

QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought

Peiguo Guo · Michael Baum · Rajeev K. Varshney · Andreas Graner · Stefania Grando · Salvatore Ceccarelli

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Abstract Drought is one of the major factors limiting barley yields in many developing countries worldwide. The identification of molecular markers linked to genes controlling drought tolerance in barley is one way to improve breeding efficiency. In this study, we analyzed the quantitative trait loci (QTL) controlling chlorophyll content and chlorophyll fluorescence in 194 recombinant inbred lines (RILs) developed from the cross between the cultivar ‘Arta’ and *Hordeum spontaneum* 41-1. Five traits, chlorophyll content, and four chlorophyll fluorescence parameters, namely initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v), and maximum quantum efficiency of PSII (F_v/F_m) which are related to the activity of the photosynthetic apparatus, were measured under well-watered and drought stress conditions

at post-flowering stage. QTL analysis identified a total of nine and five genomic regions, under well-watered and drought stress conditions, respectively, that were significantly associated with the expression of the five target traits at post-flowering stage. No common QTL was detected except one for chlorophyll content, which was identified in both growth conditions, demonstrating that the genetic control of the expression of the traits related to photosynthesis differed under different water conditions. A QTL for F_v/F_m , which is related to the drought tolerance of photosynthesis was identified on chromosome 2H at 116 cM in the linkage map under drought stress. This QTL alone explained more than 15% of phenotypic variance of maximum quantum yield of PSII, and was also associated with the expression of four other traits. In addition, another QTL for F_v/F_m was also located on the same chromosome (2H) but at 135.7 cM explaining around 9% of the phenotypic variance under drought conditions. The result presented here suggest that two major loci, located on chromosome 2H, are involved in the development of functional chloroplast at post-flowering stage for drought tolerance of photosynthesis in barley under drought stress. If validated in other populations, chlorophyll fluorescence parameters could be used as selection criteria for drought tolerance.

P. Guo · M. Baum (✉) · S. Grando · S. Ceccarelli
Biodiversity and Integrated Gene Management Program,
International Center for Agricultural Research in the Dry
Areas, Aleppo, Syria
e-mail: m.baum@cgiar.org

P. Guo
College of Life Science, Guangzhou University,
Guangzhou, China

R. K. Varshney · A. Graner
Department Genbank, Leibniz Institute of Plant Genetics
and Crop Plant Research (IPK), Corrensstr. 3, 06466
Gatersleben, Germany

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Introduction

Barley is one of the most important cereal crops in many developing countries ranging from Eritrea and Ethiopia in Africa, Yemen in the Arabian peninsula, Tibet and Nepal in the Himalaya, Peru, Bolivia, Ecuador and Colombia in the Andes, and in the countries of Near East and North Africa. In many of these countries, barley is often the only possible rainfed crop that farmers can grow, and is often subjected to extreme water deficit during the dry season (Ceccarelli 1994). Therefore, drought stress is a serious challenge for barley in these areas, because it affects simultaneously many traits through morphological, physiological, and metabolic modifications occurring in all plant organs leading to a decrease in yield (Sacks et al. 1997; Cellier et al. 1998; Cochard et al. 2002). The use of drought-tolerant cultivars is one of the most effective ways to reduce the losses caused by this stress. Although many achievements have been obtained to increase barley yield in several developing countries in past years, the progress in improving drought tolerance of barley cultivars with empirical breeding has been slow, due to the poor definition of the target environment, the complexity and difficulty of drought-evaluation procedures, the inconsistency of morpho-physiological traits as selection criteria for drought tolerance and the interaction between genotypes and environmental factors (Ceccarelli et al. 2004, 2007). In the last decade, molecular marker technologies have been successfully used to identify quantitative trait loci (QTLs) and in understanding responses to drought stress in various crop plants (Ribaut et al. 1997; Courtois et al. 2000; Baum et al. 2003; Verma et al. 2004; Grando et al. 2005). Marker-assisted selection may reduce problems associated with genotype \times environment interactions, improve the selection efficiency and facilitate combining different tolerance traits into a single genotype.

Photosynthesis is an essential process to maintain crop growth and development; photosynthetic capacity during the reproductive stage is positively correlated with crop yield (Rawson et al. 1980). Changes of some specific traits could result in the variation of photosynthetic capacity under drought stress. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate (Guo and Li 1996). Although there is an argument about whether

a higher chlorophyll content (i.e. stay green trait) contributes to yield under drought conditions or not (Blum 1998), many studies indicated that stay-green trait is associated with improved yield and transpiration efficiency under water-limited conditions in sorghum, maize and wheat (Benbella and Paulsen 1998; Baenziger et al. 1999; Borrell et al. 2000; Haussmann et al. 2002; Verma et al. 2004). Therefore, maintaining higher chlorophyll content for a longer period of time is one of the strategies for increasing crop production, particularly under water-limited conditions. In addition, Photosystem II (PSII) is an important component of plant photosynthesis, and it is particularly sensitive to water deficit conditions (Lu and Zhang 1999). The drought-induced decrease in photosynthesis has been associated with perturbations of biochemical processes (Graan and Boyer 1990) and photodamage of PSII reaction centers (He et al. 1995). While chlorophyll fluorescence is widely accepted as an indication of the energetic behavior of photosynthetic system, it is emitted mainly by PSII in the range of 680–740 nm spectra region and it can be considered as an intrinsic probe of the fate of excitation energy (Krause and Weiss 1991; Dau 1994). Several fluorescence parameters, such as initial fluorescence (F_o , fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized), maximal fluorescence (F_m , fluorescence level when Qa is transiently fully reduced), variable fluorescence (F_v , i.e. $F_m - F_o$) and maximum/potential quantum efficiency of PSII (F_v/F_m), have been widely used for such investigations in various plants under diverse growth conditions (Araus et al. 1998; Guo and Li 2000; Guo and Al-Khatib 2003; Fracheboud et al. 2004). Those parameters were observed to be closely correlated with the rate of carbon exchange in rice and maize under environmental stresses (Guo and Li 2000; Fracheboud et al. 2004). Some parameters such as F_o and F_m measured during the grain filling stage of wheat under drought stress showed high genetic correlation with grain yield (Araus et al. 1998), suggesting that those parameters can be used as reliable indicators to evaluate the energetic/metabolic imbalance of photosynthesis and yield performance across genotypes under water deficit conditions (Araus and Hogan 1994; Araus et al. 1998). Thus, these parameters may be considered as traits associated with drought tolerance. However, little is known about the genetic basis of drought tolerance in photosynthetic traits such as chlorophyll content and

the fluorescence parameters in barley. A better understanding of the genetic basis of these parameters and of their association with drought tolerance will contribute to set up a breeding program for the improvement of drought tolerance in barley based on marker-assisted selection.

In this study, a population of barley recombinant inbred lines (RILs) derived from the cross between Arta and *Hordeum spontaneum* 41-1 in which the parents differ for the stay-green trait was used to study the genetics of chlorophyll content and chlorophyll fluorescence parameters under drought stress and non-stress (well-watered) conditions during the reproductive stage. The objectives were to identify QTLs associated with photosynthesis-related physiological traits, i.e., chlorophyll content and chlorophyll fluorescence parameters, and to understand the drought tolerance of photosynthesis at genetic level.

Materials and methods

Plant material and growth conditions

A barley population of 194 F₉ RILs (Baum et al. 2003) derived from a single-seed descent of the cross between Arta and *H. spontaneum* 41-1 was used in this study. Arta is a pure-line variety of barley (*Hordeum vulgare* L.) and *H. spontaneum* 41-1 is a pure line of the wild progenitor of cultivated barley. *H. spontaneum* 41-1 has a short grain-filling period, while Arta has a long grain-filling period and high kernel weight as well as high grain yield under water deficit condition; also Arta has a higher greenness of leaves than *H. spontaneum* 41-1 under drought stress (Grando et al. 2001).

A greenhouse experiment including the 194 RILs and their parents Arta and *H. spontaneum* 41-1 was arranged in a randomized complete-block design at ICARDA (Tel Hadya, Aleppo, Syria). The experiment was repeated twice (i.e., replicate experiment 1: RP1 and replicate experiment 2: RP2), and each experiment consisted of two treatments (well-watered and drought-stress) with two replications. Three 4-week vernalized seedlings of the same entry were transplanted into a 3.0-liter pot (15 cm in height and 16 cm in diameter) filled with 2.2 kg of sterilized field soil which contained about 6% of water. Field capacity, wilting point and available water content

(AWC) of the soil were measured at ICARDA soil laboratory followed the protocol described by Ryan et al. (2001). About 70% and 10% of AWC in the soil were considered for barley as well-watered (control) and severe drought conditions, respectively (Doorenbos and Pruitt 1977). Each RIL for one treatment was planted in four pots with a total of 12 plants; all plants were grown with 16 h daylight at 28°C and 8 h dark period at 20°C under well-watered condition. The drought treatment of each replication was induced for two pots with six plants of each RIL after flowering stage. The soil moisture was maintained with required amounts of water for the pots of well-watered and drought-stress conditions by weighing pots and watering the plants everyday. The days for drought stress were counted after the AWC in the soil reached 10% to allow measurements at precise determined intervals.

Measurement of chlorophyll content and chlorophyll fluorescence parameters

Leaf chlorophyll was determined using a chlorophyll meter (SPAD-502, Minolta, Japan). Six flag leaves for each RILs and parents in well-watered and drought-stress conditions, respectively, were measured at the 12th-day after drought stress. Three measurements in the middle of the leaf were made for each plant and the average used for the analysis. About 20 leaves randomly selected with incremental chlorophyll levels (determined by SPAD-502 readings) were then harvested to construct a standard curve for quantification of chlorophyll content using the method for chlorophyll analysis described by Arnon (1949).

Chlorophyll fluorescence parameters, including initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v), and maximum quantum efficiency of PSII (F_v/F_m) were monitored on the flag leaf under both well-watered and drought-stress conditions using Handy PEA (Hansatech Instruments, Norfolk, UK) following the manufacturer's instruction. Dark adaptation period for all the measurements was about 25 min, six flag leaves from both well-watered and drought-stress conditions for each RIL and parent were selected to measure chlorophyll fluorescence parameters at 12 d after treatment.

Data analysis

Analysis of variance (ANOVA) was carried out using GENSTAT software v. 7.1 (Payne et al. 2003) to determine the significance of variation for all the traits measured for this study. A mixed model, with genotypes as random effects and treatments as fixed effects was used. The broad sense heritability (h^2) was computed on a genotype mean basis as $\sigma_G^2/(\sigma_G^2 + \sigma_e^2/2) \times 100\%$ for both well-watered and drought-stress conditions, and on an individual basis as $\sigma_G^2/(\sigma_G^2 + \sigma_{GE}^2/2 + \sigma_e^2/4) \times 100\%$ for both well-watered \times drought stress treatment. In the formulas, σ_G^2 is the genotypic variance, σ_{GE}^2 is the variance associated with the genotype by environment interaction, and σ_e^2 is the error variance.

DNA isolation

Genomic DNA was extracted following the CTAB protocol described by Saghai-Marooif et al. (1984), with minor modification. Fresh, above ground parts of 4- to 6-week-old seedlings were collected for DNA isolation from parents and each of the RILs. The DNA was quantified using a Beckman DU-65 spectrophotometer (Beckman Instruments, USA). The quality of the extracted DNA was visually checked on 1% agarose gels.

Analysis of SSRs and AFLPs

Genetic mapping was carried out by Baum et al. (2003) using amplified fragment length polymorphic (AFLP) markers and simple sequence repeat (SSR) markers. In brief, the protocol for the AFLP assay was carried out as described by Zabeau and Vos (1993) with minor modifications using combinations of *Pst*I and *Mse*I, or *Eco*RI and *Mse*I restriction enzymes and respective adapters. Pre-amplification was carried out using one base-pair extension primers and selective amplification was conducted using primers with two or three selective nucleotides. SSR markers were amplified on a PE- 9600 or 9700 system (PE Corporation, USA) using the published protocols for the respective markers (Ramsay et al. 2000). PCR amplifications were separated on 6% denaturing polyacrylamide gels, and stained with

silver nitrate stain followed the protocol described by Bassam et al. (1991), while fluorescent dyes were used on an ABI 377 sequencer (Applied Biosystems, USA).

Linkage mapping and QTL analysis

Segregation analysis was performed according to Stam and Van Ooijen (1995) with the Join Map v. 2.0 software package. Recombination fractions were converted to centiMorgans (cM) according to the Kosambi mapping function (Kosambi 1944). For QTL detection, the MQM mapping was used to identify the QTLs for traits using MapQTL 5 program (Van Ooijen 2004). To identify an appropriate threshold of LOD score for declaring a significant QTL, permutation test was conducted for 1,000 times using the program, which resulted in a LOD threshold of 2.5 to claim the presence of a QTL. The percent phenotypic variation explained by the significant intervals and estimates of their additive genetic effects were also calculated in MapQTL 5.

Results

Phenotypic distribution

Mean values and the relevant statistical parameters for all the traits are shown in Table 1 and Fig. 1. For all traits we excluded from further analysis those values which differed from the population mean more than three times the standard deviation as they were considered outliers. The parents significantly differed for all the traits under drought conditions but only for chlorophyll content when grown under well-watered conditions. The differences among RILs were significant for all the traits under both grown conditions (Table 1). The phenotypic distribution of trait values showed clear transgressive segregation under both growth conditions (Fig. 1). The exception was F_v/F_m under well-watered conditions, which showed the distribution in the range of 0.76–0.828. This distribution is in good agreement with the results of Bolhar-Nordenkampf et al. (1989) who found F_v/F_m in the range of 0.75–0.85 for non-stressed plants. In addition, significant difference between parents and a wider range of distribution for all the traits among

Table 1 Means, standard deviation and ranges of the 194 RILs and parents for fluorescence parameters and chlorophyll content (Chl, mg/g fresh weight) under well-water and drought stress conditions, respectively

Trait ^a	RP ^b	Treatment	Parents			RILs		
			Arta	<i>H. spontaneum</i> 41-1	Significance ^c	Min	Max	Mean ^f
F _o	RP1	Well-watered	233 ± 14.1	233.5 ± 11.7	ns	207	278	245.8 ± 13.3***
		Drought-stressed	238 ± 15.3	197.5 ± 13.6*** ^d	**	176	376	229.5 ± 29.6***
	RP2	Well-watered	236.3 ± 15.8	232.9 ± 13.3	ns	200	277	242.8 ± 17.1***
		Drought-stressed	235.6 ± 16.2	186.7 ± 17.9*** ^d	**	158	397	234.5 ± 35.8***
F _m	RP1	Well-watered	1145.5 ± 98.3	1237 ± 83.9	ns	980	1335	1180.0 ± 69.8***
		Drought-stressed	1183.2 ± 114.8	551.7 ± 56.7*** ^d	***	247	1270	951.0 ± 219.0***
	RP2	Well-watered	1177.4 ± 88.7	1195 ± 92.5	ns	913	1368	1165.9 ± 77.3***
		Drought-stressed	1179.2 ± 121.3	578.6 ± 80.9*** ^d	***	182	1324	932.9 ± 255.3***
F _v	RP1	Well-watered	912.5 ± 64.1	1003.5 ± 58.3	ns	766	1106	934.2 ± 65.9***
		Drought-stressed	915.2 ± 77.9	354.2 ± 43.3*** ^d	***	3	1044	721.6 ± 232.5***
	RP2	Well-watered	941.1 ± 66.9	962.1 ± 53.4	ns	712	1131	923.1 ± 74.8***
		Drought-stressed	943.6 ± 88.2	391.9 ± 72.1*** ^d	***	64	1086	698.4 ± 272.6***
F _v /F _m	RP1	Well-watered	0.797 ± 0.018	0.811 ± 0.028	ns	0.760	0.828	0.791 ± 0.013***
		Drought-stressed	0.799 ± 0.020	0.642 ± 0.032*** ^d	***	0.012	0.822	0.758 ± 0.173***
	RP2	Well-watered	0.799 ± 0.016	0.805 ± 0.021	ns	0.761	0.827	0.792 ± 0.017***
		Drought-stressed	0.800 ± 0.018	0.677 ± 0.014*** ^d	***	0.069	0.826	0.749 ± 0.221***
Chl	RP1	Well-watered	2.51 ± 0.13	1.95 ± 0.17	**	1.51	2.75	2.15 ± 0.24***
		Drought-stressed	2.27 ± 0.16* ^c	0.79 ± 0.19*** ^d	***	0.39	2.4	1.56 ± 0.44***
	RP2	Well-watered	2.49 ± 0.21	2.08 ± 0.15	**	1.47	2.87	2.21 ± 0.21***
		Drought-stressed	2.31 ± 0.12* ^c	0.81 ± 0.22*** ^d	***	0.41	2.66	1.58 ± 0.52***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^a F_o, initial fluorescence (fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized); F_m, maximal fluorescence (fluorescence level when Qa is transiently fully reduced); F_v, variable fluorescence (F_m - F_o), F_v/F_m, maximum/potential quantum efficiency of PSII

^b RP indicates replicate experiment

^{c,d} Significance between well-watered and drought stress in Arta and *H. spontaneum* 41-1, respectively

^e Significance between Arta and *H. spontaneum* 41-1 under corresponding growth conditions

^f Significance among RILs under same growth conditions

ns, not significant

RILs were also observed under drought conditions in comparison to well-watered conditions.

Relationships between chlorophyll and chlorophyll fluorescence parameters

Under well-watered conditions, the correlations among F_o, F_m, F_v and F_v/F_m were highly significant ($P < 0.01$, Table 2), and in many cases positive, except for the correlation between F_o and F_v/F_m. Chlorophyll content which was not significantly correlated with F_m, F_v and F_v/F_m, but positively correlated with F_o ($r = 0.26$ in RP1, 0.19 in RP2). A significantly positive correlation

among F_m, F_v, F_v/F_m and chlorophyll content was observed under drought conditions (Table 2). In contrast, F_o was significantly and negatively correlated with all of the other fluorescence traits under drought condition, while the relationship between F_o and chlorophyll content was not significant.

QTL analysis of chlorophyll and chlorophyll fluorescence parameters

The QTL analysis was performed on the basis of the marker linkage map constructed by Baum et al. (2003) for the Arta × *H. spontaneum* 41-1 RIL

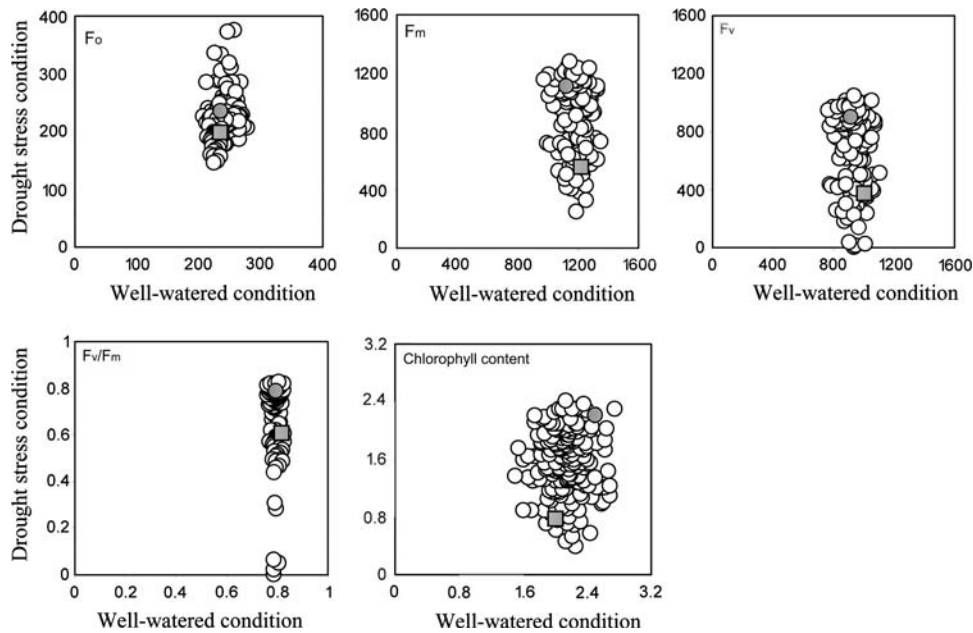


Fig. 1 Phenotypic distributions of F_0 (initial chlorophyll fluorescence), F_m (the maximum fluorescence), F_v (the variable fluorescence), F_v/F_m (the maximum quantum efficiency of PSII primary photochemistry) and chlorophyll content (mg/g fresh weight) of the flag leaf in barley plants at post-flowering stage.

The values for each trait under drought-stressed conditions (y axis) are plotted against the values of plants grown under well-watered conditions (x axis). White circles: RILs; Grey circles: Arta; grey squares: *H. spontaneum* 41-1. Values are the average of six replicates

Table 2 Simple correlation coefficients between fluorescence parameters and chlorophyll content (Chl)

Trait ^a	F_0	F_m	F_v	F_v/F_m	Chlorophyll content
F_0	–	–0.42**/–0.34**	–0.52**/–0.48**	–0.63**/–0.64**	–0.10ns/–0.11ns
F_m	0.37**/0.32**	–	0.99**/0.99**	0.91**/0.90**	0.39**/0.22*
F_v	0.19*/0.10ns	0.98**/0.98**	–	0.93**/0.92**	0.38**/0.21*
F_v/F_m	–0.48**/–0.60**	0.63**/0.56**	0.76**/0.73**	–	0.28**/0.17*
Chl	0.26**/0.19*	0.00ns/–0.05ns	–0.06ns/–0.10ns	–0.12ns/–0.1ns	–

* $P < 0.01$; ** $P < 0.001$

Upper diagonal in bold represents the plants grown under drought stress conditions and lower diagonal represents the plants grown under well-watered conditions. The first number (before /) corresponds to replicated experiment 1 (RP1) and the second (after /) to the replicate experiment 2 (RP2)

^a F_0 , initial fluorescence (fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized); F_m , maximal fluorescence (fluorescence level when Qa is transiently fully reduced); F_v , variable fluorescence ($F_m - F_0$), F_v/F_m = maximum/potential quantum efficiency of PSII; chl, chlorophyll content (mg/g fresh weight); ns, not significant

population, which contained 158 AFLP markers, 30 SSR markers and one morphological marker, covering a total map length of 890 cM (Fig. 2). QTLs for each of the five traits in both well-watered and drought stress conditions were identified using MQM analysis using the MapQTL 5 program (Tables 3, 4).

Under well-watered conditions, genetic analysis detected fifteen significant QTLs at nine loci for the five traits (Table 3). Six QTLs at four loci identified in both RP1 and RP2 were on chromosome 1H at 75–76.1 cM for F_0 , 5H at 13.7–15.9 cM for F_m , F_v and F_v/F_m , 2H at 115 cM and 4H at 81.9–82.9 cM for

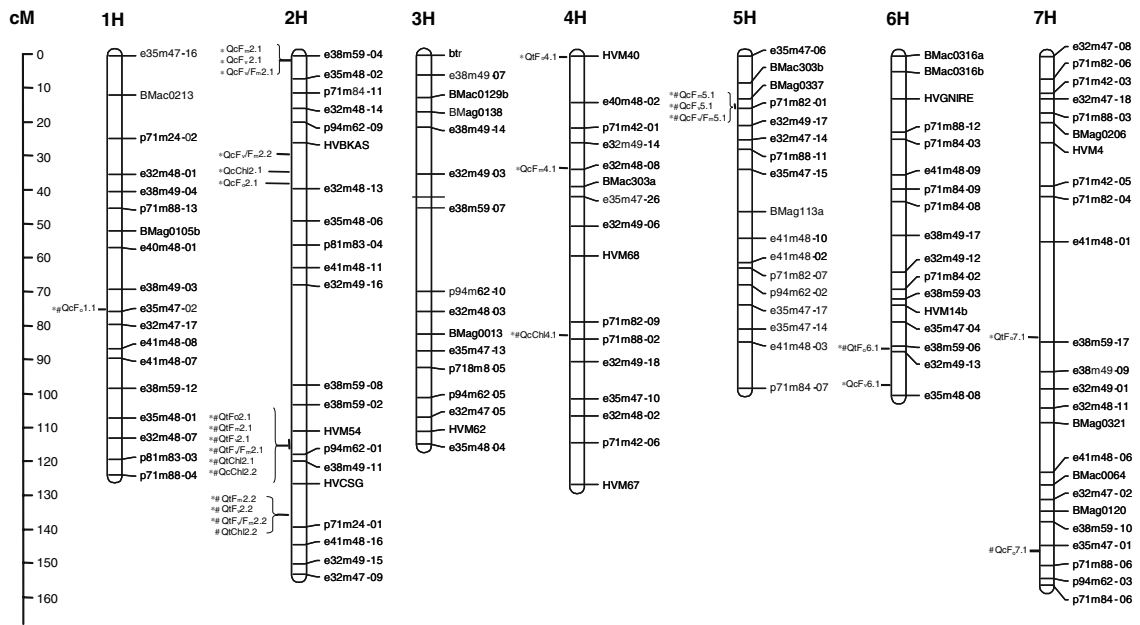


Fig. 2 Genetic linkage map of barley as established by Baum et al. (2003) for the cross *Arta* × *H. spontaneum* 41-1 population with positions. Markers are given on the right side for each chromosome. The centiMorgan scale is given on the

left. The peaks of QTLs are indicated to the left of the chromosome bars. * and # indicate that QTLs were identified in replicate experiment 1 and 2, respectively

chlorophyll content. In addition, eight QTLs with high LOD scores and R^2 values only identified in RP1 were located on chromosome 2H at 1 cM for F_m , F_v and F_v/F_m , at 30.8–38 cM for F_o , F_v/F_m and chlorophyll content, 4H at 33.7 cM for F_m , and 6H at 98.3 for F_v . The remaining one is the QTL detected in RP2 for F_o located on 7H at 148.1 cM. The positive values for additive effect indicate that the donor of the allele for the traits was *Arta*, and vice versa. The positive and negative additive effects at the different loci were in similar proportions indicating that both parents contributed alleles for such traits and confirm the transgressive segregation observed at the phenotypic level (Fig. 1).

The QTL analysis of the parameters collected on plants grown under drought conditions detected twelve significant QTLs located at five loci. A major locus in both RP1 and RP2 was identified on chromosome 2H (114–116 cM), accounting for a high proportion of phenotypic variance for F_o (13.5% in RP1 and 12.6% in RP2), F_m (15.1% in RP1 and 15.7% in RP2), F_v (16.3% in RP1 and 15.9% in RP2) and F_v/F_m (15.5% in RP1 and 11.8% in RP2). This locus was also identified for chlorophyll content, with an $R^2 = 9.9\%$ in RP1 and 8% in RP2. Another locus involved in the regulation of F_m , F_v and F_v/F_m was

also detected on chromosome 2H (135.7 cM) in both RP1 and RP2. Moreover, one QTL for chlorophyll content was identified in this locus in RP2. Additional QTLs for F_o were detected on chromosomes 4H (0 cM) and 7H (85.2 cM) in RP1, and 6H (86.5–87.5 cM) in both RP1 and RP2. In contrast to the situation under well-watered conditions where the contribution of the two parents to the expression of five traits was similar, under drought stress conditions *Arta* contributed the alleles for the traits F_m , F_v , F_v/F_m and chlorophyll content, while *H. spontaneum* 41-1 contributed the alleles for F_o thus confirming the observations at the phenotypic level. Furthermore, the major locus on chromosome 2H was also detected for chlorophyll content in plants grown under well-watered conditions, suggesting a constitutive expression for this trait.

Correlations and heritability

The heritability for all the traits was very high, ranging from 68% for F_o in RP2 to 91% for chlorophyll content in RP1 (Table 5). The heritability measured under drought conditions was only

Table 3 Main characteristics of QTLs with an LOD score > 2.5 for fluorescence parameters and chlorophyll content (Chl) in plants grown under well-watered conditions

Trait ^a	QTL	Chromosome number	RP ^b	Peak	Nearest Marker	LOD	% Expl.	Additive effect ^c
F _o	QcF _o 1.1	1H	RP1/RP2	76.1/75	e35m47-02	2.6/2.55	6.6/6.2	-6.89/-6.13
	QcF _o 2.1	2H	RP1	38	BMag0378	2.5	6.4	6.69
	QcF _o 7.1	7H	RP2	148.1	e35m47-01	2.5	6.2	6.21
F _m	QcF _m 2.1	2H	RP1	1	e38m59-04	3.5	9.5	-57.33
	QcF _m 4.1	4H	RP1	33.7	e32m48-08	2.8	7.1	-49.77
	QcF _m 5.1	5H	RP1/RP2	13.7/15.7	p71m82-01	2.7/3.1	8/8.2	53.35/55.6
F _v	QcF _v 2.1	2H	RP1	1	e38m59-04	3.5	9.4	-59.35
	QcF _v 5.1	5H	RP1/RP2	15.7/13.7	p71m82-01	2.8/3.1	7.4/8.3	53.78/66.74
	QcF _v 6.1	6H	RP1	98.3	e35m48-08	2.5	8.4	-56.04
F _v /F _m	QcF _v /F _m 2.1	2H	RP1	1	e38m59-04	2.6	7	-0.012
	QcF _v /F _m 2.2	2H	RP1	30.8	BMag0140	3	9.9	-0.014
	QcF _v /F _m 5.1	5H	RP1/RP2	15.9/14.9	p71m82-01	2.5/2.8	6.3/7.6	0.011/0.015
Chl	QcChl2.1	2H	RP1	35.1	BMag0140	3.3	9.8	1.01
	QcChl2.2	2H	RP1/RP2	115/115	HVM54	4.4/4.8	12.8/13.6	-1.15/-1.34
	QcChl4.1	4H	RP1/RP2	82.9/81.9	p71m88-02	3.5/3.4	10.2/9.6	-1.03/-1.11

^a F_o, initial fluorescence (fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized); F_m, maximal fluorescence (fluorescence level when Qa is transiently fully reduced); F_v, variable fluorescence (F_m - F_o), F_v/F_m = maximum/potential quantum efficiency of PSII

^b RP presents replicate experiment

^c Negative values indicate effects from *H. spontaneum* 41-1; positive values indicate effects from Arta

Peak, position of the peak of the QTL in centiMorgans

%Expl., % of phenotypic variance explained by the QTL

marginally lower than the one measured under well-watered conditions. The expression of the traits under well-watered conditions was not correlated with the expression of the same traits under drought stress, and as a consequence the estimates of heritability across the two growing conditions were always very low and below 2.5%.

Discussion

Plant growth depends on photosynthesis and is sensitive to both biotic and abiotic stress. Photosynthetically active radiation in plants is absorbed by chlorophyll and accessory pigments of chlorophyll-protein complexes, and it migrates to the reaction centers of PSI and II, where the conversion of the quantum photosynthetic process takes place (Horton et al. 1996). Therefore, analysis of chlorophyll content and chlorophyll fluorescence parameters (such as F_o, F_m, F_v and F_v/F_m) are considered as an important

approach to evaluate the health or integrity of the internal apparatus during photosynthetic process within a leaf (Krause and Weiss 1991; Clark et al. 2000), and provides a rapid and accurate technique of detecting and quantifying plants tolerance to drought stress (Percival and Sheriffs 2002). Several researches indicated that chlorophyll fluorescence parameters were strongly correlated with whole-plant mortality in response to environmental stresses (Greaves and Wilson 1987; Araus and Hogan 1994; Yamada et al. 1996; Hakam et al. 2000; Percival and Sheriffs 2002) and were reliable indicators of stress (Krause and Weiss 1991; Schreiber et al. 1994). In the present study, the two parents differed considerably for the chlorophyll fluorescence parameters when grown under drought stress but not under well-watered conditions (Table 1), indicating that chlorophyll fluorescence can be an efficient selection tool for drought tolerance of photosynthesis.

In the present study, the growing conditions affected the chlorophyll fluorescence parameters.

Table 4 Main characteristics of QTLs with an LOD score > 2.5 in plants grown under drought conditions

Trait ^a	QTL	Chromosome number	RP ^b	Peak	Nearest Marker	LOD	% Expl.	Additive effect ^c
F _o	QtF _o 2.1	2H	RP1/RP2	116/115.5	HVM54	3.9/3.7	13.5/12.6	-14.85/-13.9
	QtF _o 4.1	4H	RP1	0	HVM40	3.0	7.9	11.23
	QtF _o 6.1	6H	RP1/RP2	86.5/87.5	e38m59-06	2.8/2.6	7.0/7.4	-10.62/-11.73
	QtF _o 7.1	7H	RP1	85.2	e38m59-17	3.3	9.0	-12.02
F _m	QtF _m 2.1	2H	RP1/RP2	115/116	HVM54	5.4/5	15.1/15.7	85.24/105.75
	QtF _m 2.2	2H	RP1/RP2	135.7/137.7	p71m24-01	2.6/2.51	9.2/8.8	66.35/65.73
F _v	QtF _v 2.1	2H	RP1/RP2	115/116	HVM54	5.7/5	16.3/15.9	94.16/103
	QtF _v 2.2	2H	RP1/RP2	135.7/137.7	p71m24-01	2.7/2.55	9.3/8.6	71.14/70.52
F _v /F _m	QtF _v /F _m 2.1	2H	RP1/RP2	116/116	HVM54	5.2/3.6	15.5/15.2	0.069/0.067
	QtF _v /F _m 2.2	2H	RP1/RP2	135.7/137.7	p71m24-01	2.8/2.61	9.3/8.9	0.053/0.051
Chl	QtChl2.1	2H	RP1/RP2	114/116	HVM54	3.6/2.7	9.9/8	1.93/1.67
	QtChl2.2	2H	RP2	137.7	p71m24-01	2.6	8.8	1.82

^a F_o, initial fluorescence (fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized); F_m, maximal fluorescence (fluorescence level when Qa is transiently fully reduced); F_v, variable fluorescence (F_m - F_o), F_v/F_m = maximum/potential quantum efficiency of PSII, chl = chlorophyll content (mg/g fresh weight)

^b RP presents replicate experiment

^c Negative values indicate effects from *H. spontaneum* 41-1; positive values indicate effects from Arta

Peak, position of the peak of the QTL in centiMorgans

%Expl., % of phenotypic variance explained by the QTL

Table 5 Broad sense heritability on genotype mean basis and correlation analysis for fluorescence parameters and chlorophyll content measured under drought stress, well-watered

conditions, and combined. The first number (before /) corresponds to replicated experiment 1 (RP1) and the second (after /) to the replicate experiment 2 (RP2)

Trait ^a	h ²			r*
	Well-watered (%)	Drought-stress (%)	Combined (%)	
F _o	73.90/69.81	70.95/68.26	0.19/0.23	-0.015ns/0.019ns
F _m	81.86/79.73	77.53/71.35	1.42/1.78	-0.067ns/0.097ns
F _v	81.09/78.65	77.65/72.16	1.92/2.35	-0.045ns/-0.088ns
F _v /F _m	75.18/76.29	74.39/70.54	0.91/1.03	0.009ns/-0.016ns
Chl	90.10/91.03	90.04/90.08	0.72/0.63	-0.017ns/0.039ns

^a F_o, initial fluorescence (fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidized); F_m, maximal fluorescence (fluorescence level when Qa is transiently fully reduced); F_v, variable fluorescence (F_m - F_o), F_v/F_m = maximum/potential quantum efficiency of PSII, chl = chlorophyll content (mg/g fresh weight)

* Correlation coefficients of the trait between drought and well-watered conditions among RILs; ns, not significant

The values of F_o, F_m, F_v in *H. spontaneum* 41-1 decreased significantly together with F_v/F_m under drought stress, while these traits were not significantly affected by drought in Arta (Table 1). The decrease in F_o could reflect damage to regulatory processes external to P₆₈₀ (reaction center of PSII), such as impairment of the photoprotective processes that facilitates the dissipation of excess energy with

the leaf (Angelopoulos et al. 1996; Hong and Xu 1999), while a decline in F_v and F_m may indicate an increase in non-photochemical quenching (Bolhar-Nordenkamp et al. 1989), which may result from photoinhibition under stress (Baker and Horton 1987), while a reduction of F_v/F_m may represent either a reversible photoprotective down regulation or an irreversible inactivation of PSII (Baker and

Bowyer 1994; Long et al. 1994). The fact that drought affected these traits in *H. spontaneum* 41-1 but not in Arta, suggests the existence of genetic differences in the reaction of the photosynthetic apparatus to drought and that in Arta the photosynthetic process has a higher tolerance to drought stress.

The significant difference for fluorescence parameters was also observed across RILs under well-watered and drought stress conditions (Table 1 and Fig. 1). These phenomena were also observed under cold stress in maize (Fracheboud et al. 2004). The low correlations between drought and well-watered conditions and the high heritability under drought conditions indicated that chlorophyll content and chlorophyll fluorescence parameters could be efficient selection parameters for different levels of drought tolerance of photosynthesis, and suggest that selection for drought tolerance of photosynthesis should be conducted under drought-stress conditions. This is in good agreement with the result of Araus et al. (1998) that chlorophyll fluorescence could be used as a selection criterion for grain yield in wheat under drought conditions.

The QTL analysis identified a total of 27 QTLs for the fluorescence parameters measured under the two growing conditions of this study (Tables 3, 4). These QTLs were scattered along the chromosomes, and none of them was common across the two growing conditions except one, for chlorophyll content, located on chromosome 2H. Therefore, most of the QTLs detected in barley plants grown under two contrasting conditions were specific to the corresponding condition. This caused a low heritability and correlation coefficients across the two growing conditions (Table 5). The fluorescence parameters were affected by the environment at the genetic level which is in agreement with observation in maize under low temperature stress (Fracheboud et al. 2004).

Three common locations of QTLs for fluorescence parameters and chlorophyll content under well-watered conditions were detected. One of them, located on chromosome 5H at 13.7–15.9 cM for F_m , F_v and F_v/F_m , should be stable because detected in both RP1 and RP2; the other two identified only in RP1 were located on chromosome 2H at 1 cM for F_m , F_v and F_v/F_m , and 2H at 35–38 cM for F_o and chlorophyll content. In the remaining six loci, three were located on chromosome 1H for F_o , 2H and 4H

for chlorophyll content in both RP1 and RP2, other three were located on chromosome 2H and 7H for F_o , 6H for F_v in either RP1 or RP2. Therefore, fluorescence parameters and chlorophyll content could be controlled by multiple genes, and the QTLs for F_m , F_v and F_v/F_m , and QTL for chlorophyll content and F_o are tightly linked. On basis of relationship between chlorophyll content and F_v/F_m in 194 RILs under well-watered condition in this study as well as four genotypes in our previous study (Li et al. 2006), suggesting that chlorophyll content may not be a limiting factor for photosynthetic efficiency under well-watered conditions in these barley genotypes.

Under drought-stress conditions, tolerance to photoinhibition may be the key factor in the tolerance of photosynthetic apparatus to drought stress (Percival and Sheriffs 2002). The phenotypic correlations between traits (Table 2) and the identification of QTLs (Table 4) were generally in good agreement. One QTL located on chromosome 2H at 114–116 cM was common for all fluorescence parameters in two replicated experiments, and also plays an important role in chlorophyll accumulation in this population since there was a QTL with a high LOD score for chlorophyll content at this locus under both growth conditions (Tables 3, 4). This QTL seems to be of major importance because it expresses a large percentage of phenotypic variance for all the traits. The phenotypes resulting in differential expression at this QTL might be due to a single gene or to a cluster of genes. In addition, another QTL for F_v/F_m on chromosome 2H at 135.7 cM was also identified to control F_m and F_v in both RP1 and RP2. Therefore we suggest that at least two independent loci control tolerance to drought-induced chronic photoinhibition in barley. Interestingly, in a previous study on barley under Mediterranean growing conditions (Baum et al. 2003) two QTLs for kernel weight were detected on chromosome 2H at 119 cM and at 136 cM, therefore close to the QTLs for chlorophyll fluorescence parameters.

Two other QTLs on 2H (Grando et al. 2005), one at 115 cM for dry-organic matter digestibility, an important straw characteristic indicating higher amounts of easily digestible carbohydrates and less lignin at this locus, and another QTL at 135 cM for voluntary intake indicating a higher rate of passage through the digestive system of feed were also identified in this region.

The QTL analysis also showed clearly that under well-watered conditions both parents contributed alleles at the QTLs for all traits. However, under drought-stressed conditions the alleles were contributed by Arta with the only exception of F_o. Therefore, one hypothesis is that the favorable alleles from Arta at these QTLs contribute to the development of functional chloroplast under drought stress conditions. In addition, segregation for all these traits occurred across environments and therefore the identification of QTL for these traits under drought stress conditions could be exploited in a breeding program.

Interest in fluorescence parameter as a selection criterion for drought resistance has been reported in other cereals such as rice, wheat and maize, but the genetic basis for fluorescence parameter was not known in barley. The present work is the first QTL study for fluorescence parameters in barley measured at the post-flowering stage from plants grown under well-watered and drought stress conditions. The results indicated that two major loci located on chromosome 2H at 114–116 and 135.7 cM, respectively, are involved in the regulation of fluorescence parameter under drought stress conditions. The identification of these QTLs in the population of Arta × *H. spontaneum* 41-1 suggests that the improvement of drought tolerance by marker-assisted selection is feasible. However, further study of other breeding material is necessary to validate the presence of putative QTLs, and also may lead to the identification of additional useful loci for the improvement of drought tolerance of barley by marker-assisted selection.

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