

The effects of the vegetative and grain filling durations on the grain traits and the clustering of barley varieties (*Hordeum vulgare* L.)

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The effects of the vegetative and the grain-filling duration on seven quality traits of barley were studied during three growing seasons, using 17 varieties chosen to be tested for desirable balance and variability in the studied traits. Significant differences between the varieties for all measured traits were detected. The differences in grain-filling period among the varieties were mainly due to the differences in the vegetative period, rather to differences in the days to maturity. Grain-filling period was found to be positively correlated with thousand grain weight, kernel plumpness and grain yield per head and negatively correlated with hectoliter weight and grain protein content. The vegetative period was found to be positively correlated with grain protein content and hectoliter weight and negatively correlated with thousand grain weight, kernel plumpness and grain yield per head. The earliness of anthesis decreased the hectoliter weight and the grain protein content, whereas it increased the weight and the plumpness of the grains. However, a short grain-filling period increases the hectoliter weight and the grain protein content, whereas it decreased the weight and the plumpness of the grains. The current work led to identification of a number of barley varieties for further breeding and adaptation to the dramatically changing climatic conditions (due to global warming) in the Mediterranean. The measured traits (including vegetative and grain-filling periods) were used as a basis for cluster analysis. The analysis revealed four clusters on the basis of ear row number and origin. The vegetative and grain-filling periods had a great effect on this kind of grouping.

Key words: barley varieties, grain weight, hectoliter weight, kernel plumpness, grain protein content, cluster analysis.

INTRODUCTION

The length of the grain-filling period is of major importance for the yield and quality of cereal crops and a critical character for breeding adapted varieties in the drought-inflicted Mediterranean region. The earliness and the length of grain-filling period were the phenological traits that most influenced yield in water stress conditions (Gonzalez *et al.*, 2007). Evans & Wardlaw (1976) pointed out that variability exists in cereals between these traits. The relationship between the grain-filling period and the grain yield in

barley is controversial. Aksel & Johnson (1961) observed, that long sowing to heading periods were associated with high yields in barley. Bingham (1969) indicated that grain yield was dependent on the sink size (which is largely determined during the vegetative period), and on the photosynthetic capacity during the grain-filling period in bread wheat (*Triticum aestivum* L.). On the other hand, Nass & Reiser (1975) and Metzger *et al.* (1984) observed that the duration of the grain-filling period was not an important factor in determining the yield in wheat and barley. Rasmusson *et al.* (1979) suggested the acquisition of more data from the study of the correlation between the effect of the grain-filling period and the growth period in barley prior to a serious breeding project.

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Samarrai *et al.* (1987) proposed a grain-filling index, which is the ratio of the grain-filling period to the number of days from sowing to maturity. Singh (1989) reported that the vegetative period was positively correlated with the overall plant growth and that the two periods, vegetative and grain-filling, were negatively correlated with the grain weight.

Geographical origins of the varieties have been correlated with grain yield, heading date, duration of grain-filling period, and growth class (Lasa *et al.*, 2001). Samarrai *et al.* (1987) claimed that traits varied with respect to heritability being high for the vegetative period (0.94) and low for the plant height (0.36). Heritability values were intermediate for the grain-filling period (0.63) and grain-filling index (0.65). Grafius & Okoli (1974) estimated that 72% of the variation in grain yield was explained by the yield components. They suggested that the vegetative and grain-filling periods could be modified during the selection procedure. In general, the vegetative and the grain-filling periods are of great importance for the ability of a variety to differ either in constant increased growths or in constant superior quality traits. Both pre- and post-anthesis conditions operate in concert to determine the potential grain weight of barley in temperate climates (Bingham *et al.*, 2007). Besides, the vegetative and the grain-filling periods are affected by the environmental conditions. The G × E interaction is most prominent for the anthesis and maturity growth stages (McMaster & Wilhelm, 2003). Darroch & Baker (1990) found that a set of three spring wheat cultivars did not show consistent differences in grain-filling duration with respect to the environment. Thus, mean values of quantitative traits have a limited descriptive value, as they represent the behavior of the entries under a single set of the many possible environmental conditions (Lasa *et al.*, 2001).

Many researchers used cluster analysis techniques in order to determine the degree, and the corresponding patterns, of similarities between barley varieties according to morphological/agronomical and/or molecular data (Bahrman *et al.*, 1999; Molina-Cano *et al.*, 2005; Meszaros *et al.*, 2007). These statistical approaches enabled researchers not only to improve the understanding of varieties' differences, but also to assess the utility of the measured traits in varieties' groupings after characterization. An improved understanding of plant development has fundamental scientific relevance, and relationships between traits under particular production environments offer

practical ways to improve adaptation of barley in a commercial context or to formulate effective management or conservation strategies.

The objective of the present study was to determine the effects of the vegetative and the grain-filling periods on: a) grain quality traits, such as thousand grain weight, hectoliter weight, plumpness of kernel, and grain protein content, and b) on the clustering of the tested varieties, under the presence of the remaining grain traits, making a minimal set of assumptions regarding the data and without using prior information about their origin and ear row number.

MATERIALS AND METHODS

Varieties and field experiment

Three field experiments were conducted, two of which were established in the farm of the Aristotle University of Thessaloniki (fields A and B) (40° 32' N, 22° 59' E, 5 m a.s.l.) and the third in the farm of the Cereal Institute of Thessaloniki (field C) (40° 32' N, 23° 00' E, 10 m a.s.l.). Seventeen barley varieties of different origin were evaluated (Table 1). The experimental design was a randomized complete block (RCB) with four replications. Trials were established in field A (15 Nov. 1999), in field B (17 Nov. 2000), and in field C (14 Nov. 2001). Each experimental plot consisted of seven 6 m-long rows spaced 26 cm apart. All plots were sown with the same quantity of seed, i.e. 160 kg ha⁻¹. Thus, the density was 380 plants per m².

The characteristics were assessed in the experiments as follows:

Vegetative period (VP): number of days from seed germination to anthesis (anthesis was considered when 50% of the culms per plot were fully-headed); Grain-filling period (GFP): number of days from heading to physiological maturity; Days to maturity (DM): number of days from seed germination to physiological maturity (maturity was assessed when 50% of the heads per plot were ripe and had lost completely the green colour); Thousand grain weight (TGW): mean weight of ten samples per plot consisting of 1000 seeds; Hectoliter weight (HW): measured using the standard methods of two samples per plot; Kernel plumpness (KP): percentage of kernels with a size greater than 2.5 mm in five samples of 250 g per plot; Grain protein content (GPC): measured by a standard macro-Kjeldahl procedure (the conversion factor used to calculate the grain protein content was 6.25); Kernels per head (KH): estimated by the aver-

TABLE 1. Ear row number and origin of the 17 barley varieties used

Varieties	Ear row number	Origin	Pedigree
G-016252	2x	Greece	(Lignee 131 × AC2531CB82658)
Oglow	2x	Bulgaria	AccNum YG-10695
Carina	2x	Italy	(Inis × Union) × Volta
G-02020	2x	Greece	7200 × Kronos
Georgie	2x	France	Vada × Zephyr
Igri	2x	Germany	(WE 1427 × Ingrid) × Malta
Sonjia	2x	"	(Tria × Malta) × Zuchtstamm A989
ER/Apm	2x	ICARDA	
WI 2291	2x	"	
Trombillo	2x	"	Traill × 1038 × DL70
Rihane 03	6x	"	(As 46) × (Avt × Aths)
Assala 04	6x	"	Harma 03 × Beecher
Beecher	6x	"	Atlas × Vaughn
Arma	6x	France	[(3-5 × Manon) × Ager] × (259-711 × Ares)
Zenit	6x	Bulgaria	Union/Gerda
Plaisant	6x	France	Ager × Nymphe
Matico S	6x	Mexico	Cross No. CMB83A-2646-A

TABLE 2. Maximum and minimum temperatures (°C) and rainfall precipitation (mm) during the culture periods

Year	Culture Periods								
	1999/2000		Rainfall	2000/2001		Rainfall	2001/2002		Rainfall
	Temperature			Temperature			Temperature		
Month	Max	Min	Max	Min	Max	Min	Max	Min	
November	27	0	38	21	0	34	26	2	34
December	18	-1	115	12	-8	3	14	-4	40
January	16	-6	8	20	-4	5	20	-5	23
February	16	-9	53	18	-6	9	24	-7	11
March	20	-1	35	22	-1	21	23	-5	29
April	23	5	64	27	2	45	24	-2	13
May	27	8	68	29	8	83	29	7	74
June	36	10	31	35	13	50	37	9	4

age number in ten heads; Grain yield per head (GYH): calculated across a sample of ten heads per plot.

Temperature and rainfall data during the growing periods are shown in Table 2.

Statistical analysis

Analysis of variance was used to test the hypothesis that all varieties were identical (Steel & Torrie, 1980). The homogeneity of the variances was checked and all measured and derived data were subjected to analysis of variance grouped over years and locations.

Least significant difference (LSD) values were calculated and used in order to compare means of varieties. Phenotypic correlations were calculated for all studied traits with the Pearson coefficient.

The clustering of the 17 varieties according to the eight out of nine characteristics (except for DM, since DM is highly correlated with VP and GFP, DM = VP + GFP) was achieved by the application of hierarchical cluster analysis (Aldenderfer & Blashfield, 1984; Everitt, 1993) using the data of Table 4. The analysis was applied on the grouped data of the three periods in order to include all possible variation

caused by the genotype \times year interaction. The similarity of the varieties was measured through the squared Euclidean distance (Hair *et al.*, 1995; Sharma, 1996) while the Ward's method was used as a criterion for cluster formation (Ward, 1963). Prior to analysis, the values of the nine characteristics were standardized to z scores. The same methodological clustering scheme was applied on barley agronomic traits, grouped over two periods (Zakova & Bencova, 2004). Molina-Cano *et al.* (2005) also have used the Ward's method to cluster 186 barley accessions. The input order stability and validity of the resulted cluster solution was checked by applying the bootstrap methodology implemented in the PermuCLUSTER v.1.0 software (Spaans & Van der Kloot, 2004). The importance of each characteristic for cluster formation was assessed by means of the coefficient of determination r^2 . The value of r^2 expresses the percentage of variance of the examined characteristic accounted by the differences between the clusters (Sharma, 1996). This was calculated after an analysis of variance using the cluster membership as the independent variable. All analyses were performed using the SPSS version 11.5 commercial package.

RESULTS

The grain-filling period (GFP) for each variety was approached by means of two traits, the vegetative period (VP) and the days to maturity (DM). This was done for accurately measuring the differences among the varieties. The analysis of variance showed that there were differences among the varieties for all measured traits (Table 3). The mean values of each trait per variety are presented in Table 4.

The varieties Rihane 03, Beecher, Trombillo, WI-2291, Assala 04, and ER/Apm showed the shortest

VP, high, on average, KP and low HW (Table 4).

The differences in GFP among the varieties were mainly due to the differences in VP rather to differences in DM. This was suggested by the magnitude of variability (mean squares) and the correlation values between the corresponding measured traits (Table 3).

The HW showed a significant positive correlation ($r = 0.349$) with the VP and a negative one ($r = -0.266$) with the GFP (Table 3). These relations are observed in the varieties Rihane 03, Beecher and Assala 04 (Table 4). The thousand grain weight (TGW) was positively correlated with the GFP ($r = 0.347$) and negatively with the VP ($r = -0.641$).

The mean increase in grain weight per day and kernel is shown in Table 5 along with GYH, GFP and KH in all barley varieties, for the growing seasons 2000/2001 and 2001/2002. The 2-row varieties showed a higher increase of grain weight per day than the 6-row ones in the first growing season (2000/2001), but the same increase in the second season (2001/2002). This occurred because the mean GYH in the 6-row varieties decreased from 1.25 to 0.761 and the mean GYH in the 2-row varieties from 0.897 to 0.557, respectively in both culture periods. The reason was probably the low rainfall in April 2002 (13.5 mm) compared with the rainfall in April 2000 (64 mm) and in April 2001 (45 mm) (Table 2).

The cluster analysis (Fig. 1) of the 17 varieties according to the eight out of nine traits (except for DM) showed that clustering of the varieties into two groups is affected by two factors, i.e. the ear row number and the origin of the varieties. The varieties G-16252, Oglow, Carina, G-2020, Georgie, Igri, Sonjia, ER/Apm, WI 2291 and Trombillo, all 2-row varieties, belong to the 1st group. The remaining varieties (Rihane 03, Assala 04, Beecher, Arma, Zenit, Plaisant, Matico S;

TABLE 3. Analysis of variance and correlation coefficients between all studied traits

Trait	Mean square (df = 16)	CV (%)	Vegetative period (VP)	Grain-filling period (GFP)
VP	266.471**	1.14	1	-0.495**
GFP	227.719**	0.79	-0.495**	1
DM	35.148**	3.16	0.954**	-0.212**
TGW	90.031**	9.54	-0.641**	0.347**
HW	4.869**	3.97	0.349**	-0.266**
KP	57.573**	13.90	-0.604**	0.249**
GPC	5.230**	6.18	0.696**	-0.471**
KH	219.249**	16.62	0.039	0.089
GYH	0.256**	15.79	-0.244**	0.179*

significant differences at $p = 0.05^*$ and $p = 0.01^{**}$, $N = 204$

TABLE 4. Mean values of traits studied in three consecutive growing periods

Variety	VP (days)	GFP (days)	DM (days)	TGW (g)	HW (%)	KP (%)	GPC (%)	KH (number)	GYH (g)
G-16252	120.57	32.51	159.73	38.55	58.18	47.45	13.92	20.57	0.82
Oglow	119.40	31.28	157.30	36.35	57.09	45.78	13.72	23.73	0.87
Carina	124.60	28.21	159.47	33.17	59.52	31.33	14.46	21.21	0.71
G-2020	122.93	29.07	158.70	34.26	57.27	22.28	13.71	21.09	0.73
Georgie	122.40	28.60	157.67	33.25	55.26	47.02	14.11	20.15	0.68
Igri	119.33	31.10	157.10	42.89	59.01	59.95	14.63	19.68	0.84
Sonjia	124.33	29.40	160.40	44.31	56.83	63.18	14.88	19.25	0.87
ER/Apm	114.23	31.65	152.53	39.35	56.68	68.93	13.42	18.36	0.72
WI 2291	110.33	32.93	149.93	38.01	54.81	53.92	13.35	17.55	0.66
Trombillo	112.30	28.28	147.23	35.35	54.37	75.63	13.57	17.03	0.60
Rihane 03	110.77	35.68	153.13	38.53	52.62	73.87	12.91	27.39	1.05
Assala 04	114.53	32.89	154.07	38.98	52.88	56.93	12.93	26.61	1.05
Beecher	112.87	35.49	154.97	41.89	51.57	68.53	13.74	25.83	1.09
Arma	118.83	31.83	157.37	34.31	55.04	65.22	13.81	30.49	1.04
Zenit	119.10	32.33	158.13	35.77	52.85	47.00	14.02	28.10	1.01
Plaisant	120.70	31.01	158.37	34.33	56.29	38.57	14.65	30.28	1.06
Matico S	120.43	30.99	158.10	34.53	50.48	62.07	13.33	26.11	0.90
LSD _{0.05}	1.03	0.85	0.95	2.81	1.78	8.72	0.87	3.16	0.11
LSD _{0.01}	1.36	1.12	1.26	3.71	2.35	11.56	1.10	4.17	0.14

TABLE 5. Grain yield per head (GYH), grain-filling period (GFP), number of kernels per ear (KH) and weight increase per day during the growing seasons 2000/2001 and 2001/2002

Variety	Growing seasons							
	2000/2001				2001/2002			
	GYH (g)	GFP (days)	KH (number)	Weight increase per day (mg)	GYH (g)	GFP (days)	KH (number)	Weight increase per day (mg)
G-16252	0.965	35.17	21.82	1.257	0.638	34.66	20.18	0.912
Oglow	1.085	34.00	24.81	1.286	0.657	32.33	23.27	0.873
Carina	0.880	30.67	22.50	1.275	0.571	29.16	20.74	0.944
G-2020	0.894	33.00	21.68	1.250	0.600	31.50	21.79	0.874
Georgie	0.883	30.50	23.85	1.214	0.536	32.50	16.99	0.971
Igri	1.025	33.17	21.40	1.444	0.671	33.83	20.74	0.956
Sonjia	0.830	32.83	18.35	1.378	0.634	31.16	18.10	1.124
ER/Apm	0.888	33.33	19.79	1.346	0.670	33.33	18.29	1.099
WI 2291	0.790	32.50	19.06	1.275	0.589	32.50	18.98	0.955
Trombillo	0.731	30.83	19.72	1.202	0.604	31.00	17.46	1.116
Rihane 03	1.295	38.50	33.15	1.015	0.878	34.83	25.02	1.008
Assala 04	1.303	35.33	29.98	1.230	0.720	30.33	22.87	1.038
Beecher	1.307	37.67	28.69	1.209	0.815	34.50	22.79	1.037
Arma	1.180	36.83	32.70	0.980	0.911	30.33	29.85	1.006
Zenit	1.293	34.67	30.40	1.227	0.698	33.83	25.20	0.819
Plaisant	1.381	35.50	36.17	1.076	0.669	30.33	24.47	0.901
Matico S	1.036	36.33	29.42	0.969	0.636	30.83	20.10	1.026
LSD _{0.05}	0.154	1.078	3.376	0.129	0.158	1.322	5.388	0.136
LSD _{0.01}	0.204	1.430	4.476	0.171	0.210	1.752	7.144	0.180

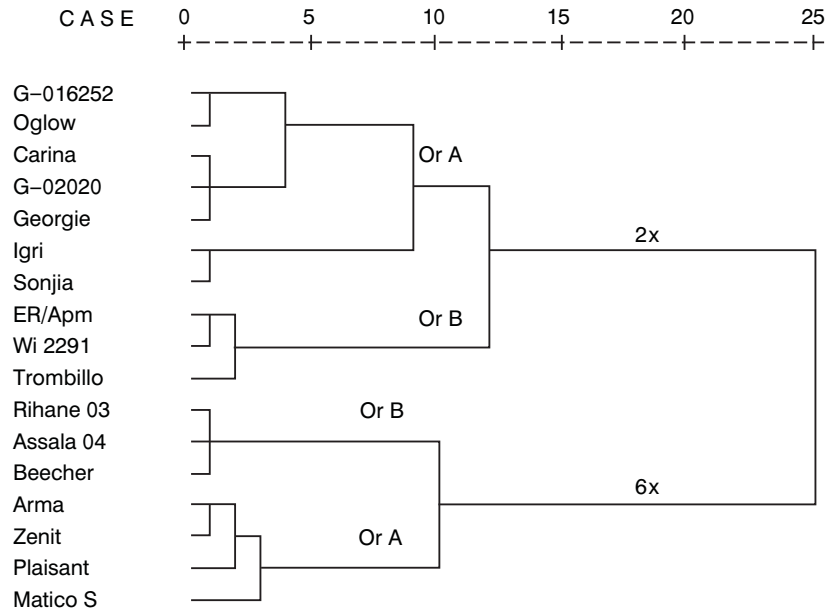


FIG. 1. Dendrogram demonstrating the clustering of 17 barley varieties on the compiled data set recorded from eight traits.

all 6-row varieties) belong to the 2nd group. The formation of the two major variety clusters was mainly caused by their significant differences in the GFP ($r^2 = 0.347, p = 0.013$), HW ($r^2 = 0.542, p = 0.001$), KH ($r^2 = 0.821, p < 10^{-4}$), and GYH ($r^2 = 0.769, p < 10^{-4}$). The VP did not affect the two-group clustering (2x and 6x) of the varieties. The GFP showed a medium discrimination effect ($r^2 = 0.347$) on the clustering of the varieties; but this effect was not very high when compared with the effects (r^2 values) of the other traits (KH, GYH and HW).

Further study of the cluster analysis dendrogram revealed that the groups of the two and six ear-row-number varieties can be divided into two subgroups. The 2-row varieties G-16252, Oglow, Carina, G-2020, Georgie, Igri and Sonjia, varieties of European origin, belong to the 1st group and 1st subgroup (2x, Or A). The 2nd subgroup (2x, Or B) consists of the 2-row varieties ER/Apm, WI 2291 and Trombillo, all originating from ICARDA. The 6-row varieties Rihane 03, Assala 04, Beecher, with origin from ICARDA, belong to the 2nd group and 3rd subgroup (6x, Or B). The 4th subgroup (6x, Or A) consists of the 6-row varieties Arma, Zenit, Plaisant from Europe and Matico S from Mexico. The division into the four subgroup varieties (with different ear row number and origin) was mainly caused by their significant differences in VP ($r^2 = 0.867, p < 10^{-4}$), GFP ($r^2 =$

$0.586, p = 0.008$), HW ($r^2 = 0.671, p = 0.002$), KH ($r^2 = 0.912, p < 10^{-4}$), GYH ($r^2 = 0.858, p < 10^{-4}$) and GPC ($r^2 = 0.505, p = 0.024$). All the above mentioned traits showed a high effect (high r^2 values) on the four group clustering solution. In this case, and on the contrary to the previous cluster solution with two groups, both VP and GFP showed a high effect in cluster formation.

DISCUSSION

Our results showed that VP is negatively correlated with GFP ($r = -0.495$) and positively with DM ($r = 0.954$) (Table 3). This is in agreement with the data by Singh (1989) and Gonzalez *et al.* (2007), while Metzger *et al.* (1984), Knott & Gebeyehou (1987), and Samarra *et al.* (1987) reported that DMs of the long and short GFP lines are almost equal and the predominant variation among them is in VP rather than in DM. Due to this behavior, the correlation between GFP and DM was very low ($r = -0.212$). There was a negative correlation between grain yield per head (GYH) and VP but the r value was low (-0.244). In the case of GYH and GFP, the correlation was positive but still the r value was low ($r = 0.179$). Kernel plumpness (KP) showed a positive correlation with the GFP ($r = 0.249$) and a negative one with VP ($r = -0.604$). Earliness, long GFP and plant density af-

fect this trait. With reference to quality traits, GFP was negatively correlated with grain protein content (GPC) ($r = -0.471$). GPC in barley is highly influenced by the environment. Bertholdsson (1999) suggested that breeding for a prolonged vegetative period might reduce the environmental effects on GPC.

This means that a long GFP creates better conditions for transportation of photosynthetic carbohydrates to the grains. Talbert *et al.* (2001) reported that a longer grain-filling period is usually associated with higher grain protein content in two environments, but with lower grain protein content in a cool, wet environment and that HW is positively correlated with VP and negatively with GFP. This might be attributed to the limited duration of GFP, which leads to the formation of kernels with low HW. This means that varieties with short VP duration and long GFP duration have low HW.

Additionally, the cluster analysis revealed two main groups of varieties (2× and 6×) that can be further divided into two subgroups each (Or A and Or B). The vegetative period and the grain-filling period seem to be important factors affecting this analytical clustering. It is worth noting that the vegetative period did not contribute to the separation of the 2× and 6× varieties. By contrast, the grain-filling period was found to be an important clustering factor, but not as important as the traits of kernels per head, the grain yield per head and the hectoliter weight. These findings resulted from the comparison of the corresponding r^2 values.

In general, the earliness of anthesis decreases the hectoliter weight and the grain protein content, whereas it increases the weight and the plumpness of the grains. However, a short grain-filling period increases the hectoliter weight and the grain protein content, whereas it decreases the weight and the plumpness of the grains. In addition, quality characteristics, such as hectoliter weight, kernel plumpness and grain protein content, were influenced not only by earliness, but also by environmental conditions during the grain-filling period. Each variety has its way to escape drought that occurs during the anthesis and the grain-filling period. For any environment there is an optimum heading date. Date of heading (rather than date of ripening), is the usual selection criterion. The above results can be explained by the extremely high temperatures during the grain-filling period as can be observed in the Mediterranean regions.

CONCLUSIONS

The experiments provide a solid characterisation of 17 varieties under field conditions in a Mediterranean environment. A significant variation was observed in the measured traits. The earliness of anthesis decreases the hectoliter weight and the grain protein content, whereas it increases the weight and the plumpness of the grains. Additionally, a short grain-filling period increases the hectoliter weight and the grain protein content, whereas it decreases the weight and the plumpness of the grains.

The measured traits were used as a basis for cluster analysis. The germplasm selected for the study is drawn from four groups: 2-row and 6-row ICARDA and European barley varieties. The cluster analysis regroups the germplasm on this basis. The analysis revealed four clusters of varieties. The vegetative and grain-filling periods posed a high effect on this grouping. Main differences were observed between 2-row and 6-row varieties.

The current work led to the identification of a number of barley varieties (Igri, G-16252, Matico S and Zenit) for further breeding and adaptation to the dramatically changing climatic conditions (due to global warming) in the Mediterranean.

The genetic basis and the physiological effects of variation in barley development are complex and in order to be better understood it is worthwhile, in a future study, to examine also the differences in key developmental genes that are known to drive adaptation to different environments (e.g. semi-dwarfing, photoperiod sensitivity, earliness), and the fundamental differences in sink/source relationships between 2-row and 6-row barley.

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