

Rapid non-destructive methods for leaf size estimations of 16 droughted and irrigated barley (*Hordeum vulgare*) genotypes

Caser G. Abdel* and Hartmout Stutzel

Institute Fur Gartenbauliche Produktions Systeme, Biologie, Leibniz Universitat, Hannover, Germany

Corresponding author: Caser G. Abdel

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 size estimation in field. The obtained equations were as below:

Leaf size (cm³) = -0.3271+1.003(base leaf width), Leaf size (cm³) = 0.3212+0.9987(mid leaf width), Leaf size (cm³) = 0.2502+0.002451(leaf L*W), Leaf size (cm³) = 0.2502+0.002451(leaf L*0.5W), Leaf size (cm³) = 0.41+0.04464 (leaf L), Leaf size (cm³) = 2.647-0.14171(L/W)+0.002628 (L/W)². L method was the most accurate method for estimating leaf thickness of irrigated and droughted barley. L, method was preferred for G30, G54, G77, G127 and G142. Mid W, method was the most accurate for G65, G74, G98, G116, G126, G144, G154, and G169. Triangle was the accurate for G83, G94, and G119. Each individual investigated irrigated and droughted genotype was mentioned its suitable estimation method.

Keywords: Barley, Leaf Size, Irrigation, Drought, Genotypes, Evaluation

INTRODUCTION

Plants produce several types of leaves during development. The first few true leaves produced are usually smaller, simpler, and anatomically different from leaves produced later in development (Poethig, 1997). Change in shape and size of successive leaves on a plant are related to physiological changes associated with increasing age of the plant (Esau, 1965), interaction between the shoot apical meristem and the developing leaf primordial (Byrne *et al.*, 2001), genetically regulated program of shoot maturation and a variety of environmental factors (Poethig, 1997). One of the leaf shape parameters is the length: width ratio. Verwijst and Wen (1996) found that in *Salix* the length: width ratio changed with leaf size and differed between different types of shoots. Sugiyama and Oozono (1999) showed that in lettuce this ratio of individual leaves decreased with time and eventually became constant. Comparable results were found in red spruce (Day *et al.*, 2001). Consequently, the ratio between leaf area and the product of length and width changes with plant age (Marshall, 1968). Persaud *et al.* (1993) suggested that in pearl millet this ratio should be calculated for each leaf position and growth phase of the plant.

When individual leaves grow older, they become more rounded resulting in a higher ratio between leaf area and LW, as was also reviewed by Marshall (1968). A model containing only LW cannot compensate for the age dependent variation in this ratio, while models with the additional leaf width terms (W, W²) can; the area of small leaves is

negatively influenced by these terms whereas larger leaves are positively influenced. Furthermore, leaf shape is dependent on leaf position as indicated by the leaf position terms (Model 2) and as known from literature (Esau, 1965; Wardlaw, 1968). Addition of separate leaf position terms for main stem and side branches gives a significantly better relation to single leaf area than the addition of a general leaf position term for all leaves. The coefficients for the main stem and side branches have significantly different values suggesting that leaves on the main stem have a shape different from that of leaves on the side branches (De Swart, 2004). The increase in individual leaf size can be the result of the higher light intensities during this experiment, as also found in Capsicum by Nilwik (1981) and Heuvelink and Marcelis (1996). In *Aglaonema commutatum* both leaf area and length: width ratio were reduced under lower radiation (Di Benedetto and Cogliatti, 1990). Furthermore, elevated CO₂ can also promote individual leaf size (Ferris et al., 2001; Taylor et al., 2001) and altered leaf shape (Thomas and Bazzaz, 1996; Taylor et al., 2003). This investigation aimed to create the most precise equations for leaf size estimation estimations of 16 irrigated and droughted barley genotypes.

MATERIALS AND METHODS

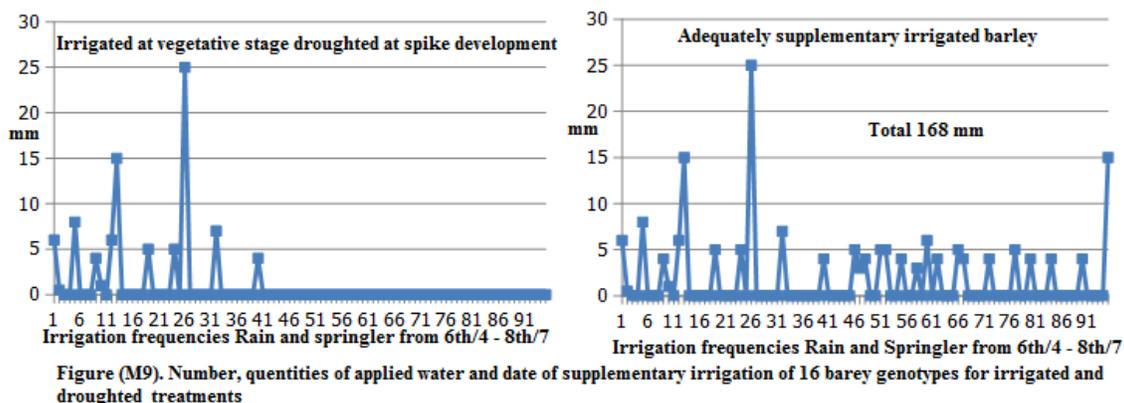
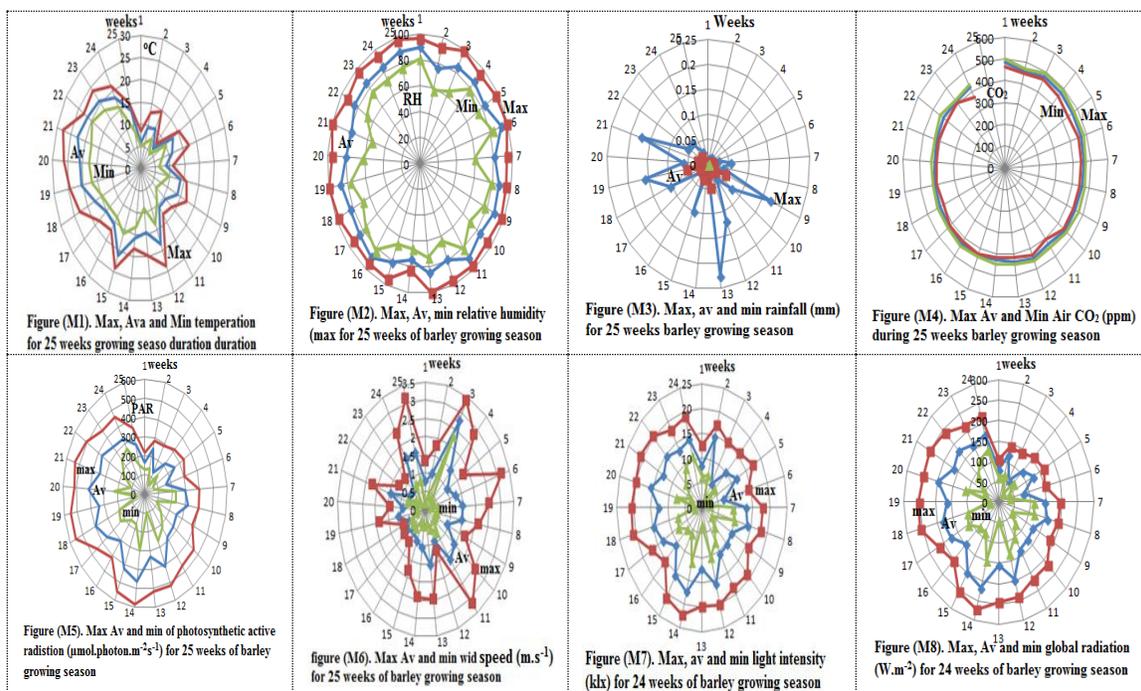
This experiment was conducted at Institute Fur Gartenbauliche Produckions Systeme, Biologie, Liebniz Universitat, Hannover, Germany. 16 Barley (*Hordeum vulgare*) genotypes, namely G30, G54, G65, G74, G77, G83, G94, G98, G116, G119, G126, G127, G142, G144, G154 and G169, to adequate irrigation and to drought during flowering and seed development stage. 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 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. Finally, water replacement method was utilized for the leaf size measurements using deionized water. Data 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].



RESULTS AND DISCUSSION

A. Effects of irrigation and drought

The most accurate estimation of leaf volume of irrigated (0.77217 cm^3) and droughted (0.86563 cm^3) barley was achieved by adopting mid leaf width, as they differed from their corresponding measured volume by 0.00946 and 0.00938 cm^3 , respectively. On the other hand, the weakest estimation was observed when L: W method was applied for both irrigated and droughted barley genotypes, since the differences with their corresponding measured leaf volume were 1.42566 and 1.3286 cm^3 , respectively. These results suggested that mid leaf width accuracy confirmed the similarity of the irrigation and drought effects on leaf uniformity. Therefore, both revealed that the most accurate estimation can be calculated by mid leaf width method. Abdel, (1994) studied the Leaf area and size estimations of *Vicia faba* L., including the first, second and third leaves below apical meristems. He concluded that estimation depend upon length and width was more accurate than that depend upon length alone, his results also confirmed that any increases in leaf area would be on the expanse of leaf thickness and vice versa, since significant differences were detected in leaf area, but not observed in leaf size.

Table R1. Leaf size estimation of irrigated and droughted 16 barley genotype based on leaf dimensions (Cm³). * **

Treatment	Measured Volume	E vol.* L	E Vol *mid W	E vol *LW	E vol Triangle	E vol L/mW
Irrigation	A 0.76271	B 0.74976	A 0.77217	B 0.74608	B 0.74593	A 2.18837
Drought	A 0.84875	A 0.86168	A 0.83937	A 0.86563	A 0.86546	A 2.17735

(*) E vol.* L = Volume estimation based on leaf length; E Vol *mid W = Volume estimation based on mid leaf width; E vol *LW= Volume estimation based on rectangle of length * mid leaf width; E vol Triangle = Volume estimation based on triangle of 0.5 mid leaf width * leaf length; E vol L/mW = Volume estimation based on leaf length: width ratio

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

B. Genotype responses

The most precise leaf volume estimation (table R2) was L method for G30 (0.8154 cm³, Δ= 0.3263 cm³), G54 (0.9009 cm³, Δ= 0.4324 cm³), G77 (0.7261 cm³, Δ= 0.1061 cm³), G127 (0.6993 cm³, Δ= 0.0507 cm³) and G 142 (0.7358 cm³, Δ= 0.1289 cm³). Mid leaf width method was paramount for leaf volume estimation of G65 (0.7274 cm³, Δ= 0.0293 cm³), G74 (0.8023 cm³, Δ= 0.0856 cm³), G98 (0.6991 cm³, Δ= 0.1026 cm³), G116 (0.8689 cm³, Δ= 0.5686 cm³), G126 (0.8107 cm³, Δ= 0.006 cm³), G144 (0.7274 cm³, Δ= 0.0274 cm³), G154 (0.7108 cm³, Δ= 0.1359 cm³), and G169 (0.6109 cm³, Δ= 0.0441 cm³). Triangle method was preferred for G83 (0.612 cm³, Δ= 0.062 cm³), G94 (0.6501 cm³, Δ= 0.1366 cm³), and G119 (0.5249 cm³, Δ= 0.1249 cm³). On the other hand, L: W was the worst method for leaf volume estimation for all genotypes. Differences between genotypes within the most precise method for prediction of leaf volume were obvious. These differences can be attributed to the genetic variations among investigated genotypes. It is well established that genes manipulate the plant defense system to fulfill the final leaf sizes. Various regulations of plant biochemical responses were found to be affected by drought stress in all three genotypes. Among them, carotenoids can reduce and eliminate the reactive oxygen damage, serve as the precursors of ABA synthesis, and also participate in photosynthesis as the pigments of chlorophyll (Goodwin and Britton, 1988 and Milborrow, 2001; Treutter, 2006). Scippa *et al.* (2004) characterized the physiological activities of plants containing an antisense *his1-s* construct, through the measurements of stomatal conductance, transpiration and net photosynthetic rate. They found that in well watered conditions, all three physiological parameters had higher values in all three transgenic lines than the wild type, with the exception of the transpiration rate of line A1 on day 62. When a water deprivation regime was applied the kinetics of decrease of the three parameters was different between the wild type and the antisense lines. During the first week of water stress treatment, all three physiological parameters were decreased in wild type plants; stomatal conductance dropped to 49% of the initial value, transpiration rate to 43% and net photosynthesis rate to 27%. By contrast, 1 week of the water-stress treatment did not alter the physiological activities of the three independent antisense lines. In the third week of the water stress treatment, all genotypes reached similar values that did not change for the remaining week of the water stress treatment. During the rewatering period, wild-type and transgenic plants showed a similar trend for recovery of physiological activities.

Table R2. Leaf size estimation of 16 barley genotype based on leaf dimensions (Cm³). * **

Genotypes	Measured Volume	E vol.* L	E Vol *mid W	E vol *LW	E vol Triangle	E vol L/mW
Geno. 30	1.1417AB	0.8154A-D	1.4099A	1.227A	12268A	2.33885A
Geno 54	1.3333A	0.9009A-C	1.2601A	1.2099A	12096A	2.29329A
Geno. 65	0.7567C-E	0.8061A-D	0.7274B-D	0.7383B-D	0.7382C-D	2.16031BC
Geno 74	0.7167C-E	0.9969AB	0.8023BC	0.9165BC	0.9163BC	2.12133C
Geno 77	0.62DE	0.7261B-D	0.794BC	0.7303C	0.7302B-D	2.21286B
Geno 83	0.55DE	0.6755CD	0.6442B-D	0.6121CD	0.612CD	2.17825BC
Geno 94	0.7867B-E	0.7067CD	0.6858B-D	0.6502CD	0.6501CD	2.1766BC
Geno 98	0.8017B-E	0.7782B-D	0.6991B-D	0.7059B-D	0.7057B-D	2.16179BC
Geno 116	1.03A-C	1.0572A	0.8689B	1.0069AB	10067AB	2.12998C
Geno 119	0.47E	0.6167D	0.5443D	0.525D	0.5249D	2.15182BC
Geno 126	0.8167B-E	0.9195A-C	0.8107BC	0.8749BC	0.8748BC	2.15918BC
Geno 127	0.75C-E	0.6993CD	0.7857B-D	0.7365B-D	0.7364B-D	2.22144B
Geno 142	0.9167B-D	0.7358B-D	0.8107BC	0.7796B-D	0.7794B-D	2.21459B
Geno 144	0.7C-E	0.7878A-D	0.7274B	0.7371B-D	0.737B-D	2.16498BC
Geno 154	0.8467B-E	0.898A-C	0.7108B-D	0.7796B-D	0.7795B-D	2.12188C
Geno 169	0.655C-E	0.7715B-D	0.6109CD	0.6638CD	0.6637CD	2.1186C

(*) E vol.* L = Volume estimation based on leaf length; E Vol *mid W = Volume estimation based on mid leaf width; E vol *LW= Volume estimation based on rectangle of length * mid leaf width; E vol Triangle = Volume estimation based on triangle of 0.5 mid leaf width * leaf length; E vol L/mW = Volume estimation based on leaf length: width ratio

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

C. Genotype responses to irrigation and drought

Triangle was the most effective method for leaf size estimation of irrigated barley genotypes, G30 (1.2052 cm³, Δ=0.0719 cm³), G77 (0.6178 cm³, Δ=0.0678 cm³), G83 (0.5171 cm³, Δ=0.1171 cm³), G116 (0.9748 cm³, Δ=0.0148 cm³), G119 (0.4205 cm³, Δ=0.0305 cm³), G126 (0.977 cm³, Δ=0.077 cm³), G144 (0.6394 cm³, Δ=0.0727 cm³), and G169 (0.4678 cm³, Δ=0.0411 cm³). Mid leaf width (md.W) method was the most accurate prediction for leaf size of irrigated genotypes meth G54 (1.3267 cm³, Δ=0.0733 cm³), G74 (0.7607 cm³, Δ=0.0774 cm³), G94 (0.7274 cm³, Δ=0.0459 cm³), and G142 (0.6442 cm³, Δ=0.0391 cm³). Leaf length method was the most precise for estimating leaf size of irrigated G65 (0.7484 cm³, Δ=0.0383 cm³), G98 (0.7239 cm³, Δ=0.1428 cm³), and G154 (1.1167 cm³, Δ=0.01 cm³). Rectangle method was preferred for G127 (0.5322 cm³, Δ=0.0245 cm³). On the other hand, triangle method was preferred for leaf size estimation of droughted G30 (1.2483 cm³, Δ=0.0983 cm³), G83 (0.7069 cm³, Δ=0.0069 cm³), G119 (0.6292 cm³, Δ=0.0792 cm³), G144 (0.8345 cm³, Δ=0.0012 cm³), and G154 (0.6086 cm³, Δ=0.0419 cm³). Rectangle method was very effective for forecasting leaf size of droughted G142 (1.0042 cm³, Δ=0.1488 cm³), and G98 (0.7317 cm³, Δ=0.005 cm³), G127 (0.9408 cm³, Δ=0.0025 cm³), and G169 (0.8597 cm³, Δ=0.0236 cm³). Leaf length method was the most potent for estimating leaf size of droughted G77 (0.8027 cm³, Δ=0.1127 cm³), and G94 (0.7968 cm³, Δ=0.0032 cm³), G116 (1.078 cm³, Δ=0.022 cm³), and G126 (0.7611 cm³, Δ=0.0278 cm³). Mid leaf method was the most suitable for leaf size estimation of droughted and G54 (1.1935 cm³, Δ=0.0732 cm³), G65 (0.7274 cm³, Δ=0.0007 cm³), and G74 (0.844 cm³, Δ=0.094 cm³). Regression analysis (figure1-6) showed that leaf volume of genotypes under irrigation and drought can be forecasted by the following regression equations:

Leaf size (cm³)= -0.3271+1.003(base leaf width), Leaf size (cm³) = 0.3212+0.9987(mid leaf width), Leaf size (cm³)=0.2502+0.002451(leaf L*W), Leaf size (cm³)=0.2502+0.002451(leaf L*0.5W), Leaf size (cm³) = 0.41+0.04464(leaf L), Leaf size (cm³) = 2.647-0.14171(L/W)+0.002628 (L/W)². L method was the most accurate method for estimating leaf thickness of irrigated and droughted barley. L, method was preferred for G30, G54, G77, G127 and G142. Mid W, method was the most accurate for G65, G74, G98, G116, G126, G144, G154, and G169. Triangle was the accurate for G83, G94, and G119. Each individual investigated irrigated and droughted genotype was mentioned its suitable estimation method. In general regardless to irrigation, all methods are suitable for estimating leaf volume of barley except L:W method (figure, 7, 8). Volume of leaves were best performed under irrigation in genotypes 30, 54,94 and 154, however, other genotypes showed their best leaf volumes under drought (figure, 9). In response to well watered conditions, the thickness of the leaf blade of the antisense plants was reduced by 20% compared to the wild type. After 2 weeks of water stress treatment, the thickness of the leaf lamella of the wild type plants was reduced by 48.4% compared to the non-stressed controls. The decrease in thickness was attributed to both a decrease in palisade cell length and a strong compression of the spongy mesophyll cells(Scippa *et al.*, 2004). They also revealed that the decrease in thickness of the spongy mesophyll was visible in the sections and was caused by a loss of intercellular spaces and a reduction in cellular diameters. These anatomical alterations were less evident in the leaf lamella of H1-S antisense plants under water-stress conditions, where the reduction in thickness of palisade and spongy mesophyll layers in response to water stress was only 23.7% and 30.3 %, respectively. The anatomical

differences observed with the light microscope between the genotypes were confirmed by electron microscopy analyses. In response to water deficit stress, the intercellular spaces between mesophyll cells were greater in the leaves of the H1-S antisense plants than the wild type.

Table R3. Leaf size estimation of irrigated and droughted 16 barley genotypes based on leaf dimensions (Cm³) * **

Geno/Irrig	Measured Volume	E vol.* L	E Vol *mid W	E vol *LW	E vol Triangle	E vol L/mW
30 W	1.1333A-C	0.7105A-E	1.5264A	1.2054AB	12052AB	2.38476A
54 W	1.4A	0.898A-E	1.3267AB	1.253A	12527A	2.30752AB
65 W	0.7867B-G	0.7484A-E	0.7274D-G	0.6926C-H	0.6925C-H	2.17624C-H
74 W	0.6833C-G	0.9203A-E	0.7607D-G	0.8272A-H	0.827A-H	2.13446E-H
77 W	0.55E-G	0.6495C-E	0.6941D-G	0.6179D-H	0.6178D-H	2.20225B-G
83 W	0.4FG	0.5899DE	0.5776E-G	0.5172F-H	0.5171F-H	2.17985C-H
94 W	0.7733B-G	0.6167DE	0.7274D-G	0.626D-H	0.6258D-H	2.2301B-D
98 W	0.8667A-G	0.7239A-E	0.6941D-G	0.68D-H	0.6799D-H	2.18166C-H
116 W	0.96A-F	1.0363A-C	0.844D-F	0.975A-F	0.9748A-F	2.1271E-H
119 W	0.39G	0.5215E	0.4611G	0.4206H	0.4205H	2.15478E-H
126 W	0.9A-G	1.078AB	0.8107D-G	0.9772A-H	0.977A-F	2.10657F-H
127 W	0.5567E-G	0.523E	0.6609D-G	0.5322E-H	0.5321E-H	2.24294B-E
142 W	0.6833C-G	0.584DE	0.6442D-G	0.555E-H	0.5549E-H	2.21374B-F
144 W	0.5667D-G	0.703B-E	0.6442D-G	0.6396D-H	0.6394D-H	2.16009E-H
154 W	1.1267A-C	1.1167A	0.7607D-G	0.9506A-F	0.9504A-F	2.06581H
169 W	0.4267FG	0.5765DE	0.4944FG	0.4679GH	0.4678GH	2.14603E-H
30 D	1.15A-C	0.9203A-E	1.2934AB	1.2486A	12483A	2.29294A-C
54 D	1.2667Ab	0.9039A-E	1.1935BC	1.1668A-C	11665A-C	2.27905A-D
65 D	0.7267B-G	0.8637A-E	0.7274D-G	0.7841A-H	0.7839A-H	2.14439E-H
74 D	0.75B-G	1.0735AB	0.844D-F	1.0058A-E	10056A-E	2.1082F-H
77 D	0.69C-G	0.8027A-E	0.8939C-E	0.8427A-H	0.8426A-H	2.22347B-F
83 D	0.7C-G	0.7611A-E	0.7108D-G	0.707C-H	0.7069C-H	2.17664C-H
94 D	0.8B-G	0.7968A-E	0.6442D-G	0.6744D-H	0.6743D-H	2.12309E-H
98 D	0.7367B-G	0.8325A-E	0.7041D-G	0.7317B-H	0.7316C-H	2.14191E-H
116 D	1.1A-E	1.078AB	0.8939C-E	1.0389A-D	10387A-D	2.13286E-H
119 D	0.55E-G	0.712A-E	0.6276D-G	0.6293D-F	0.6292D-H	2.14886E-H
126 D	0.7333B-G	0.7611A-E	0.8107D-G	0.7727C-H	0.7726D-H	2.21179B-F
127 D	0.9433A-G	0.8756A-E	0.9105C-E	0.9408A-F	0.9406A-F	2.19995B-F
142 D	1.15A-C	0.8875A-E	0.9771CD	1.0042A-E	10040A-E	2.21545B-F
144 D	0.8333B-G	0.8727A-E	0.8107D-G	0.8347A-H	0.8345A-H	2.16987E-H
154 D	0.5667D-G	0.6792B-E	0.6609D-G	0.6087D-H	0.6086D-H	2.17796C-H
169 D	0.8833A-G	0.9664A-D	0.7274D-G	0.8597A-H	0.8595A-H	2.09116GH

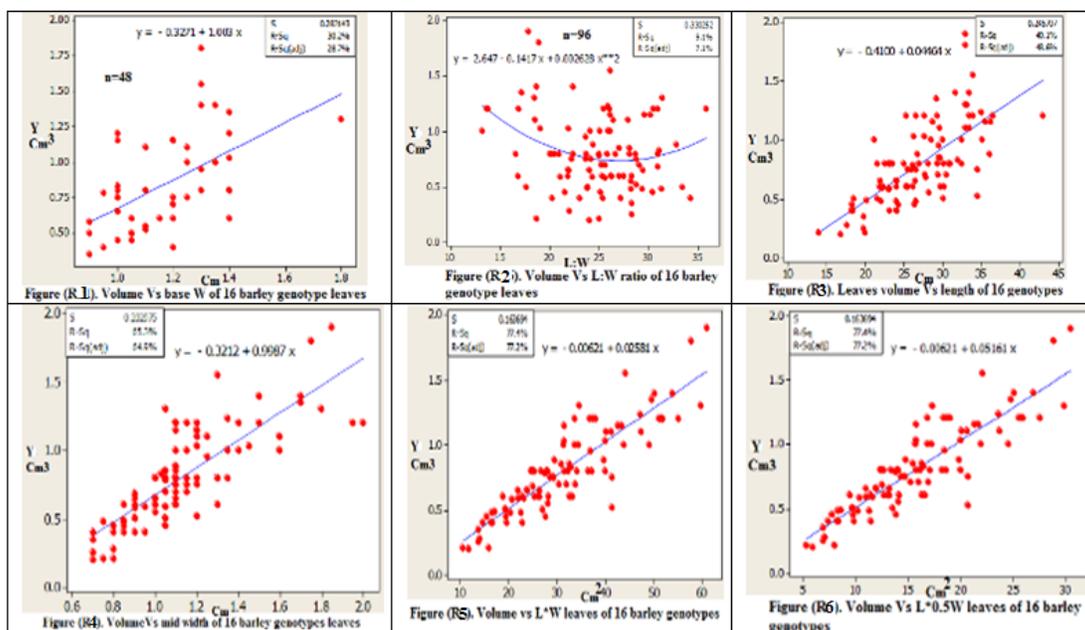
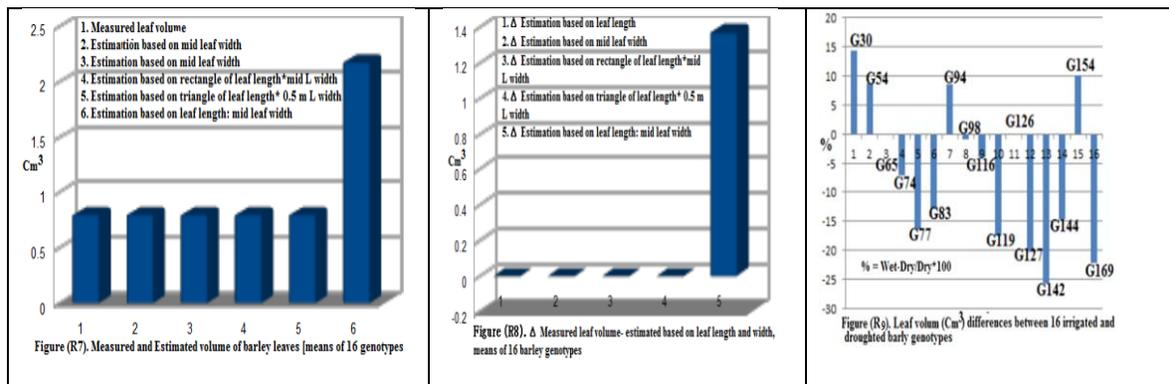
(*) E vol.* L = Volume estimation based on leaf length; E Vol *mid W = Volume estimation based on mid leaf width; E vol *LW= Volume estimation based on rectangle of length * mid leaf width; E vol Triangle = Volume estimation based on triangle of 0.5 mid leaf width * leaf length; E vol L/mW = Volume estimation based on leaf length: width ratio

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

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

Genotypes	Measured Volume	E vol.* L	E Vol *mid W	E vol *LW	E vol Triangle	E vol L/mW
Geno. 30	14.43	-1.45	-22.8	18.01	-3.46	4
Geno 54	8.79	10.52	-0.65	11.16	7.39	1.25
Geno. 65	0	8.26	-13.35	0	-11.67	1.49
Geno 74	-7.15	-8.89	-14.27	-9.87	-17.76	1.25
Geno 77	-16.44	-20.29	-19.09	-22.35	-26.68	-0.95
Geno 83	-12.9	-42.86	-22.49	-18.74	-26.85	0.15
Geno 94	8.62	-3.34	-22.6	12.92	-7.18	5.04
Geno 98	-0.97	17.65	-13.05	-1.42	-7.07	1.86
Geno 116	-4.11	-12.73	-3.87	-5.58	-6.15	-0.27
Geno 119	-17.55	-29.09	-26.76	-26.53	-33.16	0.28
Geno 126	0	22.73	41.64	0	26.47	-4.76
Geno 127	-20.27	-40.98	-40.27	-27.41	-43.43	1.95
Geno 142	-25.64	-40.58	-34.2	-34.07	-44.73	-0.08
Geno 144	-14.7	-31.99	-19.45	-20.54	-23.37	-0.45
Geno 154	10.17	98.82	64.41	15.1	56.17	-5.15
Geno 169	-22.22	-51.69	-40.35	-32.03	-45.57	2.62

(*) E vol.* L = Volume estimation based on leaf length; E Vol *mid W = Volume estimation based on mid leaf width; E vol *LW= Volume estimation based on rectangle of length * mid leaf width; E vol Triangle = Volume estimation based on triangle of 0.5 mid leaf width * leaf length; E vol L/mW = Volume estimation based on leaf length: width ratio



REFERENCES

- Abdel CG. 1994. Rapid methods for estimating leaf area and size in field bean (*Vicia faba* L.). *Tech. Res.* 7, 20: 63-70.
- Byrne M, Timmermans M, Kidner C and Martienssen R. 2001. Development of leaf shape. *Current Opinion in Plant Biology*, 4, 38-43.
- Day ME, Greenwood MS and White AS. 2001. Age-related changes in foliar morphology and physiology in red spruce and their influence on declining photosynthetic rates and productivity with tree age. *Tree Physiology*, 21, 1195-204.
- De Swart EAM, Groenwold R, Kanne HJ, Stam P, Marcelis LFM and Voorrips RE. 2004. Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. – *J. Hort. Sci. Biotechnol.* 79: 764-770.
- Dibendetto AH and Cogliatti DH. 1990. Effects of light intensity and quality on the obligate shade plant *Aglaonema commutatum*. I. Leaf size and shape. *Journal of Horticultural Science*, 65, 689-98.
- Esau K. 1965. The leaf. In: *Plant anatomy* (Esau, K., Ed.). John Wiley and sons. New York, London, Sidney, 467-80.
- Ferris R, Sambatti M, Meglietta F, Mills SR and Taylor G. 2001. Leaf area is stimulated in Populus by free air CO₂ enrichment (POPFACE), through increased cell expansion and production. *Plant, Cell and Environment*, 24, 305-15.
- Goodwin TW and Britton G. 1988. Distribution and analysis of carotenoids. In: Goodwin TW, ed. *Plant pigments*. FL: Academic Press, 61-132.
- Marshall JK. 1968. Methods for leaf area measurement of large and small samples. *Photosynthetica*, 2, 41-7.
- Milborrow BV. 2001. The pathway of biosynthesis of abscisic acid in vascular plant: a review of the present state of knowledge of ABA biosynthesis. *Journal of Experimental Botany* 52, 1145-1164.
- Treutter D. 2006. Significance of flavonoids in plant resistance: a review. *Environmental Chemistry Letters* 4, 147-157.
- Nilwik HJM. 1981. Growth analysis of sweet pepper (*Capsicum annuum* L.). 2. Interacting effects of irradiance, temperature and plant age in controlled conditions. *Annals of Botany*, 48, 137-45.
- Persaud N, Gandah M, Ouattara M and Mokete N. 1993. Estimating leaf area of pearl millet from linear measurements. *Agronomy Journal*, 85, 10-2.
- Poethig RS. 1997. Leaf morphogenesis in flowering plants. *The Plant Cell*, 9, 1077-87.
- Scippa GS, Di Michele M, Onelli E, Patrignani G, Chiatante D and Bray EA. 2004. The histone-like protein H1-S and the response of tomato leaves to water deficit. *Journal of Experimental Botany*, 55, 99-109.
- Sugiyama N and Oozono M. 1999. Leaf initiation and development in crisphead and butterhead lettuce plants. *Journal of the Japanese Society of Horticultural Science*, 68, 1118-23.
- Taylor G, Ceulemans R, Ferris R, Gardner SDL and Shao OBY. 2001. Increased leaf area expansion of hybrid poplar in elevated CO₂. From controlled environments to open top chambers and to FACE. *Environmental Pollution*, 115, 463-72.
- Taylor G, Tricker PJ, Zhang FZ, Alston VJ, Miglietta F and Kuzminski E. 2003. Spatial and temporal effects of free air CO₂ enrichment (POPFACE) on leaf growth, cell expansion, and cell production in a closed canopy of poplar. *Plant Physiology*, 131, 177-85.
- Thomas SC and Bazzaz FA. 1996. Elevated CO₂ and leaf shape: are dandelions getting toothier? *American Journal of Botany*, 83, 106-11.
- Verwijst T and Wen DZ. 1996. Leaf allometry of *Salix viminalis* during the first growing season. *Tree Physiology*, 16, 655-60.
- Wardlaw CW. 1968. Leaves and buds: further observations. In: *Morphogenesis in plants. A contemporary study* (Wardlaw, C. W., Ed.). Methuen & Co TLD, London, UK, 181-225.