Physiological traits associated with recent advances in yield of Chinese wheat

Bangwei Zhou

UNIVERSITAT DE BARCELONA

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(Rasgos fisológicos asociados con los recientes avances en el rendimiento del trigo chino)

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Doctorando	Directores de Tesis
Bangwei Zhou	Dr. José Luis Araus Ortega and Dra. M. Dolors Serret Molins

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CHAPTER 3

Agronomic and physiological responses of Chinese winter wheats to high-yielding Mediterranean conditions[a]

Bangwei Zhou1, Abdelhalim Elazab[1], Jordi Bort[1], Álvaro Sanz-Sáez[1], [2] , Maria Dolores Serret[1] and José Luis Araus[1]

[1]*Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain.*

[2]*Present address: Department of Plant Biology, University of Illinois, Urbana-Champaign, Illinois, 61801, United States of America.*

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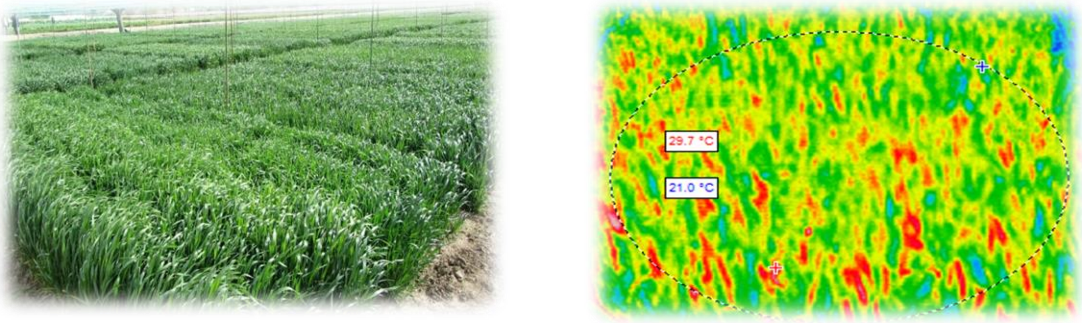

11 Chinese genotypes planted in the field of Aranjuez station (left), and the thermal image for canopy temperature analysing (right). Photo: B. Zhou at Aranjuez, 2012, Spain.

Abstract:

Nine genotypes, bred for the high input agronomical conditions of Henan Province (China) were tested under the high-yielding Mediterranean conditions of Spain. Two widely grown cultivars in the zone were included as checks. Crop growth and leaf chlorophyll content, leaf stomatal conductance (g_s) and canopy temperature (CT) were measured during the crop cycle and stable carbon, oxygen and nitrogen isotope compositions ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$) were analyzed on different plant parts. The lower yield of the Chinese genotypes compared with the checks was due to fewer kernels per unit area, associated with lower tillering. Moreover, Chinese wheats exhibited a plant height clearly below the optimal range. The physiological characteristics related to better performance under high yielding Mediterranean conditions consisted of a higher green aerial biomass, particularly during the reproductive stage, together with a better water status (higher g_s and lower CT and $\delta^{13}C$), the capacity to take up water during grain filling (higher $\delta^{18}O$) and a higher nitrogen use efficiency, related to a more efficient uptake (lower $\delta^{15}N$) and utilization (lower leaf N and chlorophyll content) of N fertilizer. We concluded that Chinese genotypes exhibited a low acclimation capacity to the moderate stress typical of the high-yielding Mediterranean conditions.

Keywords: Chinese wheats, grain yield, carbon isotopes, nitrogen isotopes, stress

1. Introduction

Wheat is the principal staple under Mediterranean conditions. The use of wheat genetic resources is essential to support future genetic progress, and is an insurance against unexpected threats to crop productivity such as diseases or abiotic stresses (Gepts 2006). Well-managed conditions in terms of agronomic inputs (mostly fertilization and support irrigation) make crops more resilient to future challenges posed by the levels of climate change predicted for the Mediterranean (Oweis *et al.* 1998). Moreover, a higher yield potential frequently translates to an increased productivity under Mediterranean drought conditions (Araus *et al.* 2002, 2008; Richards *et al.* 2002; Tambussi *et al.* 2005). This is why breeding for high-yielding Mediterranean conditions makes sense (Del Pozo *et al.* 2014). In that regard, CIMMYT germplasm developed for the high-yielding conditions of semiarid NW Mexico has been used extensively during recent decades in breeding programs aimed at increasing bread wheat productivity under Mediterranean conditions (García Del Moral *et al.* 2003). In that context, looking for alternative sources of germplasm is strategically important for the Mediterranean cultivation of wheat. Conversely, to study the performance of high-potential Chinese wheats under eventually less favorable conditions may also have implications for Chinese agriculture. Stagnation in yield for wheat and other cereals in response to climate constraints has occurred during recent decades, not only in Europe (Hawkesford *et al.* 2013), but also in China (You *et al.* 2009; Piao *et al.* 2010).

Chinese winter wheats released during the last few decades, particularly those coming from the highly fertile provinces of central China surrounding the Yellow River (such as Henan and Shandong), are very productive (Zhou *et al.* 2007). Their growth cycle in terms of duration and calendar is more similar to the situation of winter Mediterranean wheats than for example Northern European wheats. Like CIMMYT wheats, genetic gains in yield potential for wheat bred in Henan and Shandong have been largely achieved through improvements in agronomic yield components, such as harvest index (HI) and kernels per unit area (Zheng *et al.* 2011; Xiao *et al.* 2012). However, on top of that a higher biomass appears also involved in the genetic gains of these Chinese wheats (Zheng *et al.* 2011; Xiao *et al.* 2012).

This paper reports on the agronomic and physiological responses of Chinese wheat genotypes from Henan Province (China) to high-yielding Mediterranean conditions. To that end, modern varieties and advanced wheat lines from Henan, developed during the past 15 years, were grown together with a number of modern Spanish varieties as checks. Besides their yield and agronomic components, two categories of physiological traits were measured during grain filling: (1) those indicating the water status and (2) nitrogen status of the crop. Among the first category traits measured there were canopy temperature (Jones *et al.* 2009) and instantaneous gas-exchange rates (stomatal conductance) measured in the flag leaf, together with time-integrated (i.e. during grain filling) traits such as stable carbon isotope composition ($\delta^{13}C$) in the flag leaf, spike and mature kernels and stable oxygen isotope composition ($\delta^{18}O$) of the (Barbour *et al.* 2000; Araus *et al.* 2003; Cabrera-Bosquet *et al.* 2011). Among the second category of traits nitrogen concentration and nitrogen isotope composition ($\delta^{15}N$) of the flag leaf, ears and mature kernels (Serret *et al.* 2008; Yousfi *et al.* 2012), together with the chlorophyll content of consecutive leaves were evaluated. Moreover, green biomass was assessed in a non-destructive manner through a spectroradiometrical vegetation index (Marti *et al.* 2007) and nitrogen use efficiency (NUE) was assessed together with its uptake and utilization efficiency (Ortiz-Monasterio *et al.* 1997; Serret *et al.* 2008). The final aim was to identify which agronomical and physiological traits may be eventually involved in the performance of Chinese genotypes under high-yielding Mediterranean conditions.

2. Materials and methods

2.1 Plant material and growing conditions

Nine winter wheat genotypes from Henan province (China) plus two Spanish winter wheat cultivars were planted in the experimental field of Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Colmenar de Oreja (Madrid, Spain: 40°03 N, 3°36W, 552 meters above sea level) during the 2011/2012 cropping season. The Chinese genotypes included six commercial cultivars (Yumai 35, Yumai 66, Aikang 58, Lankao 198, Zhoumai 18 and Zhoumai 25) released between 1995 and 2010, and widely distributed in the winter-wheat zone of the Yellow and Huai valleys. In addition, three advanced lines (Lankao 298, Lankao 282 and Lanakao 223) were selected from the top yielding lines among ten thousands genotypes evaluated during the 2010/2011 season in the Henan Tianmin seed Co., Ltd. (Henan, China). Two Spanish genotypes chosen as checks (Gazul and Artur Nick) were recent commercial varieties with high yield performance under good Mediterranean agronomic conditions in Spain.

The experiment was carried out in a completely randomized block design with three replicates per genotype. Each replication consisted of a plot of six rows, seven meters in length and 0.25 m apart. Seeding rate was high (400 seeds/m^2), aiming to resemble cultural conditions in Henan. The trial was sown on the 14th of November 2011, achieving maturity during the first half of June and being harvested on 18th of July 2012. Soil type is sandy clay, slightly alkaline (pH=8.1), with 4.9g/kg of organic matter, 233 g/kg of carbonates and 0.38g/kg of nitrogen in the top 50 cm. Before planting, 400 kg/ha of a NPK complex fertilizer, 15-15-15, was applied. At the end of tillering the plants were top-dressed with nitrogen, using a dose of 150 kg/ha of urea (46%). Accumulated precipitation during the whole cropping season was 183.4 mm. Sprinkler irrigation was provided at booting, heading and grain filling, with one irrigation in March, two in April, three in May and one in June, totaling about 350 mm. Fungicides and pesticides were applied at booting and the beginning of grain filling to prevent diseases and pests. A net with a mesh size of approximately 15 x 15cm was used to prevent kernel loss during grain filling.

The same genotypes were grown for a second year (2012/2013 season) under identical experimental design, planting density and fertilization. Seeds were sown in 11th of November 2012. A net with a mesh size of approximately 15 x 15cm were used to prevent kernel loss by birds during grain filling. The accumulated precipitation was 265 mm from 1st of January to 1st of July. The precipitation occurred in 69 days, and most of continuous rains were concentrated during early April and middle May. Sprinkler irrigation was provided at booting, heading and anthesis with two irrigations in April and other one in early May totaling about 180mm.

2.2 ***Plant sampling and grain yield components***

In the first season (2011/2012) flag leaf blades and spikes were sampled from 5 main plant-stems per plot two weeks after anthesis and oven-dried at 70℃ for 48h, weighed and ground to a fine powder. Plant height was measured before physiological maturity from the soil surface to the top of the spike, excluding awns. At harvest a one-meter length from two rows in the middle of each plot was harvested by hand to estimate total aboveground biomass and HI, spikes per unit area and the rest of the agronomic yield components. For estimating those yield components, samples of 10 representative spikes were randomly selected to calculate thousand kernel weight (TKW), spike length, kernels per spike and the spike fertility index (defined as the quotient between the number of grains and the spike dry weight during anthesis) and kernels per unit area. The remaining plants in each plot were machine harvested and threshed on the same day. Grains were oven-dried for 48h at 60℃ to constant weight. Additionally, a subsample of kernels from each plot was ground to a fine powder.

In the second season (2012/2013) the entire plot was machine harvested and threshed and then oven-dried to constant weight as above.

2.3 *Leaf stomatal conductance and chlorophyll content*

Leaf stomatal conductance (g_s) was measured during the first season with a porometer (Decagon, SC-1, Pullman WA, USA) at tillering, heading and two weeks after anthesis. Each time measurements were performed in the upper adaxial surfaces of the last five fully expanded leaves (which correspond to the flag leaf at heading and grain filling) per plot, between 10:00 and 14:00 on sunny days. Leaf chlorophyll content (Chl) was estimated during the first season using a portable meter (Minola SPAD 520

Meter, Plainfield, IL, USA) on the same days as the porometrical measurements. During the second season, leaf chlorophyll content was also measured at tillering, heading and middle grain filling. Each time five fully expanded leaves per plot were measured. For each, leaf measurements were performed at the bottom, middle and tip of the blade and then the averaged Chl content was calculated.

2.4 Total N content and stable isotope signatures

The total N concentration per unit dry matter and the stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope signatures were analyzed during the first season, in the dry matter of the flag leaf and whole spike sampled about two weeks after anthesis, as well as in mature kernels. Finely powdered samples of 1 mg (spike and kernels) and 0.7 mg (flag leaf) dry matter were weighed into tin capsules and then analyzed by an elemental analyzer (Flash 1112 EA; Thermo Finnigan, Breman, Germany), coupled with an isotope mass ratio spectrometer (Delta C IRMS, Thermo Finnigan, Germany), operating in continuous flow mode at the Scientific Service Facilities of the University of Barcelona. Stable carbon isotope composition values were expressed in δ notation (Farquhar & Richards et al., 1984): $\delta^{13}C= [(^{13}C/^{12}C)_{\text{sample}} / (^{13}C/^{12}C)_{\text{standard}}]-1$, where: sample referred to plant material and standard to a secondary standard calibrated against Pee Dee Belemnite (PDB) calcium carbonate standard. International isotope secondary standards of known $^{13}C:^{12}C$ ratios (IAEA CH7, IAEA CH6 and USGS 40) were used for calibration with a precision of 0.1 per mil. The $^{15}N:^{14}N$ ratio was expressed as $\delta^{15}N$ using the same δ notation as above, but the standard referred to N_2 in air. International secondary isotope standards of known $^{15}N:^{14}N$ ratios (IAEA N_1, IAEA N_2 and IAEA NO_3) were used for calibration to a precision of 0.2‰. In addition the $^{18}O:^{16}O$ ratios of mature kernels were determined by Iso-Analytical Limited (Crewe, Cheshire CW2 8UY, UK). Finely powdered samples of approximately 1 mg were placed in silver capsules, oven-dried under 60°C for more than 72 h to expel moisture and loaded into an automatic sampler. Analyses were conducted by pyrolysis over glassy carbon at 1350 °C using a Thermo-Electron TC/EA (Thermo-Chemical Elemental Analyzer), coupled with a gas isotope ratio mass spectrometer (Thermo-Electron Delta Plus XL, Bremen, Germany). Results were expressed as δ notation, and calculated with the same equation as $\delta^{13}C$. International secondary isotope standards of known $^{18}O:^{16}O$ ratios (IAEA-CH-6, IAEA-C-3 and IAEA-601) were calibrated against the Vienna standard mean oceanic water (VSMOW), and the analytical precision was 0.2 per mil.

2.5 Spectroradiometrical measurements and thermal image acquisition

The normalized difference vegetation index (NDVI) was assessed using a Greenseeker portable spectroradiometer (NTech Industries, Inc., Ukiah, CA, USA). During the first season, evaluations were performed at tillering, heading and two weeks after anthesis between 10:00 h and 14:00 h, the same days as for the stomatal conductance measurements. During the second season, evaluation was performed at heading. The spectroradiometer is active; that is, it has own illumination source in both the red and near infrared (NIR) bands and measures in the red (656 nm) and NIR (770 nm) regions of the electromagnetic spectrum. Wheat canopy reflectance was obtained in order to calculate the formula NDVI = [(NIR770-R656) / (NIR770+R656)]. During the measurements, the sensor was carried parallel to the soil surface 60 cm above the crop canopy, and the operator held the trigger down for approximately 10s and walked through each plot maintaining a constant speed. Roughly 30 values were obtained per plot and data was then averaged.

Thermal image acquisition took place at tillering, heading and two weeks after anthesis of the first season. During the second year, canopy temperature was measured again during grain filling. Images were taken with a portable Midas 320L infrared camera (Dias Infrared GmbH, Germany), which has an 8-14 μm spectral range and produces pictures with a spatial resolution of 320 × 240 pixels. Measurements were carried out between 10:00 and 14:00 h in sunny and non-windy days. The operator-held camera was kept a constant 1.5 m away from the canopy, the emissivity was set at 0.95 and the trigger was pushed once the TFT-LCD touch-screen showed a complete and clear image. The images were analyzed with Pyrosoft Professional Software, averaging the temperature of the canopy area.

2.6 Statistical analyses

The effect of the different genotypes on the agronomical and physiological parameters was tested with analysis of variance (ANOVA) performed using the general linear model (GLM) procedure. Mean separation of genotypes for the measured parameters was done with a Tukey's-b multiple comparison test ($P < 0.05$). A series matrix of simple Pearson coefficient correlations was performed based on the different genotype yields and agronomical and physiological traits. For testing the association between grain yield and sets of physiological traits, linear stepwise models across genotypes were constructed with $P = 0.05$ as the criterion for variables to be either included or removed from the model. The dataset was subjected to principle component analysis (PCA) using the correlation matrix in order to standardize each variable and a Varimax rotation was applied to aid interpretation of the parameters. All the data were analyzed using the SPSS v.16 statistical package (SPSS Inc., Chicago, IL, USA). Broad sense heritabilities (*H*) were estimated as: $H = \sigma^2_g / (\sigma^2_g + (\sigma^2_{yxg}/r + (\sigma^2_e/ry))$, where σ^2_g, σ^2_{yxg} and σ^2_e are, respectively, the estimates of genotype (varieties), genotype x year interaction and error, r the number of replicates and y the number of years. The variance component estimates were obtained from the mean squares of the corresponding ANOVA (Johnson *et al.* 1955). The standard errors of the estimated heritabilities were obtained as the ratio of standard error of genotypic variance to phenotypic variance (Becker 1992). Genetic correlations were estimated each growing season by performing bivariate analysis of variance, fitting replicate as a fixed effect and variety as a random effect using the ANOVA option of SAS PROC GLM. The standard error of a genetic correlation was calculated as Falconer & Mackay (1996).

3. Results

3.1 Grain yield and related agronomical components

Grain yield ranged between 5.5 and 9 t/ha, with the two Spanish checks yielding the most (Table 1). Biomass was also the highest in the two Spanish checks. These two genotypes also exhibited the highest plant height, together with the largest number of spikes and kernels per unit ground area. For other traits such as harvest index (HI), thousand kernel weight (TKW), kernels per spike, spike length and weight and leaf area, the values of the Spanish checks did not differ from those of some of the

Chinese wheats. In fact, grain yield was positively related to biomass, plant height, number of spikes per unit area, and to a lesser extent the number of kernels per unit area, and negatively related to the spike weight and to a lesser extent the TKW (Table 2). The greatest biomass was related to taller plants with the largest number of spikes/m^2. Kernels per unit area were also positively related to the spike fertility index. Broad sense heritability (*H*) of grain yield calculated with the two-year data,was 0.50 (Supplementary Table 1).

3.2 Photosynthetic and transpirative status

Mean carbon isotope composition ($\delta^{13}C$) across genotypes was lower in the flag leaf compared with the spike and the kernels (Table 3). No genotypic differences existed for the $\delta^{13}C$ of the flag leaf and the spike, whereas the $\delta^{13}C$ of the kernels exhibited strong genotypic differences, with the two Spanish cultivars being among the four genotypes exhibiting the most negative values. Kernel oxygen isotope composition ($\delta^{18}O$) exhibited strong genotypic differences, with Artur Nick having the highest value among all the genotypes. Stomatal conductance (g_s) was not significantly different across genotypes at tillering, whereas at heading and grain filling g_s was statistically significant with the two Spanish cultivars showing the highest values. Canopy temperature (CT) did not exhibit genotypic differences at either tillering or grain filling, whereas at heading the differences across genotypes were significant with the two Spanish cultivars showing the lowest values. In agreement with the lack of genotypic variability, *H* of CT during grain filling across the two consecutive years was moderate (0.40) (Supplementary Table 1).

	Grain yield (t/ha)	Biomass (t/ha)	HI	Plant height (cm)	TKW (g)	Spikes/ area (#/m^2)	Kernels/ area (#/m^2)	Kernels/ spike (#/spike)	Spike length (cm)	Spike weight (g)	Spike fertility Index (kernels/g DM)	Leaf area (cm^2)	Heading dates (d)
Yumai 66	5.7(0.6) d	16.6(1.0) ab	0.42(0.03) abc	79(1.3) b	45(4.6) ab	575(64) a	31560(2555) a	55(2.4) a	11.8(0.2) b	0.7(0.03) ab	71.5(6.3) ab	50(2.4) b	175(0.3) a
Lankao 0347	5.7(0.8) d	17.9(1.7) ab	0.43(0.00) ab	79(3.2) b	54(0.6) a	646(120) a	19440(9055) a	31(4.0) b	9.6(0.3) c	0.8(0.04) a	46.6(6.0) b	43(3.2) ab	172(0.2) ab
Aikang 58	6.2(0.2) cd	18.5(1.4) ab	0.45(0.02) a	67(2.4) de	54(0.8) a	782(158) a	32010(9832) a	40(5.7) b	9.0(0.4) c	0.8(0.07) a	55.6(14.4) ab	43(2.8) ab	172 (0.3)ab
Lankao 282	6.4(0.3) bcd	18.8(2.0) ab	0.35(0.01) c	76(2.0) bcd	49(0.6) ab	444(155) a	18940(3411) a	43(2.2) b	10.0(0.2) c	0.8(0.01) a	53.1(2.5) ab	43(2.2) ab	172(0.6) ab
Lankao 223	6.5(0.1) bcd	17.9(2.3) ab	0.37(0.01) bc	89(0.7) a	54(4.8) a	538(54) a	32580(4363) a	59(2.9) a	13.5(0.1) a	0.6(0.01) ab	70.7(6.6) ab	50(0.6) b	174(0.3) a
Lankao 198	6.5(0.4) bcd	14.2(2.4) b	0.42(0.01) abc	66(3.9) e	47(0.4) ab	649(22) a	19970(1083) a	31(0.9) b	7.8(0.1) d	0.7(0.06) ab	53.6(3.7) ab	34(1.7) a	170(0.3) b
Lankao 298	6.7(0.2) bcd	19.4(3.0) ab	0.40(0.01) abc	69(2.4) cde	55(0.4) a	669(191) a	20150(9905) a	31(3.6) b	9.1(0.5) c	0.7(0.03) ab	47.1(6.6) b	43(3.0) ab	173(0.6) a
Zhoumai 18	6.8(0.2) bcd	17.8(2.7) ab	0.39(0.02) abc	77(0.6) bc	51(0.9) ab	848(116) a	32920(2420) a	38(2.1) b	7.5(0.3) d	0.6(0.04) ab	68.9(0.9) ab	50(2.4) b	173(0.6) a
Zhoumai 25	7.5(0.3) abc	18.8(2.5) ab	0.37(0.02) bc	74(1.1) bcde	51(1.3) ab	882(179) a	30320(6328) a	34(0.6) b	7.6(0.2) d	0.5(0.05) ab	66.5(7.8) ab	35(3.5) a	172(1.3) ab
Gazul	7.8(0.1) ab	24.7(1.7) a	0.42(0.01) abc	95(2.3) a	47(1.1) ab	983(107) a	40770(2272) a	42(3.6) b	11.5(0.5) b	0.6(0.08) ab	81.2(6.0) a	52(3.8) b	174(0.9) a
Artur Nick	8.7(0.2) a	24.8(2.6) a	0.41(0.01) abc	95(1.2) a	42(0.2) b	969(120) a	37900(4628) a	39(1.4) b	7.0(0.1) d	0.5(0.03) b	71.0(5.2) ab	54(4.0) b	172(0.4) ab
Mean	6.8	19.0	0.40	79	50	726	28778	40	9.5	0.7	62.3	45	173
S.E.M.	0.18	0.76	0.01	1.84	0.90	43.9	1987.9	0.09	0.36	0.02	2.7	1.31	0.29
Genotype	25.6	299.9	0.03	3269	564	$9.6\ 10^5$	$1.8\ 10^9$	2623	129.0	0.4	4688.7	1287.6	64
P	<0.001	0.019	0.004	<0.001	0.005	0.113	0.139	<0.001	<0.001	0.010	0.010	<0.001	<0.001

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Table 1. Mean values, standard error of means (S.E.M.) and sum of squares type III combined with analysis of variance for grain yield and agronomic yield components of 11 winter wheat genotypes assayed under high-yielding Mediterranean conditions.

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[1] **The parameters recorded are: grain yield, above ground biomass (biomass), harvest index (HI), plant height, thousand kernel weight (TKW), spikes per unit area, kernels per unit area, kernels per spike, spike length, spike weight, the spike fertility index, the area of the flag leaf blade, and the days from sowing to heading.*

[2] *†Values are the means of 3 replications of each genotype.*

	Grain yield	Biomass	HI	Plant height	TKW	Spikes/m^2	Kernels/m^2	Kernels/ spike	Spike length	Spike weight	Spike fertility index
Biomass	0.54(<0.001)										
HI	0.33(0.058)	-0.13(0.469)									
Plant height	0.51(0.002)	0.53(0.002)	-0.01(0.958)								
TKW	-0.37(0.034)	-0.27(0.122)	0.06(0.720)	-0.39(0.027)							
Spikes/m^2	0.50(0.003)	0.60(<0.001)	-0.10(0.571)	0.22(0.225)	-0.15(0.394)						
Kernels/m^2	0.43(0.014)	0.62(<0.001)	-0.08(0.652)	0.43(0.012)	-0.26(0.144)	0.80(<0.001)					
Kernels/spike	-0.05(0.796)	0.11(0.551)	0.07(0.697)	0.44(0.010)	-0.19(0.296)	-0.19(0.285)	0.41(0.018)				
Spike length	-0.30(0.092)	0.04(0.820)	-0.19(0.288)	0.36(0.043)	0.14(0.443)	-0.30(0.087)	0.16(0.363)	0.76(<0.001)			
Spike weight	-0.49(0.004)	-0.12(0.504)	-0.15(0.406)	0.00(0.989)	0.33(0.063)	-0.51(0.003)	-0.18(0.318)	0.46(0.007)	0.61(<0.001)		
Spike fertility index	0.43(0.014)	0.30(0.91)	0.18(0.308)	0.45(0.008)	-0.53(<0.001)	0.29(0.101)	0.60(<0.001)	0.57(<0.001)	0.17(0.352)	-0.45(0.101)	
Leaf area	0.17(0.331)	0.33(0.057)	0.10(0.597)	0.60(<0.001)	-0.15(0.411)	0.10(0.588)	0.33(0.060)	0.47(0.006)	0.38(0.030)	0.13(0.468)	0.35(0.043)

Table 2. Pearson correlation coefficients of grain yield and agronomic yield components across a set of 11 winter wheat genotypes and 3 replications per genotype (D.F. = 33) assayed under high-yielding Mediterranean conditions

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[3] ** yield components including: biomass, aboveground biomass; HI, harvest index; plant height, TKW, thousand kernel weight; Spikes/m2, spikes per unit area, Kernels/m2, kernels per unit area, kernels per spike, spike length, spike weight, spike fertility index, and flag leaf area.*

	δ^{13}C (‰)			δ^{18}O (‰)	g_s (mmol/m^2 s)			CT (^{o}C)		
	Flag	Spike	Kernel	Kernel	Tillering	Heading	Grain filling	Tillering	Heading	Grain filling
Yumai 66	-28.2(0.1) a	-26.8(0.3) a	-26.1(0.2) a	28.3(0.4) d	155(16) a	203(26) ab	388(26) b	17.7(0.5) a	25.6(0.5) ab	29.2(0.2) a
Lankao 0347	-28.6(0.5) a	-27.2(0.5) a	-26.7(0.1) abc	29.0(0.1) cd	228(50) a	225(30) ab	451(18) ab	18.0(0.4) a	25.6(0.6) ab	29.1(0.4) a
Aikang 58	-28.5(0.3) a	-26.9(0.2) a	-27.2(0.1) bc	28.2(0.5) d	188(17) a	271(62) ab	453(23)ab	17.7(0.6) a	25.4(0.3) ab	29.3(0.3) a
Lankao 282	-28.2(0.1) a	-27.0(0.1) a	-26.8(0.2) abc	28.7(0.4) d	227(28)a	189(62) ab	435(9) ab	18.2(0.2) a	25.4(0.3) ab	28.6(0.2) a
Lankao 223	-28.3(0.1) a	-26.7(0.1) a	-26.9(0.1) abc	29.4(0.7) bcd	168(24) a	192(30) ab	453(24) ab	17.9(0.2) a	25.7(0.2) ab	29.1(0.3) a
Lankao 198	-28.5(0.2) a	-26.7(0.2) a	-26.6(0.2) abc	28.9(0.1) cd	178(29) a	217(12) ab	414(18) ab	18.3(0.1) a	26.4(0.4) a	29.5(0.3) a
Lankao 298	-28.4(0.3) a	-26.4(0.5) a	-26.4(0.4) ab	29.0(0.1) cd	204(20) a	171(16) b	411(14) ab	17.9(0.3) a	25.9(0.4) ab	29.5(0.2) a
Zhoumai 18	-28.7(0.2) a	-27.2(0.0) a	-27.6(0.3) c	30.7(0.3) abc	207(20) a	199(10) ab	460(24) ab	18.1(0.2) a	25.9(0.2) ab	29.3(0.0) a
Zhoumai 25	-28.2(0.3) a	-27.1(0.3) a	-26.9(0.1) b	31.6(0.1) a	188(25) a	215(17) ab	464(7) ab	17.8(0.2) a	25.7(0.1) ab	28.8(0.0) a
Gazul	-28.3(0.5) a	-27.2(0.6) a	-27.4(0.4) bc	31.0(0.4) ab	232(28) a	341(23) a	510(48) a	17.7(0.2) a	25.1(0.4) b	28.9(0.3) a
Artur Nick	-28.7(0.3) a	-27.3(0.3) a	-27.5(0.0) bc	29.9(0.4) abcd	246(22) a	295(56) ab	478(30) ab	18.1(0.2) a	24.9(0.3) b	28.8(0.1) a
Mean	-28.4	-27.0	-26.9	29.5	201.9	228.9	447	17.9	25.6	29.1
S.E.M.	0.08	0.09	0.09	0.86	8.3	12.8	8.3	0.09	0.11	0.08
Genotypes	1.2	2.4	6.4**	37.7***	25609.7	80618.3*	34155.7*	1.5	5.3*	2.5
P	0.876	0.475	0.002	<0.000	0.313	0.041	0.081	0.744	0.042	0.298

Table 3. Mean values, standard error of means (S.E.M.) and sum of squares type III combined with analysis of variance for water status physiological traits of 11 winter wheat genotypes assayed under high-yielding Mediterranean conditions.
[4] [5]

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[4] ** Water status physiological traits including: the stable carbon isotope composition (δ13C) of the flag leaf, the spike and kernels, the stable oxygen isotope composition (δ18O) of kernels, the stomatal conductance (g_S) of the last fully expanded leaf at tillering, heading and two weeks after anthesis (grain filling), and the canopy temperature (CT) at tillering, heading and grain filling.*

[5] *†Values are the means of 3 replications of each genotype.*

Grain yield was negatively correlated with the $\delta^{13}C$ of kernels, whereas the relationships with the $\delta^{13}C$ of the spike were also negative but weaker and the $\delta^{13}C$ of the flag leaf did not correlate (Table 4). Total aboveground biomass at harvest did not correlate significantly with $\delta^{13}C$ even when the $\delta^{13}C$ of the spike, followed by that of the ear, tended to be better related. Kernel $\delta^{18}O$ correlated positively with grain yield and tended to correlated with total biomass too. The relationship of leaf g_s with grain yield increased in strength from tillering to grain filling, reaching significance from heading onwards, whereas the opposite pattern occurred for the relationship between g_s and biomass, with significant correlations existing with g_s at tillering and heading. Canopy Temperature correlated negatively with both grain yield and biomass at heading and two weeks after anthesis. Genetic correlation, calculated using the two-year data of CT measured at grain filling against GY,was negative and of similar magnitude than the phenotypical correlation (Supplementary Table 1). Concerning the relationships across physiological traits, the $\delta^{13}C$ of the flag leaf was positively correlated with the $\delta^{13}C$ of the spike, but only this last trait correlated significantly with the $\delta^{13}C$ of kernels. Only g_s at post anthesis correlated (negatively) with $\delta^{13}C$, with the strength of the correlation increasing from the $\delta^{13}C$ of the flag leaf to that of the kernels. Kernel $\delta^{18}O$ was negatively correlated with the $\delta^{13}C$ of kernels and positively with post anthesis g_s. Canopy temperature at heading correlated positively with the $\delta^{13}C$ of the spike and to a lesser extent with that of kernels, as well as negatively with the g_s of the flag leaf at heading and the beginning of grain filling.

Water status traits	Grain yield	Biomass	Flag δ^{13}C	Spike δ^{13}C	Kernel δ^{13}C	Kernel δ^{18}O	Tillering g_s	Heading g_s	Grain filling g_s	Tillering CT	Heading CT
Biomass	0.54(0.001)										
Flag δ^{13}C	-0.21(0.239)	-0.16(0.387)									
Spike δ^{13}C	-0.36(0.037)	-0.30(0.095)	0.59(<0.001)								
Kernel δ^{13}C	-0.42(0.014)	-0.34(0.055)	0.27(0.123)	0.56(0.001)							
Kernel δ^{18}O	0.53(0.002)	0.28(0.117)	-0.01(0.974)	-0.23(0.202)	-0.42(0.016)						
Tillering g_s	0.18(0.308)	0.41(0.018)	-0.11(0.542)	-0.17(0.344)	-0.23(0.196)	0.13(0.459)					
Heading g_s	0.35(0.043)	0.35(0.045)	-0.12(0.490)	-0.27(0.135)	-0.34(0.054)	0.18(0.311)	0.14(0.442)				
Grain filling g_s	0.50(0.003)	0.25(0.153)	-0.36(0.037)	-0.60(<0.001)	-0.72(<0.001)	0.47(0.006)	0.28(0.114)	0.57(<0.001)			
Tillering CT	-0.31(0.077)	-0.09(0.604)	0.18(0.317)	0.27(0.126)	0.13(0.478)	0.09(0.610)	0.31(0.082)	-0.35(0.047)	-0.28(0.109)		
Heading CT	-0.54(<0.001)	-0.51(0.003)	0.31(0.080)	0.61(<0.001)	0.36(0.038)	-0.06(0.756)	-0.08(0.643)	-0.42(0.016)	-0.53(0.001)	0.65(<0.001)	
Grain filling CT	-0.40(0.021)	-0.40(0.021)	0.33(0.062)	0.44(0.010)	0.20(0.271)	-0.09(0.608)	-0.13(0.469)	-0.18(0.328)	-0.30(0.095)	0.23(0.202)	0.56(<0.001)

Table 4. Pearson correlation coefficients of grain yield, biomass and water status physiological traits across the 11 winter wheat genotypes and three replications per genotype (n = 33) assayed under high-yielding Mediterranean conditions.[6]

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[6] ** Water status physiological traits including: the stable carbon isotope composition (δ13C) of the flag leaf, the spike and kernels, the stable oxygen isotope composition (δ18O) of kernels, the stomatal conductance (g_S) of the last fully expanded leaf at tillering, heading and two weeks after anthesis (grain filling), and the canopy temperature (CT) at tillering, heading and grain filling.*

3.3 N status and plant growth

Nitrogen isotope composition ($\delta^{15}N$) increased from the flag leaf and the ear to the kernels (Table 5). Genotypic differences in $\delta^{15}N$ existed for the ear and to a lesser extent for the kernels, whereas no differences existed for the flag leaf. In all three organs the lowest means were observed for Artur Nick, whereas the other Spanish check also showed low values compared with most of the other genotypes. Nitrogen concentration was the highest in the flag leaf and the lowest in the spike, with kernels exhibiting values in between. Genotypic differences existed for N concentration for the three plant parts studied. The two checks exhibited N values for the flag leaf and the ear lower, and higher, respectively than the majority of Chinese cultivars, whereas the N in kernels was placed close to the mean for the set of genotypes studied. Chlorophyll content of the last expanded leaf slightly increased from tillering to heading and then remained almost unchanged at the beginning of grain filling, with the two last measurements being performed in the flag leaf blade. Genotypic differences in chlorophyll content were evident at the three measurement times, with the two checks exhibiting relatively lower values than the rest of the genotypes, particularly for the flag leaf during grain filling. The mean values of the normalized difference vegetation index (NDVI) increased from tillering to heading and did not change after anthesis. Nevertheless, NDVI for the two checks increased from heading to two weeks after anthesis, whereas the Chinese genotypes showed a quite variable pattern. Genotypic differences in NDVI were present at heading and grain filling, with the two checks exhibiting the highest values. In agreement with the existence of genotypic variability, *H* for NDVI at heading across the two seasons was relatively high (Supplementary Table 1).

	δ^{15}N(‰)			N (%)			Chl (arbitrary units)			NDVI (arbitrary units)		
	Flag	Spike	Kernel	Flag	Spike	Kernel	Tillering	Heading	Grain filling	Tillering	Heading	Grain filling
Yumai 66	1.8(0.3) a	2.0(0.2) ab	2.9(0.9) ab	4.4(0.1) ab	1.7(0.0) ab	2.6(0.1) bc	46.4(0.7) b	56.7(0.2) a	60.6(1.2) a	0.26(0.05) a	0.73(0.03) ab	0.74(0.06) a
Lankao 0347	3.1(0.9) a	3.3(0.3) a	4.2(0.4) a	4.4(0.1) ab	1.7 (0.1)a	2.9(0.0) a	50.9(0.8) ab	56.6(0.6) a	58.1(0.4) ab	0.26(0.04) a	0.70(0.03) ab	0.76(0.03) a
Aikang 58	2.0(0.3) a	2.0(0.3) ab	2.7(1.1) ab	4.4(0.0) ab	1.6(0.0) abc	2.6(0.1) ab	51.0(0.1) ab	56.3(0.3) a	57.9(0.3) ab	0.25(0.01) a	0.71(0.02) ab	0.67(0.04) a
Lankao 282	2.2(0.2) a	1.7(0.2) ab	3.4(0.4) ab	3.9(0.0) d	1.5(0.1) abc	2.4(0.0) bcd	47.1(0.5) ab	48.4(1.9) c	52.3(0.8) cd	0.32(0.02) a	0.73(0.06) ab	0.65(0.01) a
Lankao 223	1.9(1.0) a	2.3(1.0) ab	3.4(0.8) ab	4.1(0.0) bcd	1.7(0.0) a	2.0(0.0) e	46.8(0.3) ab	50.1(1.5) bc	55.5(1.1) bc	0.29(0.02) a	0.76(0.04) ab	0.76(0.03) a
Lankao 198	1.5(0.4) a	1.7(0.4) ab	1.9(1.1) ab	4.4(0.1) a	1.4(0.1) bc	2.4(0.1) bcd	50.3(1.7) ab	54.1(1.8) abc	56.7(1.4) b	0.32(0.05) a	0.69(0.03) b	0.64(0.08) a
Lankao 298	2.3(0.7) a	2.7(0.5) ab	3.5(0.4) ab	4.2(0.1) abc	1.6 (0.1)abc	2.3(0.0) cde	46.8(1.7) ab	56.5(0.9) a	51.4(1.1) de	0.31(0.02) a	0.77(0.04) ab	0.80(0.05) a
Zhoumai 18	2.1(1.0) a	1.7(1.1) ab	3.5(1.1) ab	4.5(0.0) a	1.5(0.1) abc	2.4(0.1) bcd	48.0(1.1) ab	51.8(2.2) abc	55.5(0.4) bc	0.37(0.03) a	0.76(0.02) ab	0.77(0.06) a
Zhoumai 25	1.5(0.5) a	0.6(0.7) b	2.6(0.6) ab	4.3(0.1) abc	1.3(0.0) c	2.0(0.0) e	52.0(1.1) a	54.9(0.4) ab	54.9(0.4) bcd	0.28(0.04) a	0.75(0.04) ab	0.67(0.08) a
Gazul	1.1(0.3) a	1.0(0.2) ab	2.7(0.5) ab	4.0(0.1) cd	1.7 (0.1)ab	2.2(0.0) de	46.7(0.8) b	51.1(0.9) abc	48.4(0.5) e	0.32(0.01) a	0.81(0.02) a	0.84(0.01) a
Artur Nick	0.6(0.6) a	0.5(0.6) b	1.5(0.4) b	3.9(0.0) d	1.7(0.0) a	2.4(0.0) bcd	46.6(1.7) b	52.9(0.9) abc	51.3(1.6) de	0.32(0.05) a	0.77(0.01)ab	0.82(0.01) a
Mean	1.8	1.8	2.9	4.2	1.6	2.4	48.4	53.6	54.8	0.3	0.74	0.74
S.E.M.	0.19	0.20	0.23	0.04	0.03	0.04	0.5	0.6	0.7	0.01	0.01	0.02
Genotypes	13.3	21.5*	18.2	1.4***	0.6***	1.8***	141.0**	255.4***	389.3***	0.03	0.03*	0.15*
P	0.180	0.013	0.074	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.568	0.048	0.014

Table 5. Mean values, standard error of means (S.E.M.) and sum of squares type III combined with analysis of variance for nitrogen status physiological traits of 11 winter wheat genotypes assayed under high-yielding Mediterranean conditions.
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[7] ** Nitrogen status physiological traits including: nitrogen isotope composition (δ^{15}N) and nitrogen concentration (%N) of the flag leaf, the spike and kernels, the leaf chlorophyll content (Chl) and the canopy Normalized Difference Vegetation Index (NDVI) at tillering, heading and the beginning of grain filling.*

Grain yield was negatively correlated with the δ^{15}N of the three plant parts studied, as well as with the N concentration of the flag leaf and the kernels, and the chlorophyll content of the flag leaf and positively with the NDVI during heading and grain filling (Table 6). Genetic correlation, calculated using the two-year data of NDVI at heading against GY, was positive and high (Supplementary Table 1). In addition the genetic correlation of the leaf chlorophyll content at tillering and grain filling against GY was also negative (Supplementary Table1). Biomass at harvest also correlated negatively with the δ^{15}N of the flag leaf and the spike, as well as with the N concentration and chlorophyll content of the flag leaf, whereas it correlated positively with NDVI during the reproductive stages. In general, biomass correlated stronger with these traits than grain yield did. The δ^{15}N of the flag leaf and the spike correlated strongly between them and in a slightly lower manner with kernel δ^{15}N. The leaf chlorophyll content correlated positively with the N concentration of the flag leaf, particularly when chlorophyll content was measured in the same plant part (flag leaf) and phenological stage (early grain filling) than the N concentration. Flag leaf chlorophyll content also correlated positively with the N concentration of kernels, but less strongly than for the N concentration of the flag leaf.

N status traits	Grain yield	Biomass	Flag $\delta^{15}N$	Spike $\delta^{15}N$	Kernel $\delta^{15}N$	Flag %N	Spike %N	Kernel %N	Tillering Chl	Heading Chl	Grain filling Chl	Tillering NDVI	Heading NDVI
Biomass	0.54(<0.001)												
Flag $\delta^{15}N$	-0.40(0.020)	-0.51(0.003)											
Spike $\delta^{15}N$	-0.47(0.006)	-0.46(0.007)	0.87(<0.001)										
Kernel $\delta^{15}N$	-0.45(0.009)	-0.33(0.063)	0.63(<0.001)	0.63(<0.001)									
Flag %N	-0.49(0.003)	-0.51(0.002)	0.27(0.126)	0.21(0.235)	0.12(0.483)								
Spike %N	-0.12(0.505)	0.15(0.398)	-0.09(0.626)	0.16(0.388)	0.10(0.562)	-0.16(0.365)							
Kernel %N	-0.56(<0.001)	-0.20(0.275)	0.29(0.101)	0.35(0.047)	0.19(0.287)	0.38(0.027)	0.20(0.256)						
Tillering Chl	-0.10(0.563)	-0.03(0.881)	0.11(0.540)	0.01(0.973)	-0.06(0.751)	0.41(0.018)	-0.41(0.018)	0.21(0.247)					
Heading Chl	-0.23(0.204)	-0.24(0.178)	0.33(0.059)	0.40(0.020)	0.09(0.622)	0.46(0.006)	0.00(1.000)	0.41(0.018)	0.37(0.036)				
Grain filling Chl	-0.66(<0.001)	-0.44(0.011)	0.08(0.670)	0.14(0.451)	0.05(0.789)	0.69(<0.001)	0.04(0.819)	0.49(0.004)	0.41(0.019)	0.38(0.030)			
Tillering NDVI	0.14(0.433)	0.13(0.468)	-0.21(0.230)	-0.29(0.104)	-0.08(0.659)	0.03(0.864)	0.01(0.971)	-0.17(0.334)	-0.08(0.661)	-0.37(0.032)	-0.12(0.492)		
Heading NDVI	0.56(<0.001)	0.66(<0.001)	-0.43(0.012)	-0.46(0.007)	-0.33(0.057)	-0.35(0.048)	-0.05(0.770)	-0.39(0.025)	-0.20(0.270)	-0.40(0.021)	-0.33(0.062)	0.21(0.238)	
Grain filling NDVI	0.46(0.007)	0.56(<0.001)	-0.36(0.041)	-0.25(0.159)	-0.21(0.237)	-0.24(0.178)	0.40(0.020)	-0.18(0.317)	-0.25(0.168)	-0.06(0.750)	-0.25(0.163)	0.23(0.200)	0.64(<0.001)

Table 6. Pearson correlation coefficients of grain yield, biomass and nitrogen status physiological traits across the 11 winter wheat genotypes and three replications per genotype (n = 33) assayed under high-yielding Mediterranean conditions.[8]

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[8] ** Nitrogen status physiological traits including: nitrogen isotope composition ($\delta^{15}N$) and nitrogen concentration (%N) of the flag leaf, the spike and kernels, the leaf chlorophyll content (Chl) and the canopy Normalized Difference Vegetation Index (NDVI) at tillering, heading and the beginning of grain filling.*

3.4 Defining the ideotype: physiological characteristics associated with grain yield and biomass

A principal component analysis (PCA) was performed to assess the contribution of the different physiological characteristics explaining grain yield and biomass across genotypes. The two first principal components explained more than 40 % of the total variation. Grain yield and biomass were close to each other. Both traits were positively associated with green biomass (NDVI) and g_s, particularly during the reproductive stage, and positive associations were also found with grain $\delta^{18}O$. However, negative associations with CT and grain $\delta^{13}C$ were found during the reproductive stage, as well as with the $\delta^{15}N$ of the flag leaf, spike and kernels. In addition negative associations were observed with the N concentration and chlorophyll content during the reproductive stage (Fig. 1).

Stepwise analyses were performed with total biomass at harvest and grain yield as dependent variables and the different categories of traits related to either water status, nitrogen status or both together as independent traits (Table 7). The main water-status trait, which explained a higher genotypic total biomass and grain yield, was a comparatively low CT at heading. For grain yield, a higher kernel $\delta^{18}O$ was included as a second independent factor in the model, explaining 54 proportion of plot variability in grain yield. Concerning the traits related to N status, a higher NDVI at heading, together with a lower N concentration in the flag leaf, were the two factors involved in increased biomass. In contrast, a lower chlorophyll content of the flag leaf at post-anthesis, together with a lower kernel $\delta^{15}N$ and a higher NDVI at heading was selected, explaining 66 proportion of the overall variability in grain yield. Combining the two categories of traits, a higher NDVI at heading, together with a lower N concentration of the flag leaf and a lower kernel $\delta^{13}C$ explained nearly 60 proportion of variability in biomass. However, a lower chlorophyll content of the flag leaf, together with a lower canopy temperature at heading, as well as a higher $\delta^{18}O$ and a lower $\delta^{15}N$ of kernels explained nearly 80 proportion of variability in grain yield.

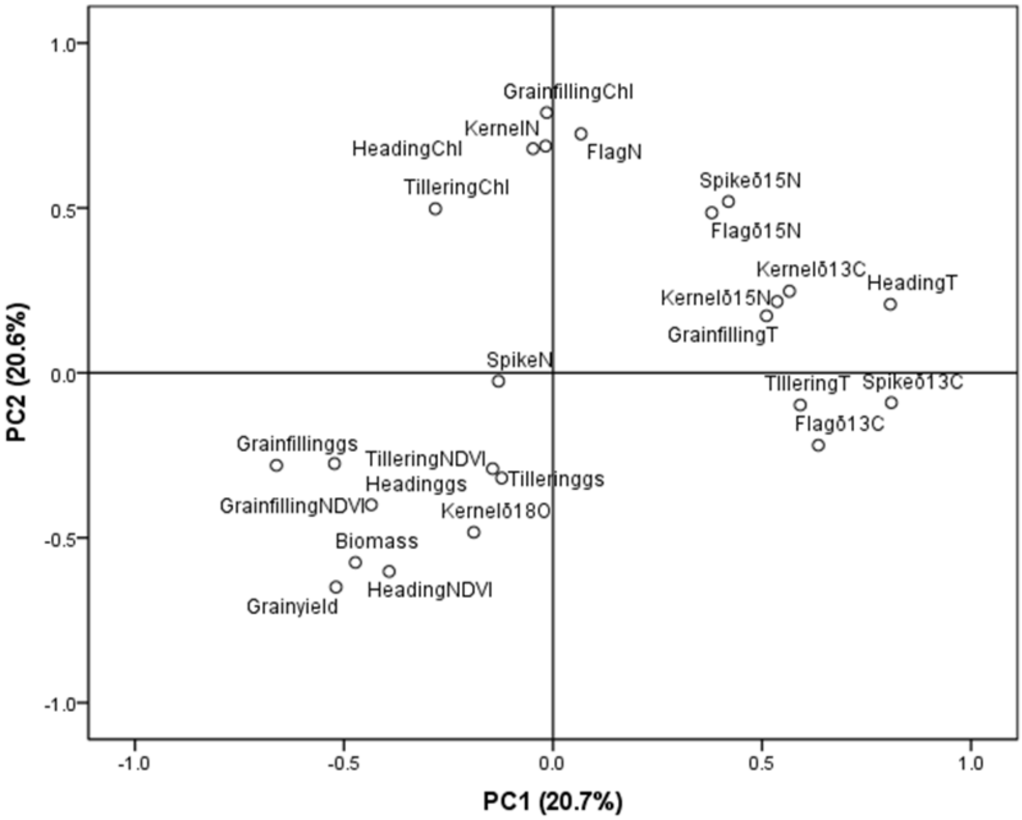

Fig. 1 *Principal component analysis of grain yield, biomass and the different physiological traits related to the photosynthetic, transpirative and nitrogen status of the crop were set for 11 winter wheat genotypes grown under high-yielding Mediterranean conditions.*[9]

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[9] *Physiological traits included as variables: stable carbon and nitrogen isotope compositions ($\delta^{13}C$, $\delta^{15}N$) and nitrogen concentration (N) of the flag leaf, the spike and the kernels, the stable oxygen isotope composition ($\delta^{18}O$) of kernels and the chlorophyll content (Chl) and stomatal conductance (gs) of the last developed leaf, and the canopy temperature (CT) and the Normalized Difference Vegetation Index (NDVI) at tillering, heading and grain filling.*

Model	Final stepwise model	r^2	P
Water status	Grain yield = 16.77 – 0.82 $CT_{heading}$ + 0.42 $\delta^{18}O_{kernel}$	0.54***	<0.001
	Biomass = 112.56 – 3.39 $CT_{heading}$	0.26**	0.003
N status	Grain yield = 16.83 – 0.16 $Chl_{grain\ filling}$ – 0.26 $\delta^{15}N_{kernel}$ + 4.59 $NDVI_{heading}$	0.66***	<0.001
	Biomass = 15.08 + 40.70 $NDVI_{heading}$ – 6.20 $\%N_{flag}$	0.53***	<0.001
Total traits	Grain yield = 19.93 – 0.13 $Chl_{grain\ filling}$ – 0.50 $CT_{heading}$ + 0.38 $\delta^{18}O_{kernel}$ - 0.22 $\delta^{15}N_{kernel}$	0.78***	<0.001
	Biomass = -37.57 + 38.61$NDVI_{heading}$ – 6.03 $\%N_{flag}$ – 1.99 $\delta^{13}C_{kernel}$	0.59***	<0.001

Table 7. Multiple linear regressions (stepwise) explaining grain yield and biomass variation across genotypes based on traits associated with water status (upper), N status (middle) or both (lower) as independent variables.

[10] [11]

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[10] **Traits related to water status and included in the analysis were: the carbon isotope composition ($\delta^{13}C$) of the flag leaf, the spike and kernels, the oxygen isotope composition ($\delta^{18}O$) of kernels, stomatal conductance (g_s) of the last fully expanded leaf and canopy temperature (CT) at tillering, heading and the beginning of grain filling.*

[11] *†Nitrogen status traits were: nitrogen isotope composition ($\delta^{15}N$) and nitrogen concentration (%N) of the flag leaf, the spike and kernels and the chlorophyll content (Chl) of the last fully expanded leaf and and the canopy Normalized Difference Vegetation Index (NDVI) at tillering, heading and the beginning of grain filling.*

4. Discussion

The modern varieties and advanced wheat lines used in the current work represent a set of the most productive wheat germplasm developed over the last 15 years for Henan and other very fertile zones of the winter wheat zone of the Yellow River and Huai Valleys. These genotypes were grown alongside two modern Spanish checks that attain a grain yield well within the range of values usually recorded for modern wheat cultivars under high yielding agronomical conditions of the Mediterranean area (Del Pozo *et al.* 2014 and references herein). The grain yield of Chinese wheats was placed below the two checks and, with exception of the two “Zhoumai” genotypes, with values clearly below the yield (potential) values reported for the modern (last decade) varieties of Henan and Shandong (Zhou *et al.* 2007; Yang *et al.* 2008; Ye *et al.* 2010; Zheng *et al.* 2011; Xiao *et al.* 2012) (Table1). In fact, yields reported in Henan (including some of the Chinese wheats tested in the current work) reached nearly 9 tones ha^{-1}, slightly higher than the checks in the current work (Zheng *et al.* 2011), whereas the crop duration was comparable. Youmai 66, together with the “Lankao” wheats and Aikang 58 had the lowest grain yield in the current study, which contrasts with studies performed in Henan where the “Lankao” wheats and Aikang 58 were among the top-yielding varieties in multitrial trials under high yielding conditions (Ma *et al.* 2008; Guo *et al.* 2009). In the case of Youmai 66, yield was lower than for the “Zhoumai” series in a multilocation study in Henan (Zheng *et al.* 2011).

In the current work the better performance in terms of grain yield of the two checks compared with the nine modern Chinese genotypes is not unexpected *a priori* considering the good “agronomical” adaptation to Mediterranean conditions of the checks. This is confirmed by the reasonable *H* of GY across the two years

(Supplementary Table 1). However, dissecting the causes of the poor performance of the genotypes from Henan under the so-called high yielding Mediterranean conditions is the aim of the current study.

In China, and probably following the success on “super rice”, wheat breeding has targeted a number of traits amenable to visual selection (morphological ideotype) in order to raise yield (Fischer 2007). Among these traits moderate tillering and height, stronger stems (i.e. lodging resistance) and large spikes have been adopted. However, in the current study the higher yield of the checks was primarily related to a higher green biomass, which was associated with taller plants and more fertile culms per plant (Table 2). In this sense heritability of NDVI at heading and its genetic correlation with GY across the two years support this statement (Supplementary Table 1). This suggests that Henan wheats experienced suboptimal conditions during growth. In fact, physiological traits such as a better water status together with a higher nitrogen utilization efficiency (in terms of a lower N concentration) of the photosynthetic organs during the reproductive stage appear to be involved in the better performance of the checks. In support of a suboptimal water status and nitrogen utilization efficiency of the Chinese varieties, the genetic correlations across the set of 11 genotypes and the two seasons between GY and both canopy temperature during grain filling and the leaf chlorophyll content at tillering and grain filling were negative (Supplementary Table 1). The agronomical conditions and the genotypic differences between the checks and the set of Chinese wheats are discussed below in terms of yield components and physiological characteristics.

4.1 Agronomical conditions

In the current study, planting density was placed in the upper range of selected planting densities under Mediterranean conditions, which may discount low planting density as being responsible for the lower yield of the Chinese cultivars. In fact, studies on the yield potential of cultivars from Henan (Zheng *et al.* 2011) and Shandong (Xiao *et al.* 2012) as well as for the whole of Northern China (Zhou *et al.* 2007) report planting densities of about 300 seeds/m^2 even when lower densities (of about 200/m^2) have been used in other studies (Guo 2009; Qu *et al.* 2009). Nevertheless, planting densities between 300-450/m^2 are usual in Henan province for modern cultivars and advanced lines like the “Lankao” series, given their relatively low tillering capacity.

Besides planting density, row distance is another factor to consider. To weaken competition between plants, it can be argued that yield could be increased by reducing row spacing to achieve an even plant-to-plant distribution. In the current study we used a distance between rows of 25 cm, which is normal practice for high-yielding Mediterranean environments. For the Chinese wheats from Henan, an optimal distance of 15 cm has been proposed (Yang *et al.* 2008), although other studies have used row distances of 20 cm (Xiao *et al.* 2012), between 20-25 cm (Zhou *et al.* 2007) or even beyond 25 cm (Zheng *et al.* 2011). In fact, this last study (Zheng *et al.* 2011) reported the highest yields among the studies evaluating wheat genotypic performance in Henan.

Concerning N fertilization, the optimal N fertilization rate for wheat in Henan ranged between a total of 180 Kg N/ha recommended for Yumai 49-198 to 270 Kg N/ha for

Lankao Aizao 8 (Ye *et al*. 2010), whereas Zheng *et al.* (2011) used 200 kg N/ha for the different trials of their study in Henan. In the current study a total of 130 kg/ha was supplied. While this is well within the recommended range for the high-yielding Mediterranean conditions of the current study, this makes the fertilization rate below the range of values used in the high yielding conditions of Henan. Finally, no incidence of pest and diseases were observed during the trial. Therefore, the lower performance of Chinese cultivars under highly productive Mediterranean conditions cannot be attributed to substantial differences in the agronomical conditions of high yielding trials in Henan, in terms of date, density and pattern of sowing. However, they were ultimately associated with the fertilization conditions. Moreover, the phenology of Chinese wheats was quite similar to the checks (Table1). However the low performance in terms of grain yield of Henan wheats under Mediterranean conditions may also be due to poor genotypic adaptation to other environmental conditions differing between Henan and Spain.

4.2 *Plant ideotype: yield components*

The two “Zhoumai” genotypes yielded at levels relatively close to those of the checks, whereas the rest of the Chinese genotypes, including the “Lankao” series, clearly had a lower yield. The “Lankao” genotypes are characterized by large spikes but low tillering, whereas the “Zhoumai” genotypes exhibit a higher tillering capacity but a lower spike size, closer to the ideotype of the checks (Table1). “Zhoumai” genotypes have been reported as exhibiting the highest yield potential in Henan (Zheng *et al.* 2011). However no “Lankao” genotypes were included in this previous study

High-yielding Chinese wheats like the “Lankao” genotypes have been selected for a low adaptation (in terms of competition for resources) to canopy environment (communalism) and a low capacity to produce fertile tillers in order to perform well under high planting densities (Fischer 2001, 2007). By producing spikes on only the main and primary culms the average size of the spikes may increase and eventually the size of the kernels. Moreover, reduced tillering is likely to be increasingly appropriate with the spread of direct seedling and higher sowing densities (Fischer 2007). In fact, whereas the “Lankao” and Aikang 58 genotypes produce a lot of tillers it is at the beginning of stem elongation when the main and primary culms develop very fast at the expense of the other tillers (Guo *et al.* 2009).

Genetic changes aimed at decreasing the number of non-productive tillers could increase the grains per spike but may cause a decrease in grain yield. It has been reported that reduced tillering boosted spike fertility but decreased the grains per unit area (Duggan *et al*. 2005; Gaju 2007). Usually a negative correlation exists between the number of kernels per unit area and kernel weight (Fischer 2007). Moreover, although selection for yield has not increased kernel weight, selection for higher kernel weight usually depresses yield (Fischer 2007). Thus in agreement with a higher tillering capacity and thus number of spikes/m^2, TKW was lower in the two checks compared with most of the Chinese genotypes (Table1 and 2). However, the TKW of the two “Zhoumai” genotypes was slightly above the average values for the whole trial. High TKW is a quality trait with a relatively high heritability (Shankarrao *et al.* 2010).

In addition it has been claimed that drought adaptation has been improved in Australia and by CIMMYT through the introgression of traits such as reduced tillering (Foulkes *et al.* 2011), which is well suited to improving a number of complex traits like potential grain size and lodging resistance. However, a reduced tillering capacity may limit the acclimation capacity of the crop even to sub-optimal growing conditions represented by mild water stress.

4.3 Plant ideotype: canopy size and N utilization

In the current work results the N concentrations of the flag leaf and kernels were negatively related to grain yield and biomass (Table 6). It has been stated that leaf N status during the beginning of the reproductive period is critical in determining final yield (Fischer 2007). Moreover, a low tiller density positively influences the accumulation of dry matter and nitrogen per stem, particularly in the spike (Qu *et al.* 2009). However, in the current study the more productive varieties were those (such as the checks) with flag leaves that exhibited lower leaf N concentrations and chlorophyll content (Table 5).

Improved nitrogen distribution and photosynthetic capacity of the canopy over the entire life cycle of the plant may contribute to a higher yield. Among other traits N distribution/partitioning and chlorophyll retention (stay green) may be involved (Reynolds *et al.* 2011). Total crop photosynthesis is dependent on the ability of the canopy to intercept and capture light for longer. Extending the duration and the amount of light captured by improving the rate of early leaf growth and also keeping a larger green area throughout the crop cycle may contribute to a higher crop photosynthesis and then yield (Parry *et al.* 2011). This may be the case for the checks where larger canopies (as concluded by the larger NDVI) were evident (Table5).

In the current study differences in nitrogen use efficiency (NUE) and its two components, N uptake efficiency and N utilization efficiency, seem to be involved. Thus, the two checks exhibited a higher biomass and grain yield per unit of N fertilizer supplied (higher NUE), and a higher amount of N accumulated in grains per unit of applied N fertilizer (higher grain N uptake efficiency: UPE) than the Chinese genotypes (Supplementary Table 2). Moreover a higher level of dry matter per unit of plant N (higher UPE) in the leaves of the checks during the reproductive stage also seems to be involved. Thus N concentration was markedly lower in the flag leaf of the checks compared to the Chinese genotypes (Table 5). In the same sense, chlorophyll content, which is usually strongly related to N content (Fox *et al.* 1994), was also lower in the leaves of checks compared to Chinese cultivars from tillering to grain filling. By contrast the checks exhibited higher NDVI during the same crop period, which means more green area. A tradeoff between a lower N (and chlorophyll) content in the leaves and a larger canopy area (Table 6) has also been found in our previous study with a similar set of genotypes under controlled pot conditions (Zhou *et al.* 2014) as well as in field conditions in response to increasing seeding rate (Spaner et al. 2005). By contrast, no clear differences existed in the N concentrations of the ears between the checks and most of the Chinese wheats. Besides its role as a photosynthetic organ during grain filling (Tambussi *et al.* 2007; Sánchez-Bragado *et al.* 2014), the ear also has a key role in supplying N to growing grains (Lopes *et al.* 2006). In fact, the N concentration of the check kernels was close to the averaged value for the whole set of genotypes tested (Table 5). The lower NUE and related components of the Chinese genotypes compared with the checks may be the consequence of a selection for high input conditions (Pask 2009). That should be not the case of breeding advance on CIMMYT wheats under high-yielding conditions of NW Mexico, where the improvement in nitrogen use efficiency (NUE) was due to better uptake capacity of (Ortiz-Monasterio et al. 1997) and utilization efficiency (Serret *et al.* 2008). The lower NUE and UPE of the Chinese genotypes also might be (at least in part) the consequence of the water stress suffered by these genotypes. In fact, the grain UPE in the current study was very high (beyond 1) but still within the range of other studies (Limon-Ortega *et al.* 2007) suggesting that aside from the fertilizer supplied, plants actually take up other N sources from the soil. Therefore, even if the amount of N fertilizer applied was somewhat lower than under the usual agronomic conditions of Henan, the total available soil N in the current study was very high.

The lower flag leaf $\delta^{15}N$ of the checks compared with the Chinese cultivars also suggests differences in N metabolism and uptake of the N fertilizer (Table5). Thus it has been reported in durum wheat that water stress increased the $\delta^{15}N$ of the leaf (Lopes & Araus 2006), even though in other studies the most tolerant genotypes have exhibited higher values (Yousfi *et al.* 2012, 2013). A lower $\delta^{15}N$ value in the most productive genotypes may be also the consequence of a more intensive use of a chemical N fertilizer like urea, which is characterized by a low $\delta^{15}N$ (Serret *et al.* 2008).

4.4 Differences in water status

Advances in grain yield in Henan Province from 1981 to 2008 have been associated with increased g_s during grain filling, with “Zhoumai” genotypes exhibiting the highest values (Zheng *et al.* 2011). In the current study the two “Zhoumai” genotypes tended to exhibit higher g_s and lower temperatures during grain filling, together with a lower grain $\delta^{13}C$ among the set of Chinese wheats assayed (Table 3). Therefore, constitutive differences in stomatal opening appear to exist among Henan wheats (Zheng et al. 2011). Besides that, the two checks showed similar or higher g_s (and thus lower canopy temperature during grain filling and grain $\delta^{13}C$) than the two “Zhoumai” cultivars.

Fischer *et al.* (1998) reported for CIMMYT wheat varieties bred under the subtropical conditions of NW Mexico between 1962 and 1988 that g_s and photosynthetic activity had increased (and $\delta^{13}C$ decreased) significantly in association with yield potential in semi-dwarf bread wheats. As in the current study, these genotypic differences were more evident after anthesis. In the high-yielding Mediterranean conditions of Chile, breeding advances in grain yield have been accompanied by concomitant increases in g_s and photosynthetic activity, together with a trend to lower $\delta^{13}C$ and $\delta^{18}O$ (Del Pozo *et al.* 2014). In the above studies reporting increases in g_s, it appears that these relatively higher g_s were associated (at last in part) with a response to a larger kernel number. However, this is not the explanation in the current study since the number and weight of kernels per spike were even lower in the checks and the area of the flag leaf blade larger compared with the Chinese wheats (Table1). Therefore in the current study the higher leaf g_s and lower kernel $\delta^{13}C$ of checks compared with the Chinese wheats are not constitutive but probably the consequence of differences in growing conditions; specifically a better water status.

Genotypes with a higher stomatal aperture, either constitutive or as a response to water stress, appear to have a more efficient use of water in terms of the amount of water transpired (Araus *et al.* 2002, 2008; Blum 2005, 2009), which may result in a higher grain yield under optimal conditions and moderate water stress. Moreover, a lower temperature is also a positive trait in terms of optimizing photosynthesis, while decreasing respiration (Reynolds *et al.* 2000) and transpirative losses.

The higher $\delta^{18}O$ of kernels of the more productive genotypes appears at first surprising, since a negative correlation between $\delta^{18}O$ and yield across genotypes has been frequently shown under water stress (Cabrera-Bosquet *et al.* 2009, 2011; Araus *et al.* 2013) (Table 4). However, a higher $\delta^{18}O$ may be the consequence of more enriched (i.e. higher $\delta^{18}O$) water being used by the plant during grain filling. An enriched $\delta^{18}O$ of water may be the consequence of higher temperature and then might indicate that plants are able to take up water late in the crop cycle (e.g. during grain filling) when temperature clearly increases, which may cause the uptake of $\delta^{18}O$ enriched water. In fact, in the current study the last irrigation was performed in early June, which corresponded to the second half of grain filling.

4.5 Plant height

Plant height somewhere between 80 and 100 m represents the optimum for maximum yield in wheat under high yield potential conditions (Richards 1992; Fischer 2007). The values have been stabilized during the last decades of wheat breeding.

Plant heights reported in field trials for modern wheat cultivars of Henan (Zheng *et al.*

2011) and Shandong (Xiao *et al.* 2012), ranged between 80-90 cm somewhat below the 95 cm achieved by the two checks in the current study. In another study with Henan genotypes (but under drip irrigation hydroponic), mean plant height was 83 cm (Zhou *et al.* 2014). Therefore, under the high yielding growing conditions of China the plant height of Henan genotypes is placed within the range of optimal plant height, even if it is on the low side. Dwarfism is a trait selected in high-yielding Chinese wheats where the aim has been for them to perform well under very intensive agronomic conditions (in terms of N fertilization and water applied) while preventing lodging. These wheats perform well under very high-input (water and fertilization) conditions (Zhou *et al.* 2007; Zheng *et al.* 2011; Xiao *et al.* 2012).

However, in the current study all Chinese genotypes except one exhibited plant heights clearly below (between 65-80 cm) the optimal range (80-100). Whereas the relatively low N fertilization in the current study compared with usual conditions at Henan might cause a decrease in culm length, the decrease (up to 15 cm) may be caused by other factors. Thus the reduced height may be the consequence of some degree of water stress experienced by the Chinese wheats, even under high-yielding Mediterranean conditions, which may cause a reduction in the plant height below the range considered as optimal. In fact, reports on modern genotypes from Henan of plant heights between 70-80 cm are not uncommon (Zhou *et al.* 2007, 2014). A plant height below the optimum may affect yield negatively. This may be the cause of Chinese cultivars from Henan having the tendency to suffer yield penalties when certain stresses affect culm growth. At the canopy level, an appropriate plant height together with proper leaf architecture may improve radiation use efficiency by permitting a light distribution profile that reduces the number of leaves experiencing wasteful and potentially destructive supersaturated light levels, while increasing light penetration to canopy levels where photosynthesis responds linearly to light.

However the fact that HI in the current study was comparable or even higher than the values reported for the Chinese wheats under adequate agronomical conditions (Zheng *et al.* 2011; Xiao *et al.* 2012) suggests that Chinese wheats only suffered a moderate water stress. Under high-yielding Mediterranean conditions some level of stress may be present, particularly during the second part of the crop cycle (reproductive stage), which may limit the growth of the culms.

Optimization of light and N distribution in the canopy may ensure not only a higher yield potential (Long *et al.* 2006; Mussgnung *et al.* 2007) but also a better performance under suboptimal conditions (which is frequently the case for high-yielding Mediterranean conditions). In fact modern cultivars selected for Mediterranean conditions are semi dwarf, with plant heights somewhat higher than in the case of Chinese wheats, and then amenable to some degree of reduction under water stress (Del Pozo *et al.* 2014).

4.6 Crop water status

A relatively better water status (or alternatively a constitutively higher stomatal conductance) of the checks compared with the Henan genotypes during the reproductive stage is inferred not only from the higher values of g_s in the flag leaf, but also from their lower kernel $\delta^{13}C$ and canopy temperature. In fact, high yielding genotypes from Shandong also exhibited lower canopy temperatures (Xiao *et al.*

2012).

Nevertheless, the lower canopy temperature of the two checks may also be the consequence of taller culms. From an energy balance consideration taller canopies are better coupled to the atmosphere, allowing much more effective exchange of latent heat and better canopy cooling. Besides the effect of taller plants and higher g_s a lower canopy temperature may be also related to a larger amount of biomass covering more of the soil (Yang *et al.* 2008). Thus in response to a lower leaf area index and more canopy openness, relative humidity decreases and canopy temperature increases (Yang *et al.* 2008).

5. Conclusions

Chinese wheats from Henan are characterized by a low tillering capacity and short stature since they have been selected for highly intensive agronomic (fertilization and irrigation) conditions. This may confer to these genotypes a low capacity of acclimation to less favorable conditions, making them prone to yield penalties even under moderate water stress conditions and/or with N fertilization levels somewhat lower than in Henan. In fact, under the conditions of the current study, Chinese genotypes exhibited poorer water status and lower NUE compared with the checks

From the physiological point of view, both N metabolism and water status may be involved. Concerning N metabolism the best genotypes were characterized by a higher uptake efficiency of the N fertilizer (lower $\delta^{15}N$) together with a higher N utilization efficiency of leaves (lower leaf chlorophyll and N concentration) and the capacity to sustain a larger canopy (larger NDVI) during the reproductive stage. Concerning water status, the best genotypes exhibited a more efficient use of water (i.e. more transpiration and thus lower canopy temperature and $\delta^{13}C$) and probably the capacity to use water (either from precipitation and irrigation) at later stages (and thus exhibiting a higher $\delta^{18}O$).

For Chinese wheats the present study does not support the conclusion that a high yield potential will translate directly to increased yield under less favorable (even moderate stress) conditions. Nevertheless genetic variability exists in terms of acclimation to suboptimal conditions. Thus the Chinese wheats that performed better under high-yielding Mediterranean conditions were those exhibiting a pattern closer to the ideotype of the checks: relatively high tillering capacity in exchange for smaller spikes, together with the highest and lowest kernel $\delta^{18}O$ and $\delta^{13}C$, respectively, across the Chinese wheats. This is the case for the “Zhoumai” series. However, these two genotypes exhibited a moderate plant height, HI and N utilization efficiency, together with a high TKW.

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Variable	*H*	r_g with GY
GY	0.50 ± 0.20	
NDVI heading	0.58 ± 0.01	1.11 ± 0.01
Chl tillering	0.41 ± 0.40	-1.04 ± 0.03
Chl grain filling	0.62 ± 1.07	-0.49 ± 0.44
CT grain filling	0.40 ± 0.05	-0.42 ± 0.13

Supplementary Table 1. Broad sense heritability (H) of grain yield (GY), normalized difference vegetation index (NDVI) at heading, leaf chlorophyll content (Chl) at tillering, and grain filling and canopy temperature (CT) during beginning of grain filling, and the correlation coefficients of the genetic correlations (r_g) between GY and the other traits of the table.

	NUE	UPE
Yumai 66	43.54 (4.4) d	1.12(0.07) c
Lankao 0347	43.69(6.2) d	1.24(0.14) abc
Aikang 58	47.64(1.7) cd	1.24(0.05) abc
Lankao 282	49.16(2.0) bcd	1.16(0.04) bc
Lankao 223	49.65(1.0) bcd	1.02(0.05) c
Lankao 198	49.65(3.4) bcd	1.19(0.07) abc
Lankao 298	51.50(1.2) bcd	1.18(0.05) bc
Zhoumai 18	52.28(1.3) abc	1.28(0.04) abc
Zhoumai 25	57.66(2.0) abc	1.17(0.04) bc
Gazul	59.95(0.5) ab	1.43(0.01) ab
Artur Nick	67.18(1.3) a	1.45(0.02) a
Mean	51.99	1.23
S.E.M.	1.34	0.03
Genotypes	1514.25	0.48
P	<0.001	<.0001

Supplementary Table 2. Mean values and sum of squares type III combined with analysis of variance for Nitrogen Use Efficiency (NUE) and Nitrogen Uptake Efficiency (UPE) of 11 winter wheat genotypes assayed under high-yielding Mediterranean conditions.

Nitrogen Use Efficiency (NUE), was calculated as grain yield divided by the total N fertilizer supplied during the experiment. Nitrogen Uptake Efficiency (UPE), was calculated as the total N accumulated in kernels divided by the amount of N fertilizer applied. Values are the means of 3 replications of each genotype and those followed by the same letter were not significantly different at p = 0.05 by the Tukey's-b test. (**, $P < 0.01$ and ***, $P < 0.001$). The genotypes are ordered according grain yield from low to high.