RELATION OF STOMATAL FREQUENCY AND SIZE ON WHEAT YIELD AND MORPHO-PHYSIOLOGICAL TRAITS UNDER VARIED DROUGHT STRESSES CONDITION

İrfan Öztürk[1], Kayıhan Z. Korkut[2]

[1]Trakya Agricultural Research Institute, Edirne, Turkey

[2]Namık Kemal University, Faculty of Agriculture, Department of Field Crops, Tekirdağ, Turkey

Abstract

Water stress is a severe abiotic stress and major constraint to wheat productivity. The objective of this study was to characterize drought tolerant and susceptible wheat genotypes on the basis of physiological and yield attributes. This research were carried out during 2008-2009 and 2009-2010 growing seasons and 15 genotypes were planted in a randomized completely blocks design in split plot with three replications. Drought treatments are placed on main parcel and genotypes on the sup-plot. Drought treatments were applied at several plant stages from stem elongation up to physiological maturating stage. Grain yield, flag leaf area, relative water content, stomata width, stomata length and stomatal number and pairwise comparison between these characters were evaluated. There were significant differences among genotypes and treatment in both years based on investigated characters. Grain yield decreased under drought condition in all cultivars. The highest grain yield with 658.3 kg da^{-1} was obtained from Bereket cultivar. Drought stress condition more affected grain yield at stem elongation stage than grain filling period. Grain yield, flag leaf area, relative water content, stomata length and width decreased under drought stress condition, while stomatal number increased. Flag leaf area varied between 18.90 cm^2 in non-stress condition and 23.46 cm^2 in fully stress. The lowest flag leaf area was 17.92 cm^2 (Kate A-1) and highest 24.09 in ÖVD26-07 line. Relative water content varied between 15.82% and 23.53%. The lowest stomatal width and length was scaled under drought stress condition. There were significant relationship between grain yield with flag leaf area (R^2=0.671), stomatal width (R^2=0.641), and length (R^2=0.681), and negative relation between grain yield and stomatal number (R^2=0.607). There was positive relation between flag leaf area and relative water content.

Keywords: *drought, bread wheat, yield, stomatal dimension and density*

1. INTRODUCTION

Mostly winter and facultative bread wheat cultivars are grown in the region and it covers 5-6% of the total wheat area of Turkey. Amount of the rainfall during growing season (October-June) is enough for wheat production but the distribution of this rainfall is not regular. The lower and fluctuation of rainfall during stem elongation and grain filling stages could causes drought problem (Öztürk and Korkut, 2017). Biotic and abiotic stresses result in decreasing yield of agricultural plants and prevent yield potential occurrence, annually. Among abiotic environmental stresses, drought stress is one of the most important factors for decreasing yield in the majority of cultivation areas for agricultural plants (Emam and Seghatoeslami, 2005). The application of indirect selection criteria in wheat breeding is a broad area of endeavour and littered with unsatisfactory results in the past. Indirect selection for Yp may be easier but it may also be more difficult. Research on indirect selection must, however, continue, for the goal is vital and the prospects have improved relative to empirical selection. While the latter has become more difficult, understanding and instrumentation for indirect selection have improved. To be successful, indirect selection must ultimately be validated for cost effectiveness in breeders' programs, compared to conventional breeding methods, or as a marginal complement to them (Reynolds et al., 2001). Water stress is a crucial climatic variable in wheat growing areas of the world under such conditions. Plant features which increase their capabilities to sustain their physiological processes may increase their growth and productivity (Heichel, 1971). To cope better with dry condition

stomata must open to allow CO_2 uptake and close during drought conditions to minimize water loss by leaves (Elizabeth and Alistair, 2007). It has been estimated that more than 95% of water entering the plant is lost by transpiration (Jianwu et al 2006; Mackay et al 2003). However, different cultivars of crop plants may have different gas exchange capabilities because they have various numbers of stomata per unit of leaf area and various stomatal sizes (Ciha and Brown 1975; Faquar et al. 2002). Significant differences have been found among plant species in the responses of their stomata to changing environment (Raschke 1975). Stomatal aperture and closure regulate the amount of CO_2 available at the Rubisco site and therefore limit photosynthesis as a consequence of stress conditions. Stomatal limitations to photosynthesis have been defined as the percentage decrease in light-saturated photosynthesis that is attributable to stomatal conductance (Farquhar and Sharkey, 1982).They found that stomatal density was negatively correlated with grain yield and with stomatal size. Stomatal frequency in wheat was shown to be greater on the adaxial than on the abaxial surface (Teare et al 1971). It has been suggested that wheat cultivars having wider somatal aperture produce higher yields without consuming more water (Smishi and Ephrat 1975). As a major crop, wheat has gained special attention in respect to morphological and physiological characters affecting drought tolerance, including stomata number, leaf area, relative water content, etc. Some agronomic traits, such as grain yield and its components have also served as criterion for drought tolerance (Dencic et al., 2000). In most wheat growing regions and especially in the Mediterranean climate, grain filling is subjected to several physical and biotic stresses. Grain filling often occurs, when temperatures are increasing and moisture supply is decreasing. Water deficit is the main environmental constraint limiting cereal yield worldwide and particularly within the Mediterranean basin, a problem likely to become even worse in the future. Cereal plants respond to drought through morphological, physiological and metabolic modifications occurring in all organs and therefore traits associated with improved performance under water limited conditions or improved survival to extremely low water availability are diverse (Slafer, 2005). Several investigations on wheat grown under water stress showed that different characters had inhibitory effects on grain yield at different levels (Leithold et al., 1997). Especially, water stress from anthesis to physiological maturity reduces grain yield (Stone and Nicolas, 1995) through reduction in the rate and duration of grain filling (Al-Khatih and Paulsen, 1984). The high negative direct effects at heading stages rate of water loss on grain yield and its high negative correlation with grain yield are explained that bread wheat genotypes with low water loss may be the high grain yield ability in stress condition. This result indicates that rate of water loss at heading stages can be used as important selection criterion for drought tolerance studies (Bilgin et al. 2011).

This study was undertaken to assess the selection criteria for identifying drought tolerance in bread wheat genotypes, so that suitable genotypes can be recommended for cultivation in the drought prone area of Trakya region. Also this experiment is an attempt to compare the usefulness of several drought stress indices for identification of cultivars with better performance at different levels of water stress. The objective of this study was to determine response of bread wheat genotypes under different drought stress and the effect on changes in yield and some drought-related characters in semi-arid regions.

2. MATERIALS AND METHODS

The research was conducted at experimental field of Trakya Agricultural Research Institution (TARI) in Edirne, Turkey (41 m above sea level, 41º64´ N, 26º59´ E), during 2008-2009 and 2009-2010 growing seasons. In the experiment 15 genotypes were used. A randomized complete block design in split block design with 3 replicate was used. Each plot had 6 meter long, 6 rows, spaced 0.17 meters apart. A seed rate of 500 seeds m^2 was used. Sowing was done by an experimental drill. Nitrogen fertilizer was applied at 40 kg ha^{-1} P_2O_5 and at 150 kg ha^{-1} three times at sowing, tillering and stem elongation stage. Also, for weed control chemical was used.

Drought applications and genotypes were main plot and sub-plot, respectively. Drought applications such as DS1 (GS30-51), DS2 (GS51-94), DS3, DS4 and DS5 (GS30-94) were performed on main plots. Five different moisture stresses were applied, these are; DS1: Drought

stress was applied from beginning of stem elongation up to heading stage and during grain filling period was irrigated with dropping system. DS2: Drought stress was applied after heading stage up to physiological maturating stage, during stem elongation irrigated with dropping system. DS3: Non-stress (well-watered, three times irrigated, at stem elongation, heading and grain filling stage). DS4: Non-treatment. DS5: Drought stress was applied from beginning of stem elongation up to physiological maturating stage.

Table 1. Climate data during experimental year in 2008-2009 and 2009-2010

Months	Years	Rainfall (mm)	Humidity (%)	Temperature (°C)		
				Min	Max	Mean
October	2008-2009	17.0	72.6	3.5	26.5	14.9
	2009-2010	112.6	82.3	2.9	28.9	15.1
November	2008-2009	29.2	77.8	-2.0	18.3	15.3
	2009-2010	51.7	89.7	-1.7	22.7	9.7
December	2008-2009	35.6	82.2	-6.9	20.4	6.4
	2009-2010	93.4	89.7	-2.1	19.6	7.3
January	2008-2009	48.6	87.8	-11.2	17.5	6.5
	2009-2010	59.6	85.2	-16.3	20.3	2.5
February	2008-2009	83.2	81.3	0.1	13.5	5.2
	2009-2010	107.0	88.1	-4.8	20.3	5.9
March	2008-2009	44.1	77.5	3.0	17.9	7.8
	2009-2010	47.6	81.9	-3.1	22.2	7.7
April	2008-2009	15.8	68.8	-0.4	25.9	12.3
	2009-2010	17.8	76.0	0.9	24.9	12.7
May	2008-2009	27.7	66.1	7.5	32.1	19.1
	2009-2010	16.0	68.6	3.3	33.6	18.1
June	2008-2009	25.9	62.5	9.3	36.4	22.6
	2009-2010	30.8	72.3	12.0	38.7	22.5
Total/Mean	2008-2009	327.1	75.2	-11.2	36.4	12.2
	2009-2010	536.5	81.5	-16.3	38.7	11.3

Some climate conditions scored during two growing cycle were given in Table 1. While amount of the rain in first year was 327.1 mm, second season was 536.5 mm. Precipitation of the rain was low in October, November, April and May in 2008-2009 growing year. Also, second growing cycle especially in April and May amount of the rain was low. The coldest month was January and the absolute lowest temperature was -16.3 °C in 2009-2010 growing cycle. The highest temperature was scored 38.7 °C in June and, mean temperature was 12.2 °C and 11.8 °C.

Grain yield, flag leaf area, number of stomata, stomata width, stomata length, leaf water potential (at heading state) were calculated under five different level of drought stress. Flag leaf area was calculated by measuring flag leaf length and width of 10 randomly selected plants and multiplying by 0.68 (Fowler and Rasmusson, 1969). Number of stomata was measured by the impression

method using clear nail polish described by Wang and Clarke (1992). Samples were taken from flag leaves for each genotype. Four to 100 microscopic fields of view (0.04 mm^2) were randomly selected from the middle of the adaxial surface for each sample. Rate of leaf water loss from the excited leaves was measured once or twice, depending on available time, heading stage in each genotype and year, using the technique described by Clarke and Mc. Caig (1982). Ten of the youngest, fully expanded leaves were sampled from each plot. Samples were immediately taken to the laboratory and weighed. The samples were weighed again after wilting for 2 h at 30 °C, and again after oven-drying to constant weight.

The analysis of variance for each character was measured followed by LSD to test significance difference between means (Steel and Torrie, 1960), and simple correlation coefficient and path analysis, which is the direct and indirect effects of each character was performed as per method of Dewey and Lu (1959). Regression analysis was done to determine relation between investigated characters (Wright, 1934). Also, regression graphs are used to predict adaptability of genotypes. Data were analysed statistically for analysis of variance following the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test. The Zadoks Decimal Code (GS) was used to describe plant growth stages of cereals.

3. RESULTS AND DISCUSSION

The results of variance analyses showed that there were significant differences ($p<0.01$) among genotypes and treatments for grain yield and other investigated traits under both stress and non-stress environments. All results showed that drought stress conditions and water stress consistently lowered the grain yield under non-irrigated rather than irrigated conditions (Table 2 and 4).

Drought resistance is usually quantified by grain yield under drought. Wheat grain yield under drought, however, depends on yield potential as well as the phenology of the genotype (Acevedo, 1991). According to results of the experiment mean grain yield of the all genotypes was 763.8 kg da^{-1} under non-stress conditions, and 457.8 kg da^{-1} under fully drought stress conditions, so grain yield decreased under drought stress by 40.1%. The highest grain yield with 894.8 kg da^{-1} determined from BBVD7 line, and also Bereket and Kate A-1 were other highest yielding cultivars under non-stress conditions. Bereket was the highest yielding cultivar with 522.6 kg da^{-1} yield under fully drought stress condition. The lowest yield was obtained from BBVD21-07 line with 519.3 kg da^{-1} grain yield under non-stress conditions and 271.5 kg da^{-1} under drought stress conditions. The highest loss of grain yield was 48.5% in Entry 14 and lowest was 33.3% in Golia (Table 3, Table 4). Drought stress condition significantly resulted in grain yield. The highest percentage of yield losses under drought stress and non-stress condition was determined in EBVD24-07 (48.5%) and BBVD21-07 (47.7%), while the lowest losses were 33.3% in Golia, 34.3% in ÖVD2/21-07 and 34.4% in ÖVD2/27-07 (Table 4).

Water content in a whole plant and water potential of its tissue and cells affect tolerance of genotypes to drought. Under stress conditions, a slowdown of biological activities is quickly observed at different level of metabolism, growth, rigidity. The drought tolerant genotypes lose less water and maintain water levels for a longer period than drought susceptible genotypes (Clarke, 1987). Clarke et al. (1991) found that rate of water content at two stages (4-5 leaves and heading) is an important selection criterion for drought tolerance. Relative water content of the plants under drought condition was less than in non-stress condition. Relative water content at the plant level largely depends on the size of the evaporating area and drought stress condition. Mean relative water content was 19.66% (Table 2), and the highest content was determined in Bereket cultivar with 21.88% and lowest in BBVD21-07 line with 15.96% (Table 3). Relative water content under non-stress condition was 23.53% and under drought stress condition was 15.82%. Water stress had an effect on relative water content. This result indicated that leaf water content of the genotypes less than under fully drought stress condition compared with all other treatment. There was positive relation between relative water content with flag leaf area, grain yield, stomatal width and length.

These finding results showed that much greater water content of leaf increased stomatal size and grain yield. When it compared non-stress and fully stresses condition the highest relative water content was determined with 41.5% in Tekirdağ cultivar and the lowest from BBVD21-07 line with 15.4%.

Table 2. Mean value based on yield and other investigated traits according to treatment

Treatment	GY	RWC	FLA	STW	STL	STN
DS1	549.9 d	18.16 d	19.62 d	26.69 c	50.95 b	10.97 c
DS2	563.9 c	21.41 b	22.68 b	26.42 b	50.12 c	10.74 cd
DS3	763.8 a	23.53 a	23.46 a	26.93 a	52.23 a	10.58 d
DS4	579.7 b	19.35 c	20.32 c	26.19 b	49.42 c	11.33 b
DS5	457.8 e	15.82 e	18.90 e	25.32 c	49.54 c	11.96 a
Mean	583.0	19.66	21.00	26.11	50.45	11.12
LSD (0.05)	12.69**	0.73**	0.44**	0.45**	0.75**	0.30**

Note: Significance at * P<0.05 and ** P<0.01, GY: Grain yield (kg da^{-1}), RWC: Relative water content (%), FLA: Flag leaf area (cm^2), STW: Width of stomata (μm), STL: length of stomata (μm), STN: Stomatal number

Flag leaf characteristics provide a potential target for selection (Blake et al. 2007). Leaf area is the main determinant of photosynthesis however it is also a common adaptive trait for drought tolerance effecting plant transpiration (Tardieu et al., 2004). Watson (1952) pointed out that flag leaf area during grain filling period was more influential on yield than net assimilation rate. For the trait, there were significant differences among genotypes in both years; according to mean value of two years flag leaf area varied between 17.92 cm^2 (Kate A-1) and 24.09 cm^2 (Table 3). There were significant increases in flag leaf area according to drought treatment in both two years. Flag leaf area based on treatment varied between 18.90 and 23.46 cm^2 (Table 2). Drought stress is also more influenced and reduced flag leaf area at stem elongation stage than other growth stage. When it compared non-stress and fully stresses condition based on mean value of the genotypes fully stress condition led to maximum decrease by 30.9% in BBVD21-07 line, however the lowest decrease by 12.6% in Gelibolu cultivar on flag leaf area (Table 4). There was positive relation between flag leaf area with grain yield, stomata width and stomata length. These finding results showed that much greater in flag leaf area resulted in an increase grain yield and stomatal size. Phenotypic correlations between leaves at different positions on the main stem are high but not perfect, and the flag leaf area may differ notably from earlier leaves (Rawson et al., 1983).

Table 3. Mean value of the genotypes according to investigated traits

Entry	Genotypes	GY	RWC	FLA	STW	STL	STN
1	Kate A-1	631,5 bc	19.06 def	17.92 h	25.50 fg	50.31 def	11.29 cd
2	Gelibolu	613,0 cde	20.95 abc	20.31 ef	26.47 a-d	50.78 cde	11.42 cd
3	Pehlivan	587,7 fg	20.07 bcd	21.82 c	25.20 g	47.70 gh	11.21 cd
4	Tekirdağ	594,5 efg	21.01 abc	22.74 b	26.29 cde	54.16 a	10.90 def
5	Selimiye	608,9 def	20.03 bcd	21.02 de	24.22 h	47.71 gh	11.62 bc
6	Aldane	551,1 ı	21.08 ab	21.19 cd	26.21 cde	51.90 bc	10.31 gh
7	Flamura-85	518,9 j	20.02 bcd	21.80 c	26.04 def	51.85 bc	10.87 d-g
8	Golia	610,4 cde	19.92 cde	20.31 ef	26.86 abc	47.05 h	13.09 a
9	BBVD7	651,0 ab	18.31 fg	20.43 ef	26.45 bcd	49.61 ef	11.02 de
10	Bereket	658,3 a	21.88 a	21.53 cd	25.27 g	52.30 b	10.18 h
11	ÖVD26-07	579,1 gh	21.06 ab	24.09 a	25.66 efg	51.19 bcd	10.50 e-h
12	ÖVD2/21-07	563,0 hı	18.87 ef	21.50 cd	27.07 ab	51.54 bcd	10.54 e-h
13	ÖVD2/27-07	617,8 cd	19.40 def	19.85 f	26.99 ab	51.81 bc	10.41 fgh
14	EBVD24-07	577,4 gh	17.21 g	19.09 g	26.27 cde	49.01 fg	11.31 cd
15	BBVD21-07	383,0 k	15.96 h	21.31 cd	27.14 a	49.85 ef	12.08 b
Mean		583,0	19.66	21.00	26.11	50.45	11.12
LSD (0.05)		21.94**	1.12**	0.73**	0.67**	1.32**	0.58**

Note: Significance at * P<0.05 and ** P<0.01, GY: Grain yield (kg da^{-1}), RWL: Relative water content (%), FLA: Flag leaf area (cm^2), STW: Width of Stomata (μm), STL: Length of stomata (μm), STN: Number of stomata

The stomatal transpiration is the main way of water loss in the plant. Stomata characteristics affecting the water-use efficiency of plants are the important factors in evaluation of genotypes for drought stress. Restriction of water loss from leaf surface during periods of severe water-stress is an important drought survival mechanism (Bilgin et al. 2011). Significant impact of drought was observed for stomatal dimensions. Results showed that there were significant differences among genotypes in terms of number of stomata. The highest number of stomata with 13.09 was determined in Golia and the lowest from Bereket cultivar with 10.18 (Table 3). Stomata numbers varied 10.58 up to 11.96 based on drought treatment and stomata number increased in drought stress condition. But, it may not be explained enough these differences according to only stomata number. Reduction in stomatal density and dimension could be used in achieving water stress tolerance (Tanzarella et al., 1984).

It was compared between non-stresses and fully stresses condition according to mean value of the genotypes and it was slightly changed in number of stomata in Tekirdağ, Aldane and ÖVD2/27-07. The highest decreasing in number of stomata was determined with 23.9% in Flamura-85 cultivar (Table 5). Stomata characteristics are poorly correlated with both grain yield and transpiration. As with many other morpho-physiological characteristics stomatal features are not in close relation with yield. This may be due to polygenic nature of yield effort. During the past decodes efforts, has been focused on finding plant characteristics which are closely correlated to yield particularly under environmental stresses such as drought. This would mean that changing those features makes it possible to change the yield (Maghsoudi and Maghsoudi, 2008).

Stomatal width and length was scaled under five different environment condition and results showed that there were differences among genotypes and treatment. Non-stress condition led to increase stomata width and length under non-stress treatment and stomata width was 26.93 μm and length 52.23 μm. According to mean value of the genotypes the largest stomata was scaled in BBVD21-07, ÖVD2/21-07 and ÖVD2/27-07 genotypes. Tekirdağ and Bereket cultivar had the longest stomata (Table 5).

Table 4. Means of grain yield, leaf water content and flag leaf area of the genotypes under different drought condition

Entry	Genotypes	Grain yield (kg da^{-1})					Relative water content (%)					Flag leaf area (cm^2)				
		DS1	DS2	DS3	DS4	DS5	DS1	DS2	DS3	DS4	DS5	DS1	DS2	DS3	DS4	DS5
1	Kate A-1	574.8	605.6	852.7	622.8	501.7	19.64	21.02	23.02	17.58	14.04	17.14	19.20	19.89	16.84	16.54
2	Gelibolu	592.9	587.1	785.7	624.5	475.0	19.23	23.47	24.90	20.22	16.93	19.77	21.17	21.59	20.18	18.86
3	Pehlivan	546.4	559.4	778.2	577.9	476.7	19.45	21.32	24.14	19.51	15.95	20.31	23.71	24.13	20.69	20.27
4	Tekirdağ	591.0	560.0	745.7	614.5	461.3	20.61	22.10	26.38	20.52	15.43	21.92	25.34	24.32	21.77	20.39
5	Selimiye	553.5	607.9	785.4	628.9	468.9	19.86	20.00	23.67	20.19	16.42	20.19	21.65	23.45	20.02	19.80
6	Aldane	495.7	568.4	716.5	541.0	433.9	16.67	23.98	25.61	21.91	17.20	20.97	22.17	23.29	20.05	19.47
7	Flamura-85	496.2	531.5	674.8	507.4	384.5	20.10	21.09	24.27	17.95	16.67	21.17	23.58	24.48	20.82	18.99
8	Golia	576.6	577.9	767.4	618.5	511.8	17.11	22.27	24.13	19.96	16.14	18.75	22.98	22.12	19.76	17.98
9	BBVD7	557.3	629.9	894.8	675.1	497.8	18.05	18.11	22.34	17.41	15.62	18.54	22.50	22.26	20.26	18.61
10	Bereket	635.1	640.1	856.7	636.7	522.6	18.21	25.06	25.90	23.63	16.59	19.31	23.88	24.49	20.32	19.66
11	ÖVD26-07	551.2	525.9	769.9	585.9	462.7	19.32	24.85	24.36	19.89	16.90	22.82	24.02	27.32	24.60	21.68
12	ÖVD2/21-07	575.1	548.1	708.5	517.5	465.7	16.77	21.62	22.44	18.20	15.34	19.86	22.73	24.04	21.81	19.08
13	ÖVD2/27-07	587.2	600.9	770.8	624.4	505.8	16.07	21.43	24.37	19.80	15.36	18.77	21.88	21.67	19.47	17.47
14	EBVD24-07	537.4	524.5	830.2	567.4	427.3	16.63	18.10	19.53	18.13	13.66	16.89	20.88	22.93	17.92	16.84
15	BBVD21-07	379.2	392.1	519.3	353.1	271.5	14.76	16.81	17.83	15.32	15.09	17.95	24.56	25.88	20.29	17.88
Average		549.9 d	563.9 c	763.8 a	579.7 b	457.8 e	18.16 d	21.41 b	23.53 a	19.35 c	15.82 e	19.62 d	22.68 b	23.46 a	20.32 c	18.90 e

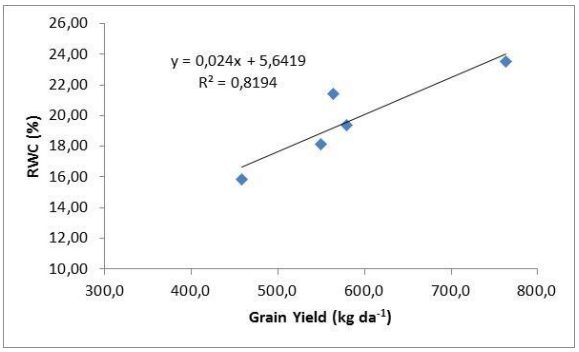
DS1 (GS30-51): Drought stress was applied from beginning of stem elongation up to heading stage and during grain filling period was irrigated with dropping system. DS2 (GS51-94): Drought stress was applied after heading stage up to physiological maturating stage, during stem elongation irrigated with dropping system. DS3: Non-stress (well-watered, three times irrigated, at stem elongation, heading and grain filling stage). DS4: Non-treatment. DS5 (GS30-94): Drought stress was applied from beginning of stem elongation up to physiological maturating stage.

Table 5. Means of stomata number, width and length of the genotypes under different drought condition

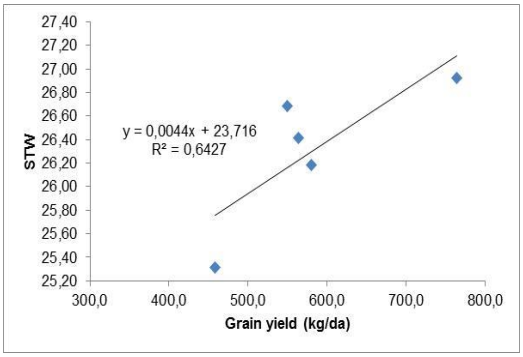
Entry	Genotypes	Stomatal width (STW) (μm)					Stomatal length (STL) (μm)					Stomatal number (STN)				
		DS1	DS2	DS3	DS4	DS5	DS1	DS2	DS3	DS4	DS5	DS1	DS2	DS3	DS4	DS5
1	Kate A-1	24.09	26.00	26.43	25.81	25.14	51.16	49.14	52.53	49.69	49.05	10.72	11.68	11.22	10.88	11.95
2	Gelibolu	26.30	26.54	27.08	26.34	26.07	50.33	50.32	53.38	50.86	49.02	12.00	10.75	10.37	11.67	12.32
3	Pehlivan	24.90	25.62	26.61	24.86	24.01	48.01	48.21	49.31	45.94	47.03	10.82	10.63	10.75	12.02	11.85
4	Tekirdağ	26.73	26.21	27.92	25.03	25.57	55.26	54.17	54.14	54.19	53.06	10.55	10.33	11.32	10.87	11.45
5	Selimiye	23.21	24.40	25.12	24.63	23.77	47.00	47.33	49.64	46.08	48.49	12.40	11.97	10.38	11.22	12.15
6	Aldane	25.82	25.96	27.64	26.81	24.81	52.60	51.98	51.73	53.27	49.93	10.32	9.07	10.83	10.60	10.72
7	Flamura-85	24.94	25.77	27.63	26.02	25.84	52.40	51.58	54.53	49.93	50.81	11.53	10.88	9.15	10.78	12.02
8	Golia	26.77	28.29	26.86	26.95	25.42	50.49	46.69	48.13	44.15	45.80	11.92	12.30	12.37	13.92	14.92
9	BBVD7	26.71	26.76	26.54	27.03	25.22	52.31	49.77	50.17	47.92	47.87	10.78	10.55	10.55	11.48	11.72
10	Bereket	25.38	25.23	25.92	25.50	24.31	53.59	52.34	53.86	50.30	51.41	9.62	9.53	9.98	10.38	11.37
11	ÖVD26-07	25.31	25.29	26.49	25.69	25.53	51.63	51.65	51.08	49.86	51.74	9.57	10.43	10.18	11.10	11.20
12	ÖVD2/21-07	26.07	27.64	28.19	27.66	25.79	50.85	50.54	55.11	50.14	51.06	10.32	10.47	9.43	10.80	11.67
13	ÖVD2/27-07	26.05	27.55	27.14	27.69	26.54	52.45	50.64	54.34	50.42	51.21	9.80	10.23	10.75	10.37	10.92
14	EBVD24-07	25.58	27.21	26.21	26.42	25.95	48.15	48.06	51.00	49.54	48.29	11.30	10.93	10.73	11.05	12.55
15	BBVD21-07	27.46	27.80	28.18	26.42	25.84	48.05	49.37	54.59	48.99	48.27	12.95	11.40	10.68	12.73	12.62
Average		26.69 c	26.42 b	26.93 a	26.19 b	25.32 c	50.95 b	50.12 c	52.23 a	49.42 c	49.54 c	10.97 c	10.74cd	10.58 d	11.33 b	11.96 a

Width of the stomata varied from 25.12 μm (Selimiye) up to 28.19 μm (ÖVD2/21-07) under non stress condition (DS3) and from 23.77 μm in Selimiye cultivar up to 26.54 μm in ÖVD2/27-07 line under fully stress condition. When it compared drought stress and non-stress condition led to maximum percentage decrease in Aldane, Pehlivan, ÖVD2/21-07 and Tekirdağ genotypes.

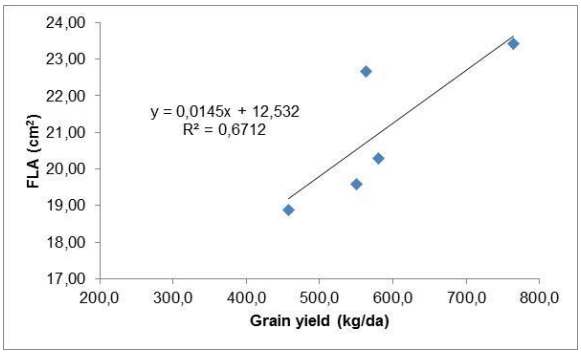
The lowest length of stomata was 48.13 μm in Golia and longest stomata 55.11 μm in ÖVD2/21-07 under non-stress (DS3) condition. Under fully drought stress condition the lowest stomata was 45.80 μm (Golia cv.) and longest was 53.06 μm (Tekirdağ cv.). The highest decreasing in length of stomata was 11.6% in BBVD21-07 line and 8.2% in Gelibolu cultivar (Table 5). Reduction in stomatal density and dimension could be used in achieving water stress tolerance (Tanzarella et al., 1984).



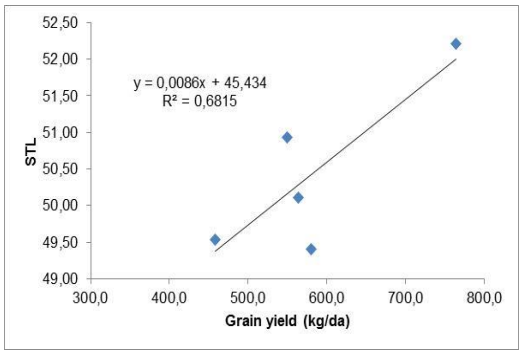
Grain yield and relative water content



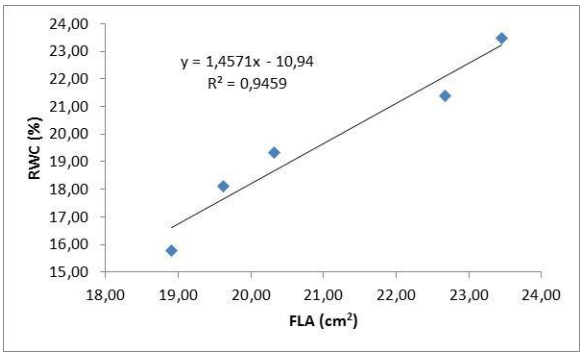
Grain yield and stomata width



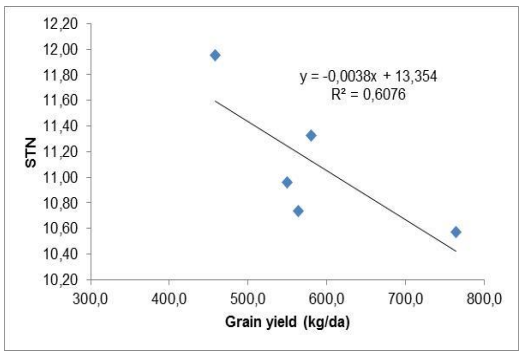
Grain yield and flag leaf area



Grain yield and stomata length



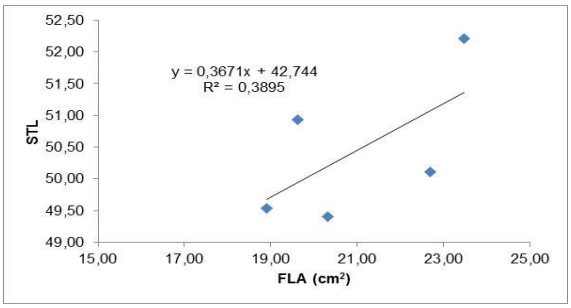
Flag leaf area and relative water content



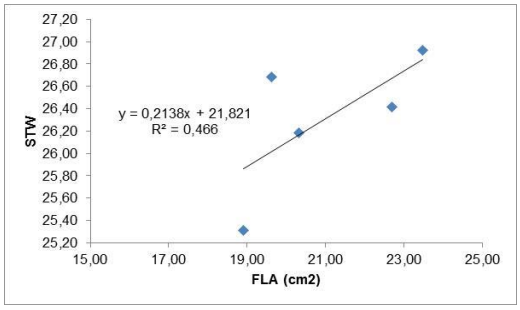
Grain yield and stomata number

Figure 1. According to treatments regression of stomatal frequency and size and other traits under various stress conditions

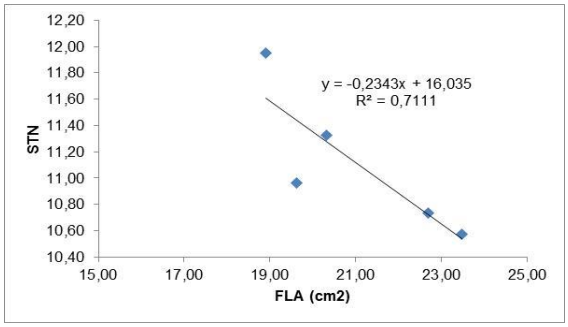
Grain yield demonstrated a positive and significant correlation with some of stomatal characters including width of stomatal and length. In this research, a general linear model regression of grain yield under drought stress revealed there were significant relationship between grain yield with flag leaf area (R^2=0.671), stomatal width (R^2=0.641), and length (R^2=0.681), and negative relation between grain yield and stomatal number (R^2=0.607). There was positive relation between flag leaf area and relative water content. These finding results showed that much greater stomatal size increased grain yield under both stress and non-stress condition. Also, it was found similar results between flag leaf area and stomatal size.



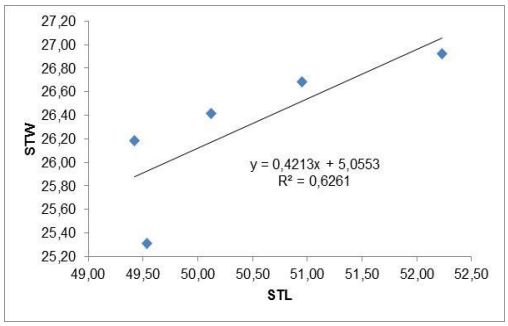
Flag leaf area and stomata length



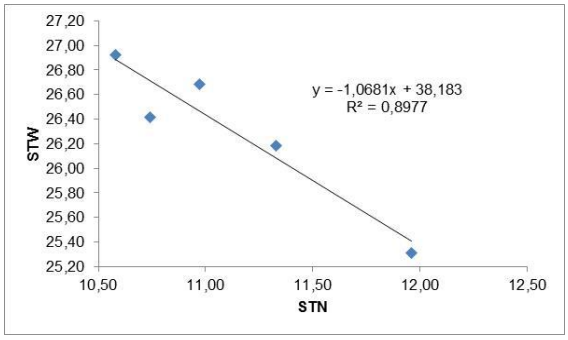
Flag leaf area and stomata width



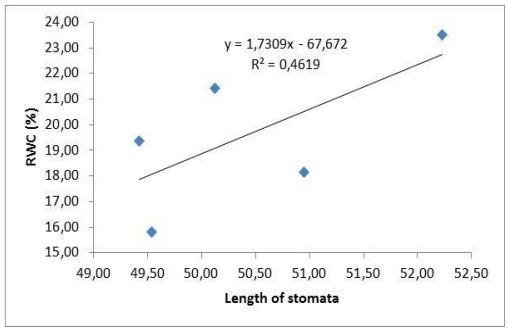
Flag leaf area and stomata number



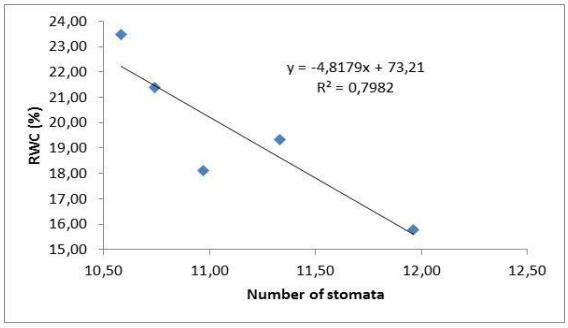
Stomata width and stoma length



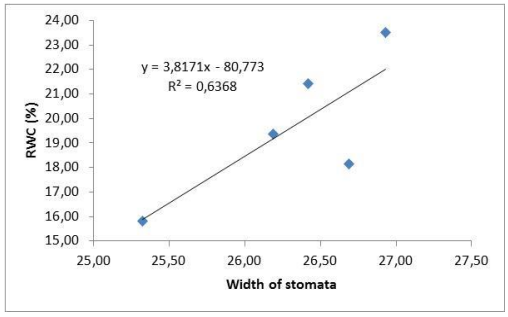
Stomata width and stoma number



Relative water content and stomata length



Relative water content and stomata number



Relative water content and stomata width

Figure 2. According to treatments relation between stomatal frequency and size and relative water lost, flag leaf area under various stress conditions

Also, it was found positive interaction between flag leaf area and stomatal length (R^2= 0.389), and stomatal width (R^2= 0.466), and negative relation between flag leaf area and stomatal number (R^2= 0.711). Regression analysis was made to determine between relative water content and stomata dimension and it was found a positive correlation between relative water content with stomatal

length (R^2= 0.461), and stomata width (R^2= 0.636). Relative water content was negatively relation with stomatal number (R^2= 0.798). These results are showed that stomatal characteristics are highly correlated with grain yield, flag leaf area and relative water content under different stress condition.

Table 6. Correlation coefficients between stomatal characters, yield and other investigated traits under stress and non-stress conditions

Traits	GY	RWC	FLA	STN	STW
RWC	0.905*				
FLA	0.819	0.973**			
STN	-0.779	-0.893*	-0.843		
STW	0.802	0.798	0.683	-0.948*	
STL	0.826	0.679	0.624	-0.739	0.791

Note: Significance at * P<0.05 and ** P<0.01, GY: Grain yield (kg da^{-1}), LWC: Relative water content (%),

FLA: Flag leaf area, STW: Width of stomata (μm), STL: length of stomata (μm), STN: Number of stomata

The correlation coefficients among characters showed varied association. The correlation between grain yield and relative water content was positive and significant (r=0.905*). There were positive correlations between grain yield and stoma width (r=0.802) and stoma length (r=0.826), while negative correlation between grain yield and number of stomata (r= -0.779). The stepwise regression revealed that the stomatal width and length and flag leaf area were more influential characters on the grain yield. The correlations were determined as 0.973**, -0.893*, 0.798, and 0.679 for rate of relative water content in flag leaf area, number of stomata, width and length of stomata, respectively. Flag leaf is of primary importance in determining for grain yield and highest direct positive effects on grain yield. Flag leaf area was negatively correlated with number of stomata (r=-0.843), while positive correlated with stomata width (r=0.683) and stomata length (r=0.624). There was a significant negative association between number of stomata with stomatal width (r=-0.948*), and stomata length (r= -0.739).

4. CONCLUSION

There were significant differences among genotypes and treatment in both years based on investigated characters. Grain yield was negatively and various degree of level affected under various drought stress conditions. Bereket had the highest grain yield. Under drought stress condition grain yield was more affected at stem elongation stage than grain filling period. Flag leaf area, relative water content, length and width of stomata negatively affected under drought stress condition. The number of stomata increased under drought stress condition. Flag leaf area had positively effect on grain yield and stomata dimension. The largest flag leaf area had higher relative water content. Drought stress condition negatively affected and decreased stomata width and length. There were significant relationship between grain yield with flag leaf area, stomata width, and stomata length and negative relation between grain yield and stomatal number. There was positive relation between flag leaf area and relative water content. The result of the research revealed that the stomatal width and length and flag leaf area were more influential characters on the grain yield.

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