

## Durum wheat ideotypes in Mediterranean environments differing in water and temperature conditions

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### ABSTRACT

Ideotypic characteristics of durum wheat associated with higher yield under different water and temperature regimes were studied under Mediterranean conditions. Six semi-dwarf cultivars with contrasting agronomic performance were grown during two consecutive years under winter-planted rainfed and winter-planted support-irrigation conditions and a late-planting trial under support irrigation, at the INIA station of Colmenar de Oreja (Madrid). Different traits were assessed to inform on: water status, root performance, phenology, photosynthetic capacity, crop growth, grain yield and agronomic yield components. Under support irrigation and normal planting, genotypes with higher grain yield exhibited better water status (lower  $\delta^{13}\text{C}$  and canopy temperature), assimilation of more superficial water (higher  $\delta^{18}\text{O}$ ), earlier heading and greater plant height and ear density. Under water-limited conditions (rainfed), the best genotypes also exhibited better water status (lower  $\delta^{13}\text{C}$ ) and earlier heading, but higher specific root length with extraction of water from deeper soil layers (lower  $\delta^{18}\text{O}$ ), more efficient N metabolism (higher  $\delta^{15}\text{N}$  and NBI) and consequently stronger growth (plant height and NDVI), and greater ear density and thousand grain weight. Under warmer conditions (late planting), the best genotypes also exhibited better water status (lower  $\delta^{13}\text{C}$ ) and greater plant height and photoprotective mechanisms (higher flavonoid content and lower chlorophyll content). However, the strong differences in drought between consecutive years determined other specific ideotypic traits within each of the three growing conditions and the particular year. Our study suggests specific ideotypes when breeding durum wheat under different agronomic scenarios, but also stresses that interannual variation in water conditions, typical of Mediterranean conditions, should be taken into account.

### 1. Introduction

Durum wheat is one of the major crops grown in the Mediterranean basin in terms of social importance and extent of cultivated area (Food and Agriculture Organization, 2019a). However, durum wheat production is usually conditioned by climate factors, particularly water availability and elevated temperatures (Araus et al., 2003; Loss and Siddique, 1994; Sabella et al., 2020; Xynias et al., 2020; Zampieri et al., 2020). To date, breeding programs have been mostly focused on

selecting genotypes based on grain yield, along with traits that include phenology and tolerance to local or regional pests and diseases. As a result, declining genetic advance has been reported in different regions of the Mediterranean basin (Chairi et al., 2018), and particularly when cultivars are confronted with weather variability (Kahiluoto et al., 2019). To deal with these circumstances, farmers are more and more directed towards tactical management, which relies on flexibility in sowing time and choice of cultivar (Hunt et al., 2019), monitoring of crops to adjust fertilizer application (basically nitrogen supply) and

*Abbreviations:* CT, canopy temperature; DTH, days to heading; HI, harvest index; INP, irrigated normal planting; ILP, irrigated late planting; NBI, nitrogen balance index; NDVI, normalized difference vegetation index; PH, plant height; RA, root angle; RNP, rainfed normal planting; SRL, specific root length; GNY, total grain nitrogen yield; TGW, thousand grain weight;  $\delta^{13}\text{C}$ , stable carbon isotope composition of mature grains;  $\delta^{15}\text{N}$ , stable nitrogen isotope composition of mature grains;  $\delta^{18}\text{O}$ , stable oxygen isotope composition of the water in the stem base.

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control the impact of biotic stresses, and wherever possible, providing support irrigation (Food and Agriculture Organization, 2019b; Mihailescu and Soares, 2020). The variability in growing conditions, together with the trends presented by ongoing climate change, call for the development and use of wheat cultivars that can adapt efficiently to the available water and withstand increased temperatures, while maintaining a relatively high yield. Therefore, there is a need for tailored breeding in terms of developing cultivars suitable to different growing conditions in the Mediterranean. Integrating phenotyping approaches within breeding strategies can pave the way to create more productive and resilient cultivars that are well adapted to specific agro-environments (Li et al., 2018).

Increasing emphasis has been given to field crop phenotyping where different remote sensing approaches are deployed, due to their high throughput and non-invasive nature, to assess crop growth, potential photosynthetic capacity or even water status (Araus and Cairns, 2014; Araus et al., 2018). Eventually the combination of remote sensing assessment with specific laboratory approaches such as stable carbon isotope signatures may improve the predictive capacity of the phenotyping process (Gracia-Romero et al., 2019; Kefauver et al., 2017; Rezzouk et al., 2020). Nevertheless, a critical hurdle for phenotyping approaches is the limited access to the belowground part of the plant under field conditions. Root phenotyping may be key when seeking to improve productivity and stability under conditions like those present in Mediterranean agro-environments where water availability limits, to a greater or lesser extent, yield and its stability (Li et al., 2019; Maccaferri et al., 2016). In fact, under elevated temperatures and water deficit, roots are reported to be more responsive in terms of growth than the aboveground parts of the plant, which may subsequently affect water uptake, plant growth and yield (Petr, 1991; Pinto and Reynolds, 2015).

Technologies developed for root phenotyping have been mainly applied to plants growing in containers under controlled conditions, making the subsequent translation of results to the real (i.e. field condition) often difficult (Atkinson et al., 2019). Under field conditions, the throughput of root phenotyping practices is still low to medium. Nevertheless, even if it is not feasible to screen the progeny of large breeding panels, these techniques may still serve to thoroughly characterize potential parents to inform strategic crosses and in general terms to define ideotypes for specific growing conditions (Maccaferri et al., 2011, 2016).

Root crown phenotyping, commonly known as “shovelomics” is a phenotyping technique to directly assess root properties. This technique was first developed for maize (Trachsel et al., 2011) and further applied to other crops, including wheat (Maccaferri et al., 2016; York et al., 2018b; York et al., 2018b). The approach consists of exploring the upper 15–30 cm of the rhizosphere via manual digging and root excavation to assess the properties of the roots (Wasson et al., 2020; York et al., 2018a). Root angle, among other root traits, was reported to be useful for improving plant productivity under drought conditions, and can contribute to breeding advances (Wasaya et al., 2018). In barley, for instance, wild genotypes exhibited a more vertical angular spread that allowed them to obtain water from deeper levels, therefore favouring survival (Bengough et al., 2004; Reynolds et al., 2007). A similar trend was observed in wheat genotypes grown under rainfed conditions, where deeper roots with higher root density at depth and lower root densities at the surface were related to higher grain yield (Passioura, 1982; Wasaya et al., 2018). Soil coring is another approach that aims to overcome some of the main limitations inherent to shovelomics, enabling exploration of the root system at deeper soil levels (Wasson et al., 2020; York et al., 2018b). However, this approach is substantially lower in throughput and much more costly than shovelomics. Other potential approaches to phenotype root architecture and/or functioning in the field were well documented, such as electrical resistance tomography (Srayeddin and Doussan, 2009), the use of electromagnetic inductance (Whalley et al., 2017) or ground penetrating radar (Liu et al., 2016, 2017) have been reported, but again the levels of throughput, cost

and/or precision are limitations.

Given the difficult nature of their direct assessment, root traits may be approached indirectly, using above-ground phenotyping as an alternative (Araus and Cairns, 2014; Wasaya et al., 2018; Tracy et al., 2020). For example, a widely considered parameter that reflects the roots' access to water resources is canopy temperature (CT). A study in wheat conducted under field conditions examined the phenotypic relationship between CT, soil moisture and the root dry weight in different soil profiles, and concluded that CT can serve as an indicator of a genotype's ability to maintain transpiration via the extraction of water from deeper soil profiles (Lopes and Reynolds, 2010). Further, in drought stressed environments and where water access is limited, transpiration and the subsequent canopy cooling effect can be supported by deeper roots (Li et al., 2019; Lopes and Reynolds, 2010). Enhanced photosynthesis due to an increase in stomatal conductance, provision of better access to water resources, and associated with genetic advance, are well documented in wheat (Roche, 2015; Li et al., 2019).

Stable isotope composition may also prove to be efficient for measuring root activities in an indirect manner. Under Mediterranean conditions (Araus et al., 2003, 2013), as well as in arid conditions under irrigation (Lopes and Reynolds, 2010), higher yielding wheat genotypes were associated with low carbon isotope composition ( $\delta^{13}\text{C}$ ) or high isotope discrimination from the surrounding  $\text{CO}_2$  atmosphere ( $\Delta^{13}\text{C}$ ) by the maturing grains. In fact, the  $\Delta^{13}\text{C}$  of plant tissues informs on the intercellular to atmospheric ratio of  $\text{CO}_2$  (Ci/Ca) within the plant (Farquhar et al., 1989), with stomatal conductance usually being the main factor determining Ci/Ca. Another approach for assessing root function is analysis of the stable water composition in plant water. Thus, oxygen isotope composition ( $\delta^{18}\text{O}$ ), when analysed in water from the base of the wheat stem (Kale Çelik et al., 2018; Millar et al., 2018; Sanchez-Bragado et al., 2019) as well as woody plants (West et al., 2006), has been proposed as a tracing method to assess the depth of soil from which the water has been extracted. In the case of plant nutrients like nitrogen, besides reflecting its source, the stable nitrogen isotope composition ( $\delta^{15}\text{N}$ ) in dry matter broadly informs about the effect of water status on nitrogen metabolism (Araus et al., 2013; Yousfi et al., 2009, 2012, 2013; Zhang et al., 2013).

Altogether, these approaches can contribute to a more efficient phenotyping, not only when supported by further simulation modelling (Condon, 2020), but even to empirically define the ideotype most suited for a particular growing condition. Therefore, the present study combines different phenotyping approaches (aboveground and belowground) to identify the ideotypic characteristics associated with a better genotypic performance in durum wheat under Mediterranean growing conditions in Spain, that vary in water availability and temperature. This range of conditions was achieved through winter planting under rainfed conditions and support irrigation and a late planting under support irrigation, and evaluating these conditions during two consecutive years.

## 2. Materials and methods

### 2.1. Plant material, field experiments and growth conditions

Field trials were located at the experimental station of Colmenar de Oreja-Aranjuez, Madrid (40°04 N. 3°31 W. 590 m a.s.l.), which belongs to the Spanish “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (INIA), and were undertaken during the 2017–2018 and 2018–2019 crop seasons. Trials were established in a complete block design with three replicates. Each plot consisted of seven rows planted 20 cm apart and a seed rate of 250 seeds  $\text{m}^{-2}$ , representing an area of  $7 \times 1.5 \text{ m}^2$ . For each of the two crop seasons, a normal (winter) planting under either rainfed conditions or support irrigation, and a late planting under support irrigation, were assessed. Hereafter, trials will be referred to as INP for irrigated normal planting, ILP for irrigated late planting, and RNP for rainfed normal planting.

In each trial, a set of 24 post green revolution commercial durum

wheat (*Triticum turgidum* L. subsp. *durum* (Desf) Husn.) cultivars were grown, from which six cultivars were selected with contrasting agronomic performance (i.e. high versus low yield). Selection of these cultivars was according to yield data from the previous crop season, together with the two crop seasons included in this study, which were evaluated at the INIA station stated above, as well as at a second INIA station located in Coria del Rio (Seville) under irrigated normal planting conditions (Data in brief Table 1). Information about the provenance of the six selected genotypes is presented in (Table 1), together with their comparative agronomic performance (grain yield) across twelve distinct environments, understood as the specific combination of crop season, growing condition and location. Grain yield in these environments ranged between slightly more than one Mg ha<sup>-1</sup> to seven Mg ha<sup>-1</sup>. Details about these twelve environments are included in the legend of Fig. 1, and their average grain yield is presented decreasingly in alphabetical order within the abscises of the same figure.

During the first season (2017–2018), INP and RNP trials were sown on November 28th (normal planting), and the ILP trial on February 26th (late planting). The normal planting season was characterized by an average temperature of 10.8 °C, and an accumulated precipitation of 326 mm for a total duration from planting to physiological maturity of 28 weeks. In contrast, the late planting season was shortened by about 13 weeks, recording an average temperature of 15.4 °C and accumulated rainfall of 228 mm. Fertilizers and phytosanitary treatments were supplied in all trials as recommended (Data in brief Table 2). Regarding the irrigation calendar, sprinklers were used on the INP trial with a total of 140 mm of water partitioned across three dates (60 mm on April 25th, 70 mm on May 7th and 10 mm on May 17th), and on the ILP trial with 220 mm of water was partitioned across four dates (60 mm on April 25th, 70 mm on May 7th, 60 mm on May 17th and 30 mm on June 19th) (Fig. 2A). The second crop season (2018–2019) was drier and relatively warmer compared to the previous one. Winter planting, for both the INP and RNP trials, took place on November 29th, 2018. The normal planting season recorded an average temperature of 11.4 °C and an accumulated precipitation of 110 mm, with the total duration from planting to physiological maturity being 26 weeks. In contrast, the late planting shortened the crop duration by 13 weeks, had an average temperature of 16.7 °C, and an accumulated precipitation of only 78 mm. Similar to the previous season, fertilizers and phytosanitary treatments were supplied as recommended (Data in brief Table 2). For irrigation, sprinklers were used for periodic watering of the INP trial (60 mm on February 7th and February 28th then 80 mm on March 22nd, April 1st, April 16th, May 9th and May 16th), totalling 520 mm; and on the ILP trial with 60 mm on February 28th, 30 mm on March 10th, 60 mm on March 22nd, April 1st and April 16th, 80 mm on May 9th, May 16th and May 24th, 90 mm on June 6th, and another 80 mm on June 17th, totalling 680 mm (Fig. 2B). As detailed below, different measurements were performed at anthesis, whereas samplings for further analyses were taken at anthesis and physiological maturity. For both seasons, trials were machine harvested during the first half of July.

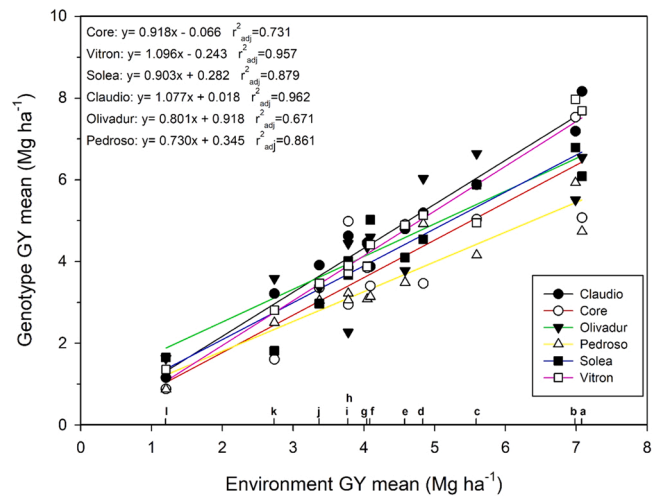
## 2.2. Leaf pigments

The content of different leaf pigments per area basis was assessed at anthesis using a portable leaf-clip sensor (Dualox, Force-A, Orsay,

**Table 1**

List of the six durum wheat varieties compared for yield performance during the study, with year of release, country of origin and available information on provenance and/or pedigree.

Variety	Selection	Year of release	Country	Pedigree/Provenance
Vitron	High yield	1987	France	TURCHIA-77/3/JORI-69(SIB)/(SIB)ANHINGA/(SIB)FLAMINGO
Claudio	High yield	1999	Italy	(Sel. Cimmyt × Durango) × (IS193B × Grazia)
Core	High yield	2009	Spain	Eurogen, PROSEME seeds
Pedroso	Low yield	1992	Spain	Battle seeds
Solea	Low yield	2005	Spain	Monsanto Agriculture Spain.
Olivadur	Low yield	2013	Spain	RAGT 2 N SAS seeds



**Fig. 1.** Linear regressions of the relationship between the genotypic mean of grain yield (GY) of the six selected durum wheat genotypes in twelve environments (each one being a specific combination of year, site and growing conditions) and the mean grain yield across the whole set of genotypes tested in each environment. Letters in the horizontal axis of the figure refer to the values of the environmental means of the 24 durum wheat genotypes: a, support irrigation and normal planting in Colmenar de Oreja (Madrid) during the 2017–2018 crop season; b, rainfed and normal planting in Coria del Rio (Sevilla) during 2016–2017; c, rainfed and normal planting in Coria del Rio during 2017–2018; d, support irrigation and normal planting in Colmenar de Oreja during 2016–2017; e, support irrigation and normal planting in Colmenar de Oreja during 2018–2019; f, support irrigation and late planting in Colmenar de Oreja during 2018–2019; g, rainfed and normal planting in Coria del Rio during 2018–2019; h, support irrigation and late planting in Colmenar de Oreja during 2016–2017; i, support irrigation and late planting in Colmenar de Oreja during 2017–2018; j, rainfed and normal planting in Colmenar de Oreja during 2017–2018; k, rainfed and normal planting in Colmenar de Oreja during 2016–2017; l, rainfed and normal planting in Colmenar de Oreja during 2018–2019. Even when annual variation in rainfall and evapotranspirative demand significantly affected the pattern, the highest yields were recorded in the support irrigation and normal planting of Colmenar de Oreja, together with that of Coria del Rio, which in spite to be theoretically a rainfed site it gets water through the water table of Guadalquivir river, while the lowest yields were recorded in the rainfed normal planting of Colmenar de Oreja.

France), which operates with a red reference beam at 650 nm and a UV light at 375 nm (Cerovic et al., 2012). This sensor produces relative measures of chlorophyll (a + b), flavonoid and anthocyanin contents, and calculates the nitrogen balance index (NBI), which is the ratio of chlorophyll/flavonoids related to the nitrogen and carbon allocation. It is a nitrogen plant status indicator that is directly correlated with nitrogen mass content and therefore to the availability of N, and it is less sensitive to the variations in leaf age and leaf thickness than the chlorophylls (Cerovic et al., 2012). For each plot, measurements were carried out on the adaxial side of flag leaves of five random plants, selected from the central rows of each plot.

**Table 2**  
Effect of water supply, planting date, and genotype on grain yield (GY), harvest index (HI), thousand grain weight (TGW), number of grains per ear (GN ear<sup>-1</sup>), ear density (ears m<sup>-2</sup>), days to heading (DTH), total grain nitrogen yield (GNY), plant height (PH) and the normalized difference vegetation index (NDVI) at anthesis in the six wheat genotypes during two successive crop seasons (2017–2018) and (2018–2019).

		Agronomic traits									
		GY (Mg ha <sup>-1</sup> )	DTH (days)	HI	TGW (g)	GN ear <sup>-1</sup>	Ear density (ears m <sup>-2</sup> )	GNY (Mg ha <sup>-1</sup> )	PH (cm)	NDVI	
<b>2017–2018</b>	Irrigated (INP)	6.86 <sup>a</sup> ± 0.29	158.8 <sup>b</sup> ± 0.7	37.42 <sup>a</sup> ± 1.00	51.59 <sup>a</sup> ± 1.32	33.79 <sup>a</sup> ± 1.51	367.5 <sup>a</sup> ± 18.3	0.171 <sup>a</sup> ± 0.007	107.3 <sup>a</sup> ± 1.8	0.79 <sup>a</sup> ± 0.01	
	Late (ILP)	3.65 <sup>b</sup> ± 0.20	80.8 <sup>c</sup> ± 0.4	34.94 <sup>ab</sup> ± 1.14	37.81 <sup>c</sup> ± 0.98	25.35 <sup>c</sup> ± 1.00	348.5 <sup>b</sup> ± 16.3	0.102 <sup>b</sup> ± 0.005	90.8 <sup>b</sup> ± 1.6	0.77 <sup>a</sup> ± 0.01	
	Rainfed (RNP)	3.37 <sup>b</sup> ± 0.13	160.0 <sup>a</sup> ± 0.5	33.28 <sup>b</sup> ± 0.80	41.00 <sup>b</sup> ± 1.07	30.56 <sup>b</sup> ± 1.29	251.2 <sup>b</sup> ± 12.9	0.097 <sup>b</sup> ± 0.003	86.8 <sup>c</sup> ± 1.4	0.66 <sup>b</sup> ± 0.01	
	<b>ANOVA</b>										
Environment	<0.001	<0.001	<0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Genotypes	<0.001	<0.001	<0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Interaction	ns	<0.001	<0.050	ns	<0.001	<0.001	<0.001	<0.050	<0.001	ns	
<b>2018–2019</b>	Irrigated (INP)	4.33 <sup>a</sup> ± 0.17	–	29.09 <sup>b</sup> ± 1.03	41.17 <sup>b</sup> ± 1.24	29.66 <sup>a</sup> ± 1.62	337.5 <sup>a</sup> ± 19.4	0.110 <sup>a</sup> ± 0.004	108.7 <sup>a</sup> ± 1.4	0.76 <sup>a</sup> ± 0.01	
	Late (ILP)	4.08 <sup>a</sup> ± 0.19	–	36.17 <sup>a</sup> ± 1.45	50.39 <sup>a</sup> ± 0.92	32.39 <sup>a</sup> ± 2.10	235.2 <sup>b</sup> ± 9.7	0.097 <sup>b</sup> ± 0.004	92.8 <sup>b</sup> ± 1.2	0.74 <sup>a</sup> ± 0.01	
	Rainfed (RNP)	1.26 <sup>b</sup> ± 0.09	–	24.42 <sup>c</sup> ± 1.70	48.98 <sup>a</sup> ± 0.94	12.74 <sup>b</sup> ± 1.21	194.0 <sup>c</sup> ± 10.1	0.037 <sup>c</sup> ± 0.003	78.5 <sup>c</sup> ± 1.3	0.45 <sup>b</sup> ± 0.01	
	<b>ANOVA</b>										
Environment	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Genotypes	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.050	<0.001	ns	ns	
Interaction	<0.010	–	ns	<0.010	ns	ns	ns	<0.010	ns	ns	

Values are means ± standard error of six genotypes with three replicates. Levels of significance for the ANOVA: P < 0.05, P < 0.01 and P < 0.001. Within each treatment, means exhibiting different letters a, b and c, are significantly different (P < 0.05) according the post-hoc test (Tukey-b) on independent samples.

### 2.3. Canopy temperature

Canopy temperature (CT) was assessed using a portable infrared (IR) thermometer (PhotoTemp™ MXSTM TD Raytek®, California; USA). The IR sensor was placed at a distance of 80 cm from the canopy, pointing the laser beam towards plant leaves with the sun towards the rear (Gracia-Romero et al., 2019).

### 2.4. Normalized difference vegetation index

The pattern of crop growth was estimated in real-time through a multispectral agronomic index known as the normalized difference vegetation index (NDVI). This index, based on the contrasting reflectance of the canopy within the visible and near infrared regions of the spectrum, is used to assess agronomic traits related to the density of green in the canopy, such as crop emergence/vigour, total biomass or the level of senescence/stay green during the last part of the crop cycle. NDVI measurements were performed using a GreenSeeker sensor (Trimble, Sunnyvale, CA, USA). This portable spectroradiometer operates through an active optical sensor in the red (660 ± 10 nm) and near infrared (NIR, 780 ± 15 nm) wavelengths (Crain et al., 2012). NDVI values were obtained by skimming the active sensor perpendicularly across the canopy of each selected plot at a constant height of 60 cm (Gracia-Romero et al., 2019). The acquired values are the average NDVI across all plants, defined as:

$$NDVI = (RNIR - Rred) / (RNIR + Rred) \tag{1}$$

### 2.5. Root image analysis

Five random plants were dug manually from the first 15 cm of soil of each selected plot, and the roots were washed carefully using a hose, then digitized in situ using a Sony ILCE-QX1 camera (Sony Europe Limited, Brooklands; United Kingdom). The digital camera has a 20.1 megapixel resolution, is equipped with a 23.2 mm × 15.4 mm sensor (type CMOS Exmor HD) and uses a 16 mm focal lens, and an exposure time of 1/60 s. The RGB images were captured zenithally at 60 cm above the roots alongside a scale reference, then saved in Tiff format for later analysis. Root angle (RA) was measured directly using a geometric protractor. Root RGB images were further analyzed using GiaRoots (General Image Analysis of Roots, Georgia Tech Research Corporation and Duke University; USA), which is an open-source software for the automated analysis of root architecture (Galkovskiy et al., 2012). Image processing was carried out using the adaptive image thresholding processing option, where around 200 images were computed per trial. The measured traits and the corresponding definition have been detailed previously in Galkovskiy et al. (2012). Briefly, GiaRoot detects pixels of the thresholded root image to estimate different root traits including: (i) crown root related parameters such as average root width (Width), number of connected components (CComp), and the maximum (MaxR) and median (MedR) number of roots; (ii) root system dimensions such as root network depth (Ndepth), root network length (Nlen), and root network width (Nwidth); (iii) root density through network area (NwA), network surface area (Nsurf), and network volume (Nvol); and (iv) root angle via network convex area (ConvA). In addition, relative traits presented as ratios were determined, such as: the ratio of network length to the network volume (specific root length (SRL)); the ratio of the maximum root number to the median root number (Network bushiness (Bush)); the total network area divided by the network convex area (Network solidity); the lower 2/3 of the root network depth (length distribution (Ldist)); and the ratio of the network width to the network depth (network width to depth ratio (NWDR)).

### 2.6. Agronomic traits

Days to heading (DTH) were determined for the first crop season

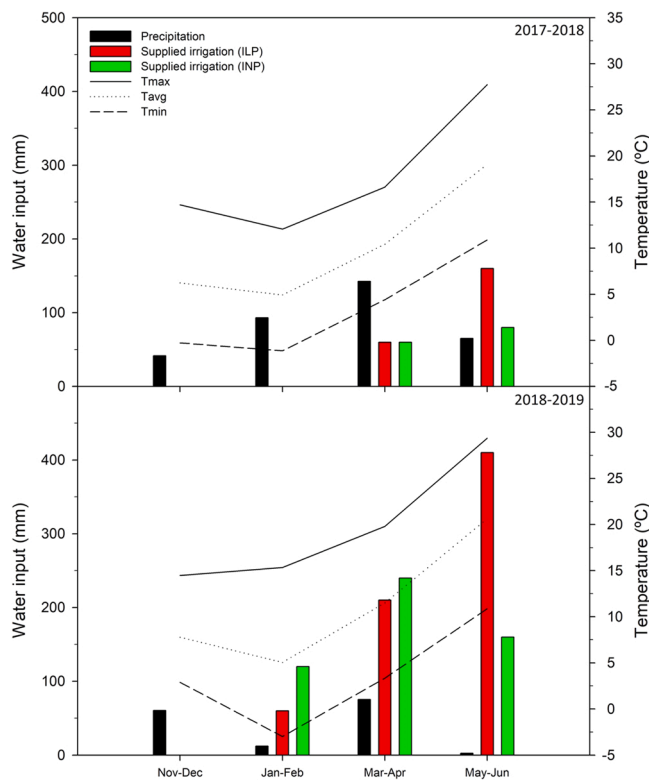


Fig. 2. Bimonthly accumulated precipitation, irrigation applied and average temperature recorded during the 2017–2018 and 2018–2019 seasons.

(2017–2018) only, for each plot, and when approximately 50% of ears had emerged. Plant height (PH) was determined at anthesis; a ruler was placed zenithally in the central rows of each selected plot, and values were taken by observing the whole canopy and averaging the distance from the ground to the overall tip of the ears, excluding the awns. At maturity, ear density (ears  $m^{-2}$ ) was determined by counting the ear density in a 1 m length of a central row. Grain number per ear (GN) and thousand grain weight (TGW) were assessed using a subset of ten representative plants from the central rows of each plot. Harvest index (HI), which is the ratio of grain weight to total aboveground biomass, was calculated from the same sampled plants.

### 2.7. Stable carbon and nitrogen isotope composition and total nitrogen content in dry matter

From each selected plot, samples of flag leaves taken at anthesis of the second crop season and mature grains collected at harvest from the two crop seasons were dried at 60 °C for a minimum of 48 h and pulverized to a fine powder, from which 1 mg was enclosed in tin capsules, and analyzed using an elemental analyser (Flash 1112 EA; ThermoFinnigan, Schwerte, Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, ThermoFinnigan), operating in continuous flow mode at the Scientific and Technical facilities of the University of Barcelona. Different secondary standards were used for carbon (IAEA-CH7, IAEA-CH6 and IAEA-600, and USGS 40) and nitrogen (IAEA-600, N1, N2, NO3, urea and acetanilide) isotope analyses. Nitrogen content in leaves and grains were expressed in percentages (%), and the corresponding isotope compositions in parts per thousand (‰), with an analytical precision (standard deviation) of 0.1‰ for  $\delta^{13}C$  and 0.3‰ for  $\delta^{15}N$  and following the Eq. (2):

$$\delta^{13}C/\delta^{15}N(‰) = \left[ \left( R_{\text{sample}}/R_{\text{standard}} \right) - 1 \right] \times 1000 \quad (2)$$

where  $R_{\text{standard}}$  is the molar abundance ratio of the secondary standard

calibrated against the primary standard Pee Dee Belemnite in the case of carbon ( $\delta^{13}C$ ) and  $N_2$  from air in the case of nitrogen ( $\delta^{15}N$ ) (Farquhar et al., 1989).

### 2.8. Stable oxygen isotope composition of stem water

At anthesis, samples of the stem base (approximately 6–7 cm length) were harvested from five random plants (main stems) of each selected plot, sealed immediately in analytical tubes and frozen at  $-80$  °C until water distillation could be undertaken. Water analysis was performed at the Department of Crop and Forest Sciences, Universitat de Lleida (Spain), using a cryogenic vacuum distillation line (Dawson and Ehleringer, 1993). Sample tubes were placed in a heated silicone oil bath (120 °C), and connected with Ultra-Torr™ unions (Swagelok Company, Solon, OH, USA) to a vacuum system ( $\sim 10$ – $2$  mbar), in series, with U-shaped collector tubes cooled with liquid  $N_2$ . Ninety minutes after commencing extraction, the extracted xylem water was transferred into 2 ml vials and stored at 4 °C until analysis. Oxygen isotope composition ( $\delta^{18}O$ ) of water was determined by isotope-ratio infrared spectroscopy using a Picarro L2120-I isotopic water analyser coupled to an A0211 high-precision vaporizer (Picarro Inc., Sunnyvale, CA, USA). Analytical precision for  $\delta^{18}O$  was 0.10‰, and the occurrence of contaminants was tested using Picarro's ChemCorrect post-processing software and corrected, when necessary, following Martín-Gómez et al. (2015).

### 2.9. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 25 (IBM SPSS Statistics 25, Inc., Chicago, IL; USA), to test the effects of year (crop season), trial, genotype and their interaction on all traits evaluated, and followed with Tukey-b tests to reveal differences within trials. A bivariate Pearson correlation was executed using the same statistical package to reveal relationships between grain yield and the assessed parameters. Principal component analyses were carried out with the open-source software, RStudio 1.2.5 (R Foundation for Statistical Computing, Vienna, Austria), to analyze all traits in a reduced bi-dimensional platform. Graphs were created using Sigma-plot 10.0 (Systat Software Inc, California; USA).

## 3. Results

### 3.1. Effects of planting date, water supply and season on grain yield, agronomic components, biomass and phenology

During the first season (2017–2018), GY was halved under irrigated late planting (ILP) and rainfed normal planting (RNP) compared to irrigated normal planting (INP). Under warmer conditions of ILP, harvest index (HI), thousand grain weight (TGW), grain number per ear (GN), ear density, total grain nitrogen yield (GNY) and plant height (PH) were decreased compared to the INP trial, with a significantly shortened days to heading (DTH) interval (Table 2). Similarly, under water-limited conditions (RNP), HI, TGW, GN, ear density, GNY and PH were lower compared to INP. In addition, NDVI was smaller under RNP than INP and even ILP. Genotypes exhibited significant differences in GY and all the other measured parameters included in the table (Table 2). Trial by genotype interaction was significant for all the traits except for GY, TGW and NDVI.

During the second season (2018–2019), GY and ear density in ILP were similar, but HI and TGW were higher, and ear density, GNY and PH were lower compared to INP. The rest of the traits were not significantly different. In contrast, under RNP, GY decreased threefold compared to the irrigated trials (INP, ILP). HI, GN, ear density, GNY, PH and NDVI were also lower in RNP than in the INP and ILP trials, while TGW increased in RNP compared to the other two trials. Genotypic differences were significant for GY, HI, TGW, GN, ear density and GNY (Table 2).

**Table 3**

Effect of planting date, water supply, and genotypes on nitrogen content (N) and stable isotope composition of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) of the flag leaf and the mature grains, the oxygen isotope composition ( $\delta^{18}\text{O}$ ) of water in the shoot base, and the canopy temperature (CT) assessed at anthesis, in six wheat genotypes during two successive crop seasons (2017–2018) and (2018–2019).

		Stable isotope composition + Canopy temperature							
		$\delta^{18}\text{O}_{\text{shoot water}}$ (‰)	$\text{N}_{\text{leaf}}$ (%)	$\delta^{15}\text{N}_{\text{leaf}}$ (‰)	$\delta^{13}\text{C}_{\text{leaf}}$ (‰)	$\text{N}_{\text{grain}}$ (%)	$\delta^{15}\text{N}_{\text{grain}}$ (‰)	$\delta^{13}\text{C}_{\text{grain}}$ (‰)	$\text{CT}_{\text{anthesis}}$ (°C)
<b>2017–2018</b>	Irrigated (INP)	-4.25 <sup>b</sup> ± 0.07	–	–	–	2.51 <sup>b</sup> ± 0.06	3.26 <sup>a</sup> ± 0.15	-26.26 <sup>c</sup> ± 0.10	29.70 <sup>b</sup> ± 0.36
	Late (ILP)	-4.19 <sup>b</sup> ± 0.05	–	–	–	2.82 <sup>a</sup> ± 0.06	0.93 <sup>b</sup> ± 0.26	-25.69 <sup>b</sup> ± 0.11	–
	Rainfed (RNP)	-3.81 <sup>a</sup> ± 0.08	–	–	–	2.87 <sup>a</sup> ± 0.03	1.26 <sup>b</sup> ± 0.14	-24.45 <sup>a</sup> ± 0.07	33.89 <sup>a</sup> ± 0.39
	<b>ANOVA</b>								
	Environment	<0.001	–	–	–	<0.001	<0.001	<0.001	<0.001
	Genotypes	<0.001	–	–	–	<0.050	<0.050	<0.010	ns
	Interaction	<0.001	–	–	–	ns	ns	ns	ns
<b>2018–2019</b>	Irrigated (INP)	-5.11 <sup>c</sup> ± 0.06	3.90 <sup>a</sup> ± 0.15	3.10 <sup>a</sup> ± 0.16	-28.26 <sup>c</sup> ± 0.23	2.56 <sup>b</sup> ± 0.07	2.99 <sup>a</sup> ± 0.08	-25.81 <sup>b</sup> ± 0.07	28.83 <sup>b</sup> ± 0.41
	Late (ILP)	-4.55 <sup>b</sup> ± 0.09	4.09 <sup>a</sup> ± 0.06	2.45 <sup>b</sup> ± 0.18	-27.45 <sup>b</sup> ± 0.11	2.40 <sup>b</sup> ± 0.04	2.49 <sup>a</sup> ± 0.14	-25.98 <sup>b</sup> ± 0.08	25.36 <sup>c</sup> ± 0.27
	Rainfed (RNP)	-3.52 <sup>a</sup> ± 0.13	3.86 <sup>a</sup> ± 0.06	-0.09 <sup>c</sup> ± 0.16	-25.40 <sup>a</sup> ± 0.12	2.97 <sup>a</sup> ± 0.05	-0.05 <sup>b</sup> ± 0.12	-23.01 <sup>a</sup> ± 0.08	35.38 <sup>a</sup> ± 0.27
	<b>ANOVA</b>								
	Environment	<0.001	ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Genotypes	ns	ns	ns	ns	ns	ns	ns	ns
	Interaction	ns	<0.050	ns	ns	ns	ns	ns	ns

Values are means ± standard error of six genotypes with three replicates. Levels of significance for the ANOVA:  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ . Within each treatment, means exhibiting different letters a, b and c, are significantly different ( $P < 0.05$ ) by the post-hoc test (Tukey-b) on independent samples.

The genotype by trial interaction was only significant for GY, TGW and GNY.

Considering the trial, crop season and genotype effects, the three-way ANOVA revealed that all the traits (GY, HI, TGW, GN, ear density, GNY, PH and NDVI) included in Table 3 showed significant effects for the three factors (Data in brief Table 3). Interactions were also significant for most of the traits, except PH and NDVI. Moreover, values for all the traits, except for PH and NDVI, were higher in the first season than the second season (Table 2).

### 3.2. Effects of planting date, water supply and season on stable isotope compositions, nitrogen content and canopy temperature

During the first season (2017–2018), grain nitrogen content ( $\text{N}_{\text{grain}}$ ) and carbon isotope composition ( $\delta^{13}\text{C}_{\text{grain}}$ ) were higher, and grain nitrogen isotope composition ( $\delta^{15}\text{N}_{\text{grain}}$ ) was lower in ILP compared with INP. However, the oxygen isotope composition of the shoot water ( $\delta^{18}\text{O}_{\text{shoot water}}$ ) exhibited similar values in ILP to INP. Under RNP, canopy temperature (CT),  $\delta^{13}\text{C}_{\text{grain}}$ ,  $\delta^{18}\text{O}_{\text{shoot water}}$  and  $\text{N}_{\text{grain}}$  were higher, and  $\delta^{15}\text{N}_{\text{grain}}$  lower, compared to INP. Genotype differences were shown in all measured traits except for CT, where values were not

available for the ILP trial (Table 3). The genotype by trial interaction was only significant for  $\delta^{18}\text{O}_{\text{shoot water}}$ .

In the second season (2018–2019), CT and the nitrogen isotope composition of the leaf ( $\delta^{15}\text{N}_{\text{leaf}}$ ) were significantly lower in ILP than INP, while the  $\delta^{18}\text{O}_{\text{shoot water}}$  and the carbon isotope composition of the leaf ( $\delta^{13}\text{C}_{\text{leaf}}$ ) were significantly higher in ILP, compared with INP. In contrast, under RNP conditions, CT,  $\text{N}_{\text{grain}}$ ,  $\delta^{18}\text{O}_{\text{shoot water}}$ ,  $\delta^{13}\text{C}_{\text{leaf}}$  and  $\delta^{13}\text{C}_{\text{grain}}$  were greater, and the  $\delta^{15}\text{N}_{\text{leaf}}$  and  $\delta^{15}\text{N}_{\text{grain}}$  lower than in the INP trial. Genotypes were not significantly different across all measured traits (Table 3). The genotype by trial interaction was only significant for  $\delta^{15}\text{N}_{\text{leaf}}$ .

The three-way ANOVA (season, trial and genotype) showed a significant trial effect for all the traits included in Table 3 (Data in brief Table 4). A genotype effect was also significant for all the traits, except for CT, while season had a significant effect for all the traits except CT and the  $\delta^{15}\text{N}_{\text{grain}}$  (Data in brief Table 4). The interaction between season and trial was significant for all the traits, while almost all the other interactions were absent. Except for the ILP trials, the  $\delta^{13}\text{C}_{\text{grain}}$  was lower (i.e. more negative) in the first season than the second season. In the case of  $\delta^{18}\text{O}$ , and except for RNP, values were higher (less negative) in the first season.

**Table 4**

Effect of planting date, water supply and genotype on leaf chlorophyll, flavonoid and anthocyanin, and the nitrogen balance index (NBI) in six wheat genotypes during two successive crop seasons (2017–2018) and (2018–2019).

		Leaf pigments (arbitrary units)			
		Chlorophyll	Flavonoid	Anthocyanin	NBI
<b>2017–2018</b>	Irrigated (INP)	49.90 <sup>b</sup> ± 0.81	1.32 <sup>b</sup> ± 0.02	0.135 <sup>b</sup> ± 0.002	38.28 <sup>a</sup> ± 0.73
	Late (ILP)	51.82 <sup>a</sup> ± 0.51	1.34 <sup>ab</sup> ± 0.02	0.125 <sup>c</sup> ± 0.001	38.94 <sup>a</sup> ± 0.80
	Rainfed (RNP)	45.94 <sup>c</sup> ± 0.81	1.39 <sup>a</sup> ± 0.02	0.149 <sup>a</sup> ± 0.004	33.49 <sup>b</sup> ± 0.76
	<b>ANOVA</b>				
	Trials	<0.001	<0.050	<0.001	<0.010
	Genotypes	<0.001	<0.001	ns	<0.010
	Interaction	ns	ns	ns	ns
<b>2018–2019</b>	Irrigated (INP)	41.26 <sup>a</sup> ± 0.66	1.500 <sup>b</sup> ± 0.023	0.035 <sup>a</sup> ± 0.002	27.76 <sup>a</sup> ± 0.56
	Late (ILP)	43.24 <sup>a</sup> ± 0.78	1.481 <sup>b</sup> ± 0.034	0.037 <sup>a</sup> ± 0.004	29.63 <sup>a</sup> ± 0.94
	Rainfed (RNP)	45.43 <sup>a</sup> ± 1.03	1.662 <sup>a</sup> ± 0.015	0.031 <sup>a</sup> ± 0.003	27.21 <sup>a</sup> ± 0.59
	<b>ANOVA</b>				
	Trials	ns	<0.001	ns	<0.050
	Genotypes	ns	<0.001	ns	<0.050
	Interaction	ns	<0.010	ns	ns

Values are means ± standard error of six genotypes with three replicates. Levels of significance for the ANOVA:  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ . Within each treatment, means exhibiting different letters a, b and c, are significantly different ( $P < 0.05$ ) according to the post-hoc test (Tukey-b) on independent samples.

### 3.3. Effects of planting date, water supply and season on leaf pigments

The effect of trial was significant during the first season (2017–2018) for all leaf pigments, whereas the genotypic effect was present for chlorophylls, flavonoids and NBI. The chlorophyll and flavonoid contents were higher in ILP than in INP, while the anthocyanin content was lower in ILP than in INP, and NBI was not significantly different. However, the flavonoid and anthocyanin values under RNP conditions were higher, and chlorophylls and NBI lower than in INP (Table 4). There was no genotype by trial interaction for any of the traits included in the table.

In the second season (2018–2019), however, significant effects of trials and genotypes were shown for flavonoids and NBI alone. In fact, no significant differences were shown between the ILP and INP trials for the measured traits, while under RNP, only the flavonoid content was higher than in the rest of trials (Table 4). No interaction between genotype and trial existed.

The three-way ANOVA (trial, genotype and season) for the pigment traits included in Table 4 exhibited significant year effects for all the traits (Data in brief Table 4). Trial and genotype effects were also significant for all the traits. Interactions were only significant for season by trial.

### 3.4. Effects of planting date, water supply and season on root traits

To investigate root characteristics, the genotypic and trial effect and their interaction were analyzed in traits derived from the RGB images from the shovelomics study (Table 5). In the first season (2017–2018), trial and genotype effects were only significant for the root network surface (Nsurf), root network volume (Nvol) and specific root length (SRL). In addition, the effect of trials was also observed for root width, root connected components (CComp), maximum roots (MaxR) and root network length (Nlen), while a genotypic effect was shown for root network area (NwA). Root CComp, MaxR, Nlen, NwA and Nsurf traits were lower in the ILP than in the INP trial; in contrast, root width, Nvol and SRL did not exhibit any differences between ILP and INP. Root width, CComp, Nlen, NwA and Nsurf were lower under RNP than in INP conditions; oppositely, MaxR and SRL were higher in the RNP than in the INP trial (Table 6). There was no interaction between trial and genotypes for any of the traits.

During the second season (2018–2019), the effect of trial was significant across all root traits, except for root angle (RA) and the NWDR ratio, while genotypic effects were significant for width, CComp, MedR, Ndepth, SRL and NWDR. The interaction exhibited significant differences only for CComp, Ndepth, ConvA and SRL. CComp, Ndepth, NwA, Nsurf, Nvol, Network solidity and Ldist were lower, and MaxR, MedR, Nwidth, ConvA, Bush and SRL were higher in the ILP than in the INP trial. However, all traits were lower in RNP than in the INP trial, except for CComp, Bush, Network solidity, SRL and Ldist, which showed no differences (Table 5).

The three-way ANOVA (trial, genotype and season) for root traits exhibited significant year and trial effects for almost all the traits (Data in brief Table 5), while genotype effects were significant for less than half of the traits (width, CComp, Ndepth, Nvol, SRL and NWDR). The interaction between year and trial was significant for most traits, except for MedR, NwA, Nsurf, Nvol and NWDR. However, the two-way interaction between year and genotype was significant for Nlen, NwA, Nsurf, Nvol, ConvA and Ldist only, and between trial and genotype was significant for Ndepth and NWDR only, whereas the three-way interaction (year, trial and genotype) was only significant for CComp, Ndepth and RA.

### 3.5. Relationships between grain yield, and agronomic, physiological and root traits

Relationships between GY and yield components, stable isotopes, CT and leaf pigments in the three growing conditions combined and separated, are presented in Table 6. Most traits exhibited significant correlations against GY when combining all trials within each crop season.

During the first season, ear density, GNY and PH were positively

correlated with GY within each growing condition (INP, ILP and RNP). Under irrigated conditions (INP and ILP), higher HI, TGW and lower  $\delta^{13}\text{C}_{\text{grain}}$  and DTH were correlated with an increased GY. Higher  $\delta^{18}\text{O}_{\text{shoot water}}$  values correlated positively with GY under INP. The NDVI and NBI correlated negatively and flavonoids positively with GY under ILP, whereas under RNP the NDVI correlated positively with GY.

During the second crop season, HI and GNY correlated positively with GY within each of the three growing conditions. In addition, TGW, ear density, PH,  $\delta^{18}\text{O}_{\text{shoot water}}$  and flavonoid correlated positively, and  $N_{\text{grain}}$  negatively with GY under INP. GN correlated positively, and nitrogen content ( $N_{\text{leaf}}$  and  $N_{\text{grain}}$ ) negatively with GY under ILP. However, under RNP the GN, PH, and NDVI correlated positively and the carbon isotope compositions ( $\delta^{13}\text{C}_{\text{leaf}}$  and  $\delta^{13}\text{C}_{\text{grain}}$ ) negatively with GY.

The correlations of root traits with GY were studied for each crop season and the three different growing conditions (Data in brief Table 6). During the first season (2017–2018), positive correlations were exhibited only for a few traits (CComp, Nvol and network solidity) and when combining all growing conditions. Therefore, no correlations were found within any of the growing conditions. In the second season (2018–2019), most root traits (except MedR, Nwidth, RA and NWDR) were correlated significantly with GY when combining all three growing conditions. Within each growing condition, no correlations existed, except for a positive correlation of CComp with GY in the INP trial, and a negative correlation of Ndepth with GY under ILP (Data in brief Table 6).

Principal Component Analysis (PCA) was performed for each growing condition and season individually (Fig. 3). For the six different environments tested (the three growing conditions and the two seasons), GY was placed opposite to  $\delta^{13}\text{C}$  and the  $\delta^{15}\text{N}$  of grains and more or less close to TGW and ear density. DTH, which was only measured in the first season, was also placed more or less opposite to GY in the three growing conditions. Except for the INP in the first season, where RA was placed opposite to GY and very close to  $\delta^{13}\text{C}$  of grains, in the other five trials it was placed rather perpendicular to GY. SLR was placed on the same side as GY in the INP and RNP trials of both seasons and in ILP of the second season. Regarding the ILP of the first season, SLR was placed opposite to GY but the eigenvector for SLR was very short. The  $\delta^{18}\text{O}_{\text{shoot water}}$  was placed close to GY in the INP trials of both seasons, whereas in the ILP trial it was placed clearly opposite to the GY in the first season and rather perpendicular to the GY in the second season. In the case of the RNP trial,  $\delta^{18}\text{O}_{\text{shoot water}}$  was placed rather perpendicular to GY in the first season and opposite to GY in the second season. PH was placed close to GY in the four normal planting trials (INP and RNP of both seasons), but it was perpendicular to the two late planting trials. Other traits such as flavonoids, or the  $\delta^{15}\text{N}$  of the grains were placed either on the same side as, opposite to or perpendicular to GY, depending on the specific environmental conditions. The set of traits used in the PCA clearly separated the two categories of genotypes for the three growing conditions in the first year as well as for the INP in the second year, while for ILP and RNP in the second year, the separation was somewhat less evident.

PCA was also undertaken per agronomic condition (RNP, INP and ILP), which meant combining the two consecutive years for each agronomic condition, and only considering the traits in common measured during the two years (Data in brief Fig. 1). Under INP conditions, higher GY was related to a higher  $\delta^{18}\text{O}_{\text{shoot water}}$ , together with a higher HI and TGW and more open (i.e. higher) RA, as well as lower (more negative)  $\delta^{13}\text{C}_{\text{grains}}$ , PH and flavonoid content, whereas the other traits were less important or not associated. Under RNP conditions, GY was positively related with  $\delta^{18}\text{O}_{\text{shoot water}}$ , NBI,  $\delta^{15}\text{N}_{\text{grains}}$ , SRL and somehow HI and RA, whereas  $\delta^{18}\text{O}_{\text{shoot water}}$ ,  $\delta^{13}\text{C}_{\text{grains}}$ , CT and flavonoids, where the other traits were weakly or not related. In the case of ILP, GY was closely (and positively) associated with HI, and negatively associated with  $\delta^{13}\text{C}_{\text{grains}}$ , and to a lesser extent with  $N_{\text{grain}}$  and the biomass (NDVI) at anthesis.

**Table 5**  
Effect of crop season (2017–2018 vs 218–2019), trials (INP, ILP, RNP) and genotypes on root characteristics.

		Width (cm)	CComp	MaxR	MedR	Ndepth (cm)	Nlen (cm)	Nwidth (cm)	NwA	Nsurf (cm <sup>2</sup> )	Nvol (cm <sup>3</sup> )	ConvA (cm <sup>2</sup> )	RA <sub>protractor</sub>	Bush	Ldist	Network Solidity	NWDR	SRL (cm <sup>-2</sup> )	
2017–2018	INP	0.061 <sup>a</sup> ± 0.001	2.55 <sup>a</sup> ± 0.16	16.7 <sup>ab</sup> ± 1.0	9.5 <sup>a</sup> ± 0.8	6.1 <sup>a</sup> ± 0.3 ± 10.3	114.3 <sup>a</sup> ± 10.3	6.22 <sup>a</sup> ± 0.37	5.77 <sup>a</sup> ± 0.55	21.78 <sup>a</sup> ± 2.09	0.40 <sup>a</sup> ± 0.04	31.37 <sup>a</sup> ± 2.62	92.28 <sup>a</sup> ± 5.34	2.00 <sup>a</sup> ± 0.11	0.88 <sup>a</sup> ± 0.09	0.18 <sup>a</sup> ± 0.01	1.08 <sup>a</sup> ± 0.07	297.1 <sup>b</sup> ± 10.4	
		0.064 <sup>a</sup> ± 0.001	1.32 <sup>b</sup> ± 0.07	13.8 <sup>b</sup> ± 0.9	7.8 <sup>a</sup> ± 0.6	5.4 <sup>a</sup> ± 0.3 ± 6.8	82.6 <sup>b</sup> ± 6.8	5.86 <sup>a</sup> ± 0.29	4.36 <sup>ab</sup> ± 0.32	16.27 <sup>b</sup> ± 1.21	0.31 <sup>a</sup> ± 0.02	24.55 <sup>a</sup> ± 1.65	84.94 <sup>a</sup> ± 4.31	1.98 <sup>a</sup> ± 0.12	0.84 <sup>a</sup> ± 0.10	0.18 <sup>a</sup> ± 0.01	1.14 <sup>a</sup> ± 0.06	267.7 <sup>b</sup> ± 10.2	
		0.053 <sup>b</sup> ± 0.001	1.77 <sup>b</sup> ± 0.11	17.6 <sup>a</sup> ± 0.9	8.5 <sup>a</sup> ± 0.5	6.2 <sup>a</sup> ± 0.3 ± 9.8	109.4 <sup>ab</sup> ± 9.8	6.51 <sup>a</sup> ± 0.25	4.91 <sup>b</sup> ± 0.42	18.23 <sup>ab</sup> ± 1.60	0.29 <sup>a</sup> ± 0.03	30.92 <sup>a</sup> ± 2.41	87.75 <sup>a</sup> ± 3.42	2.29 <sup>a</sup> ± 0.08	0.72 <sup>a</sup> ± 0.06	0.16 <sup>a</sup> ± 0.00	1.09 <sup>a</sup> ± 0.03	389.9 <sup>a</sup> ± 17.42	
	<b>ANOVA</b>																		
		Environment	<0.001	<0.001	<0.050	ns	ns	<0.050	ns	ns	<0.050	<0.050	ns	ns	ns	ns	ns	ns	<0.001
		Genotypes	ns	ns	ns	ns	ns	ns	ns	<0.050	<0.050	<0.050	ns	ns	ns	ns	ns	ns	<0.050
		Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2018–2019	INP	0.061 <sup>a</sup> ± 0.001	1.99 <sup>b</sup> ± 0.14	16.3 <sup>a</sup> ± 0.8	8.9 <sup>a</sup> ± 0.4	6.4 <sup>b</sup> ± 0.2 ± 7.0	105.3 <sup>a</sup> ± 7.0	6.35 <sup>b</sup> ± 0.31	5.30 <sup>a</sup> ± 0.35	19.74 <sup>a</sup> ± 1.3	0.35 <sup>a</sup> ± 0.03	31.72 <sup>b</sup> ± 2.25	85.81 <sup>a</sup> ± 4.85	1.89 <sup>b</sup> ± 0.06	0.71 <sup>a</sup> ± 0.04	0.17 <sup>a</sup> ± 0.01	1.03 <sup>a</sup> ± 0.04	301.5 <sup>b</sup> ± 0.1
			0.046 <sup>b</sup> ± 0.001	5.95 <sup>a</sup> ± 0.37	12.8 <sup>b</sup> ± 0.5	6.1 <sup>b</sup> ± 0.3	9.0 <sup>a</sup> ± 0.3 ± 6.9	113.8 <sup>a</sup> ± 6.9	7.96 <sup>a</sup> ± 0.35	4.48 <sup>b</sup> ± 0.26	26.42 <sup>b</sup> ± 0.97	0.23 <sup>b</sup> ± 0.01	51.22 <sup>a</sup> ± 3.73	83.69 <sup>a</sup> ± 4.74	2.30 <sup>a</sup> ± 0.10	0.13 <sup>b</sup> ± 0.01	0.09 <sup>b</sup> ± 0.00	0.93 <sup>a</sup> ± 0.04	504.8 <sup>a</sup> ± 13.7
			0.063 <sup>a</sup> ± 0.001	2.24 <sup>b</sup> ± 0.10	12.6 <sup>b</sup> ± 0.5	6.8 <sup>b</sup> ± 0.2	5.4 <sup>c</sup> ± 0.2 ± 3.5	68.3 <sup>b</sup> ± 3.5	4.97 <sup>c</sup> ± 0.17	3.60 <sup>c</sup> ± 0.15	13.23 <sup>c</sup> ± 0.58	0.24 <sup>b</sup> ± 0.01	20.93 <sup>c</sup> ± 0.99	73.31 <sup>a</sup> ± 3.85	1.95 <sup>b</sup> ± 0.06	0.66 <sup>a</sup> ± 0.04	0.18 <sup>a</sup> ± 0.01	0.96 <sup>a</sup> ± 0.04	288.6 <sup>b</sup> ± 9.9
<b>ANOVA</b>																			
		Environment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.001	<0.001	ns	<0.001
		Genotypes	<0.010	<0.050	ns	<0.050	<0.050	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.050	<0.001
		Interaction	ns	<0.001	ns	ns	<0.001	ns	ns	ns	ns	ns	<0.050	ns	ns	ns	ns	ns	<0.050

Values are means ± standard error of six genotypes with 3 replicates. Levels of significance for the ANOVA: P < 0.05, P < 0.01 and P < 0.001. Within each crop season (2017–2018 vs 2018–2019), means exhibiting different letters a, b and c, are significantly different (P < 0.05) according to the Student *t*-test on independent samples. Width, average root width. CComp, number of connected components. MaxR, maximum number of roots. MedR, median number of roots. Ndepth, network depth. Nlen, network length. Nwidth, network width. NwA, network area. Nsurf, network surface area. Nvol, network volume. ConvA, convex area. Bush, bushiness. SRL, specific root length. Ldist, network length distribution. NWDR, network width to depth ratio. RA<sub>protractor</sub>, root angle measured with a protractor. INP, Irrigated normal planting. ILP, Irrigated late planting. RNP, Rainfed normal planting. For each year, and within each treatment, means exhibiting different letters are significantly different (P < 0.05) according to the post-hoc test (Tukey-b) on independent samples.



**Table 6**

Correlation coefficients of the significant linear regressions between grain yield (GY) and days to heading (DTH), harvest index (HI), thousand grain weight (TGW), ear density, grain number per ear (GN), total grain nitrogen yield (GNY), plant height (PH), the normalized difference vegetation index (NDVI) at anthesis, root angle (RA), oxygen isotope composition in shoot water ( $\delta^{18}\text{O}_{\text{shoot water}}$ ), nitrogen content in the flag leaf ( $\text{N}_{\text{leaf}}$ ) and grain ( $\text{N}_{\text{grain}}$ ), carbon and nitrogen stable isotope compositions in the flag leaf ( $\delta^{15}\text{N}_{\text{leaf}}$  and  $\delta^{13}\text{C}_{\text{leaf}}$ ) and grain ( $\delta^{15}\text{N}_{\text{grain}}$  and  $\delta^{13}\text{C}_{\text{grain}}$ ), canopy temperature (CT) at anthesis, and leaf pigments (chlorophyll, flavonoids, anthocyanins, and NBI). Assessed traits were evaluated under three growing conditions, combined (All growing conditions) and separated (INP, RNP, ILP), during two consecutive crop seasons (2017–2018 and 2018–2019) and using individual plot values.

		Crop season (2017–2018)				Crop season (2018–2019)			
		All growing conditions	INP	ILP	RNP	All growing conditions	INP	ILP	RNP
Yield components	DTH	0.354**	-0.534*	-0.475*	ns	–	–	–	–
	HI	0.543**	0.498*	0.679**	ns	0.639**	0.473*	0.754**	0.856**
	TGW	0.810**	0.519*	0.690**	ns	ns	0.686**	ns	ns
	GN	0.329*	ns	ns	ns	0.817**	ns	0.881**	0.863**
	Ear density	0.583**	0.589*	0.599**	0.544*	0.579**	0.582**	ns	ns
	GNY	0.966**	0.847**	0.923**	0.956**	0.979**	0.849**	0.948**	0.969**
	PH	0.865**	0.696**	0.533*	0.525*	0.784**	0.561*	ns	0.531*
	NDVI	0.478**	ns	-0.546*	0.491*	0.909**	ns	ns	0.691**
Nitrogen content & Stable isotope composition	$\delta^{18}\text{O}_{\text{shoot water}}$	-0.276*	0.614**	ns	ns	-0.661**	0.593*	ns	ns
	$\text{N}_{\text{leaf}}$	–	–	–	–	ns	ns	-0.510*	ns
	$\delta^{15}\text{N}_{\text{leaf}}$	–	–	–	–	0.801**	ns	ns	ns
	$\delta^{13}\text{C}_{\text{leaf}}$	–	–	–	–	-0.750**	ns	ns	-0.637**
	$\text{N}_{\text{grain}}$	-0.668**	ns	ns	ns	-0.768**	-0.516**	-0.659**	ns
	$\delta^{15}\text{N}_{\text{grain}}$	0.719**	ns	ns	ns	0.819**	ns	ns	ns
	$\delta^{13}\text{C}_{\text{grain}}$	-0.730**	-0.540*	-0.691**	ns	-0.882**	ns	ns	-0.493*
Canopy temperature	CT	-0.759**	ns	–	ns	-0.793**	ns	ns	ns
Leaf pigments	Chlorophyll	ns	ns	ns	ns	-0.355**	ns	ns	ns
	Flavonoid	ns	ns	0.512*	ns	-0.540**	0.558*	ns	ns
	Anthocyanin	ns	ns	ns	ns	ns	ns	ns	ns
	NBI	ns	ns	-0.569*	ns	ns	ns	ns	ns

INP, Irrigated Normal Planting; RNP, Rainfed Normal Planting; ILP, Irrigated Late planting. ns,  $P > 0.05$ ;

\*  $P < 0.05$

\*\*  $P < 0.001$ .

## 4. Discussion

### 4.1. Effect of growing conditions on grain yield, agronomic components and physiological traits

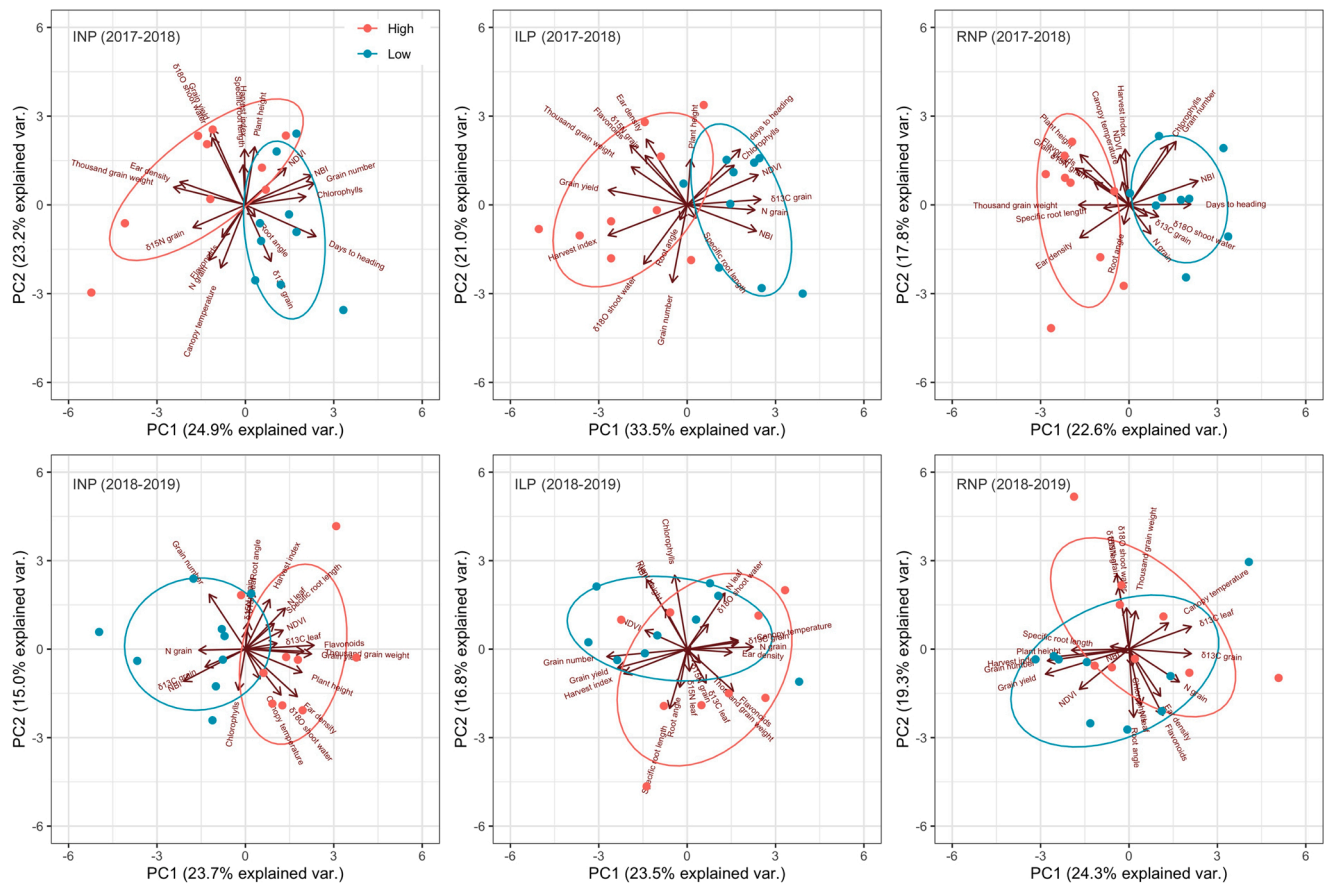
Grain yield is defined as the product of biomass and harvest index and is determined by the agronomic yield components of ear density, number of grains per ear and thousand grain weight (Donald and Hamblin, 1976). Depending on the severity and timing of stress during the crop cycle, all of the above agronomic traits may be affected to a greater or lesser degree (García del Moral et al., 2003; Giunta et al., 1993; Shpiler and Blum, 1990). In our study, the reduction in all agronomic traits, as in the case of rainfed versus irrigated conditions, was in agreement with these reports. Furthermore, interannual variability in environmental conditions was also evident, with the second crop season (2018–2019) being drier than the first one (2017–2018), as a consequence of much lower rainfall and higher temperatures (Fig. 2). As a result, grain yield was lower in the second (2018–2019) compared to the first season (2017–2018), particularly under rainfed conditions (RNP) but also under support irrigation (INP). Moreover, during the first season, the lower GY in the RNP compared with INP was associated with a major decrease in ear density as well as rather minor decreases in grain number per ear and TGW, which were the two agronomic yield components determined later in the crop cycle (Table 2). However, in the much drier conditions of the second season, ear density and GN in particular (which decreased by nearly 60%) were strongly affected under RNP compared with INP, while TGW was higher under RNP compared with INP, probably due to the strong decrease in sink capacity caused by the reduction in the number of grains per ear (Chairi et al., 2020; Slafer et al., 2005).

Increases in temperature also affected GY and its agronomic components negatively, through a shorter crop duration including grain

filling, accelerated leaf senescence and eventually a poorer grain set (García del Moral et al., 2003; Royo et al., 2000). In our study, late planting during the first season affected TGW and grains per ear negatively compared with the normal planting under support irrigation. However, under the drier conditions of the second season, the affected agronomic yield component was ear density, which is determined before TGW and grains per ear. Such results are in agreement with previous studies reporting the negative effect of late planting on GY and yield components in wheat grown under Mediterranean conditions (Joshi et al., 2016; Ma et al., 2018; Rezzouk et al., 2020). Moreover, exposure to high temperatures around anthesis induces pollen sterility, which reduces grain size and final yield in wheat (Wheeler et al., 1996). While this may have been the case in the RNP trials, late planting genotypes were grown under supplied irrigation and exhibited a canopy temperature below 30 °C (Table 3), which negates any relevance for heat decreasing the number of grains per ear.

Plant height reaches its maximum around anthesis. Provided that all genotypes have similar height in the absence of stress, this trait may be considered an indicator on how drought or shorter growth period associated with warmer temperatures may affect growth (Blum and Sullivan, 1997; De Vita et al., 2007). Thus, the late planting trials exhibited smaller plants than the normal planting trial under support irrigation, in accordance with a shorter crop duration. However, ILP produced taller plants than RNP, presumably as a consequence of the better water status in the former due to the support irrigation (Table 2). Here, PH was positively associated with high GY across the normal planting trials of the two seasons, regardless of whether they were under support irrigation or rainfed conditions (Table 6), but in the case of late planting, PH only correlated during the first season.

Canopy temperature and carbon isotope composition are physiological traits that are proposed as instantaneous (CT) and integrative ( $\delta^{13}\text{C}$ ) indicators for assessing crop water status (Araus et al., 2003;



**Fig. 3.** Principal component analysis (PCA) of 6 genotypes of durum wheat grown during two consecutive crop seasons (2017–2018 and 2018–2019), and different water regimes and planting dates: A normal (winter) planting under support irrigation conditions during the first (INP (2017–2018) and second (INP (2018–2019)) season; a late planting under support irrigation conditions in the first (ILP (2017–2018)) and second (ILP (2018–2019)) seasons; a normal (winter) planting under rainfed conditions in the first (RNP (2017–2018)) and second (RNP (2018–2019)) crop season. The variables included in the analysis are grain yield (GY), days to heading (DTH), grain number (GN), ear density (ears), plant height (PH), the normalized difference vegetation index (NDVI) at anthesis, nitrogen content in flag leaves and mature grains ( $N_{\text{leaf}}$  and  $N_{\text{grain}}$ ), nitrogen isotope composition in flag leaves and mature grains ( $\delta^{15}\text{N}_{\text{leaf}}$  and  $\delta^{15}\text{N}_{\text{grain}}$ ), carbon isotope composition in flag leaves and mature grains ( $\delta^{13}\text{C}_{\text{leaf}}$  and  $\delta^{13}\text{C}_{\text{grain}}$ ), oxygen isotope composition in stem water ( $\delta^{18}\text{O}_{\text{shoot water}}$ ), canopy temperature (CT) at anthesis, chlorophyll content (Chl), flavonoids (Flav) and the nitrogen balance index (NBI) of the flag leaf, root angle (RA) and specific root length (SRL).

Araus and Cairns, 2014; Blum, 2009; Lopes and Reynolds, 2010). The negative relationships of GY with CT (Rezzouk et al., 2020; Thapa et al., 2018; Yousfi et al., 2016; Zhou et al., 2016), and  $\delta^{13}\text{C}$  (Araus et al., 2003; Rezzouk et al., 2020; Whalley et al., 2008) across trials (Table 6), support these traits as indicators of water regime during crucial phenological stages (e.g. CT measured at anthesis) and during the complete growth cycle (e.g.  $\delta^{13}\text{C}$  in mature grains). In fact, a lower CT has been associated with higher transpiration (Blum, 2009), while a more negative  $\delta^{13}\text{C}$ , particularly in mature grains, indicates that the water input received by the crop is greater (Araus et al., 2003), and in fact it is usually the consequence of a higher stomatal conductance (Condon, 2020; Roche, 2015) associated with a better water status. In agreement with this, the  $\delta^{13}\text{C}$  of grains was clearly more negative under support irrigation than under rainfed conditions in both seasons (Table 2). In addition, under the drier conditions of the second season,  $\delta^{13}\text{C}$  values of both INP and RNP were higher (less negative), and differences in the  $\delta^{13}\text{C}$  of grains between the support irrigation and the rainfed trials were also higher compared to the first season. The CT at anthesis was also clearly higher under rainfed than support irrigation conditions, particularly during the second season. Late planting trials, even when exposed to warmer temperatures and therefore to higher water demand than the normal planting, exhibited values of grain  $\delta^{13}\text{C}$  and CT much closer to INP than RNP, due to the irrigation regime. Crop water status not only depends on the water inputs (amount of irrigation and/or precipitation) and outputs (evapotranspirative demand). Water uptake from the soil

may also be involved in the differences in water status across trials. In our study, when the data from all trials were combined,  $\delta^{18}\text{O}_{\text{shoot water}}$  correlated positively with  $\delta^{13}\text{C}$  ( $r = 0.588$ ,  $p < 0.01$ ) and CT ( $r = 0.639$ ,  $p < 0.01$ ).  $\delta^{18}\text{O}_{\text{shoot water}}$  has been proposed as indicator of how deep in the soil the roots extract water, with lower  $\delta^{18}\text{O}_{\text{shoot water}}$  values indicating greater depth of water extraction (Kale Çelik et al., 2018; Millar et al., 2018; Sanchez-Bragado et al., 2019). Therefore, the above correlations suggest that the greater the depth of water extraction from the soil (lower  $\delta^{18}\text{O}_{\text{shoot water}}$ ), the better the water status (lower  $\delta^{13}\text{C}$  and CT) of the plant.

On the other hand, the negative relationships between GY and  $\delta^{13}\text{C}$  in mature grains within at least half of the six trials assayed (the combination of the three growing conditions and the two seasons) (Table 6), as well as the opposite placement of GY and  $\delta^{13}\text{C}$  in all six PCAs (Fig. 3), suggest that the best genotypes in all tested environments were these exhibiting better water status and thus higher stomatal conductance. These results also support the fact that even when trials were conducted under good agronomic conditions (provided through supplemental irrigation and a good rainy season), and consequently rather high yields, water may still limit productivity. This was the case in the support irrigation normal planting during the first season, which attained yields close to  $7 \text{ Mg ha}^{-1}$  (Araus et al., 2008; Roche, 2015), but even in the rainfed normal planting of the second season, which was the driest of the six trials (an average yield of  $1.3 \text{ Mg ha}^{-1}$ ), there was a significant negative correlation between GY and  $\delta^{13}\text{C}$ . These results are in line with the fact that

the effective use of water (Blum, 2009) makes the difference in terms of productivity under drought conditions (Araus et al., 2008; Roche, 2015).

Root architecture is another criterion that has been widely emphasized in the literature regarding the crucial role that roots play in water and nutrient uptake (Loss and Siddique, 1994; Rogers and Benfey, 2015). Although information on a direct relationship between grain yield and root growth angle is scarce, several studies have proven the contribution of deeper root growth in providing better water status, and thus higher grain yield in wheat genotypes grown in water-limited and/or elevated temperature environments (Bai et al., 2019; Condon, 2020; Pinto and Reynolds, 2015; Rogers and Benfey, 2015). In our study, but only for the second season (which was much drier), root angle spread (assessed through the ConvA parameter) was higher in the two trials under support irrigation than in the rainfed trial (Table 5). A similar pattern (but without reaching statistical significance) was observed for the RA measured with a protractor (Fig. 3). Higher RA indicates a shallower root system, probably associated with the irrigation regime imposed, while in the case of rainfed conditions, plants were more dependent on roots that explored deeper in the soil profile. However, in the case of the first season there was no clear pattern related to the different growing conditions.

Concerning the late planting under support irrigation, these conditions produced quite a different root system pattern compared to the normal planting, depending on the crop season. During the dry conditions of the second year, plants of the ILP trial exhibited more superficial roots (higher CComp), resulting in a wider root convex hull (higher ConvA), a wider root network width (higher Nwidth), and thinner roots (lower root width and higher SRL), when compared to the INP and the RNP trials (Table 5). However, during the first season all these root traits exhibited an opposite pattern under ILP compared to the two normal planting trials. In fact, during the first season, roots were thinner (lower width and higher SRL), and root number (CComp) was reduced in the rainfed trial (RNP) compared to the two trials under support irrigation. The trend of thinner roots in response to water deficit agrees with reports for durum wheat under controlled (lysimetric) conditions (Elazab et al., 2012, 2016) and for bread wheat under field conditions (Peng et al., 2019). However, unlike the findings of these studies, the SRL during the second season of our study was higher and root width lower under ILP in comparison to both the severe water conditions of the RNP trial and also INP conditions (Table 5). These root traits may contribute to a more efficient uptake of water and nutrients under the high irrigation regime of the ILP during the second season, where water and nutrients are already accessible in the upper soil layer. It is worth mentioning that the ILP of the second season received a huge amount of irrigation (Fig. 2) and exhibited a yield comparable to that of the irrigated trial in the normal planting. These different patterns of response across seasons, and irrespective of the agronomic growing conditions (irrigation and planting time), illustrate the strong plasticity of the root system in response to the water regime.

#### 4.2. Phenology: a keystone of Mediterranean ideotypes

Phenology, and particularly heading and anthesis dates, plays a major role in the adaptation of cereals to Mediterranean environments. Phenology has been progressively shortened through breeding for adaptation to Mediterranean conditions (Loss and Siddique, 1994; De Vita et al., 2007). In addition, an earlier anthesis usually contributes indirectly to an extended grain filling period (Van Oosterom and Acevedo, 1992; Araus et al., 2002). In our study, shorter DTH measured during the first crop season (2017–2018) was correlated with increased GY in genotypes grown in the support irrigation trials (INP and ILP) of the two growing conditions (Table 5) and was further supported by the different placement of the two sets of genotypes in the PCA biplots corresponding to the 2017–2018 season (Fig. 3).

However, the future ability to exploit such phenotypic adjustment using varieties with shorter crop durations has limits (Araus et al., 2002;

Chairi et al., 2018; Prieto et al., 2020). Therefore other ideotypic traits need to be identified. In that sense, the direct role of phenology was subsequently removed by assessing the PCA within each agronomic condition with only the traits that were common across the two years, thus excluding DTH (Data in brief Fig. 1). In this case and for the three conditions, a low  $\delta^{13}\text{C}_{\text{grain}}$  was a positive trait, meaning that plants that maintained more open stomata were the most productive. A better nitrogen assimilation capacity (higher NBI under INP and RNP, together with higher  $\delta^{15}\text{N}_{\text{grain}}$  under RNP) and a lower accumulation of photo-protective pigments (Flavonoids) in both the INP and RNP seem to be good ideotype indicators. The importance of other traits changed depending on the agronomic conditions (e.g. root traits), or even shifted from positive to negative when compared between INP and RNP, such as for  $\delta^{18}\text{O}_{\text{shoot water}}$ . In that particular case, the results suggested that the capacity for water capture in the upper soil layers under irrigation conditions, or deeper soil layers under rainfed, are positive traits. The specific traits associated with a better genotypic performance within each of the six growing conditions tested are discussed below.

#### 4.3. Genotypic ideotypes under support irrigation and normal planting date

Under the relatively good growing conditions provided by normal planting under support irrigation (INP), the most productive genotypes exhibited better water status (lower  $\delta^{13}\text{C}_{\text{grain}}$ ), more superficial water extracted (higher  $\delta^{18}\text{O}_{\text{shoot water}}$ ), enhanced growth (higher NDVI and PH at anthesis), and higher values in the agronomic yield components (ear density and TGW) and HI, in addition to phenological adjustment (through shorter days to heading) (Fig. 3). However, compared to the first crop season (2017–2018), drought conditions were more evident during the second season (2018–2019), resulting in an increase in protective pigments (flavonoids) being associated with a better genotypic performance. Increases in protective pigments such as flavonoids and anthocyanins in response to drought stress are well documented in wheat (Ma et al., 2014; Naderi et al., 2020). Meanwhile, RA was positioned opposite from GY during the first season (Fig. 3), meaning that the best genotypes were those that maintained shallow roots, while in the much drier conditions of the second season the trend changed, with GY and RA placed perpendicular to each other. While this suggests a lack of a clear role concerning RA during the second season, these results may be also understood as the root system being shaped to not only extract shallow water, but to also capturing water that percolates from the upper part of the soil profile via development of deeper roots. Elazab et al. (2016) reported in a study with durum wheat grown under lysimetric conditions and a rain shelter that better genotypic performance under water deficit conditions was associated with an increase in SRL (assessed as the ratio of root length to dry biomass). Thus SRL was positively correlated with shoot biomass across genotypes under moderate water stress, but absent under full irrigation (provided by maintaining container water capacity at 100%). In our study, even the support irrigation (INP and ILP) trials were exposed to some degree of water stress under field conditions, which agrees with the fact that in five of the six PCAs (Fig. 3) the relationship between SRL and GY was linear and negative, meaning that thinner roots is a rather positive genotypic trait regardless of the growing conditions.

#### 4.4. Genotypic performance under rainfed conditions

Under the moderate water-limited conditions of the rainfed trial during the first crop season, greater GY was achieved in genotypes exhibiting a better capacity for nitrogen assimilation (higher NBI and  $\delta^{15}\text{N}_{\text{grain}}$ ), deeper water extraction (lower  $\delta^{18}\text{O}_{\text{shoot water}}$ ), better water status (lower  $\delta^{13}\text{C}$  and CT), thinner roots (high SRL), deeper root growth (lower RA), phenotypical adjustment (lower DTH) and higher flavonoid content (Fig. 3). However, during the severe water stress experienced during the second season, the best genotypes, besides exhibiting again a

better water status (lower  $\delta^{13}\text{C}$ ), showed no clear pattern in terms of root angle or the soil profile location of extracted water ( $\delta^{18}\text{O}_{\text{shoot water}}$ ), the nitrogen status (NBI and  $\delta^{15}\text{N}_{\text{grain}}$ ), or the accumulation of flavonoids. In any case, for both years the best genotypes exhibited greater growth (higher PH) and biomass (higher NDVI) at anthesis, together with higher yield components, particularly higher TGW in the first crop season and higher grain number per ear in the second crop season. Our results agree with previous studies emphasizing the pivotal role of deep root development (Condon, 2020; Lopes and Reynolds, 2010; Rogers and Benfey, 2015; Wasaya et al., 2018), but particularly under the moderate water stress conditions of the rainfed crop during the first season. Thus, when grown under water-limited conditions, the most productive genotypes adjusted their root development into narrow root angle spreads (low RA and ConVA) for better access to water resources in deeper soil sections.

#### 4.5. Genotype performance under elevated temperatures

The most productive genotypes under ILP conditions were associated with better water status (lower  $\delta^{13}\text{C}$ ). Nevertheless, the relative importance of agronomic yield components, root characteristics and protection pigments varied depending on the crop season, as did other factors such as green biomass (Fig. 3). During the first season, the best genotypes exhibited lower green biomass (lower NDVI) and higher flavonoid content at anthesis, probably associated with a lower leaf biomass. However, they also demonstrated higher ear density and TGW, along with extraction of more superficial water due to the higher  $\delta^{18}\text{O}_{\text{shoot water}}$ , while RA played no clear role. During the second season, the best genotypes exhibited more biomass at anthesis, lower flavonoid content and larger grain number per ear, but had lower ear density. RA was positioned somewhat perpendicular to GY, particularly in the first season (Fig. 3), which did not support root angle (at least measured with the shovelomics approach) as a trait conferring genotypic adaptation. However, despite the fact that rRA had no clear involvement, the apparent extraction of deeper water (lower, more

negative,  $\delta^{18}\text{O}_{\text{shoot water}}$ ) was placed on the same side as GY (Fig. 3). In a study performed under conditions comparable to our late planting trial, genotypes with cooler canopies were reported as having deep root development, which was inferred from the higher root density in the 30–60 cm soil layer, and resulted in better agronomic performance (Pinto and Reynolds, 2015). Moreover, it is possible that plants invest their resources into simultaneous development of shallow roots and deep roots to catch superficial moisture and moisture retained deep in the soil profile, respectively, as it has been proposed in a recent study on root traits contributing to higher yields in wheat (Bai et al., 2019).

#### 4.6. Conclusions

Increased water deficit and temperature remain major challenges for sustainable production of wheat under Mediterranean conditions. Here we have studied the agronomic, phenological and physiological characteristics associated with ideotypic performance of durum wheat genotypes under different Mediterranean environment conditions. A trait that was clearly associated with genotypic performance was phenological adaptation, with genotypes that reached heading earlier being the best performers, regardless of the growing conditions considered. This was the case for all of the six different scenarios studied, across which occurred a nearly seven-fold difference in grain yield. In addition, physiological traits such as the carbon isotope composition ( $\delta^{13}\text{C}$ ) of mature grains, and to a lesser extent the oxygen isotope composition ( $\delta^{18}\text{O}$ ) of the shoot water and the canopy temperature at anthesis, were key traits for characterizing water status and crop adaptation to the different growing conditions, including assessment of genotypic performance. Root angle and specific root length, as assessed through the shovelomics approach, may give some further insights, particularly when characterizing the specific water regime imposed on the trials (rainfall alone or combined with irrigation). In any case, our study proves that beyond some traits (earlier reproductive stage, lower grain

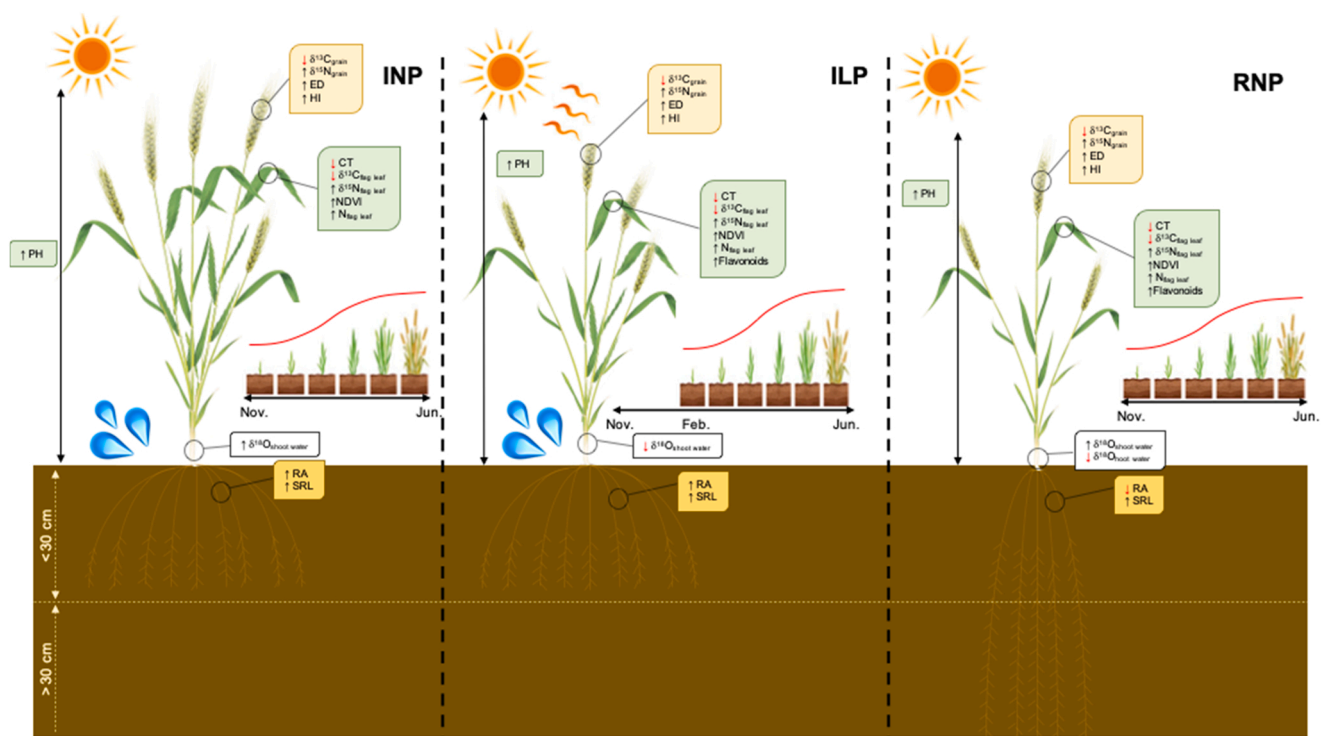


Fig. 4. A summary of potential traits contributing to the development of wheat ideotypes under different growing conditions: INP, Normal planting under irrigated conditions. RNP, Normal planting under rainfed conditions. ILP, Late planting under support irrigation conditions. ED, ear density; HI, harvest index; PH, plant height; RA, root angle; SRL, specific root length.

$\delta^{13}\text{C}$ ) associated with better genotypic performance under a wide range of Mediterranean conditions, other shoot and root traits are correlated with specific genotypic performance under a given growing condition (Fig. 4) and specific season. In this sense, rainfed and even standard support irrigation conditions are strongly affected by annual variability in precipitation and temperature, which makes it necessary to tailor the ideotype concept to the specific conditions of each environment (referred to as the particular combination of agronomic conditions and season). For this reason, introducing versatile and efficient root phenotyping techniques may contribute towards a deeper understanding of ideotype requirements within each particular environment. Nevertheless, further work is needed to improve high-throughput field phenotyping protocols to assess root performance.

### CRediT authorship contribution statement

FZR wrote the first draft, collected samples, conducted the root studies and the stable isotope and statistical analyses, drew the tables and implemented the edits; JLA participated in the field evaluation, conceived the study and implemented the edits in the consecutive drafts; MDS collaborated on the stable isotope analyses and revised the drafts; AGR and SCK ran the remote sensing measurements and collaborated in the shovelomics; MTNT conducted the field trials and collected the grain yield, the agronomic yield components and the phenological data.

### Declaration of Competing Interest

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

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