RESEARCH

Photosynthetic performance index in early stage of growth, water use efficiency, and grain yield of winter barley cultivars

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Repetitive heat and drought stress conditions have a significant impact on quantity and quality of barley (*Hordeum vulgare* L.) production in most regions of the world. Objective of this study was to determine the relationships between photosynthetic performance index (PI_{ABS}), water use (WU), grain yield-based water use efficiency (WUE_G), and grain yield per pot (GYP) of winter barley cultivars grown in a pot trial under short-term drought stress conditions and grain yield and its stability from the multi-environmental field trials. Ten winter barley cultivars were examined in two water treatments. One treatment was well watered, while the second treatment was subjected to short-term stress caused by deficiency of water in the stages of full tillering, beginning of heading, and grain filling. PI_{ABS} was measured at full tillering stage while WU, WUE_G, and GYP of barley cultivars were estimated after the whole vegetative cycle. Also, multi-environmental field trials with the same winter barley cultivars were carried out during 4 yr (2004-2007) and 3 yr (2009-2011) with two sowing densities (300 and 450 seeds m^{-2}) on multiple locations in the lowland part of the Republic of Croatia. ANOVA showed highly significant ($P \leq 0.001$) cultivar effect for all of the examined traits in the pot trial. PI_{ABS} of cultivars in both treatments was in a negative nonsignificant correlation with grain yield and grain yield stability (ecovalence) of the same cultivars in multi-environmental field trials. Winter barley cultivars with higher WU and WUE_G also had higher values of grain yield, and harvest index observed on the basis of the pot trial. WU, WUE_G, and GYP of 10 barley cultivars in pot trial showed highly positive phenotypic correlation with grain yield of all eight and 10 barley cultivars in the multi-environmental field trials. These results suggests that WU and WUE_G could be good indicators for preliminary selection of modern, high yielding, and stable winter barley genotypes which have better water management capabilities.

Key words: Grain yield, *Hordeum vulgare*, multi-environmental trials, photosynthetic performance index, water use, water use efficiency.

INTRODUCTION

Different cultivars of winter barley (*Hordeum vulgare* L.) show various genetic tolerances when they are exposed to different stress conditions. The response of a genotype to different stress conditions has a substantial impact on genotype's stability. Consequently, it is useful to apply different indices that can be calculated from values of a trait, most often yield, observed under stress and non-stress conditions (Talebi et al., 2009; Ilker et al., 2011; Kovacevic et al., 2011). Therefore, investigations of interaction among genotypes (cultivars) and environments give useful information concerning yield and yield stability (Ceccarelli et al., 2000; Lalic et al., 2009; Mohammadi et al., 2012; Altay, 2012).

Abiotic stress caused by water-limited conditions is very frequently followed by abiotic stress caused by high temperatures. Repetitive heat and drought-stress conditions have a significant impact on quantity and quality of barley production in most regions of the world. Photosynthetic performance index could be a good indicator of drought tolerance in barley cultivars (Oukarroum et al., 2007). Also, rational water use of plant cultivars could be a good indicator for plant production in water-limited conditions (Reynolds et al., 2007; Araus et al., 2008; Blum, 2009; Yong'an et al., 2010). In breeding for drought resistance, indispensable elements of agronomy are production of biomass and water use efficiency (WUE) (Blum, 1993). Several authors have studied the relationship between WUE and various agronomic traits, and they pointed out high positive correlation coefficients for WUE with grain yield and harvest index (Yong'an et al., 2010; Shamsi et al., 2010).

The purpose of this study was estimating the relationships among characteristics of winter barley

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Received: 12 November 2014.
Accepted: 13 April 2015.
doi:10.4067/S0718-58392015000400002

cultivars measured in a pot trial (photosynthetic performance index, water use, grain yield-based water use efficiency, grain yield per pot) and agronomic characters that were estimated in multi-environmental field trials (grain yield and stability of grain yield). These results could be useful for developing breeding methods for preliminary selection of barley genotypes.

MATERIALS AND METHODS

Plant materials

One year (2008) trial with 10 winter barley cultivars (A) was grown in vegetative pots according to randomized complete block design with three replicates. Seven winter barley cultivars were developed at the Agricultural Institute Osijek (45°32' N, 18°44' E), Osijek, Croatia. Five of them were two-rowed: 'Barun' (A11), 'Bingo' (A12), 'Zlatko' (A13), 'Rex' (A15), and 'Bravo' (A17) while 'Lord' (A18) and 'Titan' (A20) were six-rowed cultivars. Two-rowed barley cultivars ('A14' and 'A16') and six-rowed cultivar ('A19') were developed in other institutions.

Eight out of 10 mentioned winter barley cultivars (without new cultivars 'A17' and 'A20') were tested in the multi-environmental field trials from 2004 to 2007 (4 yr; four locations; two sowing densities; three replicates) (Lalic et al., 2009). In addition, every barley cultivar examined in the pot trial (10 cultivars) was tested in the multi-environmental field trials from 2009 to 2011 (3 yr; five locations; two sowing densities, three replicates) (Lalic et al., 2009; 2012).

The vegetative pots were filled with the upper layer (depth up to 30 cm) of soil from experimental field of the Agricultural Institute Osijek, Croatia. The soil had good fertility and same mechanical, physical, and chemical composition in every pot. The soil type was Humofluvisol chernozemic, moderately deep gleic, non-calcareous, silty-clay-loam (FAO, 2006). Pore volume was 49%, water holding capacity 39%, and air capacity 10% (Romic et al., 2005). Pots were filled with soil, saturated with water up to 39% of soil volume or 100% field capacity (FC), and weighed. Mass of each saturated pot was then used as a base for calculating water status in the pots. Pots had drainage holes and trays at the bottom for possible water surplus, which was measured and saved for next watering. The pot dimension was 22.5 cm in height, 30 cm in diameter, and 12 000 cm^3 in volume. Soil volume was 9800 cm^3 per pot, and it was measured 10 d after filling and saturation of the soil with water. Sowing was conducted 7 d after filling and saturation of the soil with water on 27 December 2008 by planting 32 seeds per vegetative pot. Seeds were arranged in a circle of 20 cm in diameter, in 16 hills (two seeds per hill) with distance of 3.9 cm between hills, and at a depth of 3.5 cm. Sowing density in pot experiment was calculated to match field sowing density of 450 seeds m^{-2} and number of tillers per pot in both treatments ranged from 28.7 to 43.3 with average value of 35.4 tillers per pot.

Growth environment and water stress treatments

Winter barley cultivars ('A11-A20') were studied in two irrigation (B) treatments: well watered as control (B1) and short-term drought stress conditions (B2). Soil water content was calculated as the difference between water content at 100% FC (39% soil volume) and soil water depletion in each vegetative pot and both irrigation treatments. Monitoring the rate of soil water depletion was done by the gravimetric method and with the help of soil moisture sensor (Watermark 30-KTCD-NL, Irrometer Company, Riverside, California, USA). Watermark sensor is hand held device designed for reading Watermark sensors in the field. Sensors were buried at 15 cm depth in the pots. The readings ranged from 0-199 kPa where 0 stands for wet soil (100% FC) while 199 kPa stands for dry soil. Soil water measurements were made every day.

The measurements of chlorophyll *a* fluorescence were conducted as follows: At the end of tillering stage (EC 29-Eucarpia Code, Reiner et al., 1992). From 7 to 12 March 2008 the soil water content in pots was maintained at 30.4% to 38.4% of the soil volume (77.9% to 98.5% FC) for B1 and from 21.3% to 28.7% of soil volume (54.6% to 73.6% FC) for B2 (Figure 1A). During the same period temperatures varied from 8 °C (early in the morning) to 26 °C (maximum daily temperature) and 80% to 99% RH. On the 12 March (day photosynthetic parameters were measured) minimum water content in the pots varied from 20.7% to 21.8% of the soil volume (53% to 56% FC) for the stressful treatment (B2), and from 37.4% to 38.6% of the soil volume (96% to 99% FC) for the control (B1).

During flag leaf stage and the beginning of heading stage (EC 49/51), from 20 to 25 April 2008, soil water content in pots was maintained at 27.2% to 35.5% (69.7% to 91.0% FC) of the soil volume for B1 and from 16.4% to 20.0% of the soil volume (42.1% to 51.3% FC) for B2 (Figure 1B). During the same period temperatures varied from 3.7 °C (minimum night temperature) to 21.6 °C (maximum daily temperature) and 30% to 99% RH. On 25 April minimum water content in pots varied from 15.2% to 17.9% of the soil volume (39% to 46% FC) for the stressful treatment (B2) and from 25.2% to 29.3% of the soil volume (65% to 75% FC) for B1.

At the end of the grain filling period (EC 75/85), from 18 to 21 May 2008, the soil moisture content in pots was maintained at 26.1% to 31.9% of the soil volume (66.9% to 81.8% FC) for B1 and from 20.8% to 24.2% of the soil volume (53.3% to 62.1% FC) for B2 (Figure 1C). During the same period temperature varied from 13.1 °C (minimum night temperature) to 32.6 °C (maximum daily temperature) and RH from 28% to 99%. On the 21 May the minimum water content in pots varied from 19.9% to 21% of soil volume (51% to 56% FC) for B2, and from 25.4% to 27.3% of soil volume (65% to 70% FC) for B1.

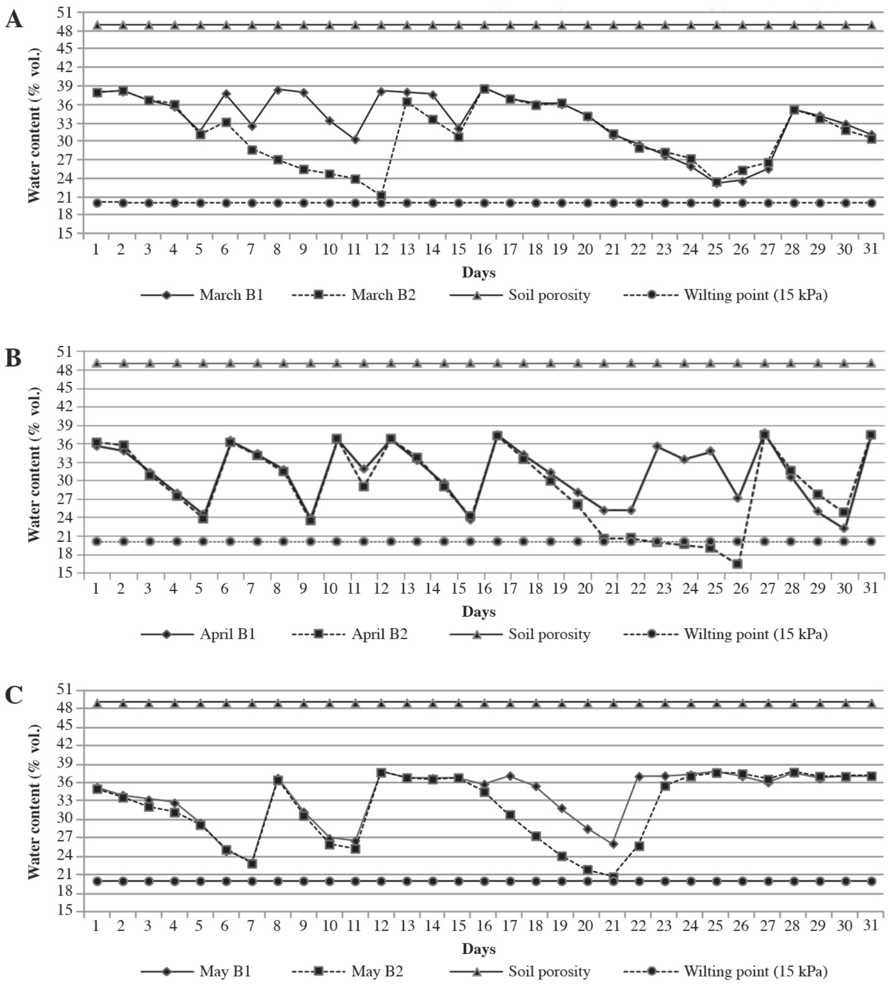


Figure 1. Soil water content in pots at different regimes of soil moisture (B1: control treatment; B2: drought stress treatment). First stress period (A) was from 7 to 12 March at the end of tillering stage, second stress period (B) was from 20 to 25 April in the flag leaf and beginning of heading stage, and third stress period (C) was from 18 to 21 May in grain filling stage.

The experiment was performed in greenhouse in the period from sowing to the beginning of stem elongation stage and after that in the open area near the greenhouse. During the experiment in the greenhouse, temperatures varied from 0.5 to 28 °C and RH from 70% to 99%. In open area temperatures varied from -3.9 to 32.9 °C and RH from 25.8% to 99%. After relocation to the open area, pluviometer was set to measure precipitation. These amounts of rainfall were accounted as a part of water use (WU). From the beginning of stem elongation to the beginning of ear emergence the amount of precipitation was 19.3 mm m^{-2} (1.33 L pot^{-1}) and from ear emergence to full maturity was 65.1 mm m^{-2} (4.5 L pot^{-1}).

Pot trial analysis

Vegetative pot analysis included photosynthetic performance index (PI_{ABS}), biomass weight per pot (total weight of air-dry plants without root) (BWP), harvest index (HI, ratio between grain weight per pot and biomass weight per pot), water use (WU, total water added for each winter barley cultivar from sowing to maturity), grain yield-based water use efficiency (WUE_G) (Viets, 1962; Passioura, 1977; Siddique et al., 1990; Boutraa, 2010; Zhang et al., 2010), and grain yield per pot (GYP).

$$WUE_G = GYP/WU.$$

WUE_G = g L^{-1} (gram of grains per liter of water evapotranspiration).

Stability indices (SI) of photosynthetic performance index (PI_{ABS}) of the cultivars were calculated by formulas for yield (Talebi et al., 2009), with reference to other characters (Kovacevic et al., 2013):

$$SI\ of\ PI_{ABS} = PI_{ABS}B2i/PI_{ABS}B1i$$

where $PI_{ABS}B2i$ is photosynthetic performance index of the i^{th} winter barley cultivar in B2 treatment (drought stress); i is from 1 to 10, $PI_{ABS}B1i$ is photosynthetic performance index of the i^{th} winter barley cultivar in the B1 treatment (well watered).

Evapotranspiration (ET) or water use (WU) for winter barley cultivars and treatments in pots was calculated using the soil water balance model according to Doorenbos and Kassam (1979). Since there was no capillary rise, downward drainage or surface runoff in vegetative pots, evapotranspiration was calculated from the following equation:

$$ET = \Delta W + I + P$$

where WU is evapotranspiration (*ET*) from emergence to maturation, ΔW is the difference in weight between two measurements of soil water content in pots at the time from sowing to maturation (kg pot^{-1}), *I* is irrigation (L pot^{-1}), and *P* is precipitation (L pot^{-1}).

Water use was calculated as addition of water (irrigation and precipitation) in every pot from sowing to maturity and was dependent on barley cultivars and water treatments (B1 and B2). Figure 2 shows water use per cultivar in the different stages of barley growth in B1 and B2 treatments.

Chlorophyll *a* fluorescence was measured both on control and stressed plants at the end of the tillering stage (EC 29) in the morning hours (07:00-09:00 h). At the time of measurement average soil water content in pots was 38.2% volume of soil for B1 and 21.3% for B2 (Figure 1A). Leaves of barley plants were adapted to darkness for 30 min using special plastic clips. The measurement was carried out on the second leaf from the top on three plants per pot by portable fluorimeter (Handy Plant Efficiency Analyser, Handy PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). After the adaptation of leaves to darkness a single one second light pulse (3500 µmol m^{-2} s^{-1}) was applied with the help of three light-emitting diodes (650 nm). The fast fluorescence kinetics (F_0 to F_M) was recorded during 10 µs to 1 s. The measured data were analyzed by the JIP test (Strasser et al., 1995), and used to calculate PI_{ABS} (Strasser et al., 2000) as follows:

$$PI_{ABS} = \frac{1-(F_0/F_M)}{M_0/V_j} \times \frac{(F_M - F_0)}{F_0} \times \frac{1-V_j}{V_j}$$

where F_0 is fluorescence intensity at 50 µs, F_M is maximum fluorescence intensity, M_0 is initial slope of fluorescence kinetics, which is derived from the equation: $M0 = 4\ (F300\mu s - F_0)/(F_M - F_0)$, V_J is relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_0)/(F_M - F_0)$, and F_J is fluorescence intensity at the *J* step (at 2 ms).

The Wricke's ecovalence (W_i) defines the share of each genotype in the sum of squares of the Genotype × Environment interaction (Bujak et al., 2014). In breeding, W_i is used as parameter of cultivar stability, and it is a reliable measure for the assessment of individual genotype adaptation to different environmental conditions. It is highly heritable, and in stable cultivars W_i value is relatively low. Grain yield and ecovalence (W_i) measured in the multi-environmental field trials were used in correlation analyses along with variables from the pot trial (PI_{ABS}, SI of PI_{ABS}, BWP, HI, WU, WUE_G, and GYP).

Statistical analysis

ANOVA was carried out and differences between treatments and cultivars were tested by the Duncan's test ($P \leq 0.05$ and $P \leq 0.01$) using SAS 9.1 statistical software (SAS Institute, Cary, North Carolina, USA). Data were also subjected to correlation analysis using Microsoft Office Excel 2010.

RESULTS

The ANOVA showed that the effect of barley cultivar was highly significant ($P \leq 0.001$) for all of the examined traits in the pot trial: PI_{ABS}, GYP, BWP, HI, WU per pot, and WUE_G (Table 1). Differences among treatments were also highly significant ($P \leq 0.001$), except for HI and WUE_G. Interactions between cultivars and treatments were not significant for all of the examined traits (Table 1).

The PI_{ABS}, WU, WUE_G, HI, and GYP values from the pot trial are shown in Figure 3 and Figure 4a-4d, respectively.

Six-rowed 'A18' had the highest value of PI_{ABS}, while two-rowed 'A12' had the lowest value of PI_{ABS}. Also, values of PI_{ABS} showed that there was no difference between treatments for each cultivar separately, except for 'A18' (Figure 3).

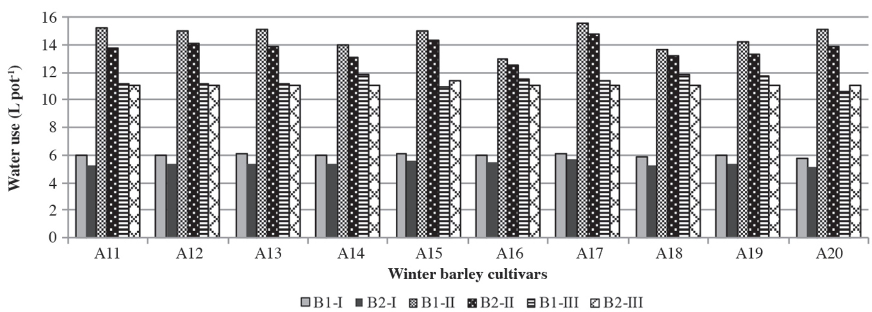


Figure 2. Quantity of water use for ten winter barley cultivars in the growth period from sowing to the end of tillering (B1-I; B2-I), end of tillering to beginning of heading stage (B1-II; B2-II), and beginning of heading to maturation stage (B1-III; B2-III) for well watered treatment (B1) and short term drought stress treatment (B2).

Table 1. The variance and F-test for photosynthetic performance index (PI_{ABS}), harvest index (HI), water use (WU), grain yield based water use efficiency (WUE_G), biomass weight per pot (BWP), and grain yield per pot (GYP) of winter barley cultivars, treatments, and interaction in the trial under control (B1) and in short-term drought stress conditions (B2).

Variance	Mean square (MS)				
Source of variability	Replicate	Cultivar	Treatment	Interaction	Error
Degrees of freedom (df)	2	9	1	9	38
PI_{ABS}	0.11443*	0.11249***	0.26075**	0.01911	0.02933⁺
HI	0.000783	0.011923***	0.000002	0.000904	0.000890
WU	0.1968	14.0136***	45.917***	0.3347	0.2120
WUE_G	0.00804	0.07466***	0.00405	0.005407	0.00603
BWP	4.183	377.100***	504.252***	16.971	14.004
GYP	5.454	92.495***	94.224***	5.111	5.878

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
⁺Error of photosynthetic performance index (PI_{ABS}) has 158 degrees of freedom because there were three measurements per pot.
HI: harvest index, WU: water use, WUE_G: grain yield-based water use efficiency, BWP: biomass weight per pot, GYP: grain yield per pot.

Two-rowed 'A17' and 'A15' had the highest values of WU per pot while six-rowed 'A18' and two-rowed 'A16' had the lowest WU values (Figure 4a). Two-rowed 'A13' had the highest WUE_G, which was highly significant in relation to other barley cultivars, in the pot trial, while two-rowed 'A16' had the lowest WUE_G (Figure 4b). Six-rowed 'A20' and two-rowed 'A11' had the highest HI while two-rowed 'A17' and 'A16' had the lowest HI (Figure 4c).

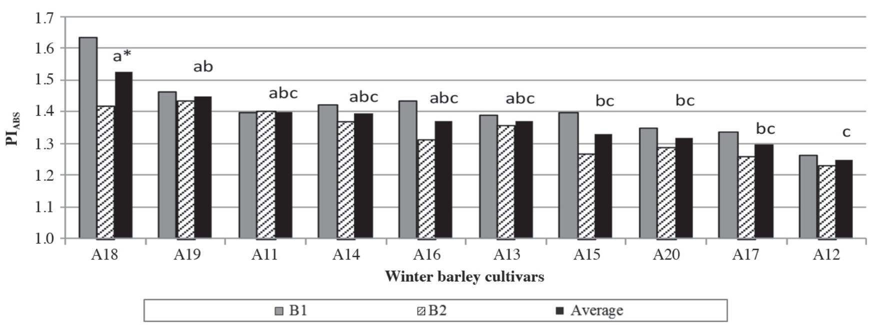
Grain yield per pot ranged similarly to WUE_G (Figures 4b and 4d). The argument for this assessment was a strong positive phenotypic correlation between WUE_G and GYP (r = 0.867 to 0.992) for B1 and B2 treatments (Table 2).

The difference of WU between B1 and B2 treatments for each cultivar was highly significant ($P \leq 0.01$), except for two-rowed 'A15' (Figure 4a). Highly significant GYP differences between treatments were estimated for six-rowed 'A19' (Figure 4d).

There were no significant phenotypic correlation coefficients of PI_{ABS} with BWP, HI and GYP for both treatments (Table 2). However, there was a tendency of positive phenotypic correlations between SI of PI_{ABS} and HI (from 0.411 to 0.584), as well as between SI of PI_{ABS} and GYP (from 0.284 to 0.465) (Table 2). Positive phenotypic correlation coefficients were estimated for WU and biomass weight per pot (BWP) (r = 0.530 to 0.747) in both treatments (B1 and B2). Also, positive phenotypic correlation was estimated between WU and GYP (r = 0.559 to 0.817) (Table 2). WUE_G in both treatments was positively correlated with BWP (r = 0.317 to 0.621), HI (r = 0.361 to 0.708), and GYP (r = 0.867 to 0.992) (Table 2).

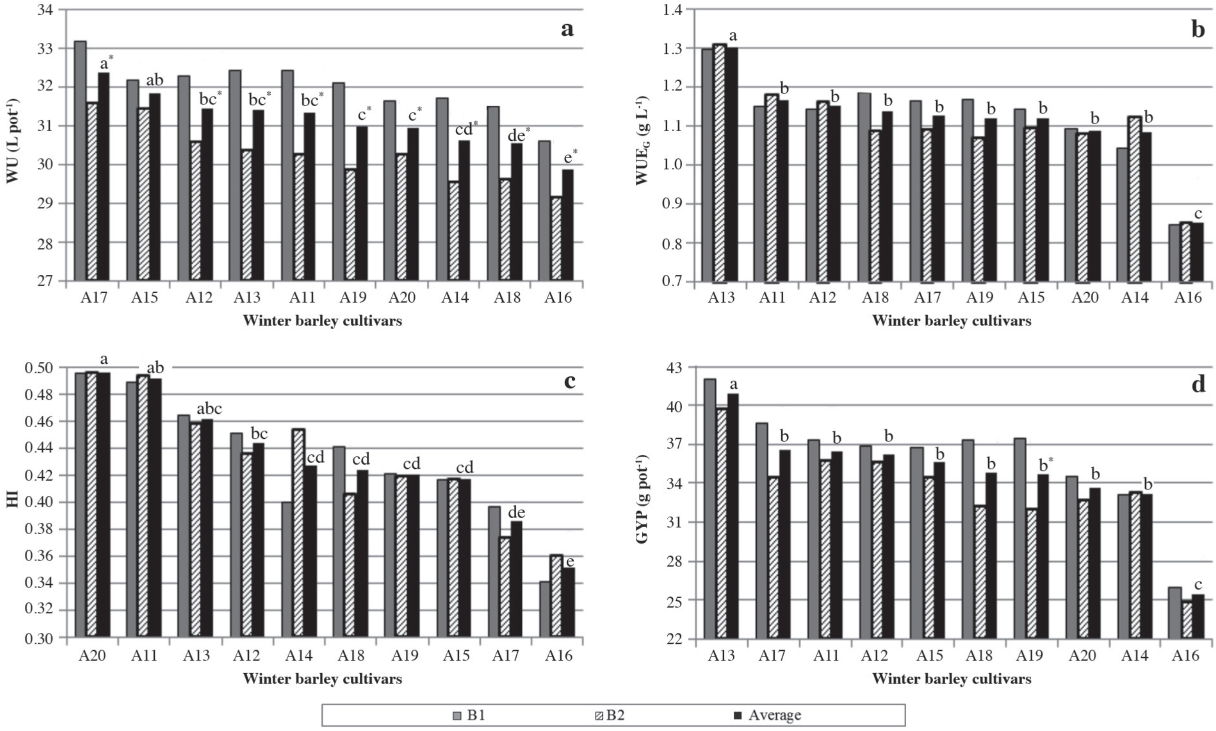
Winter barley cultivars examined in the pot trial were also examined in multi-environmental field trials in the lowland part of the Republic of Croatia during 4 yr (2004-2007, eight cultivars, four locations) and 3 yr (2009-2011, 10 cultivars, five locations) in two sowing densities (300 and 450 seeds m^{-2}) (Lalic et al., 2009; 2012). The parameters of winter barley cultivars from the pot trial were correlated with the average grain yield and W_i of the same cultivars in the multi-environmental field trials (Table 3). Positive phenotypic correlation coefficients were estimated among WU of barley cultivars in the pot trial for B1 and B2 treatments and average grain yield of cultivars in the multi-environmental field trial with eight cultivars (treatment B1: r = 0.925; treatment B2: r = 0.786) and 10 cultivars (treatment B1: r = 0.761; treatment B2: r = 0.825) (Table 3). Also, there was a tendency that winter barley cultivars with higher values of WU had better W_i (r = 0.405 to 0.709) (Table 3).

Similarly, WUE_G of eight barley cultivars in both water treatments of the pot trial was mostly in a high positive correlation with grain yield of the same cultivars in the multi-environmental field trial (treatment B1: r = 0.782; treatment B2: r = 0.823). Also, there was a tendency of positive correlations of WUE_G with grain yield stability (W_i) (r = 0.357 and 0.524 for B1 and B2 treatments respectively) (Table 3). No significant positive correlation coefficient for WUE_G and grain yield was found in the multi-environmental trial with 10 winter barley cultivars (r = 0.486 and 0.348 for B1 and B2 treatments respectively) (Table 3). However, there was also a



Differences between B1 and B2 treatments are calculated for each cultivar separately.
*Only cultivar A18 had significant difference ($P \leq 0.01$). Means with the same letter are not significantly different according to Duncan's test ($P \leq 0.01$).

Figure 3. Relative values of the photosynthetic performance index (PI_{ABS}) in the well watered treatment (B1), short term drought stress treatment (B2), and average of treatments for ten winter barley cultivars in pot experiment.



Duncan's test: Average value means with the same letter are not significantly different ($P \leq 0.01$).
*Differences between B1 and B2 treatment are significant for each cultivar separately ($P \leq 0.01$).

Figure 4. The average values of ten winter barley cultivars for: water use (WU) (a), grain yield-based water use efficiency (WUE_G) (b), harvest index (HI) (c), and grain yield per pot (GYP) (d) in control treatment (B1) and short-term drought stress treatment (B2).

tendency of positive phenotypic correlations between WUE_G and grain yield stability (W_i) (r = 0.358 and 0.697 for B1 and B2 treatments respectively) (Table 3). High significant correlation coefficients also appeared between grain yield per pot (GYP) and eight barley cultivars in the multi-environmental field trial in a similar way as WUE_G (Table 3).

Average grain yield and W_i of the multi-environmental field trials for eight and ten winter barley cultivars in two sowing densities (300 and 450 seeds m^{-2}) are presented in Table 4. Sowing densities of multi-environmental field trials (300 and 450 seed m^{-2}) did not have a significant impact on all estimated correlation coefficients (Table 3) nor the grain yield (Table 4).

DISCUSSION

Results of this paper coincide with examination of Akhter et al. (2008) and Shamsi et al. (2010). They pointed out higher positive correlation coefficients between WUE_G and grain yield of wheat cultivars. Blum (2005; 2009) suggested that high water use efficiency of plant genotypes could reduce transpiration and water use, which could be crucial for plant production and reduction of yield. These statements are confirmed in the results of this study because WU of the examined barley cultivars was also positively correlated with GYP (Table 2). Furthermore, WU had a significant correlation with grain yield of eight and ten barley cultivars in the multi-environmental field

Table 2. Phenotypic correlation coefficients for photosynthetic performance index (PI_{ABS}), stability index of PI_{ABS} (SI of PI_{ABS}), water use (WU), and grain yield-based water use efficiency (WUE_G) of different winter barley cultivars, with biomass weight per pot, harvest index, and grain yield per pot of cultivars in control treatment (B1), short-term drought stress treatment (B2), and average of treatments.

Correlated variables	Biomass weight per pot			Harvest index			Grain yield per pot		
	B1	B2	Average	B1	B2	Average	B1	B2	Average
PI_{ABS} -B1	0.043	-0.153	-0.058	-0.155	-0.232	-0.198	-0.077	-0.327	-0.199
PI_{ABS}-B2	-0.004	-0.256	-0.134	0.123	0.186	0.159	0.123	-0.038	0.048
SI of PI_{ABS}	-0.077	-0.067	-0.073	0.411	0.584	0.511	0.284	0.465	0.379
WU-B1	0.647	0.731	0.705	0.361	0.185	0.287	0.803	0.789	0.817
WU-B2	0.530	0.686	0.622	0.212	-0.017	0.107	0.574	0.559	0.581
WUE_G-B1	0.536	0.621	0.592	0.657	0.361	0.535	0.992	0.881	0.964
WUE_G-B2	0.317	0.460	0.398	0.708	0.617	0.688	0.867	0.978	0.943

$r \geq 0.632$ $P \leq 0.05$; $r \geq 0.765$ $P \leq 0.01$; $r \geq 0.872$ $P \leq 0.001$.

Table 3. Phenotypic correlation coefficients of PI_{ABS}, Wricke's ecovalence (W_i), stability index (SI) of PI_{ABS}, water use (WU), grain yield-based water use efficiency (WUE_G), and grain yield per pot (GYP) of 10 winter barley cultivars tested in pot trial (B1: control treatment; B2: drought stress treatment) with average grain yield of the same winter barley cultivars from multi-environmental field trials with two sowing densities (350 and 450 seeds m^{-2}).

Parameters on the basis of pot trial	Trial with eight cultivars: Sowing density (seeds m^{-2}) 300	450	Average	W_i	Trial with ten cultivars: Sowing density (seeds m^{-2}) 300	450	Average	W_i
PI_{ABS}-B1	-0.458	-0.458	-0.459	-0.357	-0.433	-0.675	-0.568	-0.600
PI_{ABS}-B2	-0.205	-0.205	-0.207	-0.167	-0.425	-0.541	-0.494	-0.358
SI of PI_{ABS}	0.454	0.450	0.451	-0.262	0.155	0.366	0.269	0.370
WU-B1	0.922	0.926	0.925	0.405	0.720	0.773	0.761	0.709
WU-B2	0.775	0.792	0.786	0.500	0.791	0.828	0.825	0.685
WUE_G-B1	0.776	0.791	0.782	0.357	0.506	0.447	0.486	0.358
WUE_G-B2	0.808	0.839	0.823	0.524	0.307	0.370	0.348	0.697
GYP-B1	0.812	0.827	0.819	0.228	0.568	0.529	0.559	0.419
GYP-B2	0.865	0.896	0.881	0.571	0.451	0.519	0.497	0.830

$r \geq 0.707$ $P \leq 0.05$; $r \geq 0.834$ $P \leq 0.01$; $r \geq 0.925$ $P \leq 0.001$ for trial with eight cultivars.
$r \geq 0.632$ $P \leq 0.05$; $r \geq 0.765$ $P \leq 0.01$; $r \geq 0.872$ $P \leq 0.001$ for trial with ten cultivars.

trials (Table 3). These results suggest that high correlation of WU and WUE_G with GYP of barley cultivars can be used as a selection criterion for breeding on drought tolerance. High WU can also be characteristic of drought intolerant genotypes if measured in strong long-term drought conditions. In addition, WUE_G could be a good indicator of rational WU by different winter barley cultivars.

Likewise, the results of this study also demonstrated that high WUE_G is a characteristic of high yielding winter barley cultivars like two-rowed winter barley 'A11', 'A12', and 'A13'. However, winter barley 'A17', which had the highest grain yield in the multi-environmental trials (Table 4), had the highest WU values (Figure 4a), while WUE_G was at the level of average values of all cultivars in the trial (Figure 4b). Results of this study also confirm that higher WU is a characteristic of high yielding winter barley cultivars. This characteristic of barley cultivars could be an indicator for better absorption of nutritive elements and water from soil, which is important for all physiological processes. Stomatal conductance causes leaf cooling through transpiration and thus has important impact in plant's heat stress tolerance (Reynolds et al., 1994; Vilhelmsen et al., 2001; Tuberosa, 2012; Siddiqui et al., 2014). Consequently, results of Radin et al. (1988) and Bernacchi et al. (2006; 2007) confirmed interdependence of transpiration and drought tolerance with positive correlations of WU with stomatal conductance and photosynthetic rate.

Photosynthetic performance index can be very suitable and sensitive parameter to investigate plant's overall photosynthetic efficiency under different abiotic stresses (Appenroth et al., 2001), but many authors confirm that plant's first reaction to drought stress is increase in photosynthetic efficiency parameters and thus performance index as well (Shao et al., 2005; Huseynova et al., 2010; Balouchi, 2010; Kovacevic et al., 2013).

This reaction is linked to defensive response of plant's photosynthetic apparatus on mild and moderate drought stress conditions that accurs in early stages of growth (Shao et al., 2008; Liu et al., 2010).

Negative correlations of PI_{ABS} with yield parameters indicate that barley cultivars tested in pot trial were subjected only to mild short-term drought stress conditions which did not have significant impact on photosynthetic efficiency of barley cultivars. However, stability index of PI_{ABS} (relation between PI_{ABS} of stressful and PI_{ABS} of well watered treatment for each cultivar) was in low positive correlation with grain yield of cultivars in pot trial and in both multi-environmental trials (Tables 2 and 3). Similar, but more expressed connections were estimated for winter wheat cultivars (Kovacevic et al., 2013). Inostroza et al. (2015) reported the low correlation between single physiological traits and DM production under drought conditions for 100 *Lotus tenuis* genotypes, but the combination of physiological traits in multi-physiological indices may be effective for selection of drought-tolerant genotypes.

Hejnák et al. (2011) suggest the use of photosynthesis and transpiration ratio as water use efficiency (WUE) for determination of water management capabilities of individual spring barley genotypes, especially under stress

Table 4. Average grain yield and Wricke's ecovalence (W_i) of the multi-environmental field trials for eight (2004-2007) and ten (2009-2011) winter barley cultivars in two sowing densities (300 and 450 seeds m^{-2}).

Cultivars	Grain yield 2004-2007 (t ha^{-1}) for different sowing densities (seeds m^{-2}): 300	450	Average	W_i	Grain yield 2009-2011 (t ha^{-1}) for different sowing densities (seeds m^{-2}): 300	450	Average	W_i
A12	7.410a	7.310a	7.360a	9.88	6.532b	6.707b	6.620b	1.68
A11	7.330ab	7.340a	7.335a	7.69	6.544b	6.654b	6.599b	1.16
A13	7.140ab	7.290a	7.215ab	4.72	6.368b	6.687b	6.527b	2.77
A15	7.030b	7.080a	7.055b	4.47	6.372b	6.512bc	6.442bc	2.88
A19	6.550c	6.480b	6.505c	16.41	6.216bc	6.255cd	6.236cd	9.42
A18	6.540c	6.460b	6.510c	7.81	6.227bc	5.836e	6.055de	4.35
A16	5.570e	5.550c	5.560e	38.49	5.911cd	5.954de	5.933e	10.81
A14	6.210d	6.190b	6.200d	6.55	5.643d	5.713e	5.678f	16.48
A17					7.059a	7.366a	7.213a	3.40
A20					6.477b	6.631b	6.554b	3.42

Duncan's test. Means with the same letter are not significantly different ($P \leq 0.05$) for average grain yield.
W_i: Lower values represent cultivars with higher stability.

conditions. Further research, to estimate the connection between the photosynthetic performance index (PI_{ABS}) measured in the early stage of growth, and WU, WUE, grain yield, and grain yield stability in field conditions, would be useful for breeding on stress tolerance.

Parameters of WU and WUE_G could also be used as the last phase in testing of genotypes that already showed yield and quality potential in field trials, because they are much more cost effective methods for selection of drought tolerant genotypes as compared to multi-environmental and multi-annual field trials. Possibility of testing larger number of genotypes increases if we only use stress treatment to obtain necessary data, and acknowledged cultivars as standards to compare with.

CONCLUSIONS

Cultivars 'A11', 'A12', 'A13', 'A15', and 'A17' with higher values of grain yield based water use efficiency (WUE_G) also had higher grain yield in both multi-environmental field trials. Taking into consideration high significant positive correlation coefficients between WUE_G and grain yield per pot (GYP), it is possible that this parameter could indicate genotypes with potential for drought tolerance. Values of photosynthetic performance index (PI_{ABS}) could not be connected with agronomic traits of grain yield, harvest index and biomass weight per pot, suggesting that barley cultivars were under low intensity drought stress conditions that had no influence on photosynthetic efficiency of tested barley cultivars. There are different approaches for defining water use efficiency by genotype, e.g. WUE for grain yield and WUE for biomass production. However, water use (WU) and WUE_G could be good indicators for preliminary selection of modern, high yielding and stable winter barley genotypes with better water management capabilities. Taking all into consideration, pot trials cannot substitute for multi-environmental field trials, but could be implemented as effective method for preliminary selection of desirable traits among numerous genotypes.

ACKNOWLEDGEMENTS

This paper is a part of the project entitled "Stress physiology and agricultural characteristics of wheat and barley cultivars" (073-073164-0552). The authors are grateful to the Ministry of Science, Education and Sport of the Republic of Croatia for the project support.

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