Effects of water stress on Epidermis and stomata population of sixteen water stressed and irrigated barley (*Hordeum vulgare*) genotypes

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Abstract

16 Barley (*Hordeum vulgare*) genotypes, namely G30, G54, G65, G74, G77, G83, G94, G98, G116, G119, G126, G127, G142, G144, G154 and G169, were subjected to adequate irrigation during their growing season and to drought only during spike development stage. The results revealed that upper leaf surface more affected by drought than that of lower leaf surface. Most barley genotypes increased their stomata and epidermis populations under water stress, as compared to irrigation, particularly at upper leaf surfaces. Barley genotypes categorized according to their predicted drought resistance abilities as the following: G54, G116> G142, G154, G169, G77>G74, G83, G94, G127, G144> G30, G65, G98, G119, G126.

تاثير الشد المائي على نسبة الكثافات للثغور : خلايا البشرة لستة عشر سلالة من الشعير (*Hordeum vulgare*)

قيصر جعفر عبد و هارتموت شتوتزل

الخلاصة

عرضت 16 سلاله من الشعير وهي

G30, G54, G65, G74, G77, G83, G94, G98, G116, G119, G126, G127, G142, G144, G154 and G169

الى الري المعتدل خلال مرسم النمو وعرضت ايضا الى الجفاف خلال مرحلة تطور ونمو السنابل. اضهرت النتائج ان السطح العلوي للورقة اكثر تاثرا من سطحها السفلي وان معضم سلالات الشعير المدروسة حصلت فيها زيادة معنوية في الكثافات الثغرية وكثافات خلايا البشرة عند تعريض النباتات للجفاف مقارنة مع النباتات المروية خاصة عند السطح العلوي للورقة. يمكن ترتيب السلالات حسب قدرتها على مقاومة الجفاف كما يلي:

G54, G116> G142, G154, G169, G77>G74, G83, G94, G127, G144> G30, G65, G98, G119, G126

Keywords: Barley, Drought, Irrigation, Anatomy, Stomata **caser.abdel@yahoo.com**

Introduction

The intercostal zones of all the taxa include stomata, which occur in well-defined horizontal bands. Moreover, each band consists of 1-4 rows of stomata. The stomata arranged regularly in these rows. In the genus, the number of rows of stomata in each intercostal zone varies not only from one species to another, but also in different areas of a single blade or in leaves taken from different levels of the same plant. However, each species has a maximum of two or four lines of stomata in the intercostal zones. Moreover, the rows can easily be seen in the scanning electron microscope (SEM) photos. According to Metcalfe (1960) grass stomata can be classified in terms of the shape of their subsidiary cells and they can be used for diagnostic and taxonomic purposes. From this point of view, the type of the stomata in the genus *Hordeum* possesses parallel-sided subsidiary cells. However, different taxa exhibit different stomata with great variation in their sizes (Raschke, 1979). Measurements of the length and breadth of the stomata for the showed shortest and widest stomata found in *H. bulbosum*, which means this species has the smallest stomata length/breadth ratio. Although stomatal density affected by environmental factors, its genetic background is certainly evident (Hetherington and Woodward, 2003). Comparing the stomatal densities of the taxa, *H. vulgare* has the highest value.

The responses of stomatal density to leaf water status were determined, and correlation with specific leaf area (SLA) in a photosynthetic study of a perennial grass, *Leymus chinensis*, subjected to different soil moisture contents. Moderate water deficits had positive effects on stomatal number, but more severe deficits led to a reduction, described in a quadratic parabolic curve. The stomatal size obviously decreased with water deficit, and stomatal density positively correlated with stomatal conductance (gs), net CO_2 assimilation rate (NAR), and water use efficiency (WUE). A significantly negative correlation of SLA with stomatal density observed, suggesting that the balance between leaf area and its matter may be associated with the guard cell number (Xu and Zhou, 2008). They stated that

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high flexibilities in stomatal density and guard cell size will change in response to water status, and this process may be closely associated with photosynthesis and water use efficiency. Assmann and Wang (2001) reported that the responses of guard cell size and stomatal number to environmental variables clearly depend on a time scale from milliseconds to millions of years. Actually, the physiological mechanisms of stomatal response are very complex and not yet fully understood to date (Sousa *et al*., 2006; Gudesblat *et al*., 2007). Short-term responses to humidity are fundamentally similar, that is the typical two-phase stomatal response. When humidity around a leaf reduced, gs typically increase for 5–15 min, and then declines for another 20–75 min, ultimately approaching steady state gs that is lower than the initial value (Oren *et al.*, 1999).

Leaf morphological traits, including stomatal density and distribution, and epidermal features may affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO_2 levels (Woodward, 1987; Nilson and Assmann, 2007). Several reports have shown that the stomatal density and its index increase with water stress (Yang and Wang, 2001; Zhang *et al*., 2006), but the number of stomata per leaf decreases (Quarrie and Jones, 1977). With decreasing precipitation, stomatal density also increases, whereas plant height, density, and leaf area decrease (Wang and Gao, 2003; Yang *et al.*, 2007). An increase in stomatal density was observed under moderate drought, but a decrease occurred with drought severity, which is consistent with a study of rice leaves (Meng *et al.*, 1999). Stomatal densities of leaves from several varieties of Jujube also have similar patterns under a drought severity gradient: initially increasing, then declining (Liu *et al*., 2006), similar to response patterns in rice leaves under salt stress (Zhao *et al.*, 2001), and in wheat leaves in response to plant density (Zhang *et al*., 2003). However, wheat stomatal density always increases with continually increasing drought severity (Zhang *et al.*, 2006). Stomatal densities of tree leaves rise with increasing urban integrative environmental stresses, indicating this may provoke a regulative capacity to deal with multiple simultaneous stresses including air pollution, high aerosol levels, and drought (Zhang *et al*., 2004). However, Yin *et al*. (2006) have suggested that the change in stomatal density might not be associated with drought resistance in different genetic types of wheat. Thus, how guard cell development responds to environmental stresses and/or leaf development requires further research. Leaf stomatal density and the stomatal index (the percentage of stomatal number to total cell number on a given leaf area) may be affected by cell expansion, depending on leaf development, ageing, and position (Ceulemans *et al.*, 1995; Lecoeur *et al.*, 1995). Thus, both guard cell and epidermal cell numbers per unit area of a small leaf at a later plant growth stage would be expected to increase. However, Xu and Zhou (2008) showed that the correlation of stomatal density with water potential better fitted a hump-shaped curve compared to the stomatal index, which suggests that a tradeoff may occur. Nevertheless, further research in detail at the cell development level is still needed to elicit the differing responses between guard cells and epidermal cells to water status. The objective of this study was to evaluate the performance of 16 barley genotypes for drought resistance and adequate irrigation.

Materials and Methods

This experiment was conducted at Institute Fur Gartenbauliche Produckions Systeme, Biologie, Liebniz Universitat, Hannover, Germany. 16 Barley (*Hordeum vulgare*) genotypes, namely G30, G54, G65, G74, G77, G83, G94, G98, G116, G119, G126, G127, G142, G144, G154 and G169, to adequate irrigation and to drought during flowering and seed development stage.

Experimental design

Split plot within Randomized Complete Block Design was selected for this investigation; the main plot represents irrigation (A), where adequate during whole growing season (a1) and droughted plots during flowering and seed development stage (a2). The sub plot (B) represented by 16 barley genotypes G30 (b1), G54 (b2), G65 (b3), G74 (b4), G77 (b5), G83 (b6), G94 (b7), G98 (b8), G116 (b9), G119 (b10), G126 (b11), G127 (b12), G142 (b13), G144 (b14), G154 (b15) and G169 (b16).

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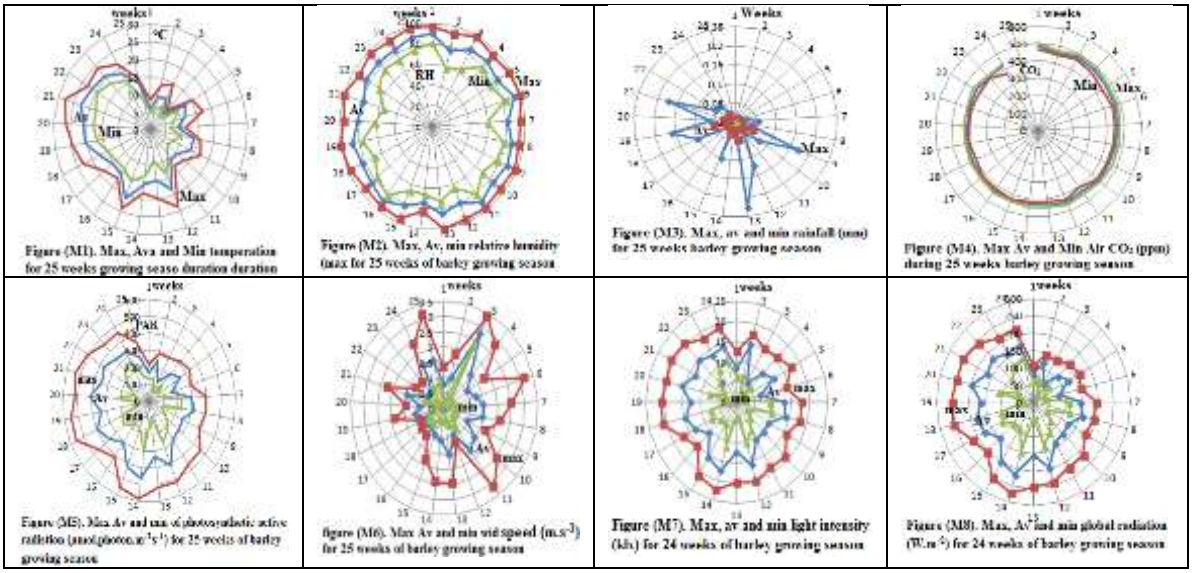
Therefore, the experiment contained 32 treatments each was repeated four times and each replicate was grown in 7m^2 at seeding rate of 300seeds.m^{-2}.

Cultural practices

Two lines driving greenhouses motivated by electrical motors were used one for adequate irrigation plots and the other one for droughted plots. Barley was covered with greenhouse whenever rainfall should be avoided during the growing season. Greenhouse land was ploughed, dissected to cope with the experimental design and then was sown with the above mentioned barley genotypes. Field meteorological data was obtained from the same institute environment control cabinet (figure, M1-8). Seeds were sown on 6th April 2014 according to the selected experimental design, seeding was fulfilled in rows with intra spaces of 15 cm and finally plants were harvested on 15th August 2014. Soil moisture content during the growing season for both irrigated and droughted greenhouses was monitored TIME DOMAIN REFLECTOMETRY (TDR). Irrigation frequencies, quantity and dates are illustrated in figure (M9). Finally, Barley leaf was sliced mounted on glass slides and they were examined under light microscope using graded slides and lenses, and then photographed.



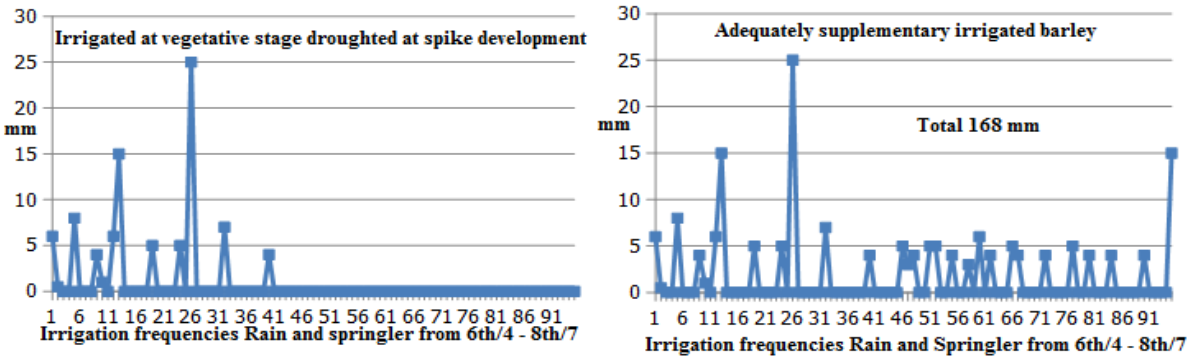


Figure (M9). Number, quentities of applied water and date of supplementary irrigation of 16 barey genotypes for irrigated and droughted treatments

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Results and Discussion

A. Effects of Irrigation

The obtained results (R1) revealed that water stressed barley substantially exceeded that of adequately irrigated barley at upper leaf surface in terms of stomata density (53.6%), epidermis density (61.83%), and at lower leaf surface in terms of stomata density (35.87%), epidermis (44.22%). However, the stomata: epidermis ratio where irrigated barley significantly exceeded that of water stressed by (35.62%). These results suggested that drought tended to increase both stomata and epidermis populations at both leaf surfaces. The responses of stomatal density to leaf water status were determined, and correlation with specific leaf area (SLA) in a photosynthetic study of a perennial grass, *Leymus chinensis*, subjected to different soil moisture contents. Moderate water deficits had positive effects on stomatal number, but more severe deficit led to a reduction, described in a quadratic parabolic curve (Xu and Zhou, 2008). Several reports have shown that the stomatal density and its index increase with water stress (Yang and Wang, 2001; Zhang *et al*., 2006), but the number of stomata per leaf decreases (Quarrie and Jones, 1977). With decreasing precipitation, stomatal density also increases, whereas plant height, density, and leaf area decrease (Wang and Gao, 2003; Yang *et al.*, 2007). Stomatal densities of tree leaves rise with increasing urban integrative environmental stresses, indicating this may provoke a regulative capacity to deal with multiple simultaneous stresses including air pollution, high aerosol levels, and drought (Zhang *et al*., 2004). However, Yin *et al*. (2006) have suggested that the change in stomatal density might not be associated with drought resistance in different genetic types of wheat. Thus, how guard cell development responds to environmental stresses and/or leaf development requires further research.

Table (R1). Stomata and Epidermis populations (St. mm^{-2}) of 16 irrigated and droughted barley genotypes leaves *

Treatment	Upper leaf Surface			Lower leaf Surface		
	Stomata density	Epidermis density	St: Epi Ratio	Stomata density	Epidermis density	St: Epi Ratio
Irrigation	B 106.103	B 154.87	A 0.9089	B 120.10892	B 144.00	A 1.4383
Drought	A 162.975	A 249.78	A 0.9133	A163.18685	A 207.97	B 1.0605

* Figures of unshared characters are significantly differs at 0.05 level; Duncan

B. Genotype responses

The highest stomata population at upper leaf surface (table, R2) was confined to G119 (190.14 St. mm^{-2}), which insignificantly differing from G74 (156.58 St. mm^{-2}), G98 (183.35 St. mm^{-2}) and G126 (171.46St. mm^{-2}). At lower leaf surface the highest stomata population was coincided with G119 (190.14 St. mm^{-2}), which insignificantly differing from G77 (169.77 St. mm^{-2}), G94 (169.77 St. mm^{-2}), G98 (183.35 St. mm^{-2}), G126 (156.18 St. mm^{-2}) and G144 (186.74 St. mm^{-2}) and G154 (176.56 St. mm^{-2}). The lowest stomata population at upper leaf surface was found in G54 and G116 (81.49St. mm^{-2}), which insignificantly differing from G142 (122.23 St. mm^{-2}), G154 (122.23 St. mm^{-2}), G169 (88.28 St. mm^{-2}). At lower leaf surface, however, the lowest stomata population was accompanied G142 (62.81St.mm^{-2}), which significantly differing from G88.23 St. mm^{-2}).

The highest epidermis population at upper leaf surface was recorded in (333.66 epidermis.mm^{-2}), which insubstantially differing from G77 (314.55 epidermis.mm^{-2}), G144 (281.94 epidermis.mm^{-2}). The highest epidermis population, at lower leaf surface was recorded in G98 (284.05 epidermis.mm^{-2}), which in significantly differing G30 (185.87 epidermis.mm^{-2}), G77 (266.45 epidermis.mm^{-2}), G83 (122.48 epidermis.mm^{-2}), G119 (207.33 epidermis.mm^{-2}), G126 (169.68 epidermis.mm^{-2}), G127 (201.8 epidermis.mm^{-2}), G142 (186.58

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epidermis.mm^{-2}) and 154 (206.51 epidermis.mm^{-2}). The lowest epidermis population, at upper leaf surface was recorded in G30 (106.88 epidermis.mm^{-2}), which in significantly differing G54 (116.82 epidermis.mm^{-2}), G74 (203.95 epidermis.mm^{-2}), G83 (196.48 epidermis.mm^{-2}), G94 (169.31 epidermis.mm^{-2}), G116 (126.52 epidermis.mm^{-2}), G119 (183.99 epidermis.mm^{-2}), G126 (200.38 epidermis.mm^{-2}), G142 (160.59 epidermis.mm^{-2}) and 169 (153.86 epidermis.mm^{-2}). At lower leaf surface, the lowest epidermis population was concomitant to G116 (98.46 epidermis.mm^{-2}), which insignificantly differing with all genotypes except G77 and G98.

The highest St: Ep ratio at the upper leaf surface was confined to G30 (2.0864), and at lower leaf surface was recorded in G144 (2.3198), which differ insignificantly with G65 (1.5295) and G94 (1.8679). On the other hand, the lowest ratio at upper leaf surface accompanied to G77 (0.8753).

These results suggested that lower leaf surface possesses higher stomata population than upper leaf surface and in most cases epidermis cell number are lower and larger than stomata number. The anatomical studies revealed numerous stomata on the lower epidermis of *Azadirachta indica*. Epidermal cell of *Chromolaena odorata* are very large with undulating cell walls. The species studied had various adaptive anatomical features. The stomatal frequency of *Azadirachta indica* was very high. With the exception of *Chromolaena odorata* the stomatal frequencies of the species were relatively high. The stomatal dimensions showed that most of the species maintained constant stomatal length during the study period except *Griffonia simplicifolia* that increased the stomatal width during the afternoon. Unlike *Morinda lucida*, *Griffonia simplicifolia* and Chromolaena odorata, that showed reduction in the breadth of stomata, the other species maintained constant stomatal width (Mensah, 2012). Varying stomata population and their ratio with epidermis was proved to influence photosynthetic rates, which then affects the cell growth rate. Both photosynthetic rate (A) and gs were closely associated with stomatal density under different water status, suggesting that stomatal density may also play an important role in CO_2 exchange under drought stress. However, Galmes *et al.* (2007) indicated that high variability and uncertainty are present among Mediterranean plants in response to changing water status. Levitt (1980) categorized crops according to their stomata behavior to spender plants that capable to maintain open stomata aperture, conservatives plants that show earlier stomata closure to sustain ample moisture in the internal tissues and semi conservatives where plants partially close their stomata in order to permit CO_2 influxes combined with transpiration reductions. Therefore, cultivar possesses higher stomata aperture and large partially opened stomata and lowest stomata populations is the best drought resistance (Abdel and Al-Salem, 2010).

C. Genotype responses to irrigation

The lowest stomata population at upper leaf surface of adequately irrigated barley (table, R3), was confined to G116 (54.32 St. mm^{-2}), which showed insignificant differences with G30 (95.07St. mm^{-2}), G54 (81.49St. mm^{-2}), G65 (81.49St. mm^{-2}), G83 (108.65St. mm^{-2}), G126 (95.07St. mm^{-2}), G127(95.07St. mm^{-2}), G142 (81.49St. mm^{-2}), G144 (95.07St. mm^{-2}), GG169 (53.42St. mm^{-2}). In contrast, the highest stomata population at upper leaf surface was detected in G119 (162.79 St. mm^{-2}), which insubstantially differing from G154 (122.23 St. mm^{-2}) and G98 (149.39 St. mm^{-2}). The lowest stomata population at upper leaf surface of droughted barley was found in G54 (81.49St.mm^{-2}), which was not profoundly differing from G77 (135.81 St. mm^{-2}), G116 (108.65 St. mm^{-2}), G154 (122.23 St. mm^{-2}), and G169 (122.23St.mm^{-2}). However, the highest stomata population at upper leaf surface of droughted barley was concomitant to G126 (247.86St.mm^{-2}), which was not differing apparently from G119 (217.3Stmm^{-2}), G98 (217.3 St. mm^{-2}), G65 (190.14St.mm^{-2}) and G30 (190.14St.mm^{-2}). At lower leaf surface, the lowest stomata population of irrigated barley was coincided to G169 (54.32St.mm^{-2}), which insignificantly differing from G142 (95.07St.mm^{-2}), G116 (95.07St.mm^{-2}), G74 (95.07St.mm^{-2}), G65 (95.07St.mm^{-2}) and G54 (81.49St.mm^{-2}). In contrast, the highest stomata population at lower

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leaf surface of irrigated barley observed in G144 (196.93St.mm^{-2}), which insubstantially differing from G77 (162.97St.mm^{-2}), G98 (162.97St.mm^{-2}) and G126 (149.39St.mm^{-2}). The lowest stomata population at lower leaf surface of droughted barley found with G142 (30.56St.mm^{-2}). However, the highest stomata population at lower leaf surface of droughted barley was detected in G119 (271.62St.mm^{-2}), which insignificantly differing from G154 (244.46St.mm^{-2}).

The lowest epidermis population at upper leaf surface of irrigated barley genotypes detected in G116 (68.85 St.mm^{-2}), which insignificantly differing from all other genotypes except G77 (306.46 Ep.mm^{-2}). However, the highest epidermis population at upper leaf surface of irrigated barley observed with G77 (306Ep.mm^{-2}), which insubstantially differing from G65 (207.97Ep.mm^{-2}) and G154 (182Ep.mm^{-2}). The lowest epidermis population at upper leaf surface of droughted barley confined to G30 (103.67Ep.mm^{-2}), which insignificantly differing from G116 (184.19Ep.mm^{-2}), G142 (192.6Ep.mm^{-2}) and G54 (141.44Ep.mm^{-2}). The highest epidermis population at upper leaf surface of droughted barley observed in G98 (443.86St.mm^{-2}), which was not profoundly differing from G144 (441.13Ep.mm^{-2}) and G77 (322.64Ep.mm^{-2}). At lower leaf surface, however, the lowest epidermis population of irrigated barley detected in G116 (63.1Ep.mm^{-2}), whereas the highest was found in G98 (314.41Ep.mm^{-2}), which insubstantially differing from G77 (284.02Ep.mm^{-2}), G83 (185.04St.mm^{-2}) and G169 (305.16Ep.mm^{-2}). On the other hand, the lowest epidermis population at lower leaf surface of droughted barley detected in G126 (101.19Ep.mm^{-2}), which insignificantly differing from G116 (133.82Ep.mm^{-2}), G169 (157.26Ep.mm^{-2}), G94 (187.59Ep.mm^{-2}), G83 (199.93Ep.mm^{-2}), G74 (156.2Ep.mm^{-2}), G65 (150.76Ep.mm^{-2}), and G54 (149.02Ep.mm^{-2}). Whereas, the highest epidermis coincided with G154 (309.94Ep.mm^{-2}), which insubstantially differing from G30 (305.16Ep.mm^{-2}), G77 (248.88Ep.mm^{-2}), G98 (253.69Ep.mm^{-2}), G119 (287.8Ep.mm^{-2}), G127 (286.55Ep.mm^{-2}), G142 (247.99Ep.mm^{-2}) and G154 (309.94Ep.mm^{-2}).

The lowest St: Ep ratio at upper leaf surface of irrigated barley was concomitant to G126 (0.6732) and the highest accompanied to G119 (1.3672). The lowest ratio at upper leaf surface of droughted barley detected in G154 (0.4187) and the highest was in 30D (3.2314). On the other hand, at lower leaf surface of droughted barley, the lowest ratio observed in G142 (0.129), which insignificantly differing from G154 (0.8515), G54 (0.82), G30 (0.5535), G116 (0.785) and G127 ((0.72). The highest ratio found in G126 (1.733), which insignificantly differing from G94 (1.014), G74 (1.237) and G65 (1.261). The lowest ratio at lower leaf surface of irrigated barley genotypes detected in G98 (0.56), which insignificantly differing from G126 (0.617). The highest ratio at lower leaf surface of irrigated barley observed in G142 (1.77). Drought increased stomata population at upper and lower leaf surfaces and their ratio in three traits in G30, G54, G77, G116, G144, G154, and four traits in G83, G94, G142, and five traits G74, G119, G126, G127 and 6 traits in G65. However, most genotypes showed tendencies to increase stomata population in relation to epidermis under drought (table, R4 and figures, R1-6). These results suggested that genotype possesses the lowest stomata and epidermis number sustained the highest cell growth rates under drought, which in other words are most drought resistance. When genotype increased stomata on the account of epidermis under drought, explained its requirements for more stomata through producing them from normal epidermis cells by tetipotencies and then dividing to sustain higher gas exchange for reasonable photosynthesis, which means that such genotypes suffered drought earlier and urged its systematic required resistance earlier. Drought resistance genotypes are capable to avoid drought adversity and sustaining higher photosynthesis rate to maintain cell growth. Enzymes concerned with removing toxic intermediates produced during oxygenic metabolism, such as glutathione reductase and superoxide dismutase, increase in response to drought stress and are probably very important in tolerance (Mittler and Zilinskas, 1994). Decreasing leaf water content and consequent stomatal closure result in reduced CO_2 availability and the

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production of active oxygen species such as superoxide radicals (Sgherri *et al.*, 1993). Increased photo respiratory activity during drought accompanied by elevated levels of glycolate-oxidase activity, resulting in H_2O_2 production (Mittler and Zilinskas, 1994). This could explain why genes encoding enzymes that detoxify active oxygen species such as ascorbate peroxidase (Mittler and Zilinskas, 1994) and superoxide dismutase found upregulated in response to drought (Perl-Treves and Galum, 1991; White and Zilinskas, 1991).

Table (R2). Stomata and Epidermis populations (St. mm^{-2}) of 16 barley genotypes leaves *

Genotypes	Upper leaf Surface			Lower leaf Surface		
	Stomata density	Epidermis density	St: Epi Ratio	Stomata density	Epidermis density	St: Epi Ratio
Geno. 30	142.6B-D	106.88F	2.0864A	129.02CD	185.87A-C	0.11380BC
Geno 54	81.49F	116.82F	0.7709B	101.86DE	117.98C	0.12502BC
Geno. 65	135.81CD	225.64B-E	0.8482B	142.6BC	144.11C	0.15295A-C
Geno 74	156.18A-D	203.95C-F	0.8546B	142.6BC	136.34C	13401BC
Geno 77	142.6B-D	314.55AB	0.5804B	169.77AB	266.45AB	0.8753C
Geno 83	129.02C-D	196.48C-F	0.7073B	122.23CD	192.48A-C	0.8953C
Geno 94	142.6B-D	169.31D-F	10041B	169.77AB	160.72BC	18679AB
Geno 98	183.35AB	333.66A	0.6031B	183.35A	284.05A	10251BC
Geno 116	81.49F	126.52EF	0.7851B	88.28FE	98.46C	13663BC
Geno 119	190.14A	183.99C-F	11923B	190.14A	207.33A-C	10300BC
Geno 126	171.46A-C	200.38C-F	13063B	156.18A-C	169.68A-C	11753BC
Geno 127	135.81CD	239.87B-D	0.7982B	142.6BC	201.8A-C	12091BC
Geno 142	122.23D-F	160.59D-F	0.8341B	62.81F	186.58A-C	0.8636C
Geno 144	127.32C-E	281.94A-C	0.7644B	186.74A	131.37C	23198A
Geno 154	122.23D-F	222.73B-E	0.5964B	176.56AB	206.51A-C	11600BC
Geno 169	88.28EF	153.86D-F	0.8454B	101.86DE	126.07C	0.945BC

* Figures of unshared characters are significantly differs at 0.05 level; Duncan

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Table (R3). Stomata and Epidermis populations (St. mm^{-2}) of 16 irrigated and droughted barley genotypes leaves *

Geno/Irrig.	Upper leaf Surface			Lower leaf Surface		
	Stomata density	Epidermis density	St: Epi Ratio	Stomata density	Epidermis density	St: Epi Ratio
30 W	95.07F-H	110.09D-G	0.9414BC	108.65G-I	66.58GH	1.7225B-D
54 W	81.49GH	92.19E-G	0.9653BC	81.49IJ	86.93F-H	0.94B-E
65 W	81.49GH	207.97B-G	0.8034BC	95.07H-J	137.47B-H	0.69B-E
74 W	149.39C-F	187.8B-G	0.907BC	95.07H-J	116.49D-H	0.816B-E
77 W	149.39C-F	306.46B	0.7342BC	162.97C-F	284.02A-E	0.574B-E
83 W	108.65E-H	127.11C-G	0.8334BC	122.23F-I	185.04A-H	0.661B-E
94 W	122.23D-G	102.07D-G	1.2762BC	149.39D-G	133.84C-H	1.116AB
98 W	149.39C-E	223.45B-F	0.7172BC	162.97C-F	314.41A	0.56C-E
116 W	54.32H	68.85G	0.818BC	95.07H-J	63.1H	1.509A-C
119 W	162.97B-E	131.91C-G	1.3672BC	108.65G-I	126.86D-H	0.875B-E
126 W	95.07F-H	178.99B-G	0.6732C	149.39D-G	238.16A-G	0.617C-E
127 W	95.07F-H	231.49C-E	0.7875BC	135.81E-H	117.06D-H	1.163B-D
142 W	81.49GH	128.57C-G	0.7657BC	95.07H-J	125.16D-H	0.76B-D
144 W	95.07F-H	122.75C-G	0.775BC	196.93CD	110.98F-H	1.774A
154 W	122.23D-G	182B-G	0.711BC	108.65G-I	103.09F-H	1.054B-E
169 W	54.32H	76.14FG	1.1163BC	54.32JK	94.88F-H	0.689C-E
30 D	190.14A-C	103.67D-G	3.2314A	149.39D-G	305.16A-C	0.5535DE
54 D	81.49GH	141.44C-G	0.5765C	122.23F-I	149.02A-H	0.82B-E
65 D	190.14A-C	243.31B-D	0.8929BC	190.14CD	150.76A-H	1.261B-D
74 D	162.97B-E	220.1B-D	0.8022BC	190.14CD	156.2A-H	1.2369B-E
77 D	135.81C-G	322.64AB	0.4266C	176.56C-E	248.88A-F	0.7235C-E
83 D	149.39C-F	265.85BC	0.5813C	122.23F-I	199.93A-H	0.7864B-E
94 D	162.97C-E	236.55B-E	0.732BC	190.14CD	187.59A-H	1.0136B-D
98 D	217.3AB	443.86A	0.489C	203.72BD	253.69A-F	0.803B-E
116 D	108.65E-H	184.19B-G	0.7522BC	81.49IJ	133.82C-H	0.785B-E
119 D	217.3AB	236.08B-E	1.0174BC	271.62A	287.8A-D	0.9808B-E
126 D	247.86A	221.77B-F	1.9394B	162.97C-F	101.19F-H	1.7335B-D
127 D	176.56B-D	248.24B-D	0.8089BC	149.39D-G	286.55A-D	0.72C-E
142 D	162.97B-E	192.6B-G	0.9025BC	30.56K	247.99A-F	0.129E
144 D	159.58B-F	441.13A	0.4041C	176.56C-E	151.76A-G	1.1634B-D
154 D	122.23D-G	263.46BC	0.4817C	244.46AB	309.94AB	0.8515B-E

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169 D	122.23D-G	231.59B-E	0.5746C	149.39D-G	157.26A-H	0.95B-E
* Figures of unshared characters are significantly differs at 0.05 level; Duncan						

Table (R4). Percentage of stomata population differences between 16 irrigated and droughted barley genotypes [Wet-Dry/Dry*100].

Genotypes	Upper leaf Surface			Lower leaf Surface		
	Stomata density	Epidermis density	St: Epi Ratio	Stomata density	Epidermis density	St: Epi Ratio
Geno. 30	-50	6.19	-70.87	-27.27	-78.18	2.11
Geno 54	0	-34.82	67.44	-33.33	-41.67	0.146
Geno. 65	-57.14	-14.52	-10.02	-50	-8.82	-0.453
Geno 74	-8.33	-14.68	13.06	-50	-25.42	-0.3398
Geno 77	10	-5.01	72.11	-7.7	14.12	-0.2066
Geno 83	-27.27	-52.19	43.37	0	-7.45	-0.15946
Geno 94	-25	-56.85	74.34	-21.43	-28.65	0.1010
Geno 98	-31.25	-49.66	46.67	-20	23.93	-0.3026
Geno 116	-50	-62.62	8.75	16.66	-52.85	0.922
Geno 119	-25	-44.12	34.38	-60	-55.92	-0.11
Geno 126	-61.64	-19.29	-65.29	-8.33	135.36	-0.64
Geno 127	-46.15	-6.75	-2.65	-9.09	-59.15	0.615
Geno 142	-50	-33.25	-15.16	211.09	-49.53	4.89
Geno 144	-40.42	-72.17	178.35	11.54	-26.87	0.5248
Geno 154	0	-30.92	47.6	-55.56	-66.74	0.2378
Geno 169	-55.56	-67.12	94.27	-63.64	-39.67	-0.2747

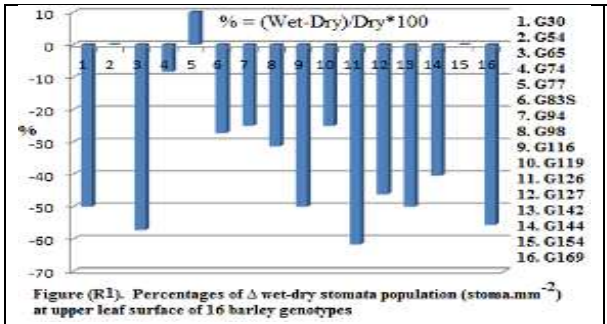

Figure (R1). Percentages of Δ wet-dry stomata population (stoma.mm^{-2}) at upper leaf surface of 16 barley genotypes

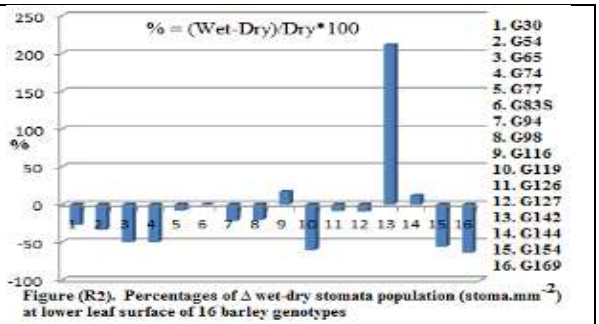

Figure (R2). Percentages of Δ wet-dry stomata population (stoma.mm^{-2}) at lower leaf surface of 16 barley genotypes

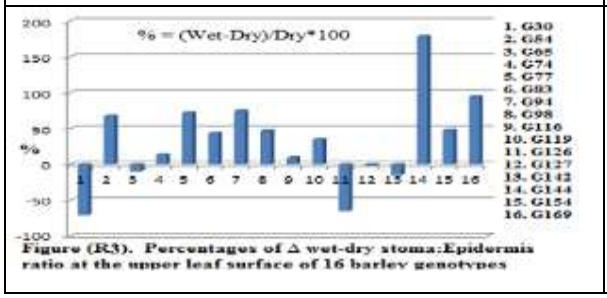

Figure (R3). Percentages of Δ wet-dry stoma:Epidermis ratio at the upper leaf surface of 16 barley genotypes

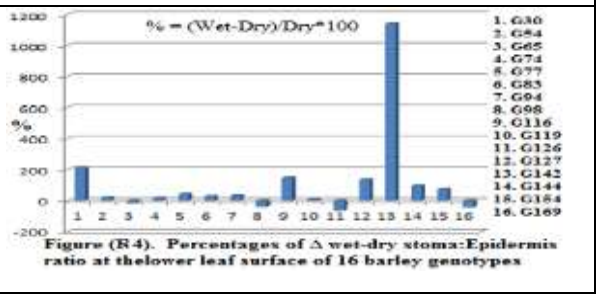

Figure (R4). Percentages of Δ wet-dry stoma:Epidermis ratio at thelower leaf surface of 16 barley genotypes

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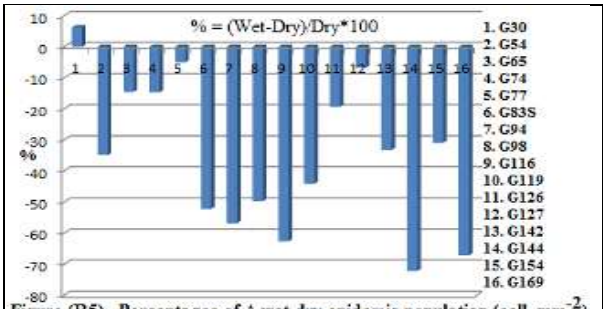


Figure (R5). Percentages of Δ wet-dry epidemis population (cell. mm^{-2}) at upper leaf surface of 16 barley genotypes

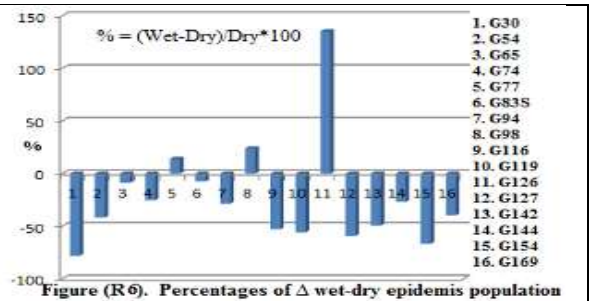
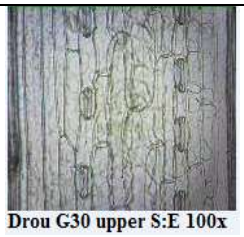
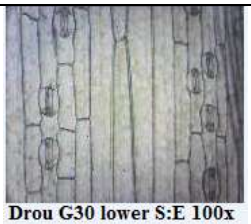
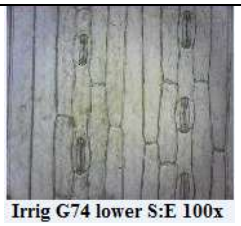
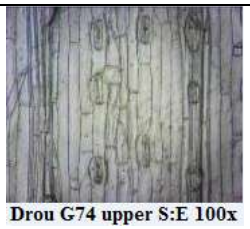


Figure (R6). Percentages of Δ wet-dry epidemis population (cell. mm^{-2}) at lower leaf surface of 16 barley genotypes

Photograph (R1). Epidermis: Stomata ratio of 16 irrigated and drought barley genotypes

	Irrigated		Drought	
	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface
30	 Irrig G30 S:E upper 100x	 Irrig G30 springularVessel	 Drou G30 upper S:E 100x	 Drou G30 lower S:E 100x
54	 Irrig G54 upper S:E 100x	 Irrig G54 lower S:E 100x	 Drou G54 upper S:E 100x	 Drou G54 lower S:E 100x
65	 Irrig G65 upper S:E 100x	 Irrig G65 lower S:E 100x	 Drou G65 upper S:L 100x	 Drou G65 lower S:E 100x
74	 Irrig G74 upper S:E 100x	 Irrig G74 lower S:E 100x	 Drou G74 upper S:E 100x	 Drou G74 lower S:E 100x

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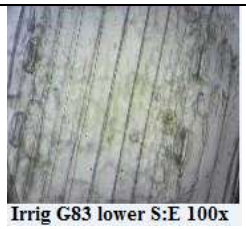
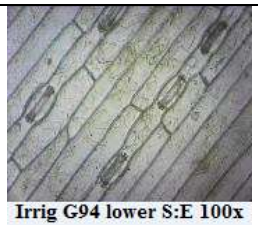
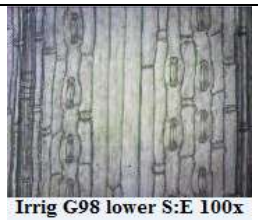
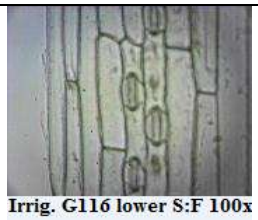
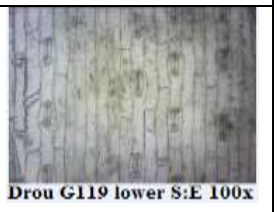
77	 Irrig G77 upper S:E 100x	 Irig G77 lower S:E 100x	 Drou G77 upper S:E 100x	 G77 Drought lower surface
83	 Irrig G83 upper S:E 100x	 Irrig G83 lower S:E 100x	 Drou G83 upper S:E 100x	 Drou G83 lower S:E 100x
94	 Irrig G94 upper S:E 100x	 Irrig G94 lower S:E 100x	 Drou G94 upper S:E 100x	 Drou G94 lower S:E 100x
98	 Irrig G98 upper S:E 100x	 Irrig G98 lower S:E 100x	 Drou G98 upper S:E 100x	 Drou G98 lower S:E 100x
116	 Irrig G116 upper S:E 100x	 Irrig. G116 lower S:F 100x	 Drou G116 upper S:E 100x	 Drou G116 lower S:E 100x
119	 Irrig G119 upper S:E 100x	 Irrig G119 lower S:E 100x	 Drou G119 upper S:E 100x	 Drou G119 lower S:E 100x
126	 Irrig G126 upper S:E 100x	 Irrig G126 lower S:E 100x	 Drou G126 upper S:E 100x	 Drou G126 lower S:E 100x

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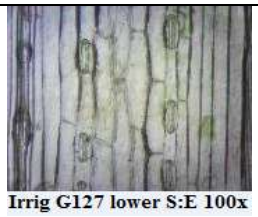
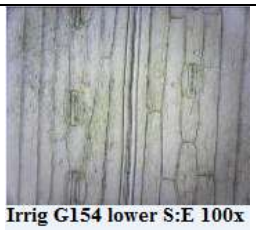
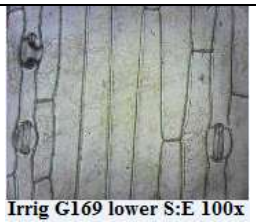
127	 Irrig G127 upper S:E 100x	 Irrig G127 lower S:E 100x	 Drou G127 upper S:E 100x	 Drou G127 lower S:E 100x
142	 Irrig G142 upper S:E 100x	 Irrig G142 lower S:E 100x	 Drou G142 upper S:E 100x	 Drou G142 lower S:E 100x
144	 Irrig. G144 upper S:E 100x	 Irrig G144 lower S:E 100x	 Drou G144 upper S:E 100x	 Drou G144 lower S:E 100x
154	 Irrig G154 upper S:E 100x	 Irrig G154 lower S:E 100x	 Drou G154 upper S:E 100x	 Drou G154 lower S:E 100x
169	 Irrig G169 upper S:E 100x	 Irrig G169 lower S:E 100x	 Drou G169 upper S:E 100x	 Drou G169 lower S:E 100x

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