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Food & Agriculture Research & Technology (IRTA), Durum Wheat Breeding Program, Lleida, Spain

Morphological Traits above the Flag Leaf Node as Indicators of Drought Susceptibility Index in Durum Wheat

D. Villegas, L. F. García del Moral, Y. Rharrabti, V. Martos, and C. Royo

Author's addresses: Dr D. Villegas (corresponding author; e-mail: dolors.villegas@irta.es), and Dr C. Royo, Food & Agriculture Research & Technology (IRTA), Durum Wheat Breeding Program, Rovira Roure, 191, 25198 Lleida, Spain; Dr L. F. García del Moral, Dr Y. Rharrabti and Dr V. Martos, Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

With 3 figures and 4 tables

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Abstract

Selection criteria for drought tolerance would be helpful tools for wheat breeding programmes. To assess the usefulness of some morphological traits above the flag leaf node as indicators of yield and the susceptibility index (SI) of Fischer and Maurer, 10 durum wheat genotypes were used in experiments conducted under two water regimes at two latitudes in Spain during 3 years. Morphological traits were measured at anthesis, and yield, yield components and quality traits were evaluated at ripening. Principal components analysis showed associations between morphological traits and yield, yield components and quality, most of them caused by differences between environments. Peduncle weight, spike weight and length and awn length were significantly related to SI within environments. Spike and peduncle weight were the traits more related to yield and SI in all the experiments together and in the rainfed sites, while in the irrigated sites spike length was better. The spike weight and length were negatively associated with SI, while peduncle weight was positively associated to SI. Genotype means across all experiments were associated with SI values. These morphological traits could be selection criteria in breeding programmes to obtain varieties with good yield stability. The genetic variability found suggests opportunity for selection.

Key words: awn length — flag leaf size — peduncle — spike length — spike weight — yield

Introduction

Drought is one of the main constraints to rainfed wheat production in the Mediterranean region, where durum wheat (*Triticum turgidum* L. var *durum* Desf.) is extensively grown. Achieving genetic increases in yield in these environments has

been recognized to be a difficult challenge for plant breeders while progress in yield gains has been much higher in favourable environments (Richards et al. 2002). Quality traits, among which some of the most important are protein, pigment and ash contents, gluten strength, vitreousness and test weight (Troccoli et al. 2000), have also to be considered because of their market importance.

Wheat breeding during the last century has been carried out by using grain yield *per se* as the most important selection criterion (Loss and Siddique 1994). In early generations of a breeding programme, however, yield still cannot be reliably evaluated, and selection has to be performed according to other criteria. Breeders usually have in their mind a specific ideotype and select according to that (Rasmusson 1987, Sedgley 1991).

It has been shown by several authors that grain yield in wheat is more closely correlated to grain number than to grain mass (Zamski and Grunberger 1995, Beharav et al. 1998). Thus, selecting for a high number of spikes per m² and a high number of grains per spike has to result in improved yield. Nevertheless, as grains have to be filled with carbohydrates produced by photosynthetic organs, both source and sink of carbohydrates have to be taken into account when breeding for drought-stressed environments. In this sense, the role of architecture, distribution and size of leaf area have been studied and discussed by several authors (Berdahl et al. 1972, Canevara et al. 1994). Some studies (Kaul 1974, Briggs and Aytenfisu 1980)

have reported that green tissues above the flag leaf node are mainly responsible for production of carbohydrates to fill the grains, because the lower leaves lose their assimilation power rapidly and die early under drought conditions. Nevertheless, the relative importance of each of these organs is not completely understood (Lukovic et al. 2001).

The flag leaf is considered to be the greatest contributor to grain yield for its short distance to spike and the fact that it stays green for longer than the rest of the leaves. Minhas et al. (1978) found that erect leaves are desirable for all environments, while for rainfed conditions, short and tough leaf-blades were important to withstand the drought stress. Contrarily, Smocek (1969) suggested that a big flag leaf area might be used in combination with yield components to obtain maximum genetic advance. Inconsistent results about the relationship between flag leaf characteristics and yield may be found in the literature as they range from strong (Minhas et al. 1978, Briggs and Aytenuf 1980, Singh and Singh 1992), to inexistent (Berdahl et al. 1972, McNeal and Berg 1977) or genotype-dependent (Chowdhry et al. 1976).

Carbohydrates are remobilized from the spike peduncle and flag leaf to the grain during the grain filling period (Zamski and Grunberger 1995). Briggs and Aytenuf (1980) found an association between short peduncles and high grain yield. In other cases such a relationship has been found inverse (Kaya et al. 2002) or depending on the environment.

Awns have also received attention because they are photosynthetically active and they have direct vascular linkage with the spike (Weyhrich et al. 1995). Moreover, awns have been shown to be advantageous during drought stress in the driest areas (Evans et al. 1972, Kaul 1974, Minhas et al. 1978, Motzo and Giunta 2002). Additionally, to their photosynthetic role, a refreshing effect of awn structure has also been reported (Ayeneh et al. 2002).

Given that most durum wheat is grown under water deficit in Mediterranean conditions, the assessment of drought tolerance is of great importance. Fischer and Maurer (1978) proposed the calculation of a simple drought susceptibility index (SI), which provides a measure of drought tolerance based on yield loss under stress when compared with optimum conditions. A low SI corresponds to high drought resistance. This index has been further used by a number of authors in wheat (Bansal and Sinha 1991, Cedola et al. 1994, Lazar et al. 1995), barley (Shakhatreh et al. 2001),

triticale (Ozkan et al. 1999) and oats (Larsson and Gorny 1988). According to Lazar et al. (1995), while yield is the main breeding objective, SI may reveal important distinctions in fundamental plant attributes. Furthermore, when yield distinctions in favourable environments are non-significant, differences among SI are equivalent to yield distinctions in the stress environment. Additionally, Blum et al. (1989) observed that the SI of Fischer and Maurer (1978) was closely and negatively related to yield stability according to the method of Finlay and Wilkinson (1963). Similarly, Bansal and Sinha (1991) reported a strong negative relationship between SI and the stability parameter described by Eberhart and Russell (1966). Therefore, the SI has been used by several authors as a measure of both drought resistance and yield stability.

A meteorological approach to the drought measurement is represented by the Palmer Drought Severity Index (PDSI, Palmer 1965). This index is based on water balance and the measurement of the drought severity (Alley 1984). Although PDSI has been criticized for several reasons, it has become a standard for measuring meteorological drought (Wells and Goddard 2004). The objective of this study was to determine the relationship between the characteristics of several organs above the flag leaf node with grain yield, yield components, grain quality and drought SI in durum wheat grown under contrasting Mediterranean conditions.

Materials and Methods

Experimental setup

Twelve field experiments were conducted under two water regimes (irrigated and rainfed) in two cereal growing zones of Spain (Ebro Valley in the north and Andalusia in the south, hereinafter referred to as latitudes), during three consecutive years in each latitude (1997–1999 in the north and 1998–2000 in the south).

Ten durum wheat genotypes were grown in a randomized complete block design with four replicates in plots of 12 m². Genotypes included four Spanish commercial cultivars (Altar-aos, Jabato, Mexa and Vitron), and six CIMMYT-ICARDA varieties or inbred lines (Awalbit, Korifla, Lagost-3, Omrabi-3, Sebah and Waha). These genotypes were chosen in an attempt to represent a wide range of genetic variability, all of them having a similar date of anthesis, near the optimum in the region.

The seeding rate was adjusted for a density of 550 viable seeds m⁻² in the north and 350 seeds m⁻² in the south, according to the standard practices at each latitude. Situation and main characteristics of the experimental sites and meteorological variables during the experiments are summarized in Table 1.

Table 1: Localization and description of the sites used in this study

	North		South	
	Irrigated	Rainfed	Irrigated	Rainfed
Site	Grimenells	El Canós	Granada	Ochichar
Coordinates	41°40'N 0°20'E	41°41'N 1°13'E	37°21'N 3°35'W	37°10'N 3°50'W
Altitude (m)	200	440	650	720
Soil characteristics	Calcixerollic Xerochrept	Fluventic Xerochrept	Typic Xerofluvent	Loamy Calcixerollic Xerochrept
Classification	Fine loamy	Loamy fine	Silty clay	Silty clay
Texture	8.1	8.2	8.0	8.2
pH	16	12	50	27
P (mg P kg ⁻¹ soil)	134	184	88	210
K (mg K kg ⁻¹ soil)	2.40	2.10	2.01	1.86
Organic matter (%)	1997	1998	1999	2000
Fertilizers (kg ha ⁻¹)	50	32	60	49
N (seed bed)	100	84	20	20
N (top dressing)	120	60	75	49
P ₂ O ₅	165	60	75	49
K ₂ O	3 December	17 November	15 December	25 November
Sowing time	23 November	3 December	15 December	12 January
	1997	1997	1998	1998
Days sowing-anthesis	143	144	138	166
Total water input (mm)	364	386	423	187
Mean temperature sowing-anthesis (°C)	8.6	8.5	9.5	9.9
Mean temperature anthesis-maturity (°C)	18.5	17.2	21.9	21.6
		17.0	17.6	19.8

Measurements

A sample of five representative main stems was collected randomly in a central row of each plot at anthesis, and the upper internode portions, including the spikes, were separated in the laboratory. For each sample, the following morphological variables were recorded and averaged: (i) length and dry weight of spike peduncle; (ii) length, area and dry weight of flag leaf blade; (iii) dry weight of flag leaf sheath; (iv) length and dry weight of spike; and (v) length of awns (considered from the terminal spikelet to the tip of awns). Samples were subsequently oven-dried for 48 h at 80°C and weighed. The specific weight of peduncles was calculated for each site with the mean data of the 3 years, as the ratio of peduncle weight to peduncle length according to Shakiba et al. (1996). Yield components were measured as detailed in García del Moral et al. (2003). Grain quality determinations (test weight, vitreousness, protein content, gluten strength, pigment content and ash content) were made as explained in Rharrabti et al. (2003).

Computations

Weekly-step Palmer Drought Sensibility Index (PDSI) was computed as described in Alley (1984) using all long-term data available, which were from 16 to 20 years depending on the site. Weekly reference evapotranspiration used in computations was calculated by the Penman–Monteith equation as detailed in Allen et al. (1998). Specific location duration factors were considered as described in Wells and Goddard (2004). Drought susceptibility index (SI) was calculated for each genotype as (Fischer and Maurer 1978):

$$SI = \frac{1 - (Y_R/Y_I)}{1 - (Y_{MR}/Y_{MI})}$$

where Y_R is the rainfed and Y_I is the irrigated genotype mean yield, while Y_{MR} is the rainfed and Y_{MI} is the irrigated site mean yield.

Analyses of variance were performed considering a fixed model in which the year factor was nested to latitude. Pearson correlation coefficients were calculated on the 3-year mean values, except in the cases indicated. To elucidate the relative importance of some morphological traits on yield and SI, stepwise regressions were calculated by using the genotype means for each site across years, either pooled together ($n = 40$) or for each site separately ($n = 10$). All statistical analyses were carried out by using the SAS-STAT statistical package (SAS Institute Inc. 2000). Principal component analyses were carried out on the correlation matrix, calculated on the mean data of the four replications by using the PRINCOMP procedure of the SAS programme, which does not allow for orthogonal rotation, leaving the original orthogonal solution.

Results

Plots of PDSI changes for each experiment are shown in Fig. 1. In the north-rainfed site, values of PDSI were negative during grain filling, while in the north-irrigated site the irrigation prevented drought to occur as a general trend. The south-irrigated site had negative values for PDSI before

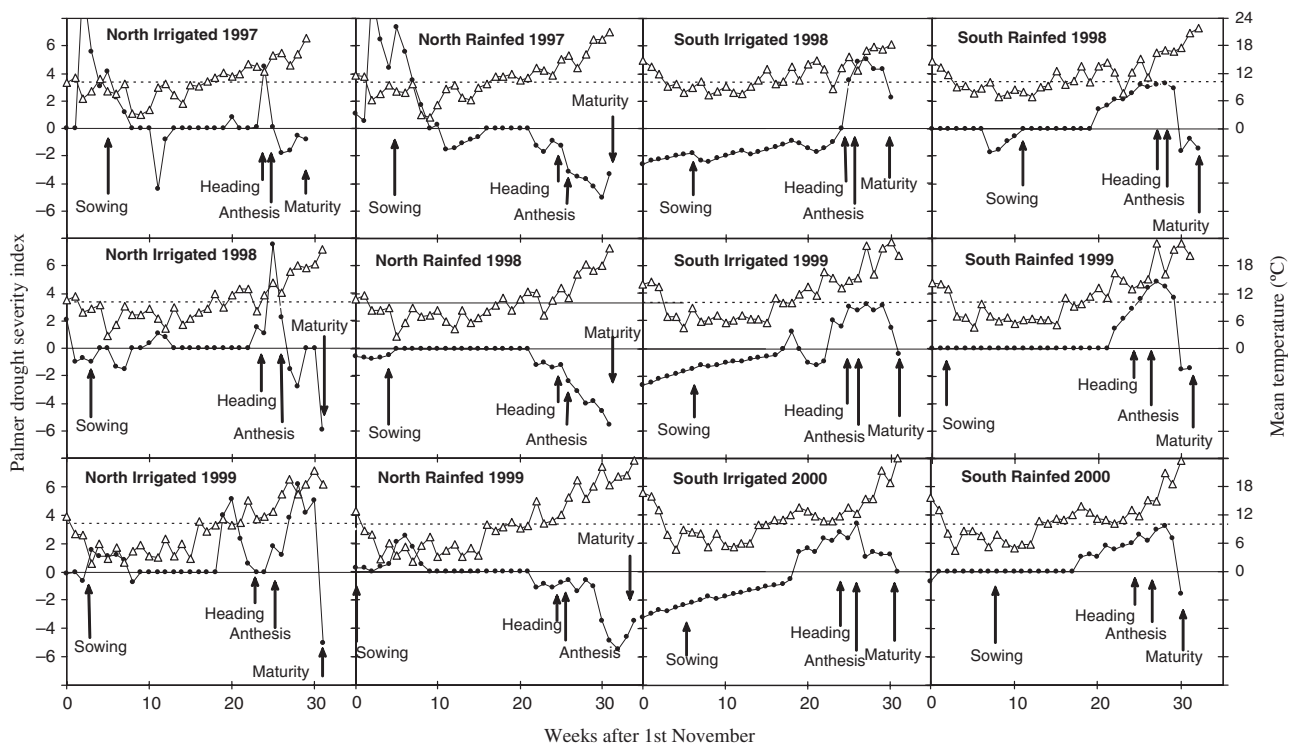


Fig. 1: Palmer Drought Severity Index (circles) and mean temperature (triangles) for each week on each experiment

anthesis, while grain filling was developed without drought stress, that is, with positive values of PDSI up to maturity. In contrast, in the south-rainfed site, PDSI was in general close to zero and with positive values during the first part of grain filling, changing its sign during the final phases of grain filling.

The analyses of variance showed that in general, the environmental factors such as latitude or water regime were much more important than the genotype in explaining the observed variation of the traits studied (Tables 2a and 2b). The latitude accounted for most of the variation observed in the length of awns and spikes and the weight of peduncles and spikes. Water regime was the main factor in explaining variations in grain yield, peduncle length, flag leaf size (Table 2a), spikes per m² and grains per spike (Table 2b). Year variations were significant for all the quality traits except pigment content, whose main source of variation was the genotype (Table 2b). Genotype accounted for important percentages of observed variation also for most yield components, gluten strength, peduncle measurements and spike size, being highly significant for all the traits studied. Double and three-way interactions were in general of lower importance than the main factors (Tables 2a and 2b).

Grain yield ranged between 1.4 t ha⁻¹ (genotype Lagost-3 in north rainfed 1997) and 7.9 t ha⁻¹ (genotype Korifla in north irrigated 1999), which can be considered a wide yield variation under Mediterranean conditions. Higher yields were recorded in the north sites (5.9 t ha⁻¹ in the irrigated and 2.8 t ha⁻¹ in the rainfed sites) irrespective of the south (4.9 and 2.6 t ha⁻¹ in the irrigated and rainfed sites, respectively). Spike weight observed in the south (0.76 g in the irrigated and 0.64 g in the rainfed) was greater than that of the north sites (0.59 and 0.49 g in the irrigated and rainfed, respectively). Grain protein content was higher in the north sites (13.9 % in the irrigated and 15.6 % in the rainfed) than in the south ones (12.1 % and 13.8 % in the irrigated and rainfed sites, respectively). Large phenotypical variability was also registered for the rest of the measured traits. Mean peduncle specific weights were 8.02, 8.85, 11.21 and 11.54 mg cm⁻¹ in the north-irrigated, north-rainfed, south-irrigated and south-rainfed sites, respectively.

Figure 2a shows the results of PCA considering the morphological traits and quality traits together. For each variable considered, the eigenvector is

Table 2a: ANOVA results for grain yield and morphological traits measured at anthesis in 10 durum wheat genotypes grown at two water regimes (WR, irrigated and rainfed) in two latitudes of Spain during 3 years

Source of variation	d.f.	Peduncle			Flag leaf			Spike			
		Grain yield	Weight	Length	Blade weight	Blade area	Blade length	Sheath weight	Weight	Length	Awn length
Latitude	1	2.8***	30.9***	0.9**	3.4***	0.0	1.3***	1.4***	28.7***	33.7***	55.6***
Year (latitude)	4	19.5***	12.4***	17.3***	4.8***	4.7***	8.0***	9.1***	7.1***	4.9***	6.3***
WR	1	61.2***	3.2***	31.3***	61.1***	72.1***	59.8***	40.9***	13.3***	22.0***	19.2***
Latitude × WR	1	1.0***	0.0	3.1***	5.5***	5.6***	3.0***	1.1**	0.0	10.1***	0.0
Year (latitude) × WR	4	5.3***	6.7***	4.2***	9.9***	5.4***	14.2***	21.5***	12.6***	11.1***	4.0***
Genotype	9	0.9**	13.1***	16.5***	4.3***	1.5***	2.9***	7.9***	15.0***	10.3***	8.8***
Latitude × genotype	9	0.9**	2.1	1.3	1.2*	0.9*	0.8*	1.2	2.1*	0.5	0.6**
WR × genotype	9	0.8*	3.0*	3.5***	1.1*	0.5	0.9*	2.9**	1.9*	0.8	0.5*
Year (latitude) × genotype	36	3.4***	8.8*	6.7**	3.4	2.6**	1.5	6.3	11.0***	2.0	1.9***
Latitude × WR × genotype	9	0.2	3.2*	0.8	0.6	0.7	1.2**	0.6	1.3	0.6	0.4
Block (latitude × year × WR)	36	4.0***	16.5***	14.4***	4.7**	6.0***	6.3***	7.1*	7.0***	3.9**	2.7***
Residual	360	11.9	35.8	25.8	17.0	11.4	13.8	29.1	25.2	16.9	8.3
Total	479										

Values presented are the percentage of the sum of squares explained by the different factors. *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2b: ANOVA results for yield components and quality traits measured in 10 durum wheat genotypes grown at two water regimes (WR, irrigated and rainfed) at two latitudes in Spain during 3 years

Source of variation	d.f.	Yield components					Quality traits				
		Spikes m ⁻²	Spikelets per spike	Grains per spike	TKW	Test weight	Vitreousness	Protein content	Gluten strength	Pigment content	Ash content
Latitude	1	13.4***	5.1***	2.1***	22.3***	8.4***	7.6***	21.0***	4.4***	24.5***	13.3***
Year (latitude)	4	12.0***	11.6***	5.9***	32.9***	26.1***	38.6***	31.8***	40.0***	6.4***	38.4***
WR	1	25.2***	11.3***	55.3***	1.2***	3***	14.5***	21.1***	3.2***	0.3	23.8***
Latitude × WR	1	0.1	40.8***	5.7***	0.5	10.1***	7.9***	0.0	0.4**	0.2	0.4**
Year (latitude) × WR	4	4.4***	13.4***	4.5***	4.8***	21.7***	23.7***	5.5***	3.0***	1.1*	15.0***
Genotype	9	15.1***	6.1***	9.7***	22.2***	17.0***	1.0***	5.7***	37.0***	48.3***	1.1**
Latitude × genotype	9	2.0	1.8***	1.3	4.4***	1.0	1.1***	0.9	2.0***	1.7*	1.0*
WR × genotype	9	1.9	0.6	0.9	2.5***	2.4**	0.8**	0.6	0.5	1.9	0.8
Year (latitude) × genotype	36	9.0*	4.0**	6.1***	6.7***	5.7**	1.7**	7.1**	7.2***	10.2***	4.1***
Latitude × WR × genotype	9	1.3	0.6	1.4*	0.4	0.9	0.7**	2.1*	0.5	2.2*	0.3
Block (latitude × year × WR)	36	15.6***	4.7***	7.1***	2.1	3.7	2.4***	4.2**	1.8***	3.4*	1.8
Residual	360	35.8	17.3	22.0	17.3	23.8	8.9	16.4	6.1	16.3	13.9
Total	479										

Values presented are the percentage of the sum of squares explained by the different factors. TKW, Thousand-kernel weight. *P < 0.05; **P < 0.01; ***P < 0.001.

represented. The length of the projection of each eigenvector on each principal component axis is proportional to its contribution to the principal component of that axis. The cosine of the angle between any two vectors is inversely proportional to the correlation between them. The first axis explained 38 % of total variability and was related with all morphological traits in its positive sense and protein content on the negative direction, without any trait clearly defining the axis. The second PCA axis explained 15 % and was mostly related to quality traits, being pigment content, test weight and vitreousness placed in its positive sense while the negative sense represented ash content. Gluten strength had a low weight in explaining global variability, and a negative association was found between protein content and spike-related measurements as well as peduncle weight.

Principal component analysis for yield and susceptibility index (Fig. 2b) explained 45 % and 19 % of variation by the first and second axis, respectively. First axis was related mainly to the number of grains per spike and flag leaf traits, especially weight, while the second axis represented mainly thousand-kernel weight, SI and with a lesser extent, yield. Some associations between morphological traits and yield or SI were observed: peduncle weight and spike-related variables were negatively associated with susceptibility index, while peduncle length was positively associated with grain yield. Thousand-kernel weight and SI also showed a strong positive association in the PCA plot.

Correlations between morphological traits and SI, yield, yield components or quality traits were calculated by each experiment for traits showing association in the PCA analyses. Only correlations with SI where consistently significant are shown in Table 3. Peduncle length and grain yield were not significantly correlated in any experiment, the same being true for protein content associations with spike length or weight. The number of grains per spike was correlated with flag leaf blade weight only in the north-rainfed experiment of 1999 ($r = 0.66$, $P < 0.05$). Protein content and awn length were negatively correlated only in the north-rainfed experiment of 1998 ($r = -0.63$, $P < 0.05$), while protein content and peduncle weight were negatively correlated in the north-rainfed experiment in 1997 ($r = -0.67$, $P < 0.05$) and positively in the south-rainfed experiment in 2000 ($r = 0.73$, $P < 0.05$). Thousand-kernel weight was correlated with peduncle weight only in the south-irrigated

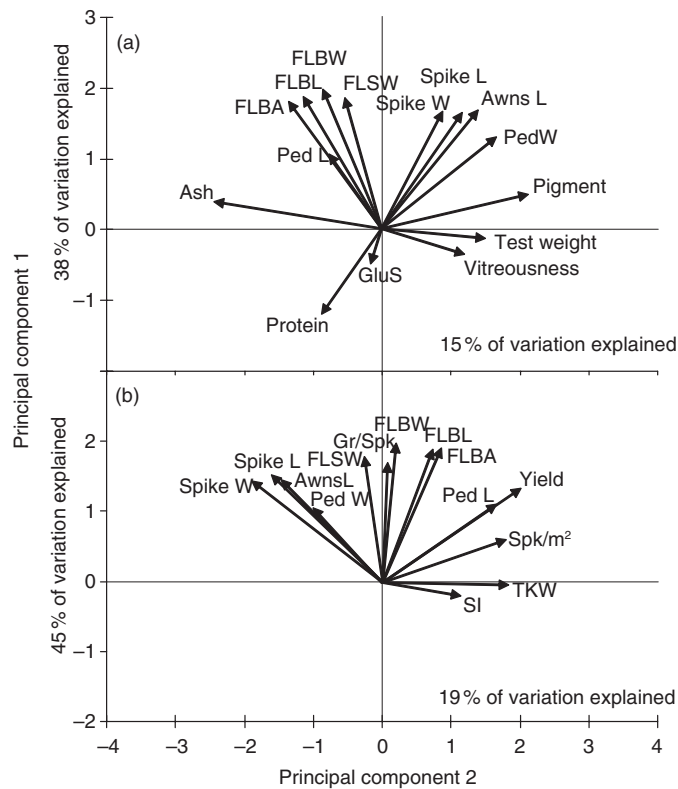


Fig. 2: Eigenvectors plotted on the first and second axes of the principal component analyses computed with morphological traits measured at anthesis and (a) quality traits and (b) yield and yield components. First and second axis explained 38 % and 15 % of total variability for quality traits (a), while for yield components the percentages were 45 % and 19 % (b). Data were obtained from 10 durum wheat genotypes grown at two water regimes (WR, irrigated and rainfed) in two latitudes of Spain during 3 years. GluS, gluten strength; Ash, ash content; Protein, protein content; Ped L, peduncle length; FLBA, flag leaf blade area; FLBL, flag leaf blade length; FLBW, flag leaf blade weight; FLSW, flag leaf sheath weight; Spike W; spike weight; Spike L, spike length; Awns L, awn length; Gr/Spk, grains per spike; Spk/m², no. spikes per m²; SI, susceptibility Index

Table 3: Pearson correlation coefficients between susceptibility index and morphological traits measured at anthesis of 10 durum wheat genotypes for each experiment (n = 10)

Experiment	Peduncle weight	Awn length	Spike length	Spike weight	Thousand-kernel weight
North irrigated 1997	0.72*	-0.80**	-0.81**	-0.35	0.02
North irrigated 1998	0.58	-0.72*	-0.75*	-0.63*	0.42
North irrigated 1999	0.83**	-0.28	-0.46	-0.48	0.27
South irrigated 1998	0.09	-0.80**	-0.46	-0.66*	0.63*
South irrigated 1999	0.64*	-0.25	-0.43	-0.36	0.06
South irrigated 2000	0.13	-0.95**	-0.75*	-0.46	0.48
North rainfed 1997	0.36	-0.49	-0.64	-0.48	-0.19
North rainfed 1998	0.55	-0.42	0.44	-0.80**	-0.02
North rainfed 1999	0.11	-0.49	-0.74*	-0.68*	-0.13
South rainfed 1998	0.48	-0.73	-0.41	-0.39	0.15
South rainfed 1999	0.50	-0.88***	-0.85**	-0.75*	-0.35
South rainfed 2000	0.72*	-0.76*	-0.21	-0.40	-0.28

*P < 0.05; **P < 0.001; ***P < 0.001.

experiment in 1999 ($r = 0.70$, $P < 0.05$), and with spike weight in the south-rainfed experiment in 1999 ($r = 0.64$, $P < 0.05$). The above mentioned correlations were not statistically significant for the

rest of the experiments. Yield and SI were neither statistically correlated (data not shown).

Mean values for each site were calculated, and association trends due to environment were

explored for traits showing relationships in PCA but without correlation within one site. The resulting correlations ($n = 4$) and trends found were as follows: Protein content of grains was negatively associated with spike weight ($R^2 = 0.96$, $P < 0.05$) and awn length ($R^2 = 0.94$, $P < 0.05$), and showed non-significant negative trend with spike length and peduncle weight. Grain yield was positively associated with peduncle length ($R^2 = 0.97$, $P < 0.05$). The number of grains per spike was associated with flag leaf blade weight ($R^2 = 0.995$, $P < 0.01$). Negative non-significant trends were found between thousand-kernel weight and either spike weight, spike length, awn length and peduncle weight.

Mean values for each genotype were calculated and selected traits according to PCA and correlation results were plotted in Fig. 3. Regression analyses were performed for all sites together and for each of them separately, considering yield and SI as dependent variables, and peduncle weight, spike weight and length, and awn length as independent variables (Table 4).

Discussion

Drought incidence

Low yields in the south could be explained by the short period of grain filling (approx. 3 weeks) contrasting with the 5–6 weeks of grain filling

duration in the north-rainfed experiments (Fig. 1). Additionally, higher temperatures were observed in the southern experiments irrespective of the north, especially by anthesis time. In the south-irrigated site, the formation of morphological characters could be limited by the drought experienced by the crop during the pre-heading phases in all experiments, while during the grain filling drought was avoided by irrigation.

Environmental effects on yield, quality and morphological traits

Yield and SI were largely independent, as other authors have pointed out (Lazar et al. 1995, Ricciardi 2001). The results of this study show that water regime was the main factor affecting yield, as differences between irrigated and rainfed sites explained 61 % of yield variations (Table 2a), and among the yield components, the number of spikes per m^2 and grains per spike resulted mostly affected by water regime (Table 2b). Many studies have reported that water availability is one of the most severe constraints for plant growth and yield production under Mediterranean conditions, being the number of spikes per m^2 one of the most affected and followed by the number of grains per spike (Blum and Pnuel 1990 and references herein). Some of the morphological traits studied here, as peduncle length and flag leaf size and weight also

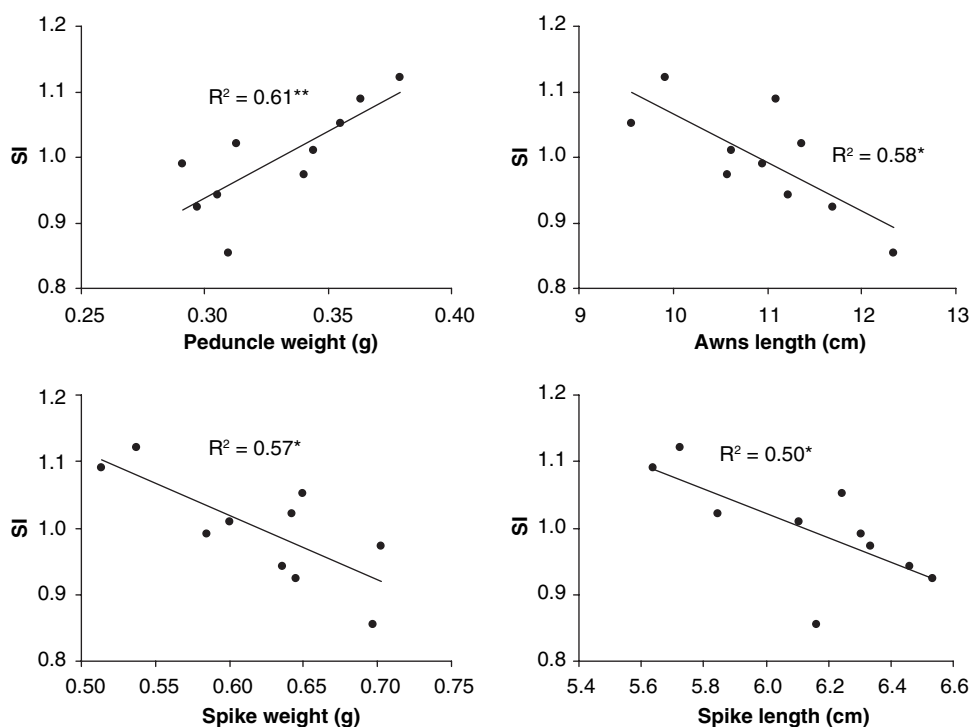


Fig. 3: Relationships between selected morphological traits measured at anthesis and susceptibility index. Each point represents the mean value of one genotype across 12 experiments (two water regimes, two latitudes and 3 years)

Table 4: Stepwise regression analysis considering susceptibility index as dependent variable, and morphological traits measured at anthesis, as independent variables

Traits included	Yield		SI	
	Partial R ²	Model R ²	Partial R ²	Model R ²
All sites				
Spike length	0.10*	0.10	–	
Spike weight			0.52***	0.52
Peduncle weight			0.15*	0.67
Final equations		Yield = 525 + 574 spike length SI = 0.96 – 0.56 spike weight + 1.16 peduncle weight		
North irrigated				
Spike length	–		0.61**	0.61
Spike weight	–		0.14*	0.75
Final equation		SI = 2.24 – 0.10 spike length – 0.52 spike weight		
South irrigated				
Spike weight	0.42*	0.42	–	
Awn length	–		0.57*	0.57
Spike length	–		0.33**	0.90
Final equations		Yield = 6767 – 2383 spike weight SI = 2.52 – 0.05 awn length – 0.11 spike length		
North rainfed				
Spike weight	0.61**	0.61	0.57*	0.57
Spike length	0.18*	0.79		
Peduncle weight			0.23*	0.80
Final equations		Yield = 4005 + 6551 spike weight – 810 spike length SI = 0.91 – 1.08 spike weight + 2.23 peduncle weight		
South rainfed				
Spike length	0.45*	0.45		
Peduncle weight			0.78***	0.78
Spike weight			0.11*	0.89
Final equations		Yield = –179 + 460 spike length SI = 0.66 + 1.70 peduncle weight – 0.43 spike weight		

Values are percentages of the total variability explained across four sites analysed together (upper part, $n = 40$) or separating sites (lower part) of experiments conducted in the north and south of Spain, considering 10 genotypes averaged over 3 years in each site.

showed their main variation associated with water regime. These results are consistent with the reported large reduction of leaf area (Ludlow and Muchow 1990, Giunta et al. 1995, Royo et al. 2004), and peduncle length (Krishnawat and Sharma 1998) with low water supply.

Contrarily, the weight of the spike and its peduncle, as well as the length of the spike and its awns, were more dependent on the latitude than on the water regime, all these traits being of higher magnitude in the south. These results may be partially explained by the lower sowing density of the southern experiments, given that low plant densities lead to less interplant competition and frequently bigger spikes (Briggs and Aytenfisu 1980, Rasmusson 1987). Increased temperatures before anthesis may have favoured the production of bigger structures above the flag leaf node in the

south (Ferris et al. 1998), especially because the spring increase of temperature took place 8–10 weeks before heading, in contrast to the 5–6 weeks in the north (Fig. 1, considering the marked arbitrary threshold in dotted line), indicating that the growth of the spikes took place under higher temperatures in the south. However, in spite of having greater spikes, grain yield in the south was significantly lower than in the north, probably because high temperatures during late spring reduced grain filling duration in the south (García del Moral et al. 2003). Maximum temperatures above 30 °C or even 35 °C, critical for grain filling (Porter and Gawith 1999) were recorded around anthesis in the south. Even in the case that the heat shock was short, the plant could not recover from the effect of heat damage during grain filling (Stone et al. 1995).

Year was the main source of variation of most quality traits, which is consistent with the statement that particular drought, high temperatures and terminal stresses during grain filling are the major factors affecting durum wheat quality (Troc coli et al. 2000, and references therein). The only exception was thousand-kernel weight, for which the genotype was the main source of variation, as stated by other authors (Sharma and Anderson 2004).

Relationship between morphological traits and quality

In Fig. 2a the relationships between the morphological characters and quality traits are shown. Axis 1 represented mainly the morphological characters, while axis 2 was related to most of the quality traits, thus indicating that morphological and quality traits are fairly unrelated. Nevertheless, a negative association was found between protein content and spike measurements. For other quality traits the relationships were considered too weak to be analysed further because in the PCA plots they were placed in different axis from the morphological traits.

The negative association between protein content and spike measurements disappeared when considering the correlation coefficients calculated by experiment. The association was related to site differences, that is, the sites where spikes had higher size (including awns) and higher peduncle weights showed also lower protein content (see results section). The lower protein contents and greater spikes were found in the south experiments, where nitrogen management was different from the southern one (Rharrabti et al. 2003). Given that the associations between protein content and spike size or peduncle weight are environmental rather than genetic, the use of these morphological traits as selection tools for increasing protein content in a breeding program may not be recommended.

Relationships between morphological traits, SI, yield and yield components

Figure 2b shows the associations between morphological traits, yield-related traits and SI. Strong positive associations were found between peduncle length and yield, which were not supported by the correlations by experiment (Table 3). Instead, the association plotted in PCA was because of environment as in the case of protein content and spike

size. Favourable conditions during growth may permit an expansion of the last internode as well as a higher yield (Gupta et al. 2001), but without an impairment of these two facts within a site or experiment, as indicated by other authors (Briggs and Aytenfisu 1980, Kaya et al. 2002). The results of this study do not suggest peduncle length as a good indicator of grain yield for breeding purposes.

The number of grains per spike and the flag leaf blade weight were also associated in PCA of Fig. 2a. Again, in the correlations by experiment, the significance was attained only at the north-rainfed site in 1999. The flag leaf and the spikes grow simultaneously before heading (McMaster 2005), and in the north experiments of 1999 a sudden increase of temperature may have caused an important growth of both organs at a time. In the irrigated experiment the subsequent water supply could enhance the growth of spikes, breaking the association between spikes and leaves and giving opportunity for yield to be expressed. This relationship is unlikely to be of importance as it was only significant in one experiment. On the contrary, an association was found between the number of grains per spike and the flag leaf blade weight, depending on the site, which can explain the PCA association. It has been reported that stress might limit growth of both vegetative and reproductive organs (Gupta et al. 2001), and given that flag leaf and spike grow concurrently (McMaster 2005), the stress observed in a given site would affect both organs at a time.

Eigenvectors for thousand-kernel weight and SI vector was plotted near SI vector in Fig. 2b. This fact suggested a possible association between these two traits. Correlation coefficients by experiment were not significant except in the south-irrigated experiment in 1998. In this experiment, PDSI was negative until heading, when the number of grains per m^2 was already defined. Given the compensation between yield components, it would be expected that the genotypes with higher thousand-kernel weight were the ones having lower number of spikes per m^2 , which is the trait most related to SI according to Fig. 2. This hypothesis is also supported by García del Moral et al. (2003), who found that the number of spikes per unit area was the limiting factor in southern conditions. Despite this fact, in each of the experiments the relationship between thousand-kernel weight and SI was rarely significant. Genotype means of thousand-kernel weight and SI were not correlated (not shown), thus indicating that thousand-kernel weight would

not be a good indicator of SI. Instead, the negative relationships between thousand-kernel weight and peduncle weight or spike measurements were tested and only in two cases they reached statistical significance. Peduncle weight and spike weight were positively correlated with thousand-kernel weight in the southern experiments of irrigated and rainfed sites in 1999. In that year, temperatures rose suddenly in the middle of the grain filling phase (Fig. 1), which could be the reason why the genotypes with carbohydrate reserves in the peduncle or the spike had advantage in following the grain filling in these conditions. Nevertheless, this association occurred only in isolated experiments, and a negative tendency was observed with mean values by site between thousand-kernel weight and spike measurements or peduncle weight; even these relationships were not significant.

PCA analyses also showed that spike measurements and peduncle weight have a negative association with SI. Susceptibility index was correlated with spike measurements and peduncle weight in four to six out of 12 experiments, as shown in Table 3. These results suggest that, while the rest of the relationships plotted in PCA may be due to differences between environments, peduncle weight, awn length, spike length and spike weight may be SI indicators. To clarify the relative importance of each trait globally and depending on the environment, the regression analyses summarized in Table 4 were calculated, and attention to these traits individually will be paid in the following sections.

Peduncle weight

Negative relationships have been reported between water-soluble carbohydrates content and dry weight of the wheat peduncle (Shakiba et al. 1996). According to this finding, a higher mass of the internodes would be related to a higher proportion of structural carbohydrates, and consequently lower water-soluble carbohydrates content to feed the grains. Thus, in the most favourable environments, where photosynthesis during grain filling is the main contributor to grain growth, a higher proportion of structural carbohydrates would be expected compared with drought stress environments, in which grain growth mostly relies on translocation of reserves accumulated in pre-anthesis (Rawson and Evans 1971, Austin et al. 1977, Bidinger et al. 1977). Furthermore, Wardlaw and Moncur (1976) found that the greater the

requirement for assimilates by the ear, the more rapid the speed of movement of the assimilates through the peduncle and the lower their concentration, which indicates that lower peduncle weights would be related to higher transport activity of the peduncle. This hypothesis is supported by the positive sign of the association between peduncle weight and SI. This observation also suggests that the genotypes with heavier peduncles would have less drought resistance and yield stability, while genotypes with lighter peduncles, and so with a higher proportion of soluble carbohydrates, would be more adapted to drought stress likely due to their higher translocation capacity.

A negative relationship between the specific weight of the peduncle and the concentration of water-soluble carbohydrates stored on it has been reported (Ehdaie and Shakiba 1996, Shakiba et al. 1996). The higher peduncle specific weights obtained in the south suggest that the water-soluble carbohydrates concentration was greater in the south than in the north. According to Wardlaw and Moncur (1976), it would indicate lower assimilate requirements by the ear in the south, which is consistent with the higher yield potentiality of northern environments.

The relationship between peduncle weight and SI increased as the water availability decreased. Thus, in the most stressed environment (south rainfed) peduncle weight explained 78 % of SI variation, but it contributed less in the north-rainfed environment and non-significantly in the irrigated environments. The strong association found between peduncle weight and SI in the environments more stressed suggests the suitability of peduncle weight as the proper estimator of the drought tolerance of genotypes under water stress. Genotypes with heavy peduncles would be less tolerant to drought showing lower yield stability than the genotypes with light peduncles (Fischer and Maurer 1978, Blum et al. 1989). Mean values of peduncle weight across several environments might also be a useful measure of SI, as shown in Fig. 3.

Spike weight and length

In the rainfed sites the spike weight at anthesis was the most consistent trait entered in SI regression models, indicating that a heavy spike was a favourable trait related to low SI. Spike weight explained 57 % of SI variations under north-rainfed conditions and 11 % under south-rainfed conditions (additional to the 78 % explained by

peduncle weight). Selection for large spike weight at anthesis, as a way to enhance sink strength, has been recommended to improve grain yield (Donald 1968). Contrarily, in the irrigated sites, spike length was the morphological trait that better tracked SI variations. Spike length and weight were also related to yield in the north-rainfed site, explaining 61 % and 18 % of the variations, respectively. In the south-irrigated site the only trait explaining variations in yield was spike weight, and in the south-rainfed site it was spike length, both having similar proportions of explained variations. This may indicate the important role of bigger spikes in the stressed environments, both for drought or temperature, as photosynthetic organs in limiting conditions (Wang et al. 2001, Motzo and Giunta 2002). Both, length and weight of the spike may be an indicator of SI when considering genotype means across a number of environments, as shown in Fig. 3, but with a lower level of significance than the peduncle weight. To explain yield variations, spike length and weight were the only variables which entered the models. In the north-rainfed site spike weight and length together explained a similar percentage of variability in yield than did spike and peduncle weight to explain SI variations. This may indicate that in drought-prone environments with mild temperatures both spikes and peduncles should be taken into account as the selection criteria.

Awn length

Negative correlation coefficients were obtained between awn length and SI, with importance especially for the southern environments (Table 3). Nevertheless, awn length was included only in the regression model for the south-irrigated site, explaining 57 % of the variations in SI. However, it did not enter in the yield models, thus suggesting that awn would exert a buffer effect on yield loss under unfavourable conditions, as pointed out by other authors (Weyhrich et al. 1995). When considering the genotype means across all environments, awn length may be an additional trait indicator of SI, jointly with peduncle weight and spike dimensions (Fig. 3).

Concluding remarks

The strong associations between some morphological traits and SI suggest that they could be useful tools to select for low SI and high drought tolerance

in Mediterranean environments. Light peduncles, large spikes and awns and weighed spikes at anthesis have shown to be suitable traits for this purpose. However, their helpfulness was environmental-dependent. Thus, for the most stressed environments spike and peduncle weights were the most useful traits, while spike length was more appropriate for irrigated sites, and the length of awns gave good indications of SI in the warmest environments. Genotype means across several environments could be used as SI indicators. The predictive value of morphological traits for grain yield was very low and inconsistent compared with SI, and they cannot be recommended as yield indicators.

Given the lack of relationship between yield and SI, the use of morphological traits as the selection criteria could complement the selection for yield in the latter generations of breeding programs, thus driving to varieties with high-yield potential and good stability. The genetic variability found for morphological traits among the durum wheat genotypes studied here suggests opportunity for selection.

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