,049*	0,777

**Significant at the level of 0.05. 1-5: applications codes

Table 5. Pearson correlation based on leaf anatomical characters of the genotypes (B)

	1	2	3	4
2	0,111			
	0,889			
3	0,167	0,131		
	0,833	0,869		
4	0,540	0,966	0,373	
	0,460	0,034*	0,627	
5	0,107	0,890	0,360	0,856
	0,893	0,045*	0,640	0,050

*Significant at the level of 0.05. 1-5: applications codes

Table 6. Pearson correlation based on leaf anatomical characters of the genotypes (C)

	1	2	3	4
2	0,691			
	0,309			
3	0,039	0,666		
	0,961	0,334		
4	0,651	0,966	0,507	
	0,349	0,004**	0,493	
5	0,348	0,412	0,950	0,685
	0,652	0,588	0,050*	0,315

**Significant at the level of 0.05. **Significant at the level 0.01. 1-5: applications codes

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