Determinants of barley grain yield in drought-prone Mediterranean environments

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Abstract

The determinants of barley grain yield in drought-prone Mediterranean environments have been studied in the *Nure* x *Tremois* (NT) population. A large set of yield and other morpho-physiological data were recorded in 118 doubled haploid (DH) lines of the population, in multi-environment field trials (18 site-year combination). Agrometeorological variables have been recorded and calculated at each site too. Four main periods of barley development were considered, vegetative, reproductive early and late grain filling phases, to dissect the effect on yield traits of the growth phases. Relationships between agrometeorological variables, grain yield (GY) and its main components (GN and GW) were also investigated by correlation. Results firstly gave a clear indication of the involvement of water consumption in determining GY and GW (r^2=0.616, P=0.007 and r^2=0.703, P=0.005, respectively) calculated from sowing to the early grain filling period, while GN showed its highest correlation with the total photothermal quotient (PQ) calculated for the same period (r^2=0.646, P=0.013). With the only exception of total PQ calculated during the vegetative period, all significant correlations with GY were associated to water-dependent agrometeorological parameters. As a second result, the NT segregating population allowed us to weight the amount of interaction due to genotypes over environments or to environments in relation to genotypes by a GGE analysis; 47.67% of G+GE sum of squares was explained by the first two principal components. Then, the introduction of genomic information at major barley genes regulating the length of growth cycle allowed us to explain patterns of adaptation of different groups of NT lines according to the variants (alleles) harbored at venalization (*Vrn-H1*) in combination with earliness (*Eam6*) genes. The superiority of the lines carrying the *Nure* allele at *Eam6* was confirmed by factorial ANOVA testing the four possible haplotypes obtained combining alternative alleles at *Eam6* and *Vrn-H1*. Maximum yield potential and differentials among the NT genotypes was finally explored through Finlay-Wilkinson model to interpret grain yield of NT genotypes together with yield adaptability (Ya), as the regression coefficient b_i; Ya ranged from 0.71 for NT77 to 1.20 for NT19. Lines simply harboring the *Nure* variants at the two genes behaved as highest yielding (3.04 t ha^{-1}), and showed the highest yield adaptability (b_i=1.05). The present study constitutes a starting point towards the introduction of genomic variables in agronomic models for barley grain yield in Mediterranean environments.

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Introduction

Rainfed agricultural areas of the Mediterranean Basin are characterized by low and erratic rainfall either in late spring and summer, or throughout the whole crop growth cycle under semi-arid conditions (with annual precipitation 250-500 mm). Since an increased depletion of soil water resources is expected from a gradual rise in temperature, the area is potentially vulnerable to global climate change (Schröter *et al*., 2005) and grain yield (GY) of small-grained cereals like durum wheat and barley can be severely influenced. Therefore, minimizing the gap between *potential* and *actual* yield, together with increasing GY stability, could be crucial for guaranteeing the cereal crop sustainability (Cattivelli *et al*., 2008). Barley is predominantly used throughout the Mediterranean region in lower yielding environments, due to its better performance in such input situations compared to durum wheat. The diploid barley is in fact commonly considered less susceptible to the water deprivation periods encountered (Ryan *et al*., 2008). Its grain yield integrates two major components, grain number per unit area (GN) and mean grain weight (GW). Although the two traits are mainly determined at different developmental stages (Miralles and Slafer, 1999), and thus influenced by different conditions and stresses during the growing season, GN and GW are interrelated and subjected to continuous evolutionary trade-off (Sadras, 2007). Nevertheless, as yield components in grain crops are generated throughout the whole crop growing season, yield seems to be much more sensitive to changes in availability of resources in some particular phases than in others. During pre-anthesis, the success of floret set defines the potential grain number (Gonzalez *et al*., 2003), while grain weight rely on the extent to which post-anthesis conditions favor grain filling (Ugarte *et al*., 2007). Knowing the critical period when GY is more strongly determined could thus be relevant for developing more adequate strategies for improving yield through either breeding or management. As the rate of physiological development is associated to drought escape, either by avoiding stress during the crop cycle or, more frequently, by avoiding the coincidence of the most sensitive growth phases with the most likely occurrence of the stress, one successful strategy for adaptation in semi-arid environments is a fast rate of development and a short time to flowering and grain maturity (Slafer *et al*., 2005). It has been proposed this being achieved through fine tuning the proportion of developmental time allocated to the different phenological phases, and allowing the available water to be used by the plant before it is lost from the soil as the temperature increases. The developmental pattern influencing GY and its components relates to the partitioning of a particular crop cycle into different proportions of vegetative and reproductive phases; by allocating different proportions of time to vegetative or reproductive growth, such *customization* is expected to fit the crop within the variable growing season conferring the ability to maintain its performance under stressful environments (Slafer *et al*., 2005). A longer time of stem elongation has been associated to an augmented yield potential in wheat and may suggest an alternative *avenue* for improving GY (Gonzalez *et al*., 2003). In barley the different developmental stages (namely vegetative, reproductive, and grain filling phases) show different sensitivity to water deficiency, and the generally faster growth before anthesis compared to wheat might explain its relative success in Mediterranean environments (Tambussi *et al*., 2005). From a physiological point of view, besides the phenological stage of the plant when the stress conditions act, the primary determinants of GY adaptation to drought in cereals can be expressed as the integrated response of distinct plant processes to limiting resources (Araus *et al*., 2008). In recent years, several approaches have been used to incorporate explicit information on environmental (ecophysiological) and genetic factors into statistical models for a better understanding of the architecture of the trait as observed across environments (Romagosa *et al*., 2009). The processes regulating the development of temperate cereals (and ultimately the actual yield) are complex due to interactions between genetic and environmental factors, of which the most important drivers are temperature and photoperiod (Miralles and Slafer, 1999). Genetically, variation at key genomic loci regulating the crop growth cycle can fine tune i) vernalization sensitivity (*Vrn* genes), ii) photoperiod response (*Ppd* genes) and iii) early maturity independent from temperature and photoperiod, or earliness *per se* (*Eam* or *Eps* genes).

Despite a large literature accumulated in the past about genotype × environment (GE) interaction for grain yield and its components in crops (van Oosterom *et al*., 1993; Kang and Gauch, 1996; van Eeuwijk, 2006), empirical studies introducing external environmental, physiological and/or genetic information in the form of co-variables useful to describe G+GE patterns are relatively few (see, *e.g.* Romagosa *et al*., 2009). One interesting example has been recently reported for a barley segregating population tested in a wide range of Mediterranean environments and characterized with molecular markers associated to four major regulators of pheonological adjustment: *Vrn-H1*, *Vrn-H2*, *Ppd-H2* and *Eam6* (Francia *et al*., 2011).

Genomics-based approaches provide access to agronomically desirable alleles present at quantitative trait loci (QTLs) and genes affecting crop responses in rainfed environments (Tondelli *et al*., 2006; Tuberosa and Salvi, 2006; Distelfeld *et al*., 2009). However, there is a continuous need to integrate disciplines such as genomics, plant physiology and the applied agricultural sciences. Without a strong link with research on plant breeding, agronomy, and crop physiology, the contribution of genomics to crop production under drought stress will remain marginal.

In this study we report a study of barley response to drought using the data generated by the *Nure* (winter) x *Tremois* (spring) mapping population, in multi-environment trials across the Mediterranean Basin. In particular, our aims have been: i) to investigate the relationships between a series of agrometeorological variables, grain yield (GY) and its main components (GN and GW), and ii) to interpret adaptation of genotypes with different alleles at major loci of phenological development (*Eam6* and *Vrn-H1*) in terms of genotype main effect and genotype x environment interaction.

Materials and methods

Plant material and field trials

Pure stock seed of the 118 doubled-haploid (DH) lines was derived by anther culture from the cross *Nure* x *Tremois*, named *NT*s, and multiplied at ICARDA according to Francia *et al*. (2004) and (2011), respectively. *Nure* - [(*Fior 40* x *Alpha*2) x *Baraka*] - is a winter, two-rowed, Italian feeding variety showing frost tolerance, wide adaptability, high yield potential and yield stability in irrigated as well as in moderately droughted conditions. *Tremois* - [(*Dram* x *Aramir*) x *Berac*] - is a spring, high yielding, two-rowed, French malting cultivar, adapted to fertile environments. In the frame of MABDE (*Mapping Adaptation of Barley to Droughted Environments*) project, a multi-environment field trial -18 site-year combinations- was conducted in six countries of the Mediterranean Basin (Table 1 and Francia *et al*., 2011) for harvest seasons 2003/2004 and 2004/2005. Locations were contrasting for water holding capacities (AWC, available water holding capacity) of soil and for natural rainfall (high *vs* low, based on past meteorological data), or for supplemental irrigation when two trials were grown at the same site. Water availability for plants in the field throughout the barley life cycle was characterized by soil water content (SWC) and by a Water Stress Index (WSI), calculated according to Francia *et al*. (2011). Each site of the multi-environment trial (MET) network was sown in a 15-by-20 rectangular grid of 6 m^2 plots. The experimental design consisted of two replicates for the 120 entries (118 NTs plus *Nure* and *Tremois*) augmented by four checks repeated 15 times in a systematic diagonal fashion to adjust for spatial variation; the first check, cv. *Harmal*, was grown at every site, whereas the other three (a landrace, a modern and an old cultivar) varied across sites being relevant to each country in which the trial was being grown.

Phenotypic data were collected in each field trial as already reported by Francia *et al*. (2011), and in the present work the analyses concentrated on grain yield (GY, t ha^{-1}), average grain weight (GW, g, calculated as thousand grain weight 10^{-3}), and number of grains per unit area (GN, m^{-2}, calculated as GY GW^{-1}).

Explicit environmental characterization and data analysis

A series of environmental co-variables was recorded on a daily basis at each site during the entire length of growing period (LGP, days from sowing to harvest). They were minimum and maximum temperature, rainfall, reference evapotranspiration (ET$_0$), and solar radiation. Since time to jointing, as well as to physiological maturity were not available for each trial, we followed the indications of Romagosa *et al*. (2009), and of Francia *et al*. (2011), after which the period of 3 weeks before heading reasonably includes the largest part of stem elongation, and the most important stages for the determination of grain number in barley, from GS 31 to GS 69. Accordingly, we divided LGP in the four following steps: 1, vegetative growth (days from sowing to heading –21 d);

2, reproductive growth (days from heading –21 d to heading); 3, early grain filling (days from heading to heading +14 d); 4, late grain filling (days from days to heading +14 d to harvest). To physically characterize the environments during the diverse developmental phases, 14 agrometeorological variables were then calculated/derived: total solar radiation (Sr, W m^{-2}); number of days with minimum temperature below 0°C (dTb0); number of days with maximum temperature above 30°C (dTa30); average minimum temperature (Tmin); average maximum temperature (Tmax); total Growing Degree Days (GDD); total photothermal quotient (PQ, calculated as [Sr (mean T $-4.5°C)^{-1}$]); total evapotranspiration (ET_0, mm); total rainfall (Rf, mm); total water input (WT, mm, calculated as Rf + irrigation); total water demand (WD, calculated as WT ET_0^{-1} 100); total water consumption (Wcons, calculated as WT + SWC); total water stress index (WSI); WSI to length of growing period (WSI/LGP, WSI d^{-1}). Statistical analysis was conducted using Genstat 11th edition software (Payne *et al*., 2008) following three main approaches:

i) Relationships between each agrometeorological variables, grain yield, grain number and grain weight were initially investigated by simple correlation calculated on the basis of environmental means of the sites. Multiple linear regressions were then performed to investigate how each set of explanatory variables was associated with the dependent variables GY, GN, and GW. To test for many alternative multiple linear models together, the *All-Subset Regression* procedure was used, and the best subset of explanatory variables identified according to three different statistics: i) the Adjusted R-squared accounted for, ii) the Mallows Cp criterion, and iii) the Akaike information criterion. Convergence between the increase in Adjusted R^2 and the decrease in Mallows Cp and AIC values was used to find the *best* combination from among the starting set of agrometeorological variables.
ii) A mixed model analysis adjusting for row and column effects was applied to generate Best Linear Unbiased Estimators (BLUEs) for GY, GN and GW data in each trial. A Sites Regression (SREG) linear-bilinear (multiplicative) model for alternative partitioning of the phenotypic variability was applied to the data in a GGE analysis that models the genotype (G) main effect and genotype x environment (GE) interaction jointly, by applying a principal components analysis to the genotype by environment two-way table of means - with the genotypes being the objects and the environments being the variables (Yan *et al*., 2001, 2007). A GGE biplot was constructed by plotting the first principal component (PCA1) scores of the genotypes and the environments against their respective scores for the second principal component (PCA2) that result from singular value decomposition of environment-centered genotype-by-environment data for GY. As an effective visual tool to examine *which-won-where* in our multi-environment trials, the genotypic classification of the NT lines according to the *Eam6–Vrn-H1* haplotype was included in the generated GGE biplot. Further details on the applied model have been given elsewhere (Romagosa *et al*., 2009).
iii) According to the procedures described by Kraakman *et al*., (2004), Finlay-Wilkinson coefficients (b_i) were estimated as a measure for yield adaptability (Ya), while Eberhart-Russell mean squared deviations from regressions (s_i^2) were estimated as a measure for yield stability (Ys). Both statistics were based on the regressions of yields for individual genotypes in a trial on an environmental index, here represented by the environment average yield supposed to express the general growing conditions in the trial.

Table 1. Trial sites of the *Nure* x *Tremois* population in six countries of the Mediterranean basin for harvest years 2004 and 2005; order follows average grain yields, with fields divided into two subgroups of wet and dry locations.

Code	Site	Country	Location (Lat - Long)	Season	Watering*	AWC	Water input°	WSI	PQ 1	GY (t ha^{-1})	GN (m^{-2})	GW (g 10^{-3})
SYR_5W	Tel Hadya	Syria	36°01'N - 36°56'E	2004/2005	Wet (rainfed)	170	192	39.5	940	5.43	12402	43.9
ITA_5W	Foggia	Italy	41°28'N - 15°33'E	2004/2005	Wet (irrigated)	130	362	57.8	1328	4.88	10305	47.7
ITA_5F	Fiorenzuola	Italy	44°55'N - 09°54'E	2004/2005	Wet (rainfed)	144	292	31.0	912	4.58	-	-
TUR_4W	Haymana	Turkey	39°26'N - 32°30'E	2003/2004	Wet (irrigated)	150	282	39.9	1237	4.44	10673	41.8
SYR_4W	Tel Hadya	Syria	36°01'N - 36°56'E	2004/2005	Wet (rainfed)	170	290	52.5	1106	4.13	8587	48.4
ITA_4W	Foggia	Italy	41°28'N - 15°33'E	2003/2004	Wet (irrigated)	130	327	27.9	1096	3.78	11169	34.2
DZA_5W	El Khroub	Algeria	36°15'N - 06°42'E	2004/2005	Wet (rainfed)	100	130	63.6	1188	3.50	-	-
JOR_5W	Rabba	Jordan	31°16'N - 35°44'E	2004/2005	Wet (rainfed)	120	217	42.8	525	0.80	2334	34.7
JOR_4W	Rabba	Jordan	31°16'N - 35°44'E	2003/2004	Wet (rainfed)	120	194	49.5	682	0.07	257	16.4
TUR_5	Haymana	Turkey	39°26'N - 32°30'E	2004/2005	Dry (rainfed)	150	174	27.2	1022	3.89	11434	34.0
ITA_5D	Foggia	Italy	41°28'N - 15°33'E	2004/2005	Dry (rainfed)	130	268	63.8	1344	3.85	10139	38.2
TUR_4D	Haymana	Turkey	39°26'N - 32°30'E	2003/2004	Dry (rainfed)	150	232	50.5	1245	3.30	7915	41.9
ITA_4D	Foggia	Italy	41°28'N - 15°33'E	2003/2004	Dry (rainfed)	130	258	40.4	1005	3.20	10123	31.8
SYR_5D	Breda	Syria	35°56'N - 37°10'E	2004/2005	Dry (rainfed)	153	143	63.5	1459	2.42	6394	37.2
SYR_4D	Breda	Syria	35°56'N - 37°10'E	2003/2004	Dry (rainfed)	153	204	63.8	1011	1.35	3352	40.4
JOR_4D	Ramtha	Jordan	32°32'N - 36°02'E	2003/2004	Dry (rainfed)	120	151	67.2	1017	1.33	-	-
JOR_5D	Ramtha	Jordan	32°32'N - 36°02'E	2004/2005	Dry (rainfed)	120	140	61.9	889	0.50	1518	31.6
ESP_5D	Foradada	Spain	41°39'N - 01°23'W	2003/2004	Dry (rainfed)	120	167	67.7	1116	0.48	-	-

*Sites are classified according to previous meteorological data; in some case the Wet site was created artificially by supplementary irrigation supplied during the growing season. °Water input, total rainfall plus irrigation (mm) from sowing to harvest. Lat, latitude; Long, longitude; AWC, available water holding capacity (mm m^{-1}) of the soil; WSI, water stress index; PQ 1, total photothermal quotient calculated for the vegetative period as [Solar radiation (mean T $-4.5°C)^{-1}$]; GY, average grain yields; GN, grain number for unit area; GW, average grain weight; N, north; E, east; W, west.

Results

Phenotypic and agrometeorological data were gathered from 18 trials conducted in six countries of the Mediterranean Basin (Francia *et al*., 2011). As summarized in Table 1, the NT population showed marked variation in GY across years and locations, ranging from more than 5 t ha^{-1} in the SYR_5W site (Tel Hadya, Syria, in harvest season 2004/2005) to nearly 0 t ha^{-1} in the JOR_4W site (Rabba, Jordan, in harvest season 2003/2004). Simple correlation analyses, calculated on the basis of environmental means, provided a clear indication of the involvement of specific agrometeorological variables related to the water resource respect to final yield (Table 2). With the only exception of total photothermal quotient PQ 1 (*i.e.*, PQ calculated from sowing to heading date –21 d), all significant correlations with yield were associated to water-dependent parameters, such as water availability (*i.e.*, Rf and WT), water consumption (*i.e.*, Wcons and WD) and water stress index either accumulated during the growing season (WSI), or calculated on an average daily basis (WSI/LGP). In total, seven out of 14 environmental co-variables were significantly correlated with GY, whereas ten and four were correlated with GN and GW, respectively. Grain yield and average grain weight showed their highest correlation (r^2=0.616, p=0.007 and r^2=0.703, P=0.005, respectively) with water consumption, calculated from sowing to the early grain filling (namely, heading date +14 d); while grain number per unit area showed its highest correlation with the total photothermal quotient calculated for the same period (r^2=0.646, P=0.013).

Among each set of explanatory variables identified by single correlation, alternative multiple linear models were tested with the *All-possible subset selection* procedure. Using GY as response variate, the model maximizing Adj-R^2 and, at the same time, minimizing the Mallows Cp and AIC criteria (67.01%,–0.88 and 17.12, respectively), was the one including PQ 1, Wcons 1+2+3, and WSI (Figure 1A). Fitted in an accumulated regression ANOVA, the three terms accounted for 21.9%, 18.3% and 32.6% of the total sum of squares, respectively. The same procedure was then applied to GN, and the subset of selected variates included Sr 1+2+3, PQ 3, WD 1 and WSI/LGP (Figure 1B), accounting for 31.4%, 10.8%, 3.5% and 47.2% of the total sum of squares, respectively. Finally, only Wcons 1+2+3 was retained as determinant of GW (Figure 1C) and it explained 49.5% of the total sum of squares.

After survey of influence of meteo variables on GY, the effect on GY of important loci that are known to regulate barley growth and development has been verified. The mechanistic importance of few simple growth cycle determinants on barley grain yield was already demonstrated in the NT dataset by introducing molecular marker information that classify allelic variation at four phenological genomic loci: *Vrn-H1*, *Vrn-H2*, *Ppd-H2* and *Eam6* (Francia *et al*., 2011). This approach revealed being suitable in understanding both the genotype main effect and the genotype x environment variability. To better interpret adaptation of genotypes, we aimed at visualizing patterns in yield-trial data with principal components G+GE (departure from location means), and thereby showing which genotype *won where*. The GGE biplot for the NT population in our multi-environment trial (Figure 2) indicates in fact the best performing genotypes in each environment and group of environments. As position of genotypes is given by the estimates for their genotypic scores and the coordinates for the environments originate from the estimates for the environmental scores, distances from the origin are proportional to the amount of interaction due to genotypes over environments or to environments in relation to genotypes. In this study, PCA1 and PCA2 accounted for 26.86 and 20.82% of the total G+GE, respectively, and a total of 47.67% of G+GE sum of squares. As expected, more differences are visible in site means than in genotypic means.

On one hand, NT lines carrying the *Nure* allele at *Vrn-H1* (with the scores of genotypes furthest from the origin along first axis) indicate their association with TUR_4W and TUR_4D scores, showing that they were most responsive to these environments. On the other hand, genotypes carrying the *Nure* early allele at *Eam6* in combination to the *Tremois* vernalization insensitive allele at *Vrn-H1* had clear association

Table 2. Correlations of agrometeorological variables with grain yield, grain number for unit area, and average grain weight. Only significant values (P≤0.05) are reported.

MeteoVar*	GY r^2	P value	MeteoVar	GN r^2	P value	MeteoVar	GW r^2	P value
PQ 1	0.468	0.050	Sr 1+2+3	0.560	0.037	Sr 1+2	0.564	0.036
Rf 2	0.556	0.017	Tmin 1+2+3	-0.627	0.016	Sr	0.617	0.019
Rf	0.526	0.025	Tmax 1+2+3	-0.634	0.015	PQ 1	0.545	0.044
WT 2	0.556	0.017	PQ 1	0.562	0.036	PQ 1+2	0.580	0.030
WT 1+2+3	0.504	0.033	PQ 1+2+3	0.646	0.013	Wcons 1	0.665	0.009
WT	0.572	0.013	Rf 2	0.592	0.026	Wcons 1+2	0.700	0.005
Wcons 2	0.475	0.046	WT 2	0.592	0.026	Wcons 1+2+3	0.703	0.005
Wcons 1+2	0.563	0.015	ET$_0$ 1	0.612	0.020	Wcons	0.687	0.007
Wcons 1+2+3	0.616	0.007	WD 1	-0.582	0.029	ET$_0$	0.577	0.031
Wcons	0.612	0.007	WSI 4	-0.636	0.015	-	-	-
WD 2	0.478	0.047	WSI/LGP	-0.636	0.015	-	-	-
WSI -	0.469	0.050	-	-	-	-	-	-
WSI/LGP	-0.528	0.024	-	-	-	-	-	-

*Each variable was calculated either for the LGP (days from sowing to harvest) or for its components. LGP, length of growing period; GY, grain yield; GN, grain number for unit area; GW, average grain weight; MeteoVar, meteorological variables; PQ 1, total photothermal quotient calculated for 1; Sr 1+2+3, total solar radiation (W m^{-2}) for 1, 2, and 3; 1, vegetative growth (days from sowing to heading -21 d); 2, reproductive growth (days from heading -21 d to heading); 3, early grain filling (days from heading to heading +14 d); Sr 1+2, total solar radiation (W m^{-2}) for 1 and 2; Rf 2, total rainfall (mm) for 2; Tmin 1+2+3, average minimum temperature for 1, 2, and 3; Sr, total solar radiation (W m^{-2}); Rf, total rainfall (mm); Tmax 1+2+3, average maximum temperature for 1, 2, and 3; WT 2, water input as total rainfall + total irrigation (mm) for 2; PQ 1+2, total photothermal quotient calculated for 1 and 2; WT 1+2+3, water input as total rainfall + total irrigation (mm) for 1, 2, and 3; PQ 1+2+3, total photothermal quotient calculated for 1, 2, and 3; Wcons 1, water consumption (WT + SWC) for 1; WT, water input as total rainfall + total irrigation (mm); Rf 2, total rainfall (mm) for 2; Wcons 1+2, water consumption (WT + SWC) for 1 and 2; Wcons 2, water consumption (WT + SWC) for 2; WT 2, water input as total rainfall + total irrigation (mm) for 2; Wcons 1+2+3, water consumption (WT + SWC) for 1, 2, and 3; ET$_0$ 1, total evapotranspiration (mm) for 1; Wcons, water consumption (WT + SWC); WD 1, water input to ET$_0$ (WT ET$_0^{-1}$ 100) for 1; ET$_0$, total evapotranspiration (mm); WSI 4, water stress index for 4; 4, late grain filling (from days to heading +14 d to harvest); WD 2, water input to ET$_0$ (WT ET$_0$–1 100) for 2; WSI, water stress index; WSI/LGP, WSI to length of growing period (WSI d^{-1}).

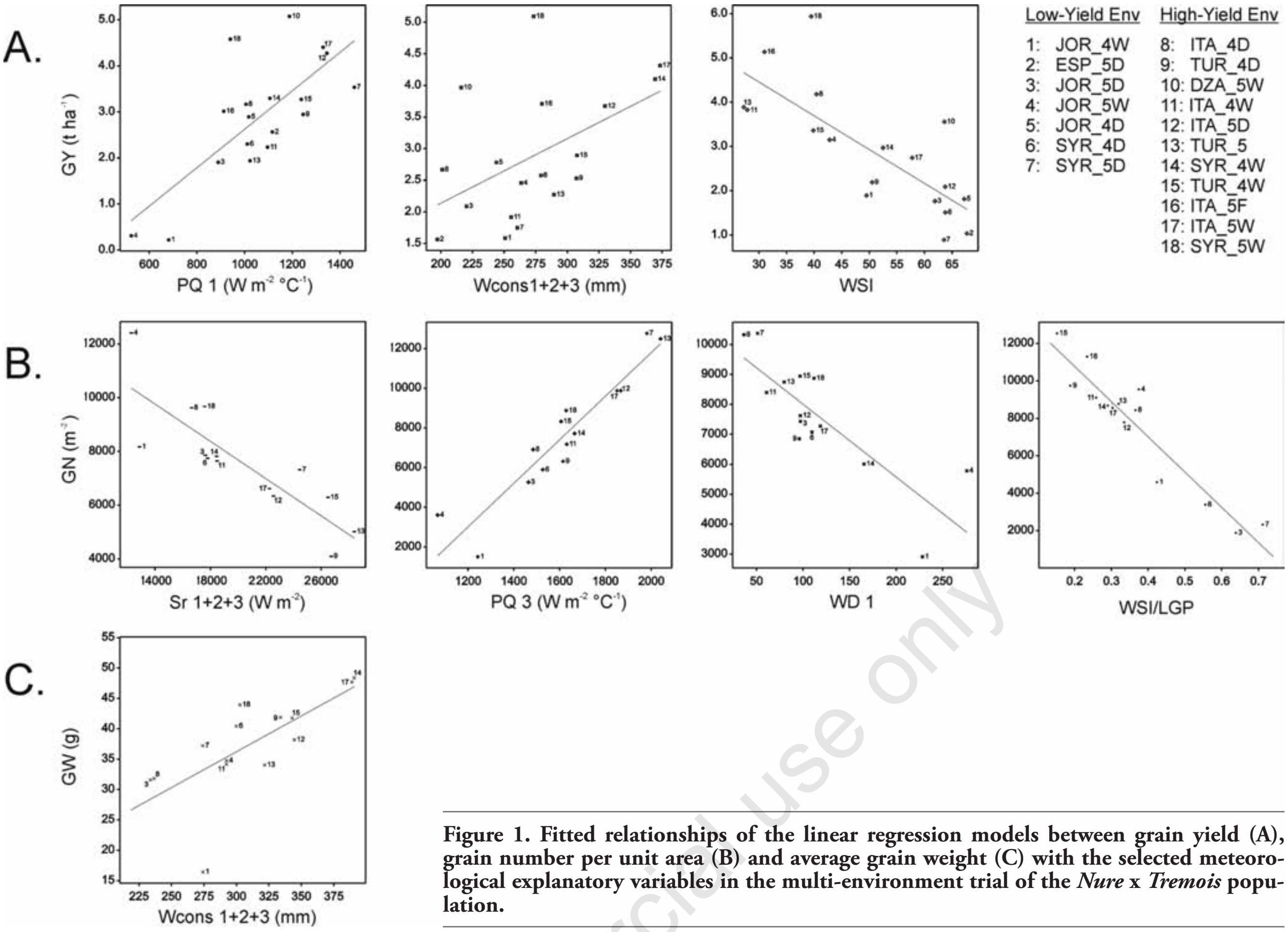

Figure 1. Fitted relationships of the linear regression models between grain yield (A), grain number per unit area (B) and average grain weight (C) with the selected meteorological explanatory variables in the multi-environment trial of the *Nure* x *Tremois* population.

with the conditions that were met in DZA_5W (planted in early spring, see Table 1). We can conclude that PCA1 is apparently driven by cold temperatures pointing to autumn-sown TUR_4W and TUR_4D trials, whereas second axis mainly showed the differential behavior of DZA_5W and, to a lesser extent, of SYR_5D, respect to the rest of environments. The conditions observed at TUR_4D could be considered an *ideal environment* for exploiting maximum yield potential and differentials among the NT genotypes. In fact, according to Yan *et al*. (2001), the best selection environments are those with small (absolute) PCA2 score (more representative of the overall environment), and large PCA1 score (more power to discriminate genotypes in terms of the genotype main effect). Finlay-Wilkinson model applied with the R joint procedure allowed to interpret grain yield of NT genotypes together with yield adaptability represented by the regression coefficient b_i. As shown in the resulting scatter plot, GY of genotypes averaged over all locations ranged from 2.04 t ha^{-1} for NT77 to 3.83 t ha^{-1} for NT101; the overall mean was 2.89 t ha^{-1} (Figure 3). Yield adaptability (Ya) ranged in NT lines from 0.71 again for NT77 to 1.20 for NT19; the average being 0.99. The Figure 3 shows the apparent superiority of those genotypes carrying the *Nure* allele at *Eam6* (indicated by black circles), and this was confirmed testing by factorial ANOVA the four possible haplotypes (*i.e.*, couples of allelic combinations) obtained combining alternative alleles at *Eam6* and *Vrn-H1* (Table 3). In our multi-environment trial, genotypes harboring the Nu-Nu haplotype at the two loci regulating the cycle behaved with the highest grain yield (3.04 t ha^{-1}) coupled with the highest yield adaptability (b_i=1.05). No significant differences were instead found in the level of yield stability (Ys) among the four haplotype groups.

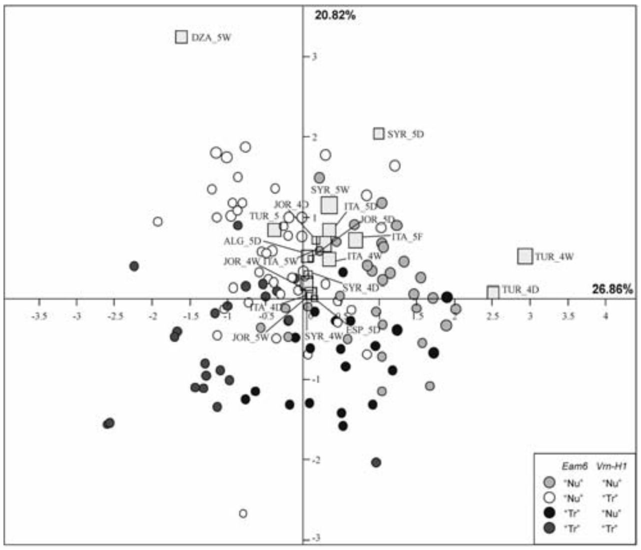

Figure 2. GGE biplot on the 120 *Nure* x *Tremois* genotypes within the 18 Mediterranean environments. The scatter plot, based on environment-centered data and scaled for genotype eigen values, derives from the genotype by environment table. Squared and circled symbols are drawn proportionally to mean site yield and average genotypic yield across sites, respectively. Genotypes are color-coded according to their *Eam6–Vrn-H1* haplotype as reported in legend.

Discussion

Climatic factors like temperature, solar radiation and water availability affect crop yield all over the world (Araus *et al*., 2003, 2008; Bingham *et al*., 2007). As an example, 0.6 to 8.9% reduction in wheat yield per 1°C rise in temperature has been reported by Lobell and Field (2007). Global climate change is also expected to make the situation worse in the near future. Therefore, it is important to understand the influence of the meteorological variables on GY and its components during the different phases of crop development, especially in fragile environments as the Mediterranean ones.

Solar radiation (Sr) might be an important environmental factor which brings positive changes in the crop growth by altering leaf architecture and light partitioning. Similarly, solar radiation activates the photosystem by which light reaction of photosynthesis started, and electrons generated by photolysis of water moves to produce energy carriers (*e.g.*, NADPH and ATP). The results showed here highlight that with the increase in solar radiation from sowing to early grain filling, GN decreased significantly. Multiple regression analysis linked this variation to a decrease in GN, as water demand in the vegetative phase (WD 1) increased, together with average water stress index during the entire crop cycle (WSI/LGP) (Figure 1B).

Photothermal quotient (PQ) portrayed the combined effect of solar radiation and temperature on crop yield. It is considered a determinant factor that affect cereal yield significantly, mainly through the GN component (Estrada-Campuzano *et al*., 2008; Sandaña and Pinochet 2011). Our results clearly indicated a strong relationship of photothermal quotient measured during both the vegetative growth (from sowing to heading –21 d) and early grain filling (from heading to heading +14 d) with grain yield and grain number determination (Figures 1A and B). Although with a lower precision in the division of the crop growth cycle, similar results have been obtained in bread wheat (Ahmed *et al*., 2011), suggesting once again that the two crops share common mechanisms of GY determination, and notably, that results obtained in barley may apply also to wheat.

Barley genomic regions significantly associated with grain yield in 28 Mediterranean environments have been identified using a germplasm collection of genotypes that represented landraces, old, and contemporary cultivars (Comadran *et al*., 2008). More recently, Francia *et al*., (2011) investigated in the same geographic range presented here the influence of the length of the different phenological phases in determining barley adaptation, and found that GY was in general more limited by GN than by GW. As stated by Savin and Slafer (1991), this can be viewed as a consequence of the crucial role played by the timing of occurrence of flowering in defining the broad adaptation of a given genotype and thus for grain yield determination. Crop ontogeny tends to be tailored to the target environment to avoid adverse conditions during the most critical stages (Slafer *et al*., 2009). The period before the start of the grain-filling is very important for the determination of grain number and despite some uncertainty about the actual beginning and the end of this critical period, it is generally accepted that it covers the period from late stem elongation to early post-flowering in wheat (Fischer, 1985; Savin and Slafer, 1991) and from early stem elongation to anthesis in barley (Bingham *et al*., 2007). The results presented here indicate a possible involvement of the early post-flowering period in determining GN, together with the pre-anthesis period, and thus appear to be in greater agreement with what was found in wheat rather than in barley (Table 2 and Figure 1). However, additional studies will be necessary to definitively clarify this issue. In all cases this entails that, as the flowering date occurs later in the season, an increased risk of (terminal) drought arises during grain filling. Therefore, extending duration of the stem elongation phase at the expense of shortening the vegetative phase has been proposed as a promising breeding tool in small grains (Borras *et al*., 2009; Francia *et al*., 2011).

Site regression model (Cornelius *et al*., 1996) with first two principal components has been widely used to generate GGE biplots that provide great insights into relationships of both the genotype and genotype x environment interaction main effects (Yan *et al*., 2001). Results obtained in our experiment demonstrate a clear pattern of adaptation for NT genotypes across Mediterranean environments explained by just two genomic loci (Figure 2). On one hand, superiority of *Eam6* allele from *Nure* across environments is suggested by the GGE biplot as open and light gray circles seem to have bigger diameter (*i.e.*, larger average yields across sites) than *Eam6* allele from *Tremois*. On the other hand, genotypes with specific adaptation are driven by mainly *Vrn-H1* as they appear best adapted to cold environments (both Turkish sites for harvest year 2004); much worst for NT genotypes harboring the vernalization insensitive *Vrn-H1* allele from *Tremois*.

After having explored G+GE main effects with different alleles at the two major loci of phenological development (*Eam6* and *Vrn-H1*), we tried to interpret yield adaptability and stability measured as regression coefficient and mean squared deviations from regressions (Finlay-Wilkinson b_i and Eberhart-Russell s_i^2, respectively). The range of Finlay-Wilkinson slopes for the relationship between the environmental index and observed yield that we obtained for the NT genotypes (0.71-1.20) agrees with that reported by Kraakman *et al*. (2004) for 146 modern European two-rowed spring barley cultivars. Genotypes with low b_i values (low adaptability) and high mean square values (low yield stability) are genotypes that yield more under drought conditions, but

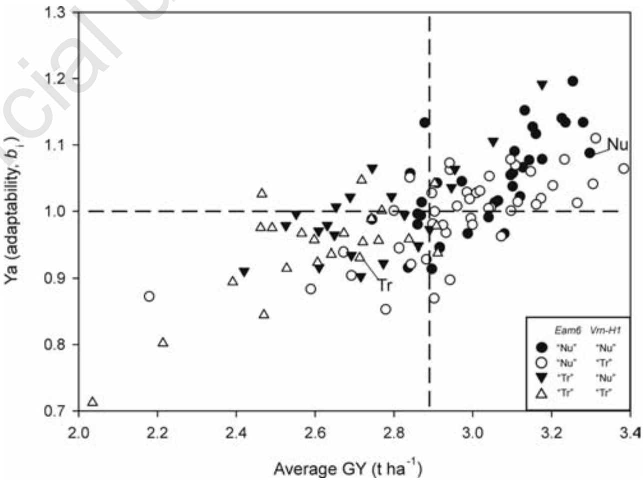

Figure 3. Scatter plot between yield adaptability (Ya) and average grain yield (GY) (environmental index) of *Nure* x *Tremois* genotypes. Horizontal and vertical dashed lines represent mean coefficient regression and mean yield across the multi-environment trial, respectively. Genotypes symbols are color-coded according to their *Eam6–Vrn-H1* haplotype as reported in legend.

Table 3. Least-square means of haplotype classes at major developmental loci *Eam6–Vrn-H1* for grain yield, yield adaptability, and yield stability.

Haplotype class°	GY (t ha^{-1})	Ya (b_i)	Ys (s^2_i)
Nu-Nu	3.04*a*	1.05*a*	0.52*a*
Nu-Tr	2.98*a*	1.00*b*	0.51*a*
Tr-Nu	2.75*b*	1.00*b*	0.46*a*
Tr-Tr	2.61*b*	0.94*c*	0.48*a*

Within column, numbers followed by the same letter are not significantly different at P≤0.05. °Multiple comparison are based on Tukey's Honestly-Significant-Difference test (P≤0.05). GY, grain yield; Ya, yield adaptability; Ys, yield stability; Nu-Nu, Nure-Nure; Nu-Tr, Nure-Tremois; Tr-Nu, Tremois-Nure; Tr-Tr, Tremois-Tremois.

are not able to respond to higher levels of moisture.

In general, our results confirm the hypothesis that genotypes selected (adapted) under high yielding environments like *Nure* perform better than those with lower yield potential when grown in a wide range of Mediterranean environments. Otherwise stated, selection for high yield in stress-free conditions gets, to a certain extent, indirectly improved yield also in many water-limiting conditions (Araus *et al*., 2008; Cattivelli *et al*., 2008). Retrospective studies on wheat indicate that the improvement in yield has more often been associated with augmented partitioning of biomass to the grain than with enhanced overall biomass (Slafer *et al*., 2009). However, it is becoming more important than ever to unravel the genetic and physiological bases of above ground biomass conversion from light and biomass determination if increased yields are to be achieved (Araus *et al*., 2008). As *-omics* sciences (*e.g*., genomics and proteomics) boosted during the past few decades, they are expected to enable systematic analysis of changes that occur in plants in response to environmental conditions (Pecchioni *et al*., 2012). However, crop scientists must remember that, as happened during the green revolution, the contribution of molecular biology cannot be separated from that of agricultural sciences.

The present characterization of barley genotypes for yield adaptability, as well as GE interaction, constitutes a starting point towards the improvement of barley yield in future Mediterranean agriculture.

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