

Rapid non-destructive methods for leaf thickness estimations of 16 droughted 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, to create new equations through regression for rapid leaf thickness estimation in field. The obtained equations were as below: Leaf thickness (mm) = 0.1039+0.5917 (base leaf width, Leaf thickness (mm) = 0.2186+0.0622 (mid leaf width), Leaf thickness (mm)=0.2502+0.002451 (leaf L*W), Leaf thickness (mm) =0.2502+0.002451(leaf L*0.5W), Leaf thickness (mm) = 0.0466+0.01668 (leaf L)- 0.000275 (leaf L)², Leaf thickness (mm) = 0.3558-0.0027 (L/W). L: W was the most accurate method for estimating leaf thickness of irrigated and droughted barley. Mid W, method was preferred for G54, G119, and G144. L: W method was the most accurate for G74, G116, G126, G127, and 142. Rectangle was the accurate for G65 and triangle for G77. L method was the best G30, G83, G94, G98, G154, and G169. Each individual investigated irrigated and droughted genotype was mentioned its suitable estimation method.

Keywords: Barley, Leaf Thickness, irrigation, drought, Genotypes, Evaluation

INTRODUCTION

Leaf thickness (LT) plays an important role in leaf and plant functioning and is related to species, strategies of resource acquisition and use. The amount of light absorbed by a leaf, and the diffusion pathway of CO₂ through its tissues depend, at least partially, on its thickness (Givnish, 1979; Agusti, 1994; Syvertsen, 1995). Negative relationships between leaf thickness (LT) and photosynthetic (Garnier, 1999) and growth (Poorter, 1990; Nielsen, 1996) rates have been observed. Thicker leaves have sometimes been associated with increased longevity and construction costs (Mediavilla, 2001; Westoby, 2002). Leaf thickness has therefore, often been used as a tool to screen species and/or cultivars for productivity (Dornhoff and Shibles, 1976; White and Montes-R, 2005) or ecological performance (Witkowski, 1992; Diaz, 2004). Leaf thickness for any given plants are dependent upon light intensities, where the higher levels of irradiation intensities are usually accompanied to thicker leaves. Owing to hormonal homeostasis for the favour of ABA that urged by plant defense systems.

The determination of leaf thickness is not straightforward, however. The wide variation in leaf morphology (presence of specialized structures on leaf surface like hairs and spines or protruding veins), the differences in thickness within individual leaves, and the fact that thickness is a relatively small dimension (sometimes <100 µm in

terrestrial plants) make leaf thickness (LT) difficult and time consuming to measure accurately (Vile , 2005). Leaf thickness has therefore often been estimated, and a number of surrogates have been proposed and used (White and Montes-R, 2005). One such estimate is the ratio of leaf fresh mass to surface area (Atkin , 1996; Wright and Westoby, 2002), but as far as is known, the validity of this approximation has not been formally tested (Sims , 1998; White and Montes-R, 2005).

The mean thickness of a laminar leaf (LT) can be calculated as the ratio of its volume (VL) to its projected area (A) (Roderick , 1999): $LT = VL/A$. Let rF be the average density of the leaf [the leaf fresh mass (MF) to volume (VL) ratio], thickness can be expressed as: $LT = (1/rF)(MF/A)$. Note that r is not the density of leaf tissues (tissue mass per tissue volume) because it includes the mass and volume of leaf water as well as the volume of intercellular spaces (Vile , 2005). They suggested that for laminar leaves, leaf thickness can be adequately estimated by $(SLA \cdot LDMC^{-1})$. Alternatively, LT could also be assessed by the computation of the saturated leaf fresh mass to surface area ratio. These findings apparently hold for a very broad range of leaf thickness encountered in species from different growth forms growing in contrasting environmental conditions. Vendramini (2002) have considered that full hydration was insured by collecting leaves in the morning immediately after rainfall, but this study (AR-Cen) was also that in which the relationship between LT and $(SLA \cdot LDMC^{-1})$ variation was the weaker ($r^2 = 0.71$), and seemed to introduce an unlikely variation among growth forms. Although, this could be the consequence of the particular flora encountered on this site, the hypothesis that this is due to an incomplete rehydration before measurement cannot be ruled out. One way to deal with these two hypotheses could be to include more studies in which traits were measured after rainfall or in a wet habitat and see whether they systematically differ from rehydrated ones. By contrast, Cunningham (1999) provided only a 10-min hydration period without any detectable impact on the fit of the linear relationship to the data. The aim of this study was to find the most accurate regression equation for estimating leaf thickness of 16 irrigated and droughted barley genotypes.

MATERIALS AND METHODS

This experiment was conducted at Institute Fur Gartenbauliche Produktions Systeme, Biologie, Leibniz 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. The objective of this study was to evaluate the genotypes performance under both adequate watering and the impacts of drought upon flowering and seed development stage.

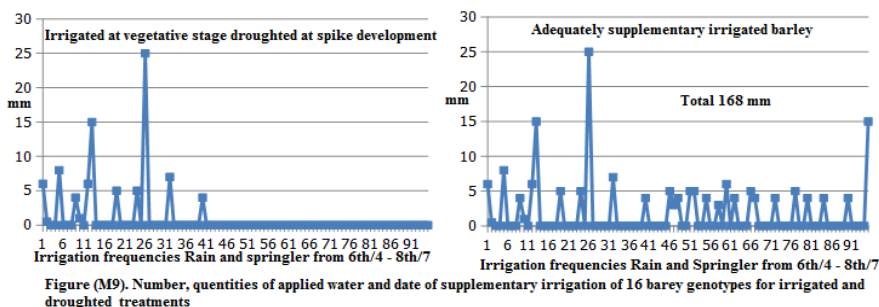
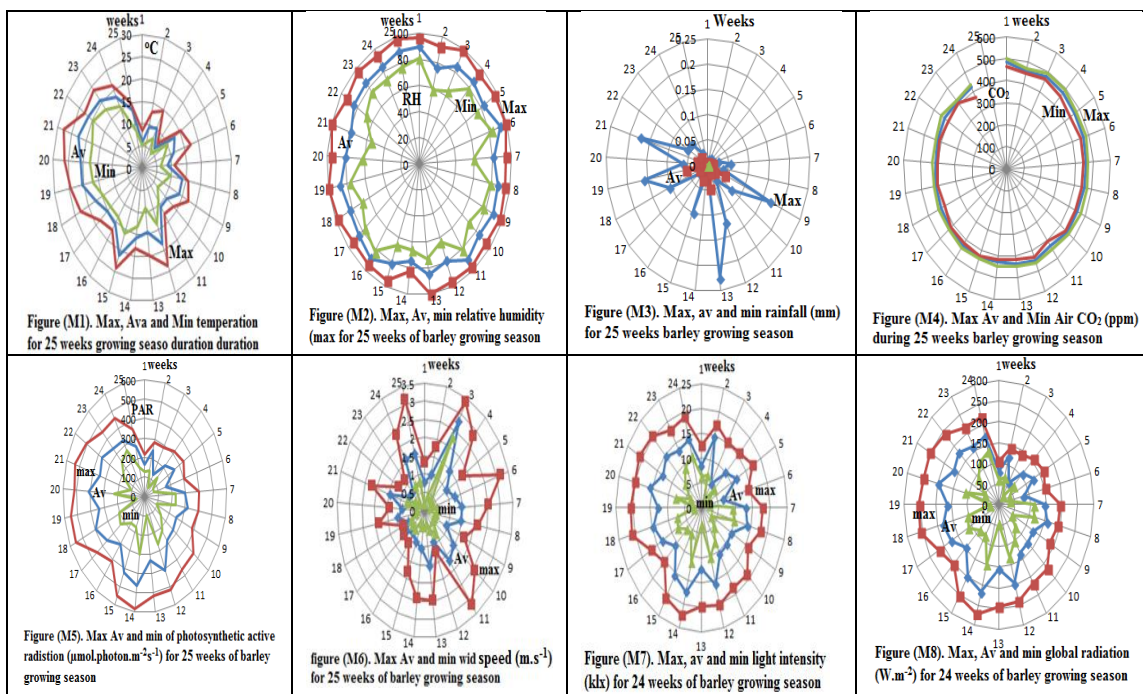
Experimental design

Split plot within Randomized Complete Block Design was selected for this investigation; the main plot represents irrigation (A), where adequate during completely 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). Therefore, the experiment contained 32 treatments each was repeated four times and each replicate was grown in 7m² 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 leaves of 16 irrigated and droughted were detached then saturated with deionized water for 12hrs in closed containers. Saturated leaves were situated between dry tissues to remove free water from leaves, and then

leaf base width, mid leaf ruler measured width and leaf length. Planometer Model LI-3100, No., measured leaf area. LAns, 36108, USA, Made. Water replacement method was utilized for the leaf size measurements using deionized water. Data was analyzed with Minitab computer program to calculated leaf area on the base of the following: Method 1, leaf base width Method 2, mid leaf width. Method 3, leaf length. Method 4, rectangle [leaf length* leaf width]. Method 5, triangle (leaf length*0.5 mid leaf width). Method 6, Leaf length: leaf width ratio [L:W]. Then leaf thickness (mm) were calculated from [(leaf saturated volume cm³/ leaf area cm²)/10].



RESULTS AND DISCUSSION

A. Effects of irrigation and drought

The most accurate estimation of leaf thickness of irrigated barley (table, R1) was L: W method (0.289654 mm), since it differed from the measured (0.29063 mm) by 0.0001026 mm. The worst was the estimation based on triangle (0.28592), which differing from the measured by 0.02153 mm. L: W was the most potent method for estimating leaf thickness of droughted barley genotypes (0.287991 mm), as it differed from measured thickness (0.28692 mm) by 0.001071mm. The worst method was that based on leaf length (0.292041mm), as it differed from measured (0.28692 mm) by 0.005121 mm. Leaf thickness is mainly dependent on light intensity and leaf growth rate, which profoundly depleted the gained assimilate. Factors affecting cell growth rate substantially reflected on leaf dimensions through their cell dimensions. Xu and Zhou (2008) found that although severe drought might lead to a reduction in stomatal density, an increase is possible under moderate drought conditions, since the response is characteristic of a parabola

rather than a linear regression. This pattern of response may also explain why a decrease in leaf area results in an increase in stomatal density under moderate drought, but an inhibition of guard cell division in relation to senescence induced by severe drought can lead to a reduction in the total stomatal number on a given leaf, i.e. stomatal density. Although our results showed that stomatal density was not significantly associated with leaf area per plant, it was negatively correlated with specific leaf area, indicating that enhanced leaf thickness may produce more guard cells for a given leaf area. Stomatal density and water status early wheat leaves, lower stomatal density could also arise because of the limitation imposed by guard cell development under stress conditions (Yin , 2006). Enlarged leaf thickness and the associated increased stomatal density may also be useful in enhancing the plasticity to a certain degree under moderate drought (Galmes , 2007). Meng (1999) reported that net photosynthetic rate (A) had a significant negative correlation with stomatal density due to a marked reduction in A induced by severe drought; this is not consistent with the present results. The disparity may be due to the age related leaf traits and soil drought severity.

Table R1. Estimation of leaf thickness (mm) of 16 irrigated and droughted barley genotypes * **

Treatment	Real Thick	L thick *L	Thick*md W	Thic rectangle	Thick Triangle	Thick*L:W
Irrigation	A 0.29068	B 0.285999	A 0.286915	B 0.285934	B 0.285920	A 0.289654
Drought	A 0.28692	A 0.292041	A 0.291117	A 0.291613	A 0.291596	A 0.287991

* Real Thick= Real thickness of leaf; L thick*L= leaf thickness estimation based on leaf length; Thick*mdW= Estimation of leaf thickness based on width of the mid leaf; Thick rectangle= estimation of leaf thickness based on length width rectangle; Thick triang= Estimation of leaf thickness based on triangle of leaf 0.5 mid width* length; Thick*L: W = Estimation of leaf thickness based on length: width ratio

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

B. Genotype responses

The most accurate estimation of barley leaf thickness (table, R2) was obtained from applying leaf length in G30 (0.293706mm), G94 (0.289264mm), G98 (0.29074mm), G154 (0.30531 mm), and G169 (0.284851mm), as they differed from their corresponding measured thickness by 0.017626, 0.028196, 0.05965, 0.015381 and 0.000951mm, respectively. Adopting the mid leaf width gave the more precise leaf thickness for G54 (0.3174 mm), G119 (0.27268mm), and G144, since they differed from their corresponding measured thickness by 0.00065, 0.0403, and 0.01343mm. L: W was the most potent method for estimating leaves thickness of G74 (0.279326mm), G116 (0.280694mm), G126 (0.285152mm), G127 (0.297841mm) and G142 (0.293835mm), they differed from their corresponding measured leaf thickness by 0.070619, 0.004084, 0.013682, 0.06579 and 0.049335mm, respectively. The best estimation of leaf thickness by rectangle method was confined to G65 (0.285567mm), as it differed from measured by 0.002493mm). Triangle derived from leaf length and mid leaf width for estimation of leaf thickness for G77 (0.285172mm), as it differed from its corresponding measured thickness by 0.020872mm. On the other hand, the highest differences from measured leaf thickness obtained by applying leaf length were found in G54 (0.293898mm, Δ=0.022852mm), G65 (0.291601mm, Δ= 0.0039mm), G74 (0.297635mm, Δ=0.06251mm), G127 (0.281501mm, Δ= 0.078749mm), G142 (0.285186mm, Δ=0.057984mm), and G144 (0.291924mm, Δ=0.021634mm). The worst estimation for leaf thickness was obtained with applying mid leaf width in G30 (0.32676mm, Δ= 0.05968mm), G98 (0.282373mm, Δ= 0.068017mm), and G154 (0.28308mm, Δ=0.02223mm). The worst estimation of leaf thickness through rectangle observed in G116 (0.298325mm, Δ= 0.021715mm). The worst estimation of leaf thickness by L: W method was detected in G77 (0.293489mm, Δ= 0.029189mm), G83 (0.288232mm, Δ= 0.05893mm) and G119 (0.284042mm, Δ= 0.051662mm). Accuracy of estimating leaf thickness with varying method might be attributed to the impact of individual genome of each genotype on magnification of leaf dimensions and its growth pattern under irrigation and drought conditions. Differences among genotypes in their capabilities in expressing genes responsible for ABA synthesis resulted in variation in leaf growth. The best studied examples of these ABRE promoter elements are Em1a from wheat and Motif I from the rice *rab 16A* gene (Marcotte , 19889; Mundy , 1990). Multiple copies of the elements fused to a minimal 35S promoter confer an ABA response to a reporter gene (Giuliano , 1990 ; Skriver , 1991) , which supports the hypothesis that ABREs are critical for the ABA induction of relevant genes (although it is difficult to explain why single copies are not sufficient for this response). The ABA effect on transcription was orientation independent in both the wheat and rice elements, which suggests that they possibly function as enhancer elements in their native genes. Electrophoretic mobility shift assays and methylation

interference foot printing have shown that both Em1a and Motif1 interact with nuclear proteins; these DNA-binding proteins are constitutively expressed in an ABA independent manner (Giuliano , 1990 , Mundy , 1990).

Table R2. Estimation of leaf thickness (mm) of 16 barley genotypes * **

Genotypes	Rea Thick	L thick *L	Thick*mdW	Thic LW	Thick Tiang	Thic*L/W
Geno. 30	0.27608B-F	0.293706AB	0.32676A	0.308778A	0.308754A	0.312456A
Geno 54	0.31675A-E	0.293898AB	0.3174A	0.307965A	0.307942A	0.305739A
Geno. 65	0.28806A-F	0.291601AB	0.28412B-D	0.285567B-D	0.285553B-D	0.285392BC
Geno 74	0.23512D-F	0.297635A	0.2888BC	0.294028BC	0.294011BC	0.279326C
Geno 77	0.2643C-F	0.288086AB	0.28828BC	0.285186B-D	0.285172B-D	0.293489B
Geno 83	0.2293F	0.283723AB	0.27892B-D	0.279572CD	0.27956CD	0.288232BC
Geno 94	0.31746A-D	0.289264AB	0.28152B-D	0.281379CD	0.281367CD	0.287934BC
Geno 98	0.35039AB	0.29074AB	0.282373B-D	0.284024B-D	0.284011B-D	0.28568BC
Geno 116	0.27661B-F	0.296998AB	0.29296B	0.298325AB	0.298305AB	0.280694C
Geno 119	0.23238EF	0.277597B	0.27268D	0.275431D	0.275421D	0.284042BC
Geno 126	0.27147B-F	0.28768AB	0.28932BC	0.292056BC	0.292039BC	0.285152BC
Geno 127	0.36025A	0.281501AB	0.28776B-D	0.28548B-D	0.285466B-D	0.294871B
Geno 142	0.34317A-C	0.285186AB	0.28932BC	0.287526B-D	0.28751B-D	0.293835B
Geno 144	0.27029B-F	0.291924AB	0.28412B-D	0.285505B-D	0.285494B-D	0.286193BC
Geno 154	0.30531A-E	0.289929AB	0.28308B-D	0.287529B-D	0.287513B-D	0.279259C
Geno 169	0.2839A-F	0.284851AB	0.27684CD	0.282027CD	0.282014CD	0.278866C

* Real Thick= Real thickness of leaf; L thick*L= leaf thickness estimation based on leaf length; Thick*mdW= Estimation of leaf thickness based on width of the mid leaf; Thick rectangle= estimation of leaf thickness based on length width rectangle; Thick triang= Estimation of leaf thickness based on triangle of leaf 0.5 mid width* length; Thick*L: W = Estimation of leaf thickness based on length: width ratio

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

C. Genotype responses to irrigation and drought

Leaf length method was the most accurate estimating for leaf thickness of irrigated barley G30 (0.28977 mm, Δ=0.00016 mm), G65 (0.29317 mm, Δ=0.03169 mm), G119 (0.26746 mm, Δ=0.05615 mm), G169 (0.26956 mm, Δ=0.03387 mm). Mid leaf width was the best method for estimating leaf thickness of irrigated barley G54 (0.29317 mm, Δ=0.0076 mm), G77 (0.28204 mm, Δ=0.00109 mm), G (0.28977 mm, Δ=0.00016 mm), G83 (0.27476 mm, Δ=0.06452 mm), G142 (0.293736 mm, Δ=0.057095 mm), and G154 (0.295648 mm, Δ=0.051022 mm). Rectangle method was the best for predicting leaf thickness of irrigated barley G144 (0.27892 mm, Δ=0.03458 mm). L: W method was preferred for the estimation of leaf thickness of barley G74 (0.28145 mm, Δ=0.0317 mm), G94 (0.296234 mm, Δ=0.046116 mm), G98 (0.288802 mm, Δ=0.1102808 mm), G116 (0.280254 mm, Δ=0.019654 mm), G126 (0.276855 mm, Δ=0.005045 mm), G127 (0.29819 mm, Δ=0.01403 mm). On the other hand, leaf length method was preferred for estimating droughted barley G30 (0.29764 mm, Δ=0.0306 mm), G77 (0.28865 mm, Δ=0.04318 mm), G94 (0.2955 mm, Δ=0.00293 mm), G98 (0.29539 mm, Δ=0.00379 mm), and G144 (0.29819 mm, Δ=0.03579 mm). Mid leaf width method was suitable for estimating leaf thickness of droughted barley G83 (0.28308 mm, Δ=0.03472 mm), G116 (0.29452 mm, Δ=0.00189 mm), G119 (0.27788 mm, Δ=0.03043 mm), G127 (0.29556 mm, Δ=0.13199 mm), and G142 (0.29972 mm, Δ=0.03579 mm). Rectangle method was the most accurate for estimating leaf thickness of droughted barley G65 (0.282968 mm, Δ=0.0031708 mm), and G74 (0.277203 mm, Δ=0.056713 mm). Triangle method was suitable for estimating leaf thickness of droughted barley G54 (0.30589 mm, Δ=0.001555 mm), G126 (0.287186 mm, Δ=0.016066 mm), G154 (0.279398 mm, Δ=0.01548 mm), and G169 (0.291316 mm, Δ=0.04715 mm). Regression analysis (figure, R1-6) revealed that leaf thickness can be estimated by the following equations: Leaf thickness (mm) = 0.1039+0.5917 (base leaf width), Leaf thickness (mm) = 0.2186+0.0622 (mid leaf width), Leaf thickness (mm)=0.2502+0.002451 (leaf L*W), Leaf thickness (mm) =0.2502+0.002451(leaf L*0.5W), Leaf thickness (mm) = 0.0466+0.01668 (leaf L)- 0.000275 (leaf L)², Leaf thickness (mm) = 0.3558-0.0027 (L/W). L: W was the most accurate method for estimating leaf thickness of irrigated and droughted barley. Mid W, method was preferred for G54, G119, and G144. L: W method was the most accurate for G74, G116, G126, G127, and 142. Rectangle was the accurate for G65 and triangle for G77. L method was the best G30, G83, G94, G98, G154, and G169. Each individual investigated irrigated and droughted genotype was mentioned its suitable estimation method. The obtained result (figure, 7-9; table, R4) showed that genotypes 30, 54, 65, 74, 98 and 154 performed the best leaf thickness under irrigation ,however, under drought, the best thickness performances were confined to genotypes 83, 116, 119, 127, 144 1nd 169. Variation among genotypes within irrigation and drought were due to the combination of gene expressions of genotypes to match with ambient environment of varying water availabilities such combination mainly affects the assimilate production and portioning among varying leaf cells, which reflected on the final leaf dimensions.

It was noted that stomatal density increased with increasing water stress, and g_s was positively correlated with stomatal density, but stomatal size decreased with increasing water stress (Xu and Zhou, 2008). They suggested that a greater g_s may appear under water stress concurrent with high stomatal density and small guard cell size. Moreover, small guard cells may cause stoma to remain open under drought to some extent (Spence, 1986) or when the effects of Abscisic acid are felt (Quarrie and Jones, 1977), indicating that there is greater g_s with a small guard cell size, which seems to be confirmed by our results. However, a parallel increase in g_s and photosynthetic rate (A) with stomatal density might not imply higher g_s and photosynthetic rate (A) under water stress, because severe drought might cause simultaneous declines in g_s , photosynthetic rate (A), as well as stomatal density. Just as g_s is not always closely associated with photosynthetic rate (A) (Maherali, 2002; von Caemmerer, 2004), the relationships of stomatal density and size with gas exchange may be complex, suggesting that some compromises can occur during plant adaptation to varying degrees of water status.

Table R3. Estimation of leaf thickness (mm) of 16 irrigated and droughted barley genotypes * **

Geno/Irrig	Rea Thick	L thick *L	Thick*mdW	Thic LW	Thick Tiang	Thic*LW
30 W	0.28961B-F	0.28977AB	0.33404A	0.307753AB	0.307729AB	0.319207A
54 W	0.32916A-F	0.29651A	0.32156AB	0.310012A	0.309989A	0.30788AB
65 W	0.32486A-F	0.29317AB	0.28412D-G	0.283394C-H	0.28338C-H	0.287816C-H
74 W	0.24974C-F	0.29943A	0.2862D-G	0.289785A-H	0.289769A-H	0.28145E-H
77 W	0.28313F	0.28752AB	0.28204D-G	0.279847D-H	0.279835D-H	0.291939B-G
83 W	0.21024F	0.28141AB	0.27476E-G	0.275063F-H	0.275053F-H	0.288534C-H
94 W	0.34235A-D	0.28303AB	0.28412D-G	0.280229D-H	0.280217D-H	0.296234B-E
98 W	0.40161AB	0.28609AB	0.28204D-G	0.282797D-H	0.282784D-H	0.288802C-H
116 W	0.2606C-F	0.29706A	0.2914D-F	0.296806A-F	0.296788A-F	0.280254E-H
119 W	0.21731EF	0.26746B	0.26748G	0.270474H	0.270466H	0.284382E-H
126 W	0.27181C-F	0.28408AB	0.28932D-G	0.296911A-F	0.296892A-F	0.276855F-H
127 W	0.29295B-F	0.27355AB	0.27996D-G	0.275776E-H	0.275766E-H	0.298196B-E
142 W	0.35083A-C	0.2794AB	0.27892D-G	0.276857E-H	0.276847E-H	0.293736B-F
144 W	0.24434C-F	0.28566AB	0.27892D-G	0.280867D-H	0.280862D-H	0.285441D-H
154 W	0.34667A-C	0.29491AB	0.2862D-G	0.295648A-F	0.295629A-F	0.270506H
169 W	0.23569C-F	0.27695AB	0.26956FG	0.272722GH	0.272712GH	0.283235E-H
30 D	0.26254C-F	0.29764A	0.31948AB	0.309804A	0.30978A	0.305704A-C
54 D	0.30434B-F	0.29129AB	0.31324BC	0.305918A-C	0.305895A-C	0.303598A-D
65 D	0.25126C-F	0.29003AB	0.28412D-G	0.28774A-H	0.287725A-H	0.282968E-H
74 D	0.22049D-F	0.29584A	0.2914D-F	0.298271A-E	0.298252A-E	0.277203F-H
77 D	0.24547C-F	0.28865AB	0.29452C-E	0.290525A-H	0.290509A-H	0.295039B-F
83 D	0.24836C-F	0.28604AB	0.28308D-G	0.284081C-H	0.284067C-H	0.287929C-H
94 D	0.29257B-F	0.2955AB	0.27892D-G	0.28253D-H	0.282516D-H	0.279634E-H
98 D	0.29918B-F	0.29539AB	0.282707D-G	0.285251B-H	0.285237B-H	0.282557E-H
116 D	0.29263B-F	0.29694A	0.29452C-E	0.299843A-D	0.299823A-D	0.281135E-H
119 D	0.24745C-F	0.28773AB	0.27788DG	0.280388D-H	0.280376D-H	0.283701E-H
126 D	0.27112C-F	0.29128AB	0.28932D-G	0.287201B-H	0.287186B-H	0.293448B-F
127 D	0.42755A	0.28945AB	0.29556C-E	0.295184A-G	0.295166A-G	0.291545B-G
142 D	0.33551A-E	0.29098AB	0.29972CD	0.298194A-E	0.298174A-E	0.293933B-F
144 D	0.29623B-F	0.29819A	0.28932DG	0.290143A-H	0.290127A-H	0.286945D-H
154 D	0.26394C-F	0.28495AB	0.27996D-G	0.279409D-H	0.279398D-H	0.288013D-H
169 D	0.3321A-F	1.28495AB	0.28412DG	0.291332A-H	0.291316A-H	0.274498GH

* Real Thick= Real thickness of leaf; L thick*L= leaf thickness estimation based on leaf length: Thick*mdW= Estimation of leaf thickness based on width of the mid leaf; Thick rectangle= estimation of leaf thickness based on length width rectangle; Thick triang= Estimation of leaf thickness based on triangle of leaf 0.5 mid width* length; Thick*L:W = Estimation of leaf thickness based on length: width ratio

** Figures of unshared characters are significantly differs at 0.05 levels, Duncan

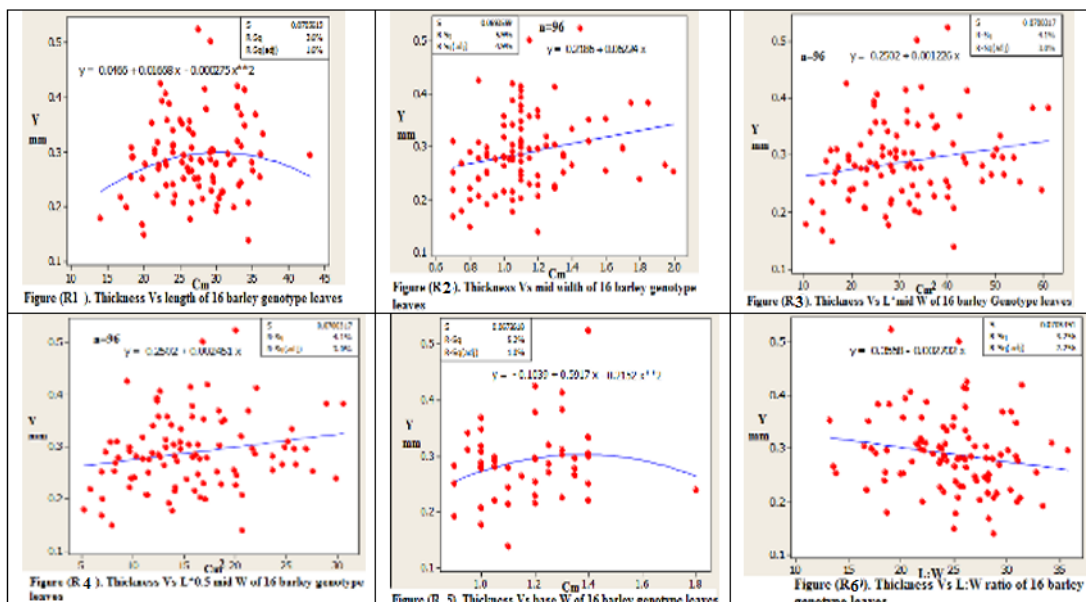
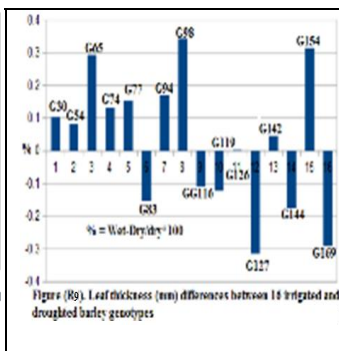
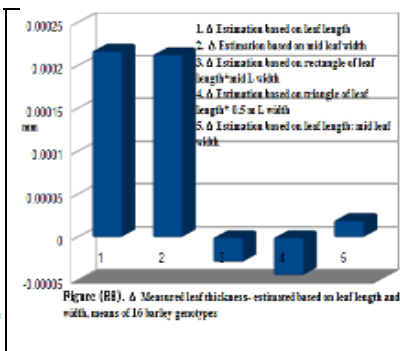
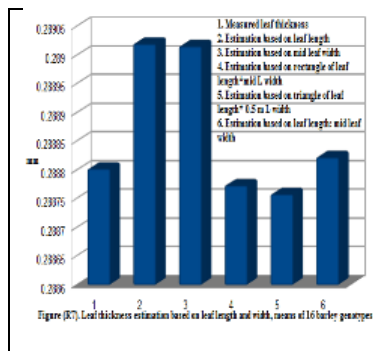


Table R4. Percentage differences in estimated leaf thickness between irrigated and droughted 16 barley genotypes [Wet-Dry/Dry*100] (*)

Genotypes	Rea Thick	L Thick *L	Thick*mdW	Thic LW	Thick Tiang	Thic*L/W
Geno. 30	0.1	-2.64	4.56	-0.66	-0.66	4.42
Geno 54	0.08	1.79	2.66	1.34	1.34	1.41
Geno. 65	0.29	1.08	0	-1.51	-1.51	1.71
Geno 74	0.13	1.21	-1.78	-2.85	-2.84	1.53
Geno 77	0.15	-0.39	-4.24	-3.68	-3.67	-1.05
Geno 83	-0.15	-1.62	-2.94	-3.17	-3.17	0.21
Geno 94	0.17	-4.22	1.86	-0.81	-0.81	5.94
Geno 98	0.34	-3.15	-0.24	-0.86	-0.86	2.21
Geno 116	-0.11	0.04	-1.06	-1.01	-1.01	-0.31
Geno 119	-0.12	-7.04	-3.74	-3.54	-3.53	0.24
Geno 126	0	-2.47	0	3.38	3.38	-5.65
Geno 127	-0.31	-5.49	-5.28	-6.57	-6.57	2.28
Geno 142	0.05	-3.98	-6.94	-7.16	-7.15	-0.07
Geno 144	-0.18	-4.2	-3.59	-3.2	-3.19	-0.52
Geno 154	0.31	3.5	2.23	5.81	5.81	-6.08
Geno 169	-0.29	-5.4	-5.12	-6.39	-6.39	3.18

* Real Thick= Real thickness of leaf; L thick*L= leaf thickness estimation based on leaf length;Thick*mdW= Estimation of leaf thickness based on width of the mid leaf; Thick rectangle= estimation of leaf thickness based on length width rectangle; Thick triang= Estimation of leaf thickness based on triangle of leaf 0.5 mid width* length; Thick*L:W = Estimation of leaf thickness based on length: width ratio.



REFERENCES

- Agusti S, Enriquez S, Frostchristensen H, Sandjensen K and Duarte CM. 1994. Light harvesting among photosynthetic organisms. *Functional Ecology* 8: 273–279.
- Cunningham SA, Summerhayes B and Westoby M. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs* 69: 569–588.
- Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen JHC and Jalili A. 2004. The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* 15: 295–304.
- Galmes J, Flexas J, Save R and Medrano H. 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant and Soil* 290,139–155.
- Garnier E, Salager JL, Laurent G and Sonie L. 1999. Relationships between photosynthesis, nitrogen and leaf structure in 14 grass species and their dependence on the basis of expression. *New Phytologist* 143: 119–129.
- Giuliano G, Pichersky E, Malik VS, Timko MP, Scolnick PA and Cashmore AR. 1988. An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene. *Proc. Natl. Acad. Sci. USA* 85: 7089–93.
- Givnish TJ. 1979. On the adaptive significance of leaf form. In: Solbrig OT, Jain S, Johnson GB, Raven PH, eds. *Topics in plant population biology*. New York: Columbia University Press, 375–407.
- Maherali H, Reid CD, Polley HW, Johnson HB and Jackson RB. 2002. Stomatal acclimation over a subambient to elevated CO₂ gradient in a C3/C4 grassland. *Plant, Cell and Environment* 25, 557–566.
- Marcotte WRJR, Russell SH and Quatrano RS. 1989. Abscisic acid responsive sequences from the Em gene of wheat. *Plant Cell*, 1: 969–76.
- Mediavilla S, Escudero A and Heilmeyer H. 2001. Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiology* 21: 251–259.
- Meng QM, Zou J, Zou JH, Jiang WS and Liu DH. 2007. Effect of Cu²⁺ concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*Allium sativum* L.). *Acta Biol Cracov Bot.*, 49:95-101.
- Mundy J, Yamaguchi-Shinozaki K and Chua NH. 1990. Nuclear proteins bind conserved elements in the abscisic acid responsive promoter of a rice rab gene. *Proc. Natl. Acad. Sci. USA* 87:1406–10.
- Nielsen SL, Enriquez S, Duarte CM and Sand-Jensen K. 1996. Scaling maximum growth rates across photosynthetic organisms. *Functional Ecology* 10: 167–175.
- Poorter H and Remkes C. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*.83, 553-559.
- Quarrie SA and Jones HG. 1977. Effects of abscisic acid and water stress on development and morphology of wheat. *Journal of Experimental Botany* 28,192–203.
- Roderick ML, Berry SL, Noble IR and Farquhar GD. 1999. A theoretical approach to linking the composition and morphology with the function of leaves. *Functional Ecology* 13: 683–695.
- Skriver K, Olsen PL, Rogers JC and Mundy J. 1991. Cis-acting DNA element responsive to gibberellin and its antagonist abscisic acid. *Proc. Natl. Acad. Sci. USA* 88: 7266–70.
- Spence RD, Wu H, Sharpe PJH and Clark KG. 1986. Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells. *Plant, Cell and Environment* 9, 197–202.
- Syvrtsen JP, Lloyd J, McConchie C, Kriedemann PE and Farquhar GD. 1995. On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant Cell and Environment* 18: 149–157.
- Vendramini F, Diaz S, Gurvich DE, Wilson PJ, Thompson K and Hodgson JG. 2002. Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytologist* 154: 147–157.
- Vile D, Garnier ER and Siple B. 2005. Specific Leaf Area and Dry Matter Content Estimate Thickness in Laminar Leaves. *Annals of Botany* 96: 1129–1136.
- von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ and Raines CA. 2004. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. *Journal of Experimental Botany* 55, 1157–1166.
- Westoby M, Falster DS, Moles AT, Vesk PA and Wright IJ. 2002. Plantecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- Witkowski ETF, Lamont BB, Walton CS and Radford S. 1992. Leaf demography, sclerophylly and ecophysiology of two Banksias with contrasting leaf life spans. *Australian Journal of Botany* 40: 849–862.
- Xu Z and Zhou G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, 59, 12: 3317–3325.
- Yin X, Wang J, Duan Z, Wen J and Wang H. 2006. Study on the stomatal density and daily change rule of the wheat. *Chinese Agricultural Science Bulletin* 22,237–242.