Grain filling parameters of two- and six-rowed barley genotypes in terminal drought conditions

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Abstract: *After the kernel number per spike has been formed during the vegetative stage, grain weight depends on the rate and duration of grain filling. Drought and high temperatures during the grain filling stage frequently occurs in the majority of barley growing regions worldwide. To investigate impact of terminal drought stress on grain filling parameters and grain yield, 15 two- and 10 six-rowed barley genotypes were tested in two-year field trials, set in two locations with two treatments. One treatment was control (C), while in the other treatment (D) terminal drought was simulated by mechanical removal of all leaf blades 7 days after heading of each genotype. In average, defoliation caused reduction of yield (33%), grain filling rate (12%) and 1000-kernel weight (11%). In both treatments, grain yield and the absolute grain filling rate were higher in two-rowed barley genotypes than in six-rowed ones. In both treatments, the correlation was stronger between yield and grain filling duration than between yield and the grain filling rate. The correlation between average yield and grain filling duration was stronger in the D than in the C treatment. According to the results obtained for terminal drought conditions, breeders should create early maturing genotypes of two-rowed type, with a longer grain filling duration and the gradual accumulation of dry matter.*
Keywords: *Barley type, defoliation, grain filling, grain yield, terminal drought.*

Riassunto: *Mentre il numero di cariossidi per spiga si determina durante la fase vegetativa, il loro peso dipende dal tasso e dalla durata della fase di riempimento della granella. La siccità e le alte temperature durante la fase di riempimento della granella si verificano frequentemente nella maggior parte delle regioni in cui l'orzo viene coltivato, causando fenomeni di stress idrico. Per studiare l'impatto di tale stress sui parametri di riempimento e sulla resa, sono stati testati 15 genotipi di orzo a due ranghi e 10 a sei ranghi in prove sperimentali in campo per due anni, in due luoghi e con due trattamenti. Un trattamento era il controllo (C), mentre nell'altro trattamento (D) è stata simulata una condizione di siccità mediante rimozione meccanica delle foglie 7 giorni dopo la fase di spigatura. In media, la defogliazione ha causato una riduzione della resa (33%), del tasso di riempimento della granella (12%) e del peso dei 1000 semi (11%). In entrambi i trattamenti, la resa in granella e il tasso assoluto di riempimento della granella erano più alti nei genotipi di orzo a due ranghi che in quelli a sei. In entrambi i trattamenti, la correlazione era più marcata tra la resa e la durata di riempimento della granella rispetto alla resa e al tasso di riempimento della granella. La correlazione tra resa media e durata di riempimento della granella era più forte nel trattamento D che nel trattamento C. In base ai risultati ottenuti, sarebbe opportuno produrre dei genotipi a due ranghi a maturazione precoce, con una durata di riempimento della granella più lunga e un accumulo graduale di sostanza secca.*
Parole chiave: *Varietà di orzo, defogliazione, riempimento della granella, resa, siccità terminale.*

1. INTRODUCTION

Drought is one of the most important environmental stresses that reduces barley grain yield (Forster, 2004), depending not only of the duration and intensity of water stress, but also on the developmental phase at which stress occurs (Szira *et al.*, 2008; Martiniello and Teixeira da Silva, 2011). Moreover, drought and heat stress during terminal stages of the barley growth cycle limit its productivity world-wide through the interruption of carbohydrate supply from the source organs, which leads to reduced biomass accumulation, a large number of aborted flowers and grains, and reduced weight of the individual grains and the seed size (Setter *et al.*, 2001; Boyer and Westgate, 2004, Prasad *et al.*, 2008; Rajala *et al.*, 2011; Seiler *et al.*, 2011; De Storme and Geelen, 2014; Stratonovitch and Semenov, 2015). Several authors reported a significant decrease of barley yield under terminal drought conditions ranging from 27 to 41% (Zare *et al.*, 2011, Gonzales *et al.*, 2010, Przulj and Momcilovic, 2012). Individual grain weight can be considered as a result of two growth parameters: grain filling rate and grain filling duration, and from the physiological aspect these two parameters explain two different phenomena (Voltas *et al.*, 1999). Drought following flowering is known to have little effect on grain filling rates, but the grain filling duration is shortened leading to a small grain size or lower grain yield (Wardlaw and Willenbrink, 2000),

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while Sanchez *et al.*, (2002) reported that even grain filling rate had been reduced by 40%.

As abiotic stresses are unpredictable, the best way to cope with them is to develop tolerant varieties that perform well under stress and under optimum environments (Prasad *et al.*, 2008; Nouri *et al.*, 2011; Hossain and Teixeira da Silva, 2012). Selecting different genotypes under environmental stress conditions is one of the main tasks of plant breeders for exploiting the genetic variations to improve the stress tolerant cultivars (Talebi *et al.*, 2009, Lopes *et al.*, 2012). Agronomic traits such as grain yield and its components are the major selection criteria for evaluating drought tolerance under field conditions (Lopes *et al.*, 2012, Fayaz *et al.*, 2011). Regarding the barley row type, there are different results concerning grain yield in stress environments of the Mediterranean region. For example, Samarah *et al.*, (2009), Bavei *et al.*, (2011), Vaezi *et al.*, (2010) obtained that under late drought and heat conditions in the semiarid Mediterranean six-rowed genotypes achieved better yield than two-rowed genotypes. On the other side, Garcia del Moral *et al.* (2003) reported where terminal drought and large inter-annual variation are present, the yield of two-rowed barley proved to be more responsive to environmental variations, while six-rowed barley had more stable behaviour.

Terminal drought during grain filling becomes more common in Mediterranean environments. Thus, the objectives of this study were to: i) compare 2- and 6-rowed barley genotypes in terms of grain filling parameters under non-stress and terminal drought conditions and ii) investigate the relationship between grain filling parameters and the yield and yield components.

2. MATERIAL AND METHODS

The experimental material consisted of 25 winter barley genotypes (15 two-rowed and 10 six-rowed), including cultivars and breeding lines (Tab. 1). Genotypes were grown in two experimental sites (5km apart), in north Serbia, Zemun

	Genotype	Status	Row type	Origin	Earliness
1	NS 565	C	2	NS, RS	early
2	Rekord	C	2	KG, RS	mid- early
3	NS 519	C	2	NS, RS	early
4	Bingo	C	2	OS, HR	early
5	Nectaria	C	2	SECR, FR	mid- early
6	Maksa	C	2	KG, RS	early
7	ZP 12/I	L	2	ZP, RS	early
8	Boreale	C	2	SECR, FR	mid- early
9	Nektar	C	2	ZP, RS	mid- early
10	Vanessa	C	2	SJB, DE	late
11	PKB Pivan	C	2	PKB, RS	early
12	IBSP/04-22	L	2	ICARDA, SY	mid- early
13	Jagodinac	C	2	KG, RS	mid- early
14	Kristal	C	2	ZA, RS	early
15	NS 525	C	2	NS, RS	mid early
16	ZP 34/II	L	6	ZP, RS	early
17	Leotar	C	6	NS, RS	late
18	Sremac	C	6	NS, RS	late
19	ZP 33/II	L	6	ZP, RS	late
20	Grand	C	6	KG, RS	early
21	ZP 154/II	L	6	ZP, RS	mid- early
22	NS 313	C	6	NS, RS	late
23	Nonius	C	6	NS, RS	mid- early
24	Ozren	C	6	NS, RS	mid- early
25	Atlas	C	6	NS, RS	mid- early

C- cultivar; L-breeding line; ZP- Maize Research Institute, "Zemun Polje", Belgrade; NS - Institute of Field and Vegetable Crops, Novi Sad; PKB - Agroekonomik, Belgrade; KG - Small Grains Research Centre, Kragujevac; ZA - Centre for Agricultural and Technological Research, Zajecar; OS - Agricultural Institute, Osijek; SECR- SECOBRA Recherches S.A., Centre de Bois-Henry, Maule; SJB- Saatzucht Josef Breun GmbH & Co, Herzogenaurach, ICARDA - The International Centre for Agricultural Research in the Dry Areas, Aleppo

Tab. 1 - Name, row type, origin and earliness of 15 two-rowed and 10 six-rowed barley genotypes.
Tab. 1 - Nome, tipo di rango, origine e precocità di 15 genotipi di orzo a due ranghi e 10 a sei ranghi.

Season	Month	Temperature (°C) Aver.	Max	Min	Days > 30°C	Precipitation (mm)	Relative humidity (%)
2010-2011	Nov.–Feb.	4,8	7,5	0,9	0	190,4	80,5
	March	8,0	11,9	2,2	0	18,6	70,2
	April	14,4	19,1	7,6	0	14,1	58,5
	May	17,5	22,6	11,2	0	94,8	68,5
	June	22,2	27,3	15,4	7	23,0	63,3
Aver. (March.–June)		15,5	20,2	9,1	7,0	150,5	65,1
2011-2012	Nov.–Feb.	2,2	5,2	-0,7	0	168,2	79,5
	March	8,9	15,3	1,7	0	2,5	55,3
	April	13,5	19,3	7,7	0	73,3	65,2
	May	17,3	22,7	11,5	1	81,8	70,6
	June	24,3	29,9	16,4	14	16,1	56,4
Aver. (March–June)		15,9	21,8	9,3	15,0	173,3	61,9

Tab. 2 - Meteorological data for two barley cycles, Zemun Polje, Serbia.
Tab. 2 - Dati meteorologici per due cicli vegetativi di orzo, Zemun Polje, Serbia.

Polje - ZP (44°86'N20°33E) and Skolsko dobro - SD (44°86'N20°28E), during two growing seasons: 2010-2011 and 2011-2012. The experimental design was a randomized block with two replications. Each plot consisted of five 1-m long rows, spaced at 20 cm, with a typical seeding rate of 350 seeds m^{-2}. Genotypes were sown in late October and harvested in late June of the following year. The plots were irrigated manually from early March (beginning of tillering) to the end of April (beginning of heading) when water in the top 0.75 m of soil had declined below 50% of field capacity. Standard cropping practices were applied to provide adequate nutrition and keep plots free of pests and diseases. Summarized meteorological data for winter barley crop cycle in spring (March – June) is provided in Tab. 2. Daily maximum, minimum and mean temperature, number of days with maximum temperature over 30°C, precipitations, relative air humidity and sunshine hours/day were recorded from the nearest weather station of the Republic Hydrometeorological Service of Serbia (5 km southwest of experimental site). Average daily mean temperatures during grain filling (May and June) were 19.9°C (2011) and 20.8°C (2012), while the number of days with the maximum temperature of over 30°C in the May-June period was 7 (2011) and 15 (2012). Higher temperatures in 2012 were combined with a lower sum of precipitation during grain filling (98 mm in 2012 vs 118 mm in 2011).

The intact plants (control, C) represented the first treatment, while the physical removal of all leaf blades, performed seven days after heading of each genotype, represented the second treatment (defoliation, D). Through the inhibition of current photosynthesis (as a result of defoliation), the treatment simulated drought conditions during grain filling (Blum *et al.*, 1983a,b). The technique has been used successfully in wheat to breed for improved grain filling under terminal drought (Blum *et al.*, 1991). Spike sampling was initiated at the moment of defoliation, and continued at 5 day interval until full maturity (a total of nine samples were completed per each plot). Five uniform spikes per plot were collected from the center of the plot at each sampling and then dried at 105ºC for 24 hours on each fifth day. After drying, 15 and 20 kernels from the central part of the spike were used to measure the grain filling parameters in two- and six-rowed barley, respectively (Tab. 3). Grain yield (YLD), kernel number per spike (KN) and thousand kernel weight (TKW) were calculated at full maturity for each plot. The parameter days to heading (DH) was estimated from 1st January to the moment when 50% of main stems in a plot had at least half of emerged ears.

To evaluate significant differences between genotypes, treatments and environments (year-location combination), the three-way analysis of variance (ANOVA) was performed. The significance of differences between the C and D treatment and between two- and six-rowed barley was carried out by the t-test. The differences between genotype means of grain filling parameters were tested by the Tuckey test. Letter groupings were generated by using a 5% level of significance. The broad sense heritability was estimated from the ANOVA table by calculating the variance components after Hallauer

Grain filling parameter	Acronym	Measurement units	References	Equations
The maximum grain weight	MGW	mg		Estimated on the basis of single grain weight at different sampling points
The grain filling rate	GFR	mg 100 GDD day-1		GFR= final grain dry weight/ GFD
The grain filling duration	GFD	°C		Accumulated GDD from anthesis
Growing degree days-summation of daily degree days (Tn)	GDD	°C		Tn = ((Tmax + Tmin)/2) – Tb)
The absolute intensity of grain filling rate	AFI	mg day^{-1}	Radford, 1967	$AFI = \frac{W_2 - W_1}{T_2 - T_1}$
The maximum grain filling rate	MFR	mg 100 GDD day^{-1}	Brdar et al., 2004	Estimated on the basis of AFI for each genotype
The relative grain filling rate	EF	mg mg^{-1} day^{1}	Hunt, 1978	$EF = \frac{LnW_2 - LnW_1}{T_2 - T_1}$

Tn = daily degree day, Tmax = maximum daily temperature, Tmin = minimum daily temperature, Tb = base temperature (0°C); W1 and W2= dry weights of sample at time T1 and T2, respectively.

Tab. 3 - List of calculated grain filling parameters.
Tab. 3 - Elenco dei parametri di riempimento della granella considerati.

			YLD	KN	TKW	MGW	GFD	GFR	MFR
2011	2-row	D	5706^{a}	28.2^{a}	46.8^{a}	51.0^{a}	845^{a}	0.0609^{a}	2.77^{a}
		C	8239^{b}	27.9^{a}	52.3^{b}	56.7^{b}	822^{a}	0.0694^{b}	3.11^{b}
		D/C	0.69	1.0	0.89	0.90	1.02	0.87	0.89
	6-row	D	4704^{a}	57.4^{a}	39.1^{a}	42.5^{a}	861^{a}	0.0497^{a}	2.27^{a}
		C	7432^{b}	57.6^{a}	44.1^{b}	47.1^{b}	830^{a}	0.0572^{b}	2.5^{b}
		D/C	0.63	0.99	0.88	0.90	1.03	0.87	0.91
2012	2-row	D	4853^{a}	24.1^{a}	42.4^{a}	45.5^{a}	845^{a}	0.0542^{a}	2.85^{a}
		C	6599^{b}	23.9^{a}	46.8^{b}	51.4^{b}	875^{b}	0.0591^{b}	3.36^{b}
		D/C	0.73	1.0	0.90	0.88	0.96	0.91	0.84
	6-row	D	3204^{a}	48.9^{a}	35.0^{a}	37.0^{a}	837^{a}	0.0444^{a}	2.47^{a}
		C	5241^{b}	49.6^{a}	39.4^{b}	43.5^{b}	850^{a}	0.0517^{b}	3.03^{b}
		D/C	0.61	0.98	0.89	0.85	0.98	0.86	0.81
2011/2012	2-row	D	5279^{a}	26.2^{a}	44.6^{a}	48.3^{a}	845^{a}	0.0575^{a}	2.81^{a}
		C	7419^{b}	25.9^{a}	49.6^{b}	54.0^{b}	849^{a}	0.0642^{b}	3.23^{b}
		D/C	0.71	1.0	0.90	0.89	0.99	0.89	0.87
	6-row	D	3954^{a}	53.2^{a}	37.1^{a}	39.8^{a}	849^{a}	0.0470^{a}	2.37^{a}
		C	6337^{b}	53.6^{a}	41.7^{b}	45.3^{b}	840^{a}	0.0543^{b}	2.76^{b}
		D/C	0.62	0.99	0.89	0.88	1.0	0.86	0.86
Aver. across treatm.		D	4617^{a}	39.7^{a}	40.8^{a}	44.1^{a}	847^{a}	0.0523^{a}	2.59^{a}
		C	6878^{b}	39.8^{a}	45.6^{b}	49.6^{b}	845^{a}	0.0593^{b}	2.99^{b}
		D/C	0.67	0.99	0.89	0.88	1.0	0.88	0.86

Values of the same trait followed by the same letter are not significantly different at the 0.05 probability level. C - control, D - terminal drought condition, YLD - grain yield, KN - kernel number, TKW - thousand-kernel weight, MGW- maximum grain weight, GFD - grain filling duration, GFR - grain filling rate, MFR- maximum absolute grain filling rate.
Valori dello stesso tratto seguiti dalla stessa lettera non sono significativamente diversi al livello di probabilità di 0.05. C-controllo, D – condizione di siccità terminale, YLD – resa, KN – numero di cariossidi, TKW – peso dei 1000 semi, MGW – peso massimo della granella, GFD – durata di riempimento della granella, GFR – tasso di riempimento della granella, MFR – tasso di riempimento della granella massimo assoluto.

Tab. 4 - Mean values of 2- and 6- row barley genotypes in control (C) and stress conditions (D) during 2011 and 2012, across the years and across the treatments.
Tab. 4 - Valori medi per i genotipi a 2 e 6 ranghi nel controllo (C) e in condizioni di stress (D) durante il 2011 e il 2012, tra località e anni.

et al., (2010). In order to visually display relations of observed traits and genotypes multivariate biplot analysis (genotype by trait biplot), described by Yan and Rajcan, (2002), was used. A positive correlation between two traits is represented by an acute angle between them, while obtuse angle represents a negative correlation. Separate biplots were constructed for C and D treatments. All computations were accomplished within the Minitab 17 software package and for data visualizations R 2.9.0 program was used (R Development Core Team 2010).

3. RESULTS AND DISCUSSION

The results showed that defoliation, i.e. stress caused by the removal of all leaf blades significantly ($P<0.05$) influenced all the traits except GFD and KN (Tab. 4). The greatest average reduction due to defoliation was recorded for YLD (33%), followed by MFR (14%), GFR (12%), MGW (12%) and TKW (11%). Other authors also reported significant yield decrease (24-35%) in barley under post anthesis drought stress (Gonzales *et al.*, 2010, Zare *et al.*, 2011, Pureisa *et al.*, 2013). Also, the reduction of TKW by 11%, was similar to that of 8.2% (Fard *et al.*, 2013) and a little bit lower than 22.1% (Zare *et al.*, 2011) reported for barley under terminal drought. In this study, GFR in D was lower by 12% than in C, while Gonzales *et al.*, (2008), investigating barley genotypes in well-watered *vs*. terminal drought conditions, reported the decrease in the grain filling rate by 20%. The mentioned comparisons with other authors suggest that we have achieved a reliable simulation of terminal drought by defoliation in terms of quantitative traits reduction.

Yield reduction in first season due to defoliation treatment was higher in six-rowed than in two-rowed barley (37 *vs*.31%, respectively). In the second season the difference in yield reduction between six-rowed and two-rowed barley was even higher (39% *vs*. 27%, respectively). Also, while reduction in GFR in two-rowed and six-rowed barley was similar in the first season (about 13%), in the second season six-rowed barley had higher GFR reduction compared to two-rowed barley (14% *vs.* 9%, respectively). Other traits had similar reduction for two-rowed and six-rowed barley in both seasons. The second season can be regarded more unfavourable for grain filling and final yield than the first season as it combined lower precipitations and higher temperatures after flowering. This situation is under future climate scenarios in agricultural areas subjected to Mediterranean weather patterns (Trnka *et al.*, 2011; Morgunov *et al.*, 2013). These results indicate the two-row barley would have advantage over six rowed barley in future climate change.

The three factorial ANOVA (Tab. 5), showed that the effects of genotype (G), treatment (T) and year-location combination (E) was significant ($P < 0.01$)

Trait		G	T	E	G x T	G x E	T x E	G x T x E
YLD	F-test	**	**	**	**	**	**	**
	σ^2 (%)	16.8	27.3	25.0	2.6	16.3	0.8	6.8
KN	F-test	**	ns	**	ns	**	*	ns
	σ^2 (%)	93.7	0.0	4.2	0.0	1.2	0.0	0.2
TKW	F-test	**	**	**	**	**	**	ns
	σ^2 (%)	63.4	12.5	15.4	1.1	2.9	0.4	1.1
MGW	F-test	**	**	**	**	**	**	ns
	σ^2 (%)	61.7	13.6	13.6	1.2	2.9	1.3	1.5
GFD	F-test	*	ns	**	**	**	**	**
	σ^2 (%)	5.9	0.0	5.4	7.6	21.2	5.6	24.8
GFR	F-test	**	**	**	**	**	**	**
	σ^2 (%)	52.5	10.7	14.0	2.3	5.4	0.6	5.6
MFR	F-test	**	**	**	ns	*	**	ns
	σ^2 (%)	29.0	8.3	3.3	2.5	13.3	3.8	12.4
Aver.	σ^2 (%)	46.1	10.3	11.6	2.5	9.0	1.8	7.5

** indicate significance at the 0.01 probability level; * indicate significance at the 0.05 probability level; ns - not significant; YLD - grain yield, KN - kernel number, TKW - thousand kernel weight, GFD - grain filling duration, GFR - grain filling rate.
*** indica la significatività al livello di probabilità 0,01; * indica la significatività al livello di probabilità 0,05; ns: non significativo; YLD - resa in granella, numero di cariossidi KN, TKW - peso dei 1000 semi, GFD - durata di riempimento della granella, GFR - tasso di riempimento della granella.*

Tab. 5 - The three factorial ANOVA for yield, agronomic traits and grain filling parameters of 25 barley genotypes.
Tab. 5 - ANOVA fattoriale per la resa, i caratteri agronomici e i parametri di riempimento della granella dei 25 genotipi d'orzo.

for all the traits, except KN and GFD. Several other studies (Naghaii and Asgharipour, 2011; Zare *et al.*, 2011; Noshadifard and Zare, 2012) also showed that drought-induced stress after the flowering of barley, significantly influenced the yield and 1000 kernels weight. On average, the most significant influence on the variability of the investigated traits had the factor genotype (46.1%), followed by environment (11.6%) and treatment (10.3%). The influence of the interactions (G x T, G x E and T x E and G x T x E) in average was weaker than the influence of the main factors. The highly significant influence of G x T interaction on YLD, TKW, MGW, GFD and GFR indicate that there was a difference among the investigated genotypes in their response to stress caused by the defoliation.

To evaluate 25 barley genotypes for the intensity of grain filling in control and defoliation conditions, the absolute (AFI) and relative (EF) intensity of the grain filling is calculated (Figg. 1 and 2, respectively). The highest AFI in two-rowed genotypes, in both treatments, occurred between the 17th and the 22nd day after heading. The highest increase of the grain filling intensity in six-rowed genotypes in the C treatment was in the period from the 22nd to the 27th day, but in terms of defoliation ten days earlier, between the 12th and the 17th day after heading. Hence, the absolute grain filling increase occurrence was more stable between treatments in two-rowed than in six-rowed genotypes.

There were no significant differences in AFI between two-barley types in both treatments until 27th day after heading. On the 37th day after heading, the differences were most noticeable. In the C treatment, for both types of barley AFI was significantly more intense ($P < 0.05$) than in the D treatment in which ongoing assimilation was reduced by the removal of leaf blades. The difference in AFI was statistically significant ($P < 0.05$) in favour of two-rowed genotypes in both treatments. At the final stage, on the 47th day after heading, two-rowed barley genotypes under control conditions continued to have significantly ($P < 0.05$) higher AFI in relation to stress conditions, but also in relation to six-rowed forms. Overall, the results are consistent with those obtained by Ho and Jui, (1989) that the grain filling rate was higher in two-rowed barley genotypes than in to six-rowed barley genotypes under both stress and non-stress conditions. Comparing with six-rowed genotypes, they reached maximum values of AFI later and grain filling process lasted longer.

The relative intensity of the grain filling (Hunt, 1990) is a parameter which is interpreted as an index of the efficiency of filling rate (EF), (mg/mg

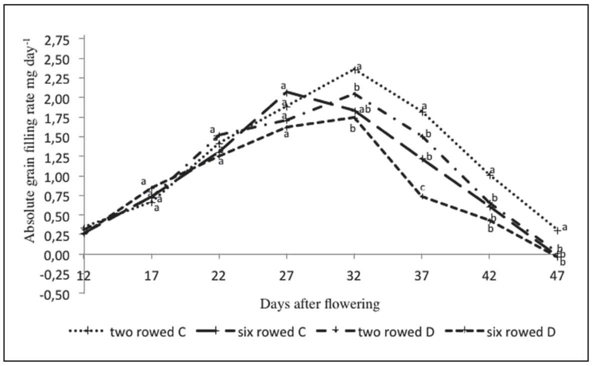

Fig. 1 - The mean values of the absolute intensity of the grain filling rate of 25 two- and six-rowed barley genotypes in the control (C) and the stress treatment (D) across locations and years. Values at the same day followed by the same letter are not significantly different at the 0.05 probability level.

Fig. 1 - Valori medi dell'intensità assoluta del tasso di riempimento della granella di 25 genotipi di orzo a 2 e 6 ranghi nel controllo (C) e nel trattamento con stress (D) nelle diverse località nei due anni. I valori nello stesso giorno seguiti dalla stessa lettera non sono significativamente diversi al livello di probabilità di 0.05.

daily) and allows more objective comparison than absolute intensity of grain filling (Fig. 2). Significant differences between genotypes in EF within groups were already achieved in the interval II (12-17 days after heading). However, neither of groups of genotypes, showed a significant decrease ($P < 0.05$) of EF in the D treatment comparing to the C treatment. Except in the initial stage (up to interval II), six-rowed barley genotypes had lower values of EF than two-rowed barley genotypes during the entire filling period. The two- rowed genotypes, that achieved the highest average yield under stress conditions, also had the highest values of the EF.

According to their earliness (date of heading) all barley genotypes evaluated in this study were classified into the following three groups: early, mid-early and late. These groups were compared in terms of GFR and GFD, to realize to which should be given a priority for growing under terminal drought conditions (Fig. 3). The value of GFR (6.08 mg 100 GDD day^{-1}) was on average the same in mid-early and late genotypes in the C treatment, while this value amounted to 5.94 mg 100 GDD day^{-1} in the group of early genotypes. In the D treatment, the highest average grain filling rate was recorded in the group of late genotypes (5.40 mg 100 GDD day^{-1}), followed by the group of early (5.33 mg 100 GDD day^{-1}) and mid-early genotypes (5.31 mg 100 GDD day^{-1}). In terms of GFD, the value of this parameter, in both treatments, was lowest for the group of late genotypes (842 GDD in C and 837

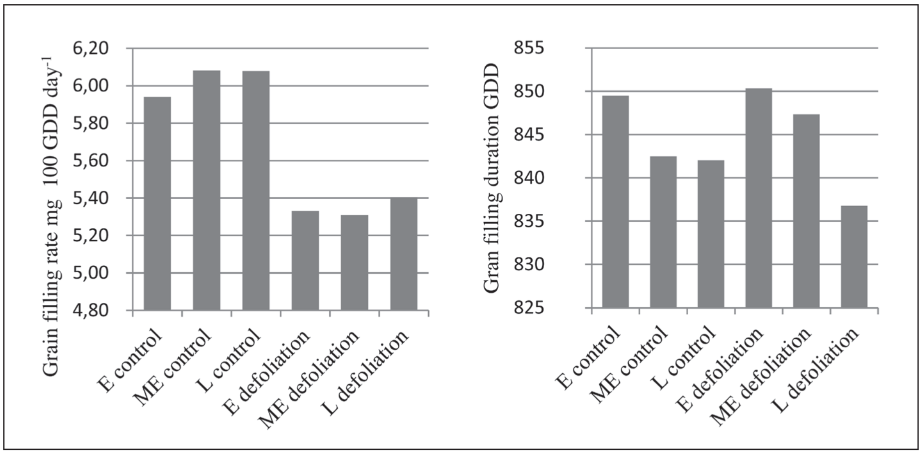

Fig. 2 - Grain filling rate and grain filling duration of early (E), mid-early (ME) and late (L) barley genotypes in control (C) and stress treatment (D), in both experimental years and locations.
Fig. 2 - Tasso e durata di riempimento della granella di genotipi di orzo precoci (E), medio-precoci (ME) e tardivi (L) nel controllo (C) e nel trattamento con stress (D), nei diversi anni e località.

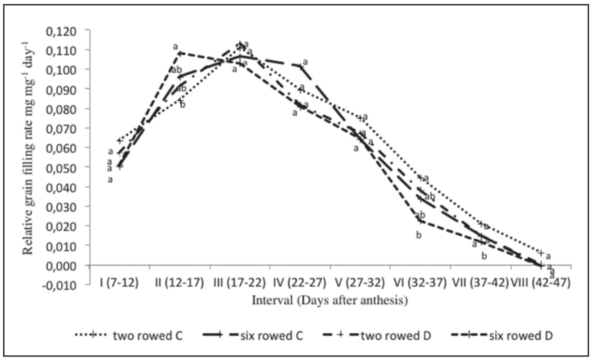

Fig. 3 - The average relative grain filling rate of 25 two- and six-rowed barley genotypes in the control (C) and the stress treatment (D) across locations and years. Values in the same interval followed by the same letter were not significantly different at the 0.05 probability level.
Fig. 3 - Tasso medio relativo di riempimento della granella di 25 genotipi di orzo a due e sei ranghi nel controllo (C) e nel trattamento di stress (D) tra località e anni. I valori nello stesso intervallo seguiti dalla stessa lettera non sono significativamente diversi al livello di probabilità 0,05.

GDD in D), as well as for mid-early genotypes in control (842 GDD).

One of the important aspects of plant breeding is evaluation of genotypes based on multiple traits. The technique of multivariate analysis - genotype by trait (GT) biplot design, can be used for comparison of genotypes based on several traits, in order to isolate those particularly good with certain properties important for the breeding process (Yan and Rajcan, 2002). The Fig. 4 shows the GT biplot for 15 two- rowed and 10 six rowed-genotypes, four grain filling parameters (GFR, GFD, MFR, MGW) and four agronomic traits (YLD, DH, KN, TGW) under the control and stress conditions. The similar pattern of correlations among traits and genotype grouping were observed in both treatments. The highest positive correlation with YLD had GFD, which is in accordance with Ahmadi *et al.*, 2016;

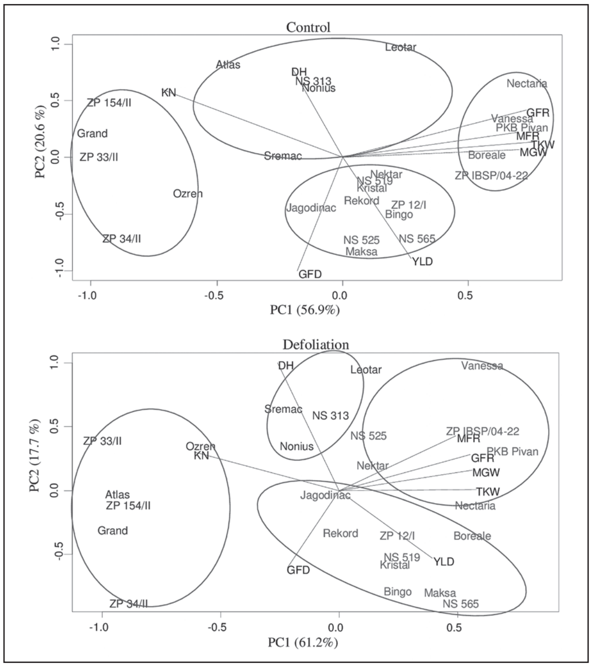

Fig. 4 - Genotype by the trait biplot showing the interrelationship among traits in control (C) and defoliation (D) treatments based on mean values for 25 genotypes in both experimental years and locations. DH - days to heading; KN - kernel number; GFD - grain filling duration; GFR - grain filling rate; MFR - maximum absolute grain filling rate; TKW - 1000-kernel weight; YLD - Yield, MGW - maximum grain weight.
Fig. 4 - Analisi del genotipo che mostra l'interrelazione tra i trattamenti di controllo (C) e di defogliazione (D) sulla base di valori medi per 25 genotipi nelle stagioni e aree sperimentali. DH - giorni di raccolto; KN - numero di cariossidi; GFD - durata della maturazione; GFR - velocità della maturazione; MFR: massima velocità assoluta di maturazione; TKW: peso di mille semi; YLD - produzione; MGW - peso massimo della granella.

Samarah *et al.*, 2009; González *et al.*, 2007, while the correlation of YLD with MGW, TKW, MFR and GFR was also positive but lower. In general, high yielding genotypes were early maturing. Separation between two- and six-rowed genotypes was primarily based on KN and DH. In general, two-rowed barley genotypes had a lower number of kernels per spike (caused by different morphological structure of the spike), larger kernels and a shorter growing season. Richards, (2006) and Akkaya *et al.*, (2006) reported that an optimum flowering time in relation to seasonal variations of solar radiation and water availability is a critical factor to maximize grain yield.

Two-rowed genotypes could be divided in two groups in both treatments. The first group consists of two-rowed genotypes that had the earliest heading, while yield and grain filling duration were above the trial average (lower right quadrant on the biplot). Grain filling parameters and 1000-kernel weight for this group were about trial average. The second group consists of genotypes with the average heading date and yield, but with extremely large values for grain filling parameters and 1000-kernel weight (upper right quadrant on the biplot). Six-rowed barley could be also divided in two groups in both treatments. First group is consisted of genotypes which had late heading date, low yield and short grain filling duration, while grain filling parameters and 1000-kernel weight were close to the trial average (located in the upper part of the biplot). Second six-rowed group (located on the left side of the biplot) consists of genotypes with medium earliness. Compared to the third group with also predominant six-rowed genotypes, genotypes of this group had higher yield and a longer length of the filling period, but had a lower 1000-kernel weight and grain filling parameters.

Although the pattern of trait interrelationship in control and the defoliation treatment seems rather similar, there were some inconsistencies in genotype grouping. In most cases high yielding genotypes in the C treatment (such as Maksa, NS 565, Bingo) also had high yields in the D treatment. The reason for this could be that high yield potential, expressed in favourable environments, can also be expressed in less favourable environments (Richards, 1996). However, the cultivar Boreale improved its yield under stress compared to non-stress conditions, while maintaining relatively high grain filling parameters and 1000-kernel weight. Duration of its grain filling period in the stress treatment was longer than in control. On the other hand, genotypes NS 525 and Nektar had relatively high yield under control conditions, but failed to maintain their yield under stress conditions. It seems that stress prolonged their growing season (they flowered later compared to control) and their grain filling period were more exposed to high temperatures. Results from genotype x trait biplots, indicate that earlier genotypes with a longer grain filling period and the high grain filling rate should be selected for terminal drought conditions. Przulj, (2001) also favours genotypes with a higher rate, but with a shorter duration of grain filling for semiarid conditions. In general, our findings are consistent with Vaezi *et al.*, (2010) who suggested that high potential yield, earliness and high TKW are the most significant traits in the selection of barley plants to improve the yield under terminal drought.

4. CONCLUSIONS

Terminal stress caused by the removal of all leaf blades seven days after anthesis significantly ($P < 0.05$) influenced grain yield, 1000- kernel weight and the grain filling rate in both barley types. The two-row barley genotypes in comparison to the six-rowed barley genotypes generally had a higher absolute grain filling rate, with maximum values of the grain filling rate achieved later and the process of grain filling lasted longer. The gradual accumulation of dry matter showed to be of more importance than intensity of grain filling rate under post-anthesis stress. Another advantage of two-rowed barley over six rowed barley is earlier heading time, which is important for the future climate scenario to avoid high temperature and low precipitations during grain filling. Therefore, for terminal drought conditions breeders should develop early maturing two-rowed genotypes with a prolonged grain filling duration.

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