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Agronomic performance of durum wheat landraces and modern cultivars and its association with genotypic variation in vernalization response (*Vrn-1*) and photoperiod sensitivity (*Ppd-1*) genes

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ABSTRACT

This study analyzed the relationship between important agronomic traits and major genes regulating flowering time in a panel of 151 Mediterranean durum wheat landraces and 20 modern cultivars. Field experiments were conducted under rainfed conditions during six crop seasons in northeastern Spain. Multivariate analysis of agronomic traits and genotypic data allowed the modern cultivars to be differentiated from the landraces and germplasm pools to be identified within the landraces associated with their geographic origin. The high frequency of the *Vrn-A1c* allele and the photoperiod insensitive alleles *GS105* and *GS100* at *Ppd-A1* reduced time to anthesis and enlarged the grain filling period of the modern cultivars compared with the landraces. Ancient durums collected close to the domestication area of wheat showed a high frequency of the winter allele *vrn-B1* and the photoperiod sensitivity allele *Ppd-B1b*. None of the allele variants or allelic combinations accounted significantly for variations in any agronomic trait of modern cultivars. Vernalization and photoperiod genes acted additively in explaining the genotypic variance for the agronomic traits of the landraces. *Vrn-A1* alleles and *Vrn-A1* + *Vrn-B1* allelic combinations significantly affected the number of grains per spike (NGS), thousand kernel weight (TKW) and grain filling rate (R), accounting for 9%–12% of the genotypic variance for these traits. *Ppd-1* accounted for 6%–21% of the genotypic variance for R, grain filling duration (GFD), plant height (PH), biomass at anthesis (CDW) and harvest index (HI). *Vrn-1* + *Ppd-1* allelic combinations accounted for 21%–26% of the genotypic variance for these traits. Except for NGS, the effect of vernalization and photoperiod genes on the agronomic traits was linked to their effect on anthesis time. The three-day delay in anthesis time caused by the allele *Vrn-A1d* irrespective of the allele *Vrn-A1c* resulted in increases of 10 % in R and 7% in TKW. The eight-day delay in anthesis time caused by the allele *Ppd-A1*(*DelCD*) compared with *Ppd-A1*(*GS105*) increased R by 19 % and PH by 28 %, but reduced GFD and HI by 10 %. None of the allele variants or allelic combinations at the *Vrn-1* or *Ppd-1* genes accounted significantly for variations in yield or number of spikes m^{-2} (NSm^2).

1. Introduction

Wheat is grown on about 219 million hectares worldwide with a global consumption of around 720 million tons per year (FAO, 2017). The tetraploid cultivated species, durum wheat (*Triticum turgidum* L. ssp. *durum*) represents about 6% of the global wheat production. The Mediterranean Basin is the largest durum producing area worldwide, the largest consumer of durum wheat products and the most important import market.

Wheat was domesticated about 10,000 years ago in the Fertile Crescent, a region stretching from the coast of Israel to southeastern Turkey and westwards through Syria, Iraq and western Iran (Feldman, 2001). From the east, wheat spread through the western Mediterranean Basin, reaching the Iberian Peninsula around 7000 years BP (Feldman, 2001). The Mediterranean Basin includes countries between 27° and 47 °N and 10 °W and 37 °E, shoring on three continents with 46,000 km of coast line and a marked climate heterogeneity between zones and years (Nicault et al., 2008; Royo et al., 2014). During the expansion of its cultivation area, as a result of natural and human selection, wheat underwent a progressive adaptation to a variety of environmental

Abbreviations: NSm^2, number of spikes m^{-2}; NGS, number of grains per spike; TKW, thousand kernel weight; R, grain filling rate; GFD, grain filling duration; PH, plant height; CDW, crop dry weight at anthesis; HI, harvest index; AC, allelic combination

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conditions, including a diversity of climatic variables and soil properties (Mercer and Perales, 2010). After establishing in a given area, the crop gradually adopted new features for phenology fitting and yield formation that probably conferred specific adaptive advantages in the new cultivation areas (Moragues et al., 2006). This process resulted in the evolution of local landraces, which are considered endemic to specific areas where they are very well adapted (Peng et al., 2011). The pool of Mediterranean landraces is regarded as the largest source of genetic diversity within the species due to its high level of polymorphism (Moragues et al., 2006; Nazco et al., 2012; Soriano et al., 2016). Landraces and pure line cultivars derived from them were widely cultivated until the middle of the 20th century, when the advent of the homogeneous and more productive semi-dwarf cultivars led to their virtual disappearance from farmer's fields. Landraces are a source of the putative genetic variability lost during the process of breeding, and their characterization is essential for their use in widening the genetic basis of virtually any trait in modern breeding programs.

Flowering time is a critical feature for wheat adaptation because it regulates the length of period of spike formation and determines the conditions under which the transition to the grain-filling period occurs (Snape et al., 2001; Worland, 1996). Several characteristics critical to yield formation are defined after anthesis, such as the number of grains per spike (NGS), the grain weight and the allocation of resources in vegetative and reproductive organs. Fitting flowering time to the environmental conditions prevailing in a given cultivation area allows the crop to avoid the negative impact of any abiotic stress that may occur during growth.

The length of the pre-anthesis period in wheat is controlled mainly by three groups of loci: vernalization requirement genes (*Vrn-1*), photoperiod sensitivity genes (*Ppd-1*) and narrow-sense earliness or earliness *per se* genes (*Eps*) (Distelfeld et al., 2009). Vernalization requirement determines the need for a minimum exposure to low temperature following germination to transit from vegetative to reproductive growth. It is controlled in durum wheat by two major homologs of the *Vrn-1* locus, *Vrn-A1* and *Vrn-B1*, which have been mapped onto the long arm of chromosomes 5A and 5B, respectively (Fu et al., 2005). Spring types exhibit one or more dominant alleles and the presence of recessive alleles in the two genes leads to a winter growth habit. The frequency and geographical distribution of vernalization genes is mostly determined by the temperature of the area of adaptation. Iwaki et al. (2001) reported that *Vrn-A1* confers adaptability of common wheat landraces to warmer environments. Though vernalization genes exert the greatest influence on crop phenology (Kamran et al., 2014), most of the elite durum wheat gene pools have a spring growth habit, showing no major vernalization requirements. It has been reported that the major *Vrn* genes have a differential effect on flowering time, plant height (PH) and various yield components of common wheat (Stelmakh, 1993).

The sensitivity of wheat to photoperiod indicates the dependence of flowering on daylength. There are two known genes of photoperiod response (*Ppd-1*) in durum wheat, *Ppd-A1* and *Ppd-B1*, located on the short arm of chromosomes 2A and 2B (Maccaferri et al., 2008; Wilhelm et al., 2009). Photoperiod insensitivity in durum wheat results from mutations in the *Ppd-1* genes on the A or B genomes. The *Ppd-A1* gene has three known alleles: the wild type conferring photoperiod sensitivity (*Ppd-A1b*) and two mutations (*GS100* and *GS105*) conferring insensitivity (Wilhelm et al., 2009). The *Ppd-B1* gene has two alleles: the wild type conferring photoperiod sensitivity (*Ppd-B1b*) and *Ppd-B1*a causing photoperiod insensitivity (Maccaferri et al., 2008; Royo et al., 2016). Alleles at *Ppd-A1* causing photoperiod insensitivity shorten the pre-flowering period of durum wheat to a greater extent than the insensitive *Ppd-B1* alleles (Royo et al., 2016). Moreover, the allele *Ppd-A1*(*GS100*) has a stronger effect than the allele *GS105* (*GS100* > *GS105* > *Ppd-B1a*) (Arjona et al., 2018; Royo et al., 2016; Wilhelm et al., 2009). The photoperiod insensitivity commonly identified in bread wheat from southern latitudes has been found to produce higher yields under certain conditions, such as in southern Europe (Worland, 1996). It has been suggested that the selection for the most suitable genotypes in terms of photoperiod response may have resulted from the selection for agronomically important traits (Kosner and Zurkova, 1996). Actually, pleiotropic effects of insensitive *Ppd* alleles observed in bread wheat include reduced plant height (Marshall et al., 1989; Worland, 1996), reduced final leaf number and reduced tillering (Miralles and Richards, 2000), and lesser green leaf area (Foulkes et al., 2004). Models for the genetic control of flowering in cereals suggest a functional relationship between the *Vrn-1* and *Ppd-1* genes (Distelfeld et al., 2009) that leads to differences in agronomic traits influenced by flowering date (Snape et al., 2001).

Earliness *per se* is the difference in flowering time of genotypes whose vernalization and photoperiod requirements have been fulfilled. It is a quantitatively inherited trait controlled by a number of minor genes (Kato and Wada, 1999).

A number of studies have analyzed the allele composition, the geographical distribution and the separate effect of major genes regulating flowering time in bread wheat (Kamran et al., 2014 and references herein). However, much less knowledge has been obtained for durum wheat, particularly regarding the effect of allelic combinations at these genes on the agronomic performance of germplasm from different historical periods.

This study was conducted with the following objectives: 1) to analyze the relationship between the allelic composition at the *Vrn-1* and *Ppd-1* loci and the agronomic performance of a panel of Mediterranean landraces and some representative modern cultivars, and 2) to assess the effect of individual alleles and allelic combinations on the yield formation and biomass production and allocation of both germplasm sets.

2. Material and methods

2.1. Plant material

A panel of 171 durum wheat genotypes including 151 landraces from 21 Mediterranean countries and 20 modern cultivars of diverse geographical origin was assembled for this study (Supplementary Table 1). The aim was to sample a large portion of the unexplored genetic diversity of ancient durums from the Mediterranean Basin and a fraction of the major spring durum wheat gene pools cultivated at present: Mediterranean cultivars, CIMMYT (International Maize and What Improvement Center) derived lines, and North American cultivars (Royo et al., 2009). The landraces were selected from a larger collection comprising 231 accessions extracted from public Gene Banks (Centro de Recursos Fitogenéticos INIA-Spain, ICARDA Germplasm Bank and USDA Germplasm Bank) based on their genetic variability (Nazco et al., 2012). The landraces were bulk purified to select the dominant type (usually with a frequency above 80 % of the bulk), and the seed was increased first in spike-rows and then in plots planted in the same field in the years before each experiment to ensure a common origin for seeds of all lines. The collection was characterized with a set of molecular markers associated with the *Vrn-1*, *Vrn-3* and *Ppd-1* loci. A complete description of the methods used for this characterization, the allele variants recorded on each genotype and their effect on crop phenology is found in Royo et al., 2020 (Available at https://www.frontiersin.org/articles/10.3389/fpls.2020.00838/full). The marker associated to *Vrn-B3* was monomorph in the collection and so it is not further mentioned in the current paper.

2.2. Experimental field setup

Field experiments were conducted under rainfed conditions during six crop seasons in Lleida (41°40′N, 0°20′E, 260 m.a.s.l.), northeastern Spain (Table 1). Experiments were performed in augmented designs with three replicated checks (*cv* 'Claudio', 'Simeto' and 'Vitron') and

Table 1
Overall statistics calculated for the six field experiments of the study.

Year	2007	2008	2009	2013	2014	2015
Water input (mm)						
Sowing-anthesis	165	152	183	185	95	163
Anthesis-maturity	24	133	9	52	9	6
Mean photoperiod (h)						
Sowing-anthesis	11.4	11.3	11.4	11.6	11.2	11.2
Anthesis-maturity	15.1	15.2	15.1	15.3	14.7	14.8
Days from sowing to anthesis	161^{b}	161^{b}	163^{a}	153^{d}	146^{e}	155^{c}
Days from sowing to physiological maturity	192^{c}	206^{a}	190^{d}	193^{b}	176^{f}	184^{e}
Mean yield (kg ha^{-1})	3262^{d}	3972^{c}	3361^{d}	4771^{a}	3077^{e}	4256^{b}
Number of spikes m^{-2} (NSm^2)	371^{b}	317^{c}	440^{a}	320^{c}	366^{b}	326^{c}
Number of grains $spike^{-1}$ (NGS)	19.7^{e}	22.0^{d}	20.4^{c}	35.1^{a}	30.2^{b}	27.5^{c}
Thousand kernel weight (TKW, g)	45.8^{c}	47.4^{ab}	38.5^{d}	48.4^{a}	37.0^{e}	46.5bc
Grain filling rate (R, mg d^{-1})	1.58^{b}	1.36^{d}	1.46^{c}	1.28^{e}	1.26^{e}	1.71^{a}
Plant height (PH, cm)	104^{cd}	102^{d}	111^{a}	99^{e}	108^{b}	105^{bc}
Crop dry weight at anthesis (CDW, g m^{-2})	787^{b}	950^{a}	953^{a}	718c	737^{c}	797^{b}
Harvest index (HI)	0.39^{bc}	0.44^{a}	0.29^{d}	0.40^{b}	0.38^{c}	0.39^{bc}

Means within rows with different letters are significantly different at $P = 0.05$, following Tukey test.

plots of 6 m^2 for the first three years, and two replicated checks (*cv* 'Avispa' and 'Euroduro') and plots of 3.6 m^2 for the last three years. In all experiments, the plots consisted of eight rows spaced 0.15 m apart. The ratio of checks to test-genotypes was 1:5. Sowing density was adjusted to 250 germinable seeds m^{-2}. Agronomic management (including fertilization and pest, disease and weed control) was carried out according to standard agriculture practices.

2.3. Data recording

Maximum and minimum temperatures and water input were measured daily at weather stations located at a distance of about 2 km from the experimental fields. Soil moisture was monitored in one of the repeated checks from the seedling stage by means of soil probes (model EC-20, ECH2O Dielectric Aquameter, Decagon Devices, Inc.) located at three depths (0–10, 10–25 and 25–40 cm). Dates of anthesis and physiological maturity were recorded when more than 50 % of the main spikes within a plot had reached Zadoks growth stages 65 (GS65) and 87 (GS87), respectively (Zadoks et al., 1974). Crop dry weight (CDW, g m^{-2}) was determined at GS65 from weighed samples of the plants contained in a 0.5-m-long stretch of a central row of each plot after oven-drying them at 70 °C for 48 h. At ripening, samples of the plants in a 1-m-long stretch were pulled up in a central row of each plot and the number of spikes, grains per spike (NGS), plant weight and grain weight were recorded. Harvest index (HI) was computed by dividing the grain weight by the total plant weight of the 1-m-long row sample. Plant height (PH, cm) was measured at GS87 in three main stems per plot from the tillering node to the top of the spike, excluding the awns. Grain yield was determined by mechanically harvesting the plots at ripening, and was expressed at a 12 % moisture level. Thousand kernel weight (TKW, g) was determined by counting the grains in 10 g drawn randomly from harvested grains of each plot. The mean rate of grain filling (R, mg d^{-1}) was calculated for each plot as the ratio between grain weight and grain filling duration (GFD).

2.4. Statistical analysis

Phenotypic data were fitted to a linear mixed model and restricted maximum likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs). Principal component analysis (PCA) was performed on the correlation matrix using the allele frequencies of the *Vrn-1* and *Ppd-1* loci and the mean agronomic traits across the six field experiments for each geographic origin. Data were analyzed with the MIXED procedure of the SAS statistical package (SAS Institute Inc, Cary, NC, USA) with the Kenward-Roger correction due to the unbalanced number of cultivars for each germplasm type, allele variant and allelic combination (AC). In order to test the differences between landraces and modern cultivars on the analyzed traits, the sum of squares of the genotype effect in the ANOVA was partitioned into differences between the two types of germplasm and the genotypic variance retained within them, which was used as error term. The percentage of the genotypic variance explained by each allele or allelic combination was assessed by conducting successive ANOVAs. In each of them, the sum of squares of the genotype effect was partitioned into differences between allele variants in each gene or allelic combinations and the genotypic variance retained within them that was used as error term. Mean comparisons were carried out using the Tukey-Kramer correction with the SAS statistical package (SAS Institute Inc, Cary, NC, USA). Pearson correlation coefficients were calculated between traits and linear regression models were fitted to the relationships between the number of days to anthesis and the agronomic traits.

3. Results

3.1. Environmental conditions

The experimental site has a typical Mediterranean climate characterized by an uneven distribution of rainfall during the season, low temperatures in December and January, a sharp rise in temperature in spring, and high temperatures continuing until the end of the crop cycle (Fig. 1). Water input received by the experiments ranged between 104 mm in 2014, when the lowest yield, R and TKW were recorded, and 285 mm in 2008, when HI reached the highest value, probably as a result of the intense rains in May, just after flowering (Table 1 and Fig. 1). The highest average yield (4771 kg/ha) was recorded in 2013, when the greatest NGS and the heaviest grains were achieved, probably as a result of the rain that fell during the first stages of the grain filling period (Fig. 1).

3.2. Traits differentiating germplasm pools

The first two axes of the PCA accounted for 54.6 % of the total variance (axis 1, 38.6 %, axis 2, 16.0 %, Fig. 2). The winter allele *vrn-A1* had the lowest effect of all the variables considered in the analysis as shown by its short eigenvector in Fig. 2a. Principal component 1 (PC1) was positively related to the frequency of alleles *Vrn-A1b*, *Vrn-A1d*, and *Vrn-B1a* but negatively related to that of alleles *Vrn-A1c* and *vrn-B1*. PC1 also separated alleles *Ppd-A1*(*DelCD*), *Ppd-A1b,* and *Ppd-B1a*, located in the positive direction of this axis, from alleles *Ppd-A1*(*GS105*), *Ppd-A1*(*GS100*) and *Ppd-B1b*, located in the negative direction (Fig. 2a). In terms of the agronomic traits, PC1 was positively associated with days to anthesis (DA), PH, CDW, R and TKW and negatively associated with GFD, HI, NGS, yield and number of spikes m^{-2} (NSm^2). The angles between vectors indicated strong positive correlations of DA with R, CDW and PH, but a negative relationship of DA with GFD (Fig. 2a). Negative relationships were also found between GFD and R and between NSm^2 and TKW, as shown in Table 2.

The points representing the geographic origin of genotypes, depicted in Fig. 2b, clearly separate the two types of germplasm. Modern cultivars are located in the upper left part of the figure in the direction of the vectors representing the frequency of alleles *Vrn-A1c*, *Ppd-A1*(*GS105*) and *Ppd-A1*(*GS100*) and the agronomic traits GFD, HI, NGS and yield. Modern cultivars from CIMMYT, Spain and Italy are closer to the direction of axes related to yield and *Ppd-A1*(*GS105*), while cultivars from USA are closer to the vectors representing GFD and *Vrn-A1c*. The points representing landraces are much more dispersed from the

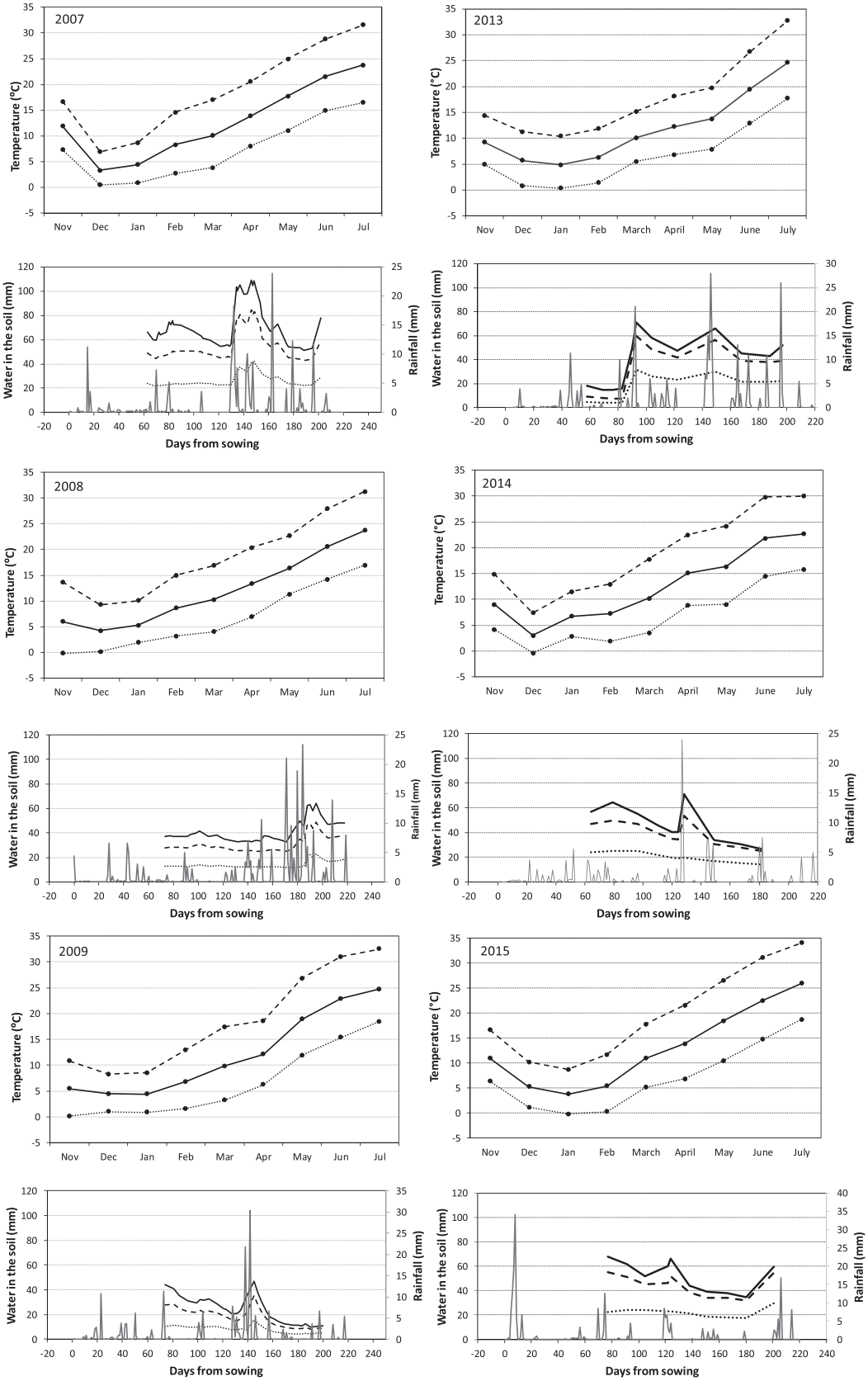

Fig. 1. Maximum (dashed line), mean (solid line) and minimum (dotted line) temperatures during the growth cycle of each crop season. The bottom figures for each year indicate the water soil content at three depths (0 − 40 cm solid line, 10 − 40 cm dashed line and 25 − 40 cm dotted line) and the rainfall (grey line).

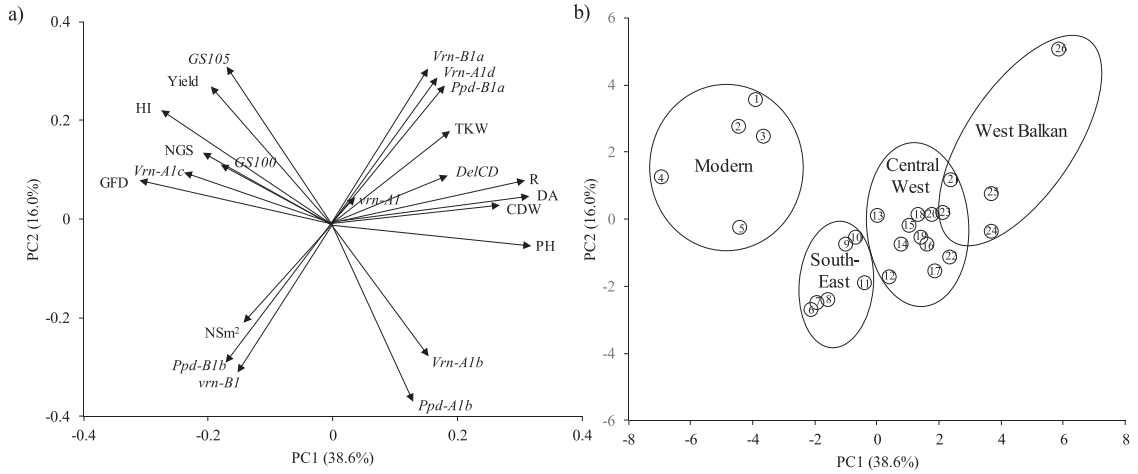

Fig. 2. Biplot of the first two axes of the principal component analysis summarizing the relationships between days to anthesis (DA), agronomic traits and allele frequency for the *Vrn-1* and *Ppd-1* genes of each on each geographical origin of the 151 Mediterranean landraces and 20 modern cultivars. Each allele was considered as a different variable and the data used for the analysis were the frequencies of each allele within the genotypes belonging to each country. a) Eigenvalues of the correlation matrix symbolized as vectors representing the variables. TKW, thousand kernel weight; R, grain filling rate; CDW, crop dry weight at anthesis; PH, plant height; NSm^2, number of spikes m^{-2}; GFD, grain filling duration; NGS: number of grains $spike^{-1}$; HI: harvest index. b) Plot of the points corresponding to the origin of the landraces and the modern cultivars. 1-5 modern cultivars (1, CIMMYT; 2, Spain; 3, Italy; 4, USA; 5, France) and 6-26 landraces (6, Libya; 7, Israel; 8, Jordan; 9, Egypt; 10, Syria; 11, Lebanon; 12, Cyprus; 13, Italy; 14, Bulgaria; 15, Algeria; 16, Morocco; 17, Tunisia; 18, France; 19, Greece; 20, Portugal; 21, Spain; 22, Turkey; 23, Serbia; 24, Macedonia; 25, Montenegro; 26, Croatia).

bottom-left to the upper-right parts of the figure, but three clusters related to the geographic origin of landraces can be identified. The first one, in the direction of the vectors representing the frequency of the *vrn-B1* and *Ppd-B1b* alleles and NSm^2, (Fig. 2a) includes landraces collected in countries located in the southeastern Mediterranean Basin (Libya, Israel, Jordan, Egypt, Syria and Lebanon) (Fig. 2b). Points corresponding to the western Balkan countries (Serbia, Macedonia, Montenegro and Croatia) are located in the right part of Fig. 2b on a cluster partially overlapping with a third one that includes the remaining countries. The point representing Croatian landraces is located in the upper right part of the figure in the direction of the vectors related to *Vrn-A1d*, *Vrn-B1a*, *Ppd-B1a* and TKW.

The ANOVAs conducted to analyze the differences in agronomic traits between landraces and modern cultivars revealed a significant effect of the year and the genotype on all of them (Table 3). The year effect accounted for 4.2 % (for PH) to 87.5 % (for GFD) of the total variance of the model. The partitioning of the SS of the genotype effect into its components revealed statistically significant differences between landraces and modern cultivars for all traits except NSm^2 and TKW. Most of the variability induced by the genotype effect was explained by differences within landraces (41.2 %−94.1 %), as variability within modern cultivars contributed to a much lesser extent (0.4 %−14.0 %). The landraces showed a longer duration to anthesis, a shorter GFD, taller plants, more CDW at anthesis and more R than modern cultivars, but less NGS, HI and yield (Fig. 3). The average number of DA across years ranged between 151 and 166 days in landraces and between 152 and 156 days in modern cultivars. Ranges of average yield across years were 3004 − 4124 kg ha^{-1} in landraces and 4024 − 4548 kg ha^{-1} in modern cultivars. Correlation coefficients between traits indicated that the yield of landraces was negatively associated with NSm^2 but positively associated with R and TKW, but it was not significantly affected by DA or by GFD, NGS or HI (Table 2).

3.3. Effect of Vrn-1 and Ppd-1 loci on the agronomic performance

The effect of *Vrn* and *Ppd* genes on the agronomic performance was assessed separately for landraces and modern cultivars. The results of the ANOVA showed that the year and the genotype effects significantly affected all the agronomic traits for the landraces (Table 4). The contribution of the *Vrn-1* and *Ppd-1* genes to explaining the phenotypic variability induced by the genotype effect was assessed through successive ANOVA. In each ANOVA, the genotype effect was partitioned into differences between alleles at each gene (or allelic combinations, shown jointly below the genotype effect in Table 4) and differences within allelic combinations, which was used as error term.

None of the allele variants or allelic combinations at the *Vrn-1* or

Table 2
Pearson's correlation coefficients for the relationships between traits. Data above diagonal correspond to landraces + modern cultivars (n = 171) and those below it to landraces (n = 151). DA, days to anthesis; NSm^2, number of spikes m^{-2}; NGS, number of grains $spike^{-1}$; TKW, thousand kernel weight; R, grain filling rate; GFD, grain filling duration; PH, plant height; CDW, crop dry weight at anthesis; HI, harvest index.

	DA		Yield		NSm^2		NGS		TKW		R		GFD		PH		CDW		HI	
DA			−0.22	**	−0.29	***	−0.15	**	0.25	**	0.63	***	−0.81	***	0.80	***	0.70	***	−0.66	***
Yield	0.11				−0.14		0.32	***	0.32	***	0.04		0.42	***	−0.28	***	−0.08		0.48	***
NSm^2	−0.29	***	−0.27	***			−0.36	***	−0.41	***	−0.34	***	−0.01		−0.40	***	−0.34	***	0.22	**
NGS	0.02		0.14		−0.44	***			−0.36	***	−0.46	***	0.28	***	−0.18	**	−0.13		0.41	***
TKW	0.26	***	0.49	***	−0.43	***	−0.32	***			0.85	***	0.02		0.36	***	0.35	***	−0.28	***
R	0.58	***	0.37	***	−0.35	***	−0.36	***	0.87	***			−0.54	***	0.64	***	0.61	***	−0.56	***
GFD	−0.75	***	0.13		−0.05		0.13		−0.02		−0.47	***			−0.66	***	−0.61	***	0.62	***
PH	0.75	***	0.23	**	−0.47	***	0.12		0.42	***	0.60	***	−0.50	***			0.74	***	−0.76	***
CDW	0.65	***	0.25	**	−0.37	***	0.01		0.40	***	0.60	***	−0.54	***	0.73	***			−0.54	***
HI	−0.58	***	−0.03		0.27	**	0.22	**	−0.39	***	−0.54	***	0.43	***	−0.58	***	−0.43	***		

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

Table 3
Percentage of the sum of squares (SS) explained by the factors included in the ANOVA model for days to anthesis (DA), yield and associated traits. The SS of the genotype effect was partitioned into differences between landraces and modern cultivars and differences within them. NSm^2, number of spikes m^{-2}; NGS, number of grains $spike^{-1}$; TKW, thousand kernel weight; R, grain filling rate; GFD, grain filling duration; PH, plant height; CDW, crop dry weight at anthesis; HI, harvest index.

Source of variation	df	DA		Yield		NSm^2		NGS		TKW		R		GFD		PH		CDW		HI	
Year	5	68.7	***	67.9	***	42.2	***	56.9	***	41.1	***	38.2	***	87.5	***	4.2	***	22.9	***	53.9	***
Genotype	170	27.5	***	12.3	***	22.8	***	14.0	***	34.3	***	35.9	***	7.4	***	82.9	***	24.2	***	23.9	***
Between landraces and modern	1	20.9	***	41.1	***	0.8		15.1	***	0.5		11.7	***	27.5	***	42.4	***	15.3	***	55.2	***
Within landraces	150	77.7	***	55.2	***	94.1	***	70.9	***	92.4	***	84.6	***	70.0	***	57.2	***	78.4	***	41.2	***
Within modern cultivars	19	1.4	***	3.7		5.1		14.0	**	7.2	***	3.7	***	2.5	*	0.4	***	6.3		3.6	*
Year x Genotype	850	3.8		19.8		35.1		29.1		24.6		25.8		5.0		12.9		52.9		22.2	

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

Ppd-1 loci accounted significantly for variations in yield or in NSm^2 (Table 4). However, differences between alleles at *Vrn-A1* and between allelic combinations at the *Vrn-A1* + *Vrn-B1* genes accounted significantly for 8%–12% of the genotype effect for NGS, TKW and R. The comparison of the mean values of the four allelic variants identified at the *Vrn-A1* gene revealed that the two landraces harboring the winter allele *vrn-A1* showed the largest NGS (Table 5a). Moreover, genotypes harboring allele *b* or *d* exhibited the lowest NGS but the heaviest grains and the greatest R. The comparison of the mean values for the six *Vrn-A1* + *Vrn-B1* allelic combinations followed a similar trend because of the smaller effect of the allele variant at *Vrn-B1* (Table 5b). The regression line fitted to the relationship between DA and the mean values of NGS for the four *Vrn-A1* alleles and the six *Vrn-A1* + *Vrn-B1* allelic combinations showed no significant trend (Fig. 4a). On the other hand, the relationships between DA and TKW or R indicated positive associations (Figs. 4b and 4c). Although they were not statistically significant when the mean values of the four alleles were used, the coefficients of determination indicated that the number of days to anthesis, associated with the presence of *Vrn-A1* alleles or allelic combinations, accounted for more than half of the variability in TKW and R.

The ANOVA conducted to test the variability of the genotype effect explained by the *Ppd-1* alleles and their combinations showed a significant effect of the *Ppd-A1* alleles on R, GFD, PH, CDW and HI, accounting for 5.6 % (for R) to 17.7 % (for HI) of the genotypic variance (Table 4). The comparison of the mean values of these traits for the three allele variants identified at the *Ppd-A1* gene revealed that landraces harboring the allele *Ppd-A1*(*DelCD*) had the highest R and pH but the lowest GFD and HI (Table 6a). The allele *Ppd-A1*(*GS105*) caused the opposite effect, and the wild type led to intermediate values. The *Ppd-B1* alleles accounted significantly for 10.3 %, 3.2 % and 2.5 % of the genotype effect for GFD, PH and CDW, respectively (Table 4), with the presence of allele *Ppd-B1a*, causing photoperiod insensitivity, resulting in the lowest GFD but the highest PH and CDW (Table 6a). The allelic combinations *Ppd-A1* + *Ppd-B1* explained a larger percentage (up to 21 % for PH and GFD) of the variation induced by the genotype effect (Table 4). The mean comparison showed that allelic combinations harboring the *Ppd-A1*(*DelCD*) allele led to the highest values of R and the tallest plants but the lowest HI, independently of the allele present at *Ppd-B1* (Table 6b). The lowest R and pH and the highest GFD and HI were recorded in the two landraces carrying the allelic combination *Ppd-A1*(*GS105*) + *Ppd-B1a*. On the other hand, the presence of the *Ppd-A1b* allele led to different results depending on the allele present at *Ppd-B1*, but for all variables the values were intermediate between those resulting from the presence of alleles *DelCD* and *GS105* at *Ppd-A1* (Table 6b).

The study of the relationship between DA and agronomic traits through the effect of *Ppd-1* genes indicated that extending time to anthesis was associated with increased R, PH and CDW but reduced GFD and HI (Fig. 5). Though the coefficients of determination of the regression lines fitted to the relationships between DA and the agronomic traits using the mean values of the three individual alleles at *Ppd-A1* were $R^2 \geq 0.94$ (Fig. 5 left), they were not statistically significant because of the low number of points. However, the same tendencies were observed, and the R^2 values were statistically significant, when the regression lines were fitted to the mean values of the five *Ppd-A1* + *Ppd-B1* allelic combinations (Fig. 5 right). The number of DA accounted for at least 90 % of all these traits.

The 16 *Vrn-1* + *Ppd-1* allelic combinations (named AC-1 to AC-16) identified accounted significantly for 18 % of the genotype variance in the ANOVA for NGS and for more than 21 % for R, GFD, PH and HI (Table 4). The NGS ranged between 22.5 (AC-2) and 32.2 (AC-12), but there was no clear pattern associated with allelic combinations (Table 7). Grain filling rates ranged from 1.28 to 1.64 mg d^{-1}, and the highest values recorded in AC-1, AC-2 and AC-3 were statistically different from the lowest ones obtained in AC-13, AC-14, AC-15 and AC-16, while all other combinations gave intermediate values. Three of the four allelic combinations carrying the *Vrn-A1d* were associated with the highest R values, while those carrying the allele *Vrn-A1b* tended to give intermediate figures, and those harboring the allele *Vrn-A1c* lower ones. Regarding *Ppd-A1,* the *DelCD* allele was the most frequent in combinations, associated with the highest R means (1.49–1.64 mg d^{-1}); the allele *Ppd-A1b* was present in combinations leading to intermediate values (1.32–1.58 mg d^{-1}), and the two landraces carrying the allele *GS105* were characterized by the lowest one (Table 7). *Vrn-1* + *Ppd-1* allelic combinations resulted in range of approximately five days in GFD, and the largest differences were recorded between AC-1 and AC-6 (both carrying the alleles *DelCD* and *Ppd-B1a*), which was associated with the shortest GFD, and AC-16 harboring the alleles *GS105* and *Ppd-B1a*, as no statistical differences existed between all other allelic combinations. Plant height ranged between 94 cm (AC-16) and 126 cm (AC-1 and AC-2), with the shortest plants associated with the presence of the allele *Ppd-A1*(*GS105*) and the tallest ones from combinations carrying the allele *Ppd-A1*(*DelCD*) (Table 7). Harvest index ranged from 0.34 (AC-2 and AC-4) to 0.40 (AC-16), the latter recorded in the only combination harboring the allele *Ppd-A1*(*GS105*) (Table 7).

The regression line fitted to the relationship between time to anthesis and the mean values of the 16 *Vrn-1* + *Ppd-1* allelic combinations for NGS was not statistically significant ($R^2 = 0.04$, $P > 0.05$). However, positive and significant relationships were found for R and PH (Figs. 6a and 6c) as well as negative ones for GFD and HI (Figs. 6b and 6d). The percentage of the variability of these four traits explained by the number of DA using the mean values of the six allelic combinations ranged between 47 % for HI and 71 % for PH.

Table 3 shows that differences among modern cultivars were statistically significant for all agronomic traits except for yield, NSm^2 and CDW. As in the case of the landraces sequential ANOVA were conducted for the traits showing significant differences between cultivars in order to estimate the effect of *Vrn-1* and *Ppd-1* on the phenotypic variability. The results showed that none of the allele variants or allelic combinations accounted significantly for variations in any agronomic trait for this reduced set of modern cultivars.

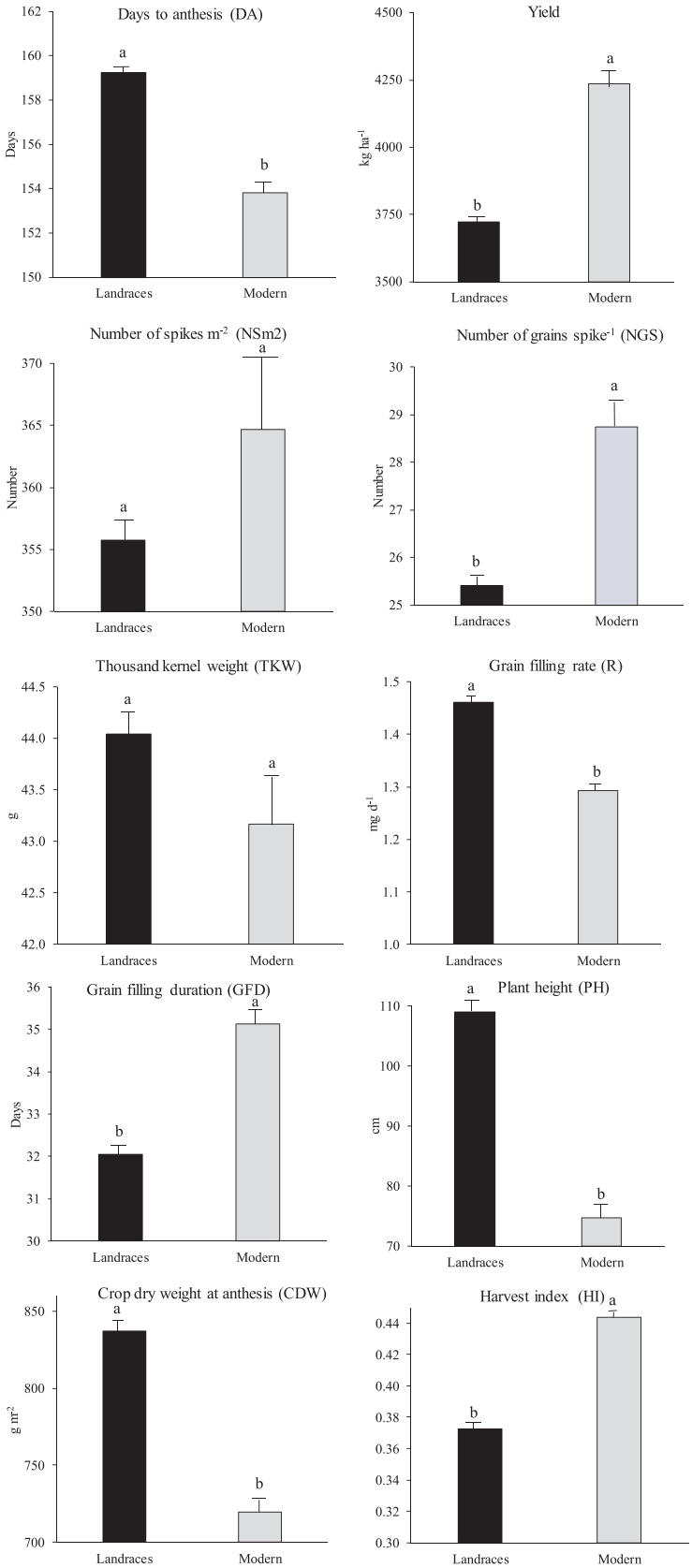

Fig. 3. Mean values ± SE of phenology, yield and associated traits for the 151 landraces and 20 modern cultivars. Data are means across six crop seasons. Means with the same letters are not significantly different for a Tukey-Kramer test at $P < 0.05$.

Table 4
Percentage of the sum of squares (SS) explained by the factors included in the ANOVA model for yield and associated traits in landraces. The effect of each allele and allelic combination was assessed by partitioning the genotype effect on successive ANOVA. NSm^2, number of spikes m^{-2}; NGS, number of grains $spike^{-1}$; TKW, thousand kernel weight; R, grain filling rate; GFD, grain filling duration; PH, plant height; CDW, crop dry weight at anthesis; HI, harvest index; NT, not testable.

Source of variation	df	Yield		NSm^2		NGS		TKW		R		GFD		PH		CDW		HI	
Year	5	72.17	***	40.65	***	59.58	***	41.12	***	45.06	***	89.10	***	6.44	***	26.20	***	62.55	***
Genotype	150	8.52	***	24.37	***	12.02	***	36.98	***	33.22	***	6.10	***	74.74	***	21.79	***	13.49	***
Between *Vrn-A1* alleles	3	0.03		0.56		11.33	***	8.27	**	8.82	**	0.95		4.20		2.89		0.51	
Between *Vrn-B1* alleles	1	0.96		0.22		0.07		0.38		0.54		0.21		1.20		1.00		0.51	
Between *Vrn-A1* + *Vrn-B1* allelic combinations	5	3.96		1.15		12.19	**	8.58	*	9.01	*	1.03		4.75		3.44		1.93	
Between *Ppd-A1* alleles	2	2.31		1.34		0.39		1.01		5.58	*	10.10	***	17.47	***	8.17	**	17.74	***
Between *Ppd-B1* alleles	1	0.64		0.07		0.03		0.48		0.81		10.33	***	3.18	*	2.52	*	0.37	
Between *Ppd-A1* + *Ppd-B1* allelic combinations	4	3.73		1.51		0.44		1.65		6.54	*	20.81	***	21.12	***	10.64	**	19.66	***
Between *Vrn-A1* + *Vrn-B1* + *Ppd-A1* + *Ppd-B1* allelic combinations	15	11.08		9.07		18.14	*	14.72		21.02	**	23.67	***	26.12	***	15.04		25.69	***
Year x Genotype	750	19.31	NT	34.97	NT	28.40	NT	21.89	NT	21.72	NT	4.80	NT	18.82	NT	52.02	NT	23.96	NT

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

Table 5
Mean values ± SE for a) *Vrn-A1* allele variants, and b) *Vrn-A1* + *Vrn-B1* allelic combinations significantly affecting the number of grains per spike (NGS), thousand kernel weight (TKW) and grain filling rate (R) of 151 durum wheat landraces from 21 Mediterranean countries. Data are means across six crop seasons. Means within columns with different letters are significantly different at $P < 0.05$, following a Tukey-Kramer test.

a) *Vrn-A1* allele variants

Vrn-A1 allele	N	NGS		TKW (g)		R (mg d^{-1})	
vrn-A1	2	29.51 ± 2.78	a	44.50 ± 1.84	ab	1.48 ± 0.07	ab
Vrn-A1b	72	24.88 ± 0.33	c	45.06 ± 0.34	a	1.49 ± 0.01	a
Vrn-A1c	67	26.09 ± 0.37	b	42.71 ± 0.33	b	1.41 ± 0.01	b
Vrn-A1d	10	24.01 ± 0.81	c	45.57 ± 0.73	a	1.55 ± 0.04	a

b) *Vrn-A1* + *Vrn-B1* allelic combinations

Vrn-A1 allele	*Vrn-B1* allele	N	NGS		TKW (g)		R (mg d^{-1})	
vrn-A1	*vrn-B1*	2	29.51 ± 2.78	a	44.50 ± 1.84	ab	1.48 ± 0.07	ab
Vrn-A1b	*vrn-B1*	71	24.85 ± 0.34	b	45.08 ± 0.34	a	1.49 ± 0.01	a
Vrn-A1b	*Vrn-B1a*	1	27.19 ± 2.14	ab	44.30 ± 3.01	ab	1.48 ± 0.08	ab
Vrn-A1c	*vrn-B1*	67	26.09 ± 0.37	ab	42.71 ± 0.33	b	1.41 ± 0.01	b
Vrn-A1d	*vrn-B1*	9	24.18 ± 0.85	b	45.28 ± 0.77	a	1.54 ± 0.04	a
Vrn-A1d	*Vrn-B1a*	1	22.5 ± 2.90	b	48.21 ± 2.29	a	1.64 ± 0.14	a

4. Discussion

4.1. Environmental conditions

The current study was conducted under rainfed conditions over six years at a site with a typical Mediterranean climate. The high yields recorded considering the low water input, particularly in 2014, could be attributed to the high soil fertility (about 3% of organic matter) and the superficial sub-soil water layer at this site (Moragues et al., 2006). The total amount of rain and its distribution was probably the main cause of the strong year effect on the agronomic performance of the durum wheat panel. The trait most affected by environmental variation was GFD, likely due to the strong effect of temperature and water availability on this trait at the end of the grain filling period (Royo et al., 2006). By contrast, the attribute with least environmental effect was PH, in agreement with the strong genetic control of this trait (Hedden, 2003).

4.2. Geographic origin and germplasm pools

The results of the multivariate analysis conducted using allelic composition at *Vrn-1* and *Ppd-1* loci together with the mean values of agronomic traits across years, for each type of germplasm and geographic origin, showed that most of the information produced could be summarized in the plane determined by the first two axes of the PCA. This analysis demonstrated the clear divergences between modern cultivars and landraces in the frequency of vernalization and photoperiod alleles and between the agronomic performances of the two germplasm groups. The modern cultivars presented a high frequency of the spring growth-habit allele *Vrn-A1c* and the photoperiod insensitive alleles *GS105* and *GS100* at *Ppd-A1* (Royo et al., under revision). The different combinations of these frequent alleles resulted in modern cultivars being generally earlier than landraces but with a longer GFD. This is an expected result given the fact that most modern genotypes included in this study were related to or derived from the CIMMYT germplasm pool that was developed using the shuttle-breeding strategy between two Mexican locations. This strategy is known to select against alleles conferring strong vernalization requirement or photoperiod sensitivity, and is considered one of the main basis for wide adaptation of this germplasm pool (Pfeiffer and Payne, 2005). The reduced time to flowering resulting from photoperiod insensitivity was accompanied with longer GFD and reduced R. These results are consistent with the recent findings of experiments conducted at different latitudes with semi-dwarf durum wheat lines harboring different alleles at *Ppd-1* (Arjona et al., 2020). Results of the present study showed that the compensation between the higher R of landraces and the longer GFD of modern cultivars led to similar grain weight in both germplasm sets. In addition, as the NSm^2 was similar in both types of germplasm, the higher yield of the modern cultivars can be attributed to their superior NGS, in agreement with previous studies (Álvaro et al., 2008a; Royo et al., 2007; Subira et al., 2015). Grain weight has been reported to remain unchanged or to slightly decrease as a result of breeding (Waddington et al., 1987; De Vita et al., 2007; Royo et al., 2007), which is observed herein. The earlier anthesis, shorter plants and higher HI and yield of modern durum cultivars in comparison with landraces has been well documented in previous studies (De Vita et al., 2007; Isidro et al., 2011; Royo et al., 2007, 2008; Subira et al., 2016). Moreover, it has been shown that aboveground biomass is reduced in semi-dwarf

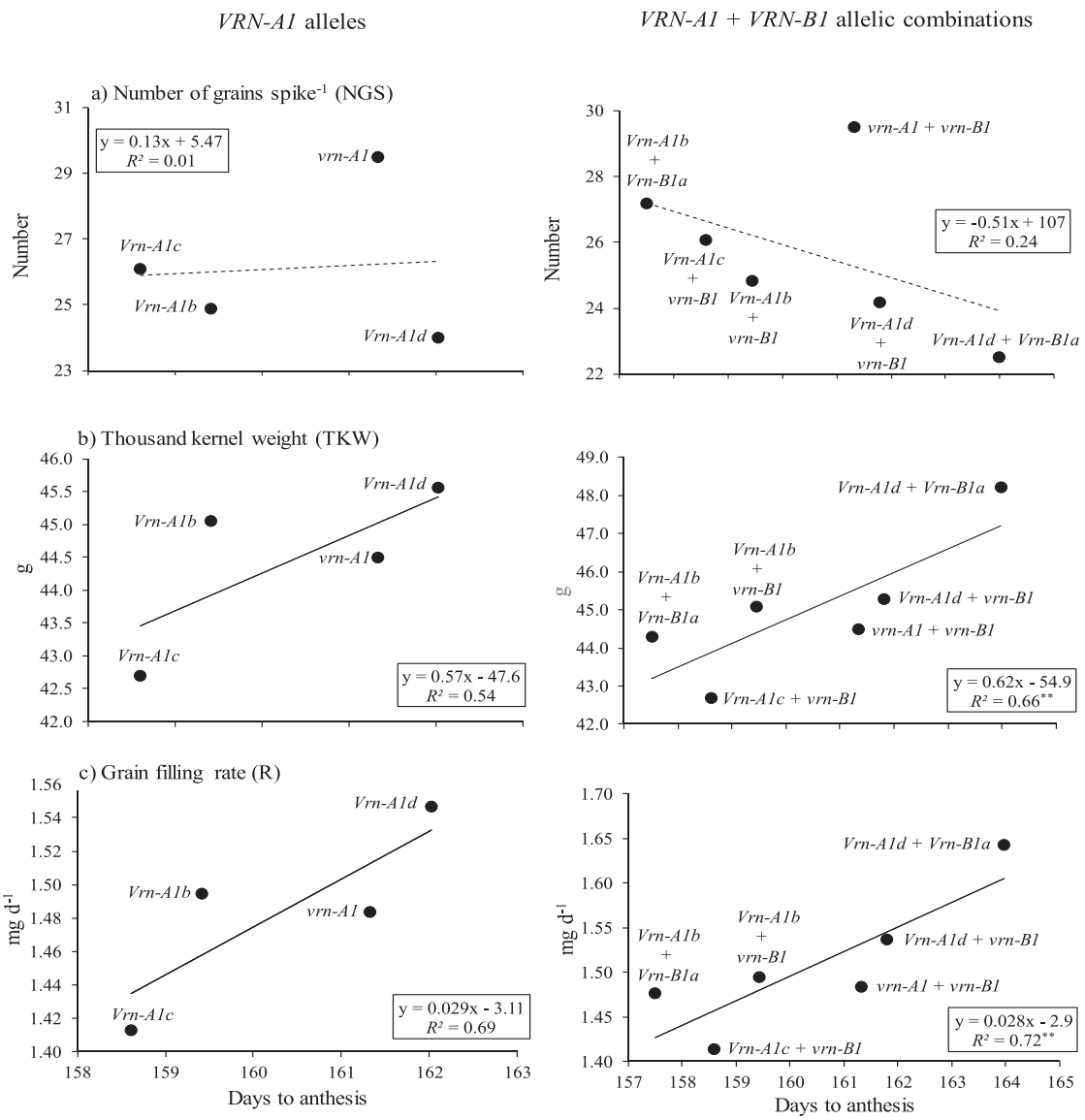

Fig. 4. Relationship between the number of days to anthesis and a) number of grains per spike, b) thousand kernel weight and c) grain filling rate for the four allele variants at *VRN-A1* (left) and the six *VRN-A1* + *VRN-B1* allelic combinations (right) identified in 151 Mediterranean durum wheat landraces.

Table 6
Mean values ± SE for a) allele variants at *Ppd-1*, and b) *Ppd-A1* + *Ppd-B1* allelic combinations significantly affecting grain filling rate (R), grain filling duration (GFD), plant height (PH), crop dry weight at anthesis (CDW) and harvest index (HI) of 151 durum wheat landraces from 21 Mediterranean countries. Data are means across six crop seasons. Means within columns with different letters are significantly different at $P < 0.05$, following a Tukey-Kramer test.

a) *Ppd-1* allele variants

Gene	Allele	N	R (mg d^{-1})		GFD (days)		PH (cm)		CDW (g m^{-2})		HI	
Ppd-A1	*DelCD*	30	1.52 ± 0.02	a	31.2 ± 0.45	c	120 ± 0.97	a	889 ± 16	a	0.36 ± 0.004	b
	Ppd-A1b	119	1.45 ± 0.01	b	32.2 ± 0.25	b	106 ± 0.57	b	825 ± 7.1	a	0.38 ± 0.002	a
	GS105 Ppd-A1a	2	1.28 ± 0.05	c	34.6 ± 2.29	a	94 ± 2.53	c	789 ± 40	a	0.40 ± 0.013	a
Ppd-B1	*Ppd-B1b*	63			32.7 ± 0.35	a	106 ± 14	b	820 ± 186	b		
	Ppd-B1a	88			31.6 ± 0.28	b	111 ± 17	a	850 ± 201	a		

b) *Ppd-A1* + *Ppd-B1* allelic combinations

Ppd-A1 allele	*Ppd-B1* allele	N	R (mg d^{-1})		GFD (days)		PH (cm)		CDW (g m^{-2})		HI	
Del CD	*Ppd-B1b*	11	1.52 ± 0.03	a	31.8 ± 0.74	c	120 ± 1.40	a	883 ± 21	a	0.35 ± 0.007	b
Del CD	*Ppd-B1a*	19	1.53 ± 0.03	a	30.8 ± 0.57	d	120 ± 1.31	a	893 ± 22	a	0.36 ± 0.005	b
Ppd-A1b	*Ppd-B1b*	52	1.43 ± 0.02	c	32.9 ± 0.39	b	103 ± 0.73	c	807 ± 11	a	0.38 ± 0.003	a
Ppd-A1b	*Ppd-B1a*	67	1.46 ± 0.01	b	31.7 ± 0.33	c	109 ± 0.81	b	839 ± 9.5	a	0.37 ± 0.003	a
GS105	*Ppd-B1a*	2	1.28 ± 0.05	d	34.6 ± 2.29	a	94 ± 2.52	c	789 ± 40	a	0.40 ± 0.013	a

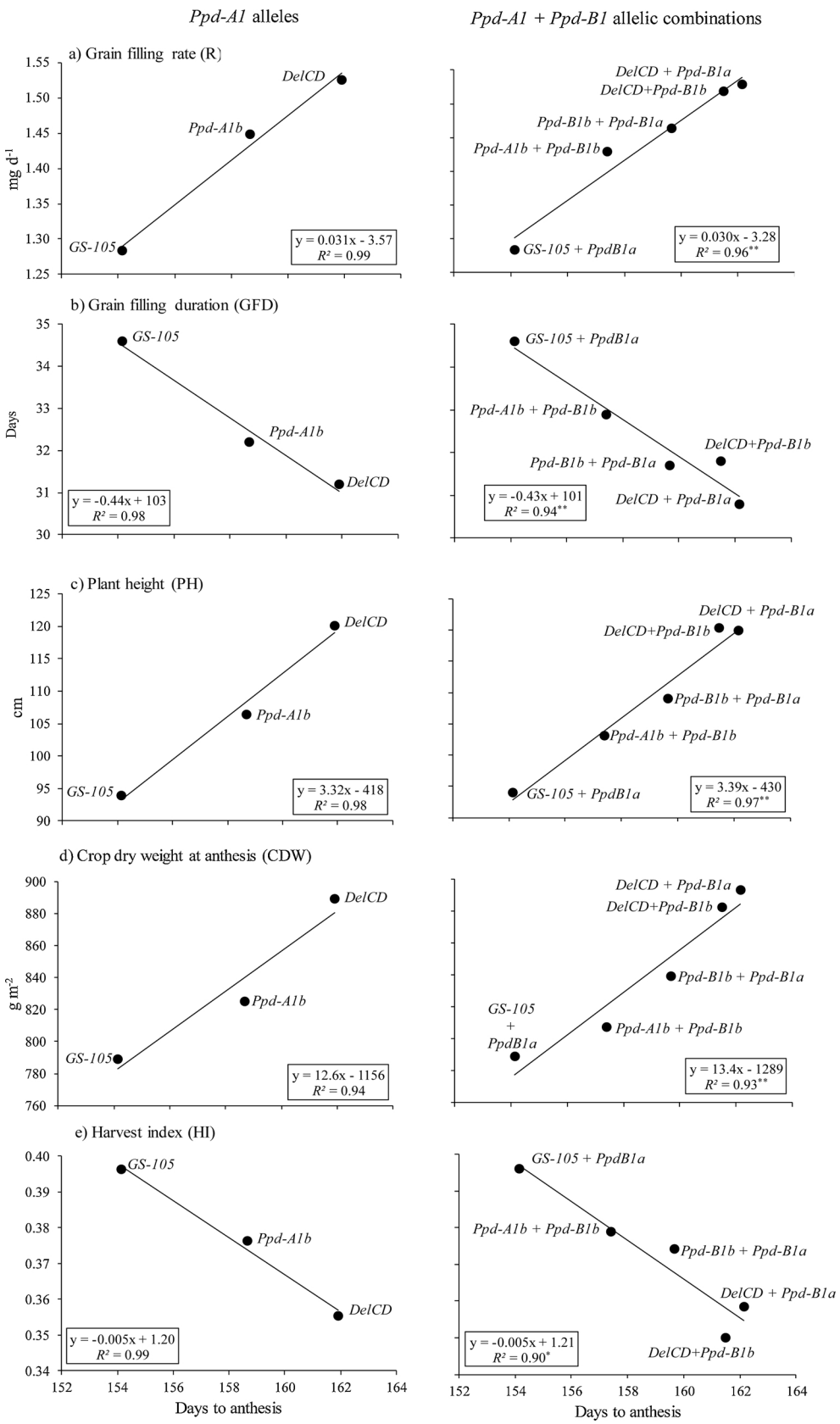

Fig. 5. Relationship between the number of days to anthesis and a) grain filling rate, b) grain filling duration, c) plant height, d) crop dry weight at anthesis and e) harvest index for the three allele variants at *Ppd-A1* (left) and the five *Ppd-A1* + *Ppd-B1* allelic combinations (right) identified in 151 Mediterranean durum wheat landraces.

Table 7
Mean values ± SE for the *Vrn-1* + *Ppd-1* allelic combinations significantly affecting the number of grains per spike (NGS), grain filling rate (R), grain filling duration (GFD), plant height (PH) and harvest index (HI) of 151 durum wheat landraces from 21 Mediterranean countries. Data are means across six crop seasons. Means within columns with different letters are significantly different at $P < 0.05$, following a Tukey-Kramer test.

Allelic combination number	*Vrn-A1* allele	*Vrn-B1* allele	*Ppd-A1* allele	*Ppd-B1* allele	N	NGS		R (mg d^{-1})		GFD (days)		PH (cm)		HI	
AC-1	*Vrn-A1d*	*vrn-B1*	*Del CD*	*Ppd-B1a*	2	23.65 ± 1.66	b	1.64 ± 0.09	a	29.8 ± 1.7	c	126 ± 2.8	a	0.36 ± 0.016	ab
AC-2	*Vrn-A1d*	*Vrn-B1a*	*Del CD*	*Ppd-B1a*	1	22.50 ± 2.90	b	1.64 ± 0.14	a	31.3 ± 2.4	bc	126 ± 2.6	ab	0.34 ± 0.028	b
AC-3	*Vrn-A1d*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1a*	5	23.50 ± 1.13	b	1.58 ± 0.05	a	31.4 ± 1.2	bc	119 ± 1.8	abc	0.38 ± 0.010	ab
AC-4	*Vrn-A1b*	*vrn-B1*	*Del CD*	*Ppd-B1b*	1	22.69 ± 1.82	b	1.56 ± 0.11	ab	32.5 ± 2.7	abc	109 ± 3.2	cdef	0.34 ± 0.019	b
AC-5	*vrn-A1*	*vrn-B1*	*Del CD*	*Ppd-B1b*	1	26.87 ± 2.73	ab	1.53 ± 0.10	abc	32.3 ± 2.6	abc	123 ± 2.0	abc	0.39 ± 0.027	ab
AC-6	*Vrn-A1b*	*vrn-B1*	*Del CD*	*Ppd-B1a*	7	24.18 ± 1.13	b	1.52 ± 0.04	abc	30.6 ± 1.0	c	121 ± 2.1	abc	0.36 ± 0.008	ab
AC-7	*Vrn-A1b*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1a*	28	24.61 ± 0.54	b	1.52 ± 0.02	abc	31.5 ± 0.5	bc	110 ± 1.2	cde	0.37 ± 0.004	ab
AC-8	*Vrn-A1c*	*vrn-B1*	*Del CD*	*Ppd-B1b*	9	25.37 ± 1.07	b	1.51 ± 0.03	abc	31.6 ± 0.8	bc	121 ± 1.6	abc	0.35 ± 0.007	ab
AC-9	*Vrn-A1c*	*vrn-B1*	*Del CD*	*Ppd-B1a*	9	26.66 ± 1.11	ab	1.49 ± 0.04	abc	31.1 ± 0.8	bc	117 ± 2.0	bcd	0.36 ± 0.007	ab
AC-10	*Vrn-A1b*	*Vrn-B1a*	*Ppd-A1b*	*Ppd-B1a*	1	27.19 ± 2.14	ab	1.48 ± 0.08	abcd	31.5 ± 2.6	bc	118 ± 6.2	abcd	0.38 ± 0.017	ab
AC-11	*Vrn-A1b*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1b*	35	25.24 ± 0.47	b	1.46 ± 0.02	abcd	32.9 ± 0.5	ab	103 ± 0.9	ef	0.38 ± 0.004	ab
AC-12	*vrn-A1*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1b*	1	32.16 ± 4.89	a	1.44 ± 0.10	abcd	31.1 ± 2.6	bc	113 ± 3.0	bcde	0.38 ± 0.024	ab
AC-13	*Vrn-A1c*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1a*	33	26.26 ± 0.53	ab	1.39 ± 0.02	bcd	32.0 ± 0.5	bc	106 ± 1.2	ef	0.38 ± 0.004	ab
AC-14	*Vrn-A1c*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1b*	14	25.50 ± 0.74	b	1.36 ± 0.02	cd	33.0 ± 0.8	ab	101 ± 1.5	fg	0.38 ± 0.006	ab
AC-15	*Vrn-A1d*	*vrn-B1*	*Ppd-A1b*	*Ppd-B1b*	2	26.41 ± 1.94	ab	1.32 ± 0.05	cd	33.3 ± 2.1	ab	107 ± 2.8	def	0.38 ± 0.014	ab
AC-16	*Vrn-A1c*	*vrn-B1*	*GS105*	*Ppd-B1a*	2	26.71 ± 1.47	ab	1.28 ± 0.05	d	34.6 ± 2.3	a	94 ± 2.5	g	0.40 ± 0.013	a

genetic backgrounds (Richards, 1992).

The current study allowed us to differentiate three sub-groups within the modern germplasm, namely, the CIMMYT-derived and Mediterranean cultivars, the North American cultivars, and the French cultivar. The relatedness between CIMMYT-derived and modern Mediterranean cultivars is not surprising given the strong impact of CIMMYT germplasm on the release of new durum wheat varieties in Spain and Italy (Royo, 2005, 2009). The only modern French cultivar, 'Arment', was separated in the cluster, probably because it was the only semi-dwarf genotype harboring photoperiod sensitive alleles at both genes (Royo et al., 2020).

Landraces collected from countries of the Fertile Crescent region were clustered in the negative part of the two axes in the PCA, while those from all other origins grouped in the positive direction of PC1. Differences in DA and agronomic performance between these two groups of landraces reflect the incorporation of novel adaptive traits advantageous in the new environments encountered during the spread of domesticated wheat from the east to the west of the Mediterranean Basin. The high frequency of the winter allele *vrn-B1* and the photoperiod sensitivity allele *Ppd-B1b* in landraces collected from countries of the Fertile Crescent region are in agreement with the winter growth habit and photoperiod sensitivity of wheat ancestors (Yan et al., 2004; Laurie, 1997). The high NSm^2 of landraces from eastern Mediterranean countries has been recognized as an adaptive trait to the warm and dry environmental conditions of this area (Royo et al., 2014) and was reported to be linked to molecular marker wPt-5385 on chromosome 1B (Soriano et al., 2018).

Landraces from central and western Mediterranean countries showed a later anthesis date, taller plants, more aboveground biomass at anthesis and higher R and TKW, coinciding with the presence of the *DelCD* allele at *Ppd-A1*, recently identified in durum wheat (Royo et al., under revision). The late anthesis of the western Balkan landraces is in agreement with the low temperatures and high rainfall of this region (Royo et al., 2014). Croatian landraces were distinguished within this cluster due to their heavy grains and to the presence of the alleles *Vrn-B1a* and *Vrn-A1d* conferring spring growth habit, and the photoperiod insensitive allele *Ppd-B1a*.

Whereas there was substantial and significant differences within the landraces for all agronomic traits, there was no significant difference for yield, NSm^2 and CDW within the modern group. This could be attributed to the different size of the two germplasm groups and to the fact that the landraces were selected to maximize variability and representativeness of the entire Mediterranean region, while the modern cultivars were selected as representative of the germplasm adapted to the local target environment. Our results showed that the allelic composition at *Vrn-1* and *Ppd-1* could not clearly relate to the variability observed for any agronomic trait in the modern cultivar group, which is consistent with the narrow selection for local adaptation implemented in assembling this group.

4.3. Effect of the Vrn-1 and Ppd-1loci on the agronomic performance of the landraces

The results suggest that photoperiod had a much greater differentiating effect on agronomic performance than vernalization within the landraces group, as suggested by the larger number of traits significantly affected by *Ppd-1* versus *Vrn-1* and the greater fraction of genotypic variance explained by their allelic combinations at *Ppd-1* versus *Vrn-1*. This is because only two of the landraces, the Italian 'Carlantino' and the Spanish 'Verdial', were true winter types, harboring the recessive alleles at both vernalization genes. These results indicate that although spring growth-habit is the most common among Mediterranean wheats, winter landraces were present in western Mediterranean countries. However, the eleven Turkish landraces included in the current study were spring types in spite of winter wheat landraces being frequent in regions close to the origin of wheat,

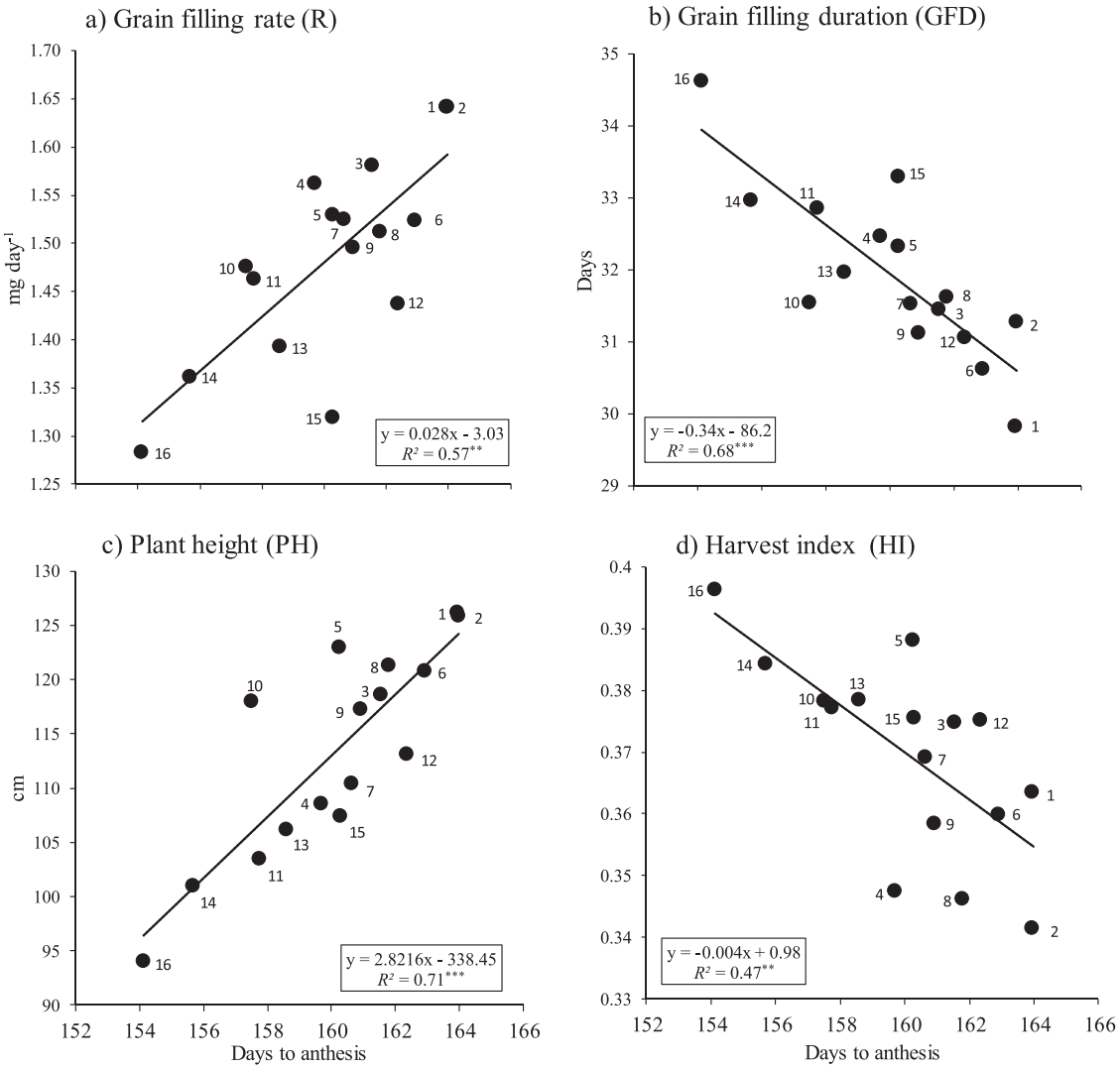

Fig. 6. Relationship between the number of days to anthesis (DA) and a) grain filling rate, b) grain filling duration, c) plant height and d) harvest index for the 16 *Vrn-A1 + Vrn-B1 + Ppd-A1 + Ppd-B1* allelic combinations identified in 151 Mediterranean durum wheat landraces. See Table 7 to identify the allelic combinations corresponding to the numbers shown in the figures.

particularly in Turkey.

The fraction of genotypic variance explained by individual alleles amounted to 17.7 % of the genotype effect in the ANOVA models, that explained by allelic combinations for either *Ppd-1* or *Vrn-1* loci to 21.1 %, and that explained by the *Vrn-1 + Ppd-1* allelic combinations to 26.1 % indicating that the effects of these genes regulating wheat phenology are additive in nature.

4.3. Yield formation

None of the individual alleles at *Vrn-1* or *Ppd-1* or the allelic combinations identified in this study significantly affected the yield of the landraces. This is consistent with our observation that yield was very marginally correlated with phenology traits (DA, GFD) within this group. The *Vrn-1 + Ppd-1* allelic combinations accounted for 11.1 % of the genotypic components of yield variability. This is a very low fraction of the total variance observed, especially when it is compared to the environmental component in the ANOVA, which accounted for 72.2 % of yield variations. This latter figure is within the range of values reported for field experiments with durum wheat, a species that has a greater G × E interaction than bread wheat (Subira et al., 2015).

The lack of a significant effect of the allelic variation at *Vrn-1* and *Ppd-1* on NSm^2 may be explained by the fact that this yield component is determined by the tiller number, which in turn is established very early during the vegetative stages, well before anthesis and therefore minimally affected by phenology. The negative relationship between DA and NSm^2 found in this study is consistent with the contrasting yield formation strategies of Mediterranean landraces from different climatic zones (Royo et al., 2014). Landraces from the warmest and driest areas within the region, the southeastern Mediterranean Basin, have a short pre-flowering period and a high NSm^2, while landraces from colder and wetter areas, such as the Balkans, have a later anthesis and a lower NSm^2 (Royo et al., 2014; Soriano et al., 2018).

The only allelic effect that was significant on NGS was that of the *Vrn-A1* locus, but as noted earlier, this locus was close to monomorphic in the present panel, with only two landraces harboring the winter type allele. With this limitation in mind, our results seem to suggest that the winter allele at *Vrn-A1* may be associated with higher NGS, but this hypothesis needs further investigation. Other allelic combinations at *Vrn-1* or/and *Ppd-1* loci had a significant effect on NGS but seemingly independently from DA, given the absence of correlation between NGS and DA (r = 0.02). The comparison of the mean NGS of sub-groups defined based on *Vrn-A1* alleles or *Vrn-A1 + Vrn-B1* allelic combinations suggested an association between the presence of *Vrn-A1d* and low NGS. However, the lack of a clear pattern in the analysis of the *Vrn-1 + Ppd-1* allelic combinations in general suggests large epistatic

interactions in the determination of NGS in the landraces, as reported previously in durum wheat (Sharma and Sain, 2004).

Our results revealed a significant effect of the *Vrn-A1* allele variants and the *Vrn-A1* + *Vrn-B1* allelic combinations on TKW. The effect of the *Vrn-B1* gene on grain weight was negligible in this panel where it was substantially underrepresented (only two landraces harbored the *vrn-B1* allele). *Vrn-A1* has been demonstrated to exhibit the strongest effect on inhibiting vernalization requirement in bread wheat (Loukoianov et al., 2005). The positive correlation coefficient between DA and TKW could be illustrated through the effect of the *Vrn-A1* alleles on DA, with a three-day delay caused by the allele *Vrn-A1d* resulting in a 6.8 % increase in TKW. The low NGS recorded in landraces harboring the *Vrn-A1d* allele gave rise to the heaviest grains because of a compensation between the two yield components, in agreement with the negative correlation coefficient between them.

A thorough analysis of grain growth was conducted by dissecting kernel weight into its two components, R and GFD. The ANOVAs showed that the phenotypic variability explained by the genotype effect for R was more than five times that for GFD. The greater genetic control of R relatively to GFD observed in the current study is in agreement with the high heritability of this trait (Mou and Kronstad, 1994). Grain filling rate was the only trait significantly affected by both vernalization and photoperiod genes and by their allelic combinations. The response of R to the presence of vernalization genes followed the same pattern as that of TKW, with the allele *Vrn-A1d* associated with an accelerated R and the allele *Vrn-A1c* associated with a slowing down of R. This finding suggests that the effect of allelic variation at *Vrn-A1* on grain weight was through its effect on R, which is consistent with the high association between TKW and R. The effect of each of the three *Ppd-A1* alleles on R was evident, with *DelCD* leading to the greatest values, *GS105* to the lowest and the wild type *Ppd-A1b* to intermediate values. Accordingly, as expected, the landraces simultaneously carrying the alleles *Vrn-A1d* and *Ppd-A1*(*DelCD*), both enhancing R, resulted in the highest R-values, while the landraces harboring the alleles *Vrn-A1c* and *Ppd-A1*(*GS105*) resulted in the lowest ones.

The effect of vernalization and photoperiod genes on R was associated with the anthesis date, in agreement with the positive and significant correlation coefficient between DA and R. The range of anthesis dates associated with *Vrn-A1* alleles and *Vrn-A1* + *Vrn-B1* allelic combinations coincided with R increases of about 10 % (deduced from Fig. 4). The range of anthesis dates associated with *Ppd-1* allelic groups coincided with an increase in R of about 19 % (deduced from Fig. 5). The analysis of the *Vrn-1* + *Ppd-1* allelic combinations revealed that the 10 days difference in DA of landraces harboring the alleles *Vrn-A1d* and *Ppd-A1*(*DelCD*) irrespective of those harboring *Vrn-A1c* + *Ppd-A1*(*GS105*) were associated with a 28 % rise in R (deduced from Fig. 6). These results suggest that the additive nature of the genes regulating wheat phenology was related with an additive effect on R increases.

GFD was the trait most affected by environmental variations. This result is in agreement with the relatively low heritability of this trait and the great environmental influence on it (Egli, 2004; Royo et al., 2016). Alleles regulating photoperiod response, their combinations and combinations of the *Vrn-1* + *Ppd-1* alleles had the opposite effect on GFD compared to those observed for R, in agreement with the negative correlation between these two traits found in this and previous studies (Alvaro et al., 2008b; Royo et al., 2006). Thus, the allele *Ppd-A1*(*DelCD*), which was related to the highest R-values, was associated with the shortest GFD, and the opposite was true for *Ppd-A1*(*GS105*). Though significant, the effect of the *Ppd-B1* gene was smaller, as only one day of difference was observed in the mean values of the GFD of landraces carrying either the wild type or the mutation at this locus. Consequently, the five *Ppd-A1* + *Ppd-B1* allelic combinations followed the same pattern as that observed for the individual alleles at *Ppd-A1*. However, even though the longest grain filling was recorded in the two landraces harboring the allele *Ppd-A1*(*GS105*) and the shortest in the two carrying the allele *Ppd-A1*(*DelCD*), there was no clear pattern for GFD in terms of its association with *Vrn-1* + *Ppd-1* allelic combinations. The effect of photoperiod on GFD could be related to its influence on anthesis date, as a lengthening of the pre-flowering period was associated with significant reductions in GFD. However, GFD had no effect on TKW, which depended rather strongly on R. It is conceivable that the increase in R and TKW associated with a delayed anthesis was compensated by the low NSm^2 in late landraces, thereby canceling any relationship between anthesis and yield. This may explain the lack of significant effects of vernalization and photoperiod genes on yield in this set of landraces. Compensation between yield components often occurs as a result of competition for limited resources (Simane et al., 1993).

4.4. Biomass production and allocation

CDW at anthesis was mostly affected by year and the year × genotype interaction, in agreement with the highly environment-dependent control of biomass availability (Giunta et al., 1995; Simane et al., 1993; Villegas et al., 2001). The *Vrn-1* genes had no effect on the traits related to biomass production and allocation analyzed herein, confirming that photoperiod sensitivity regulating genes have a much greater effect on biomass than those involved in determining vernalization requirements. For the three traits related to biomass, the differences associated with alleles at *Ppd-A1* were much higher than those related to allelic variation at *Ppd-B1*.

The effect of the *Ppd-A1* alleles and *Ppd-A1* + *Ppd-B1* allelic combinations on PH followed the same pattern as that observed for R, with the presence of allele *Ppd-A1*(*DelCD*) associated with increased PH and the presence of allele *Ppd-A1*(*GS105*) related to a generally reduced PH. The effect of *Ppd-A1* alleles on PH could be related to anthesis date. In the current study, PH was 28 % greater in landraces harboring allele *Ppd-A1*(*DelCD*) than in those carrying allele *Ppd-A1*(*GS105*), coinciding with an 8 days delay in anthesis between the two allelic groups, with the wild type leading to intermediate height values. The analysis of *Ppd-A1* + *Ppd-B1* allelic combinations revealed similar results, confirming that the strong anthesis-delaying effect of *Ppd-A1*(*DelCD*) was indeed associated with greater PH. Although the mean CDW of landraces belonging to different photoperiod allelic groups was not statistically significant, the pattern of variation was similar to that observed for PH. It has been demonstrated that the growth of durum wheat follows a logistic curve that reaches a plateau after the milk-grain stage of grain filling (Villegas et al., 2001). Accordingly, a delay in anthesis could conceivably allow for more time for vegetative biomass production, possibly resulting in increases of height and CDW.

The effect of *Ppd-A1* allele variants on HI followed a similar pattern to that observed for GFD, but contrast with that observed for PH and CDW. This is consistent with the positive association between HI and GFD and the negative relationships between HI and PH on one side and CDW on the other. The eight days average difference in anthesis date between the group of landraces carrying *Ppd-A1*(*DelCD*) and those harboring *Ppd-A1*(*GS105*) coincided with a 10 % reduction in HI. The negative relationship between DA and HI is probably due to a limitation in the allocation of biomass to grains as anthesis was delayed making coincide grain filling with higher temperatures and reduced water availability. This obliged the plants to invest energy for transpiration, thereby reducing the energy available for accumulating photosynthates in the grain (Royo et al., 2018). The strong environmental influence on HI, as shown by the ANOVA, is in line with this assumption. A conceptual physiological framework expresses yield as the product of biomass production and the fraction of it allocated to grains. The results of the current study demonstrated that the greater HI observed in landraces with early anthesis did not necessarily result in a higher yield, conceivably because the lower biomass characterizing these early landraces resulted in an under-compensation in terms of grain biomass production.

5. Conclusions

This is the first study that relates the allelic variation for major genes regulating flowering time with the agronomic performance of a representative set of Mediterranean durum wheat landraces. The wide genetic background of the panel used here allowed us to identify several germplasm pools. The largest differences were detected between landraces taken as a group and the sub-panel of locally adapted modern cultivars included as checks. These two groups differed in the allelic frequencies observed for major genes regulating phenology and in yield formation strategies. Allelic variants for the *Vrn-1* and *Ppd-1* genes and agronomic traits were associated with the geographic origin of the landraces, revealing the type of changes that occurred during the dispersal of wheat throughout the Mediterranean Basin and emphasizing the importance of flowering time in wheat adaptation.

The contribution of vernalization and photoperiod genes in the variability of phenotypic traits of landraces was additive. Although the *Vrn-1* and *Ppd*-1 loci had a strong effect on flowering time within the landraces group, the higher biomass accumulation and grain filling rate associated primarily with the presence of alleles *Vrn-A1d* and *Ppd-A1*(*DelCD*) conferred no advantage in terms of grain yield due to compensatory effects between traits.

Author contributions

CR led the projects, conceived the manuscript and wrote the manuscript. CR and DV performed field evaluations and data analyses. CR, DV and KA assembled and purified the germplasm collections. SD performed the molecular analyses. CR, DV, SD and KA edited and provided a critical review of the manuscript and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2020.126129.

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