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Physiological Stress Indices: A Valuable Tool to Mitigate the Impacts of High Temperatures and Drought on Durum Wheat Yield and Quality

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ABSTRACT

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is one of the world's most important and widely grown cereals, playing a fundamental role in global food security. In countries where it is traditionally cultivated, abiotic stress caused by high temperatures and limited precipitation, exacerbated by the ongoing climate crisis, is the main cause of production losses quality deterioration, and uncertainty in meeting market demand. The objective of this study was to monitor, across five experimental sites, the effect of climatic variability on phenological, morpho-physiological, and agronomic parameters, using physiological stress indices such as RWC, NDVI, and SPAD. The research was conducted during the 2023/2024 season across the southern Italian landscape of Sicily, a region representative of the durum wheat cultivation scenario in hot-arid environments, and one of the main production areas. Analysis of variance (ANOVA) revealed significant differences in most parameters, with the exceptions of the number of spikelets per spike, harvest index, and test weight. In general, a decline in yield and quality was observed across all the sites, with notable variability. High temperatures and the absence of precipitation shortened the elongation-flowering and flowering-maturation stages, influencing the accumulation of growing degree days (GDD) in the more inland sites. In most environments, plants showed reduced growth (average culm height: 41.5 cm), while yields ranged from 10.0 to 27.0 q ha^{-1}. Quality parameters, such as yellow pigment (22.9%–24.3%), protein content (13.6%–15.8%), and gluten content (8.8%–11.9%), were variable depending on environmental conditions. Finally, Pearson's correlation analysis showed strong positive correlations between physiological parameters (SPAD, RWC, NDVI) and yield ($r \geq 0.8$), between the yellow index and yield ($r > 0.8$), and strong negative correlations between proteins, dry gluten, and yield ($r < -0.8$).

Although durum wheat is drought-tolerant, it is affected by water and thermal stress, which causes spatial and temporal variability in production. Monitoring cereal systems and adopting appropriate techniques could mitigate this vulnerability, supporting cereal farming businesses.

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1 | Introduction

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is one of the most important and commonly cultivated cereals worldwide, playing a crucial role as a commercial crop for the food industry (Tortorici et al. 2024; Tidiane Sall et al. 2019). According to the Global Yield Gap Atlas (GYGA), the potential yield of durum wheat exceeds 7.0 t ha^{-1} in major producing countries, thanks to genetic gains made since the 20th century (Xynias et al. 2020). However, current data suggests a significant gap between actual and potential yield, widespread across all countries. This production gap is mainly attributed to abiotic stress traditionally affecting durum wheat and the climate change that is intensifying its effects. Predictive models indicate an intensification of the frequency and severity of droughts, associated with a decrease in precipitation and an increase in temperatures, even during the winter period, which will lead to desertification of many areas, especially those already vulnerable (Vicente-Serrano et al. 2014). Italy, considered a major producer of durum wheat and a key player in the global pasta market, is among the areas most vulnerable to climate change, often identified as a climate change hotspot. (Carbone and Henke 2023; Ciliberti et al. 2022). According to ISMEA data, in many production areas in Southern Italy, in 2024, yields were generally below 2.0 t ha^{-1} (www.ismea.it, accessed January 2025). This scenario of yield uncertainty and reduced quality poses risks to food security for an increasing global population and to agricultural economies, as well as a risk of abandonment of durum wheat cultivation in traditionally cultivated areas. The current condition also imposes limits on the 100% Italian durum wheat pasta supply chain, where growing consumer demand is driven by a growing interest in sustainable products (Ciliberti et al. 2022).

Researchers have always investigated the effects of abiotic stresses on crop production, and now more than ever, in the context of the climate crisis, to find mitigation and adaptation solutions that address current and future challenges. Drought and heat are the two main abiotic stresses limiting durum wheat production, with the final result arising from a complex interaction between these climatic extremes (Zhu et al. 2022).

Both water availability and temperature regulate the duration of phenological stages; in fact, they are the main factors controlling crop physiological and metabolic processes (Grosse-Heilmann et al. 2024). Generally, water stress and high temperatures lead to a shorter growing season, accelerated leaf senescence, and reduced biomass and yield (Figueiredo et al. 2015; Pal et al. 1996). Furthermore, higher temperatures increase the demand for evapotranspiration, reducing the crops' water use efficiency, thus aggravating water stress (Saadi et al. 2015; Zampieri et al. 2017). On the other hand, temperature tolerance somewhat depends on experimental conditions, particularly the rate and intensity of exposure to extreme temperatures and soil water levels. Zampieri et al. (2017) identified excess or deficit heat and water stress as explanations for more than 40% of the interannual variability in wheat yield globally.

Vulnerability to drought increases during the durum wheat biological cycle, with higher sensitivity during the reproductive phase (Entz and Fowler 1988); however, if the soil water content is low at sowing, germination slows or stops, reducing plant establishment with negative effects on yield (Passioura 2006; Anjum and Arif 2022).

In fact, to date, due to a lack of precipitation during the winter season, wheat sowing is delayed, which exposes phenological stages to higher temperatures, causing problems during flowering and shortening the maturation period, ultimately leading to decreased yield and grain quality (Anjum and Arif 2022; Zhang and Oweis 1999; Barnabás et al. 2008; Tari 2016).

Considering the variability of climatic extremes during the cycle and their effects on crops, continuous monitoring of agricultural systems is crucial. Early detection of stress facilitates the mitigation of the negative impacts of stress factors, preserving the potential crop production from irreversible damage and improving resource use efficiency (Liu et al. 2015). To date, several parameters are used to monitor stress, some of which are measured in parallel to provide a clearer understanding of the physiological state of the crop. Proximal tools allow the detection of physiological parameters such as gas exchange, water potential, relative water content (RWC), leaf rolling, chlorophyll content, and canopy temperature, which, combined with remote sensing techniques, represent a practical and efficient way to monitor plant responses to environmental stress factors over large areas (Roy et al. 2023). Some of these indices are non-destructive, noninvasive, quick, cost-effective, well correlated with important agronomic and physiological traits of crops, and they could also be used at the farm level.

The research presented in this paper was conducted in Southern Italy, in Sicily, an island characterized by significant spatial and temporal climatic variability and where the effects of climate change are evident (Ferrara and Lindberg 2023), in addition to being one of the regions most involved in durum wheat cultivation. The aim of this study was to monitor, at five experimental sites, the effect of climatic variability on the yield and quality of durum wheat using physiological stress indices. These indices were selected because they are highly correlated with yields, scientifically reliable, and allow easy and rapid field stress monitoring, providing a comprehensive and adequate understanding of plant stress. Specifically, indices such as RWC, SPAD, and NDVI were chosen because of their proven ability to reflect water stress, plant health, and vegetative growth, respectively, all of which are strongly influenced by climatic factors such as temperature and precipitation. GDD (Growing Degree Days) was also considered due to its capacity to track thermal accumulation, an important factor influencing wheat development in regions with variable temperatures.

2 | Materials and Methods

2.1 | Experimental Sites

The experiment was conducted in the field during the 2023/2024 growing season. Five experimental stations in Sicily (Italy) were selected: Castelvetrano (TP) at the "Campo Castelvetrano" farm, owned by the Agricultural Development Authority of the Sicilian Region (ESA), managed by the Department of Agricultural, Food, and Forest Sciences of the University of Palermo

(37°48′49.67“N 12°33′25.58“E), Marsala (TP) at the private “Marino” farm (37°48′47.2“N 12°33′20.9“E), San Giovanni Gemini (AG) at the “Sparacia” farm, owned by the Agricultural Development Authority of the Sicilian Region (ESA), managed by the Department of Agricultural, Food, and Forest Sciences of the University of Palermo (37°38′08.8“N 13°45′46.4“E), Alia (PA) at the private “Di Marco” farm (37°75′05.1“N 13°73′46.0“E), and Caltanissetta (CL) at the private “Sollami” farm (37°26′6.04“N 14°01′44.87“E) (Figure 1). The five experimental sites were selected because they represent traditional durum wheat cultivation areas and reflect the pedoclimatic variability of the region. Supporting information S1: Table 1 shows, for each experimental site, the soil types according to the United States Department of Agriculture (USDA) classification.

2.2 | Plant Material, Crop Management, and Experimental Design

The tested crop was the Antalis (Semìa) variety of *Triticum turgidum* subsp. *durum*. This variety, with a medium-early growth cycle, is reliable for its agronomic profile and high production potential. It is suitable for traditional and challenging environments in Italian cereal farming due to its resilience and tolerance to plant diseases. Additionally, it has desirable quality characteristics (gluten) for the milling industry.

The seeding density was set to 350 plants per m^2, and sowing occurred at the different experimental sites between November 20 and December 20, depending on rainfall, as indicated in Supporting information S2: Table 2.

The fields were managed according to the traditional agricultural system prevalent in Sicily, which involved plowing (0.30 m) in early autumn followed by surface tillage before sowing. The fertilization plan involved applying 120 nitrogen (N) units, 60 phosphorus (P_2O_5) units, and 40 potassium (K_2O) units. Phosphorus and potassium were applied during sowing, while nitrogen was applied in two stages: 30.0% at sowing in the form of ammonium nitrogen and the remaining 70.0% during the tillering phase in the form of urea (46.0%).

Weed control was carried out during the third leaf phenological stage using the active ingredients Diflufenican (2.2%) and Chlortoluron (35.5%) at a dose of 3.5 l ha^{-1}.

Fungal disease control was performed between the late tillering and early stem elongation phases using the active ingredient Pyraclostrobin at a dose of 0.7 l ha^{-1}.

The experimental design adopted was a randomized block design with three replications, each measuring 50.0 m^2.

2.3 | Agrometeorological Data

Daily data for maximum temperature (T_{max}) and minimum temperature (T_{min}), precipitation, wind speed, relative humidity, and solar radiation were provided by the Agro-Meteorological



FIGURE 1 | Experimental sites.

Information Service of the Sicily Region (http://www.sias.regione.sicilia.it/frameset_rete_new.htm, accessed October 2024). The stations are equipped with data loggers and sensors for measuring air temperature (MTX FAR120LB) (°C), relative humidity (MTX FAR 134BA) (%), precipitation (MTX MA-B023AA) (mm), wind speed at 2.0 meters height (Gill Windsonic Option 1) ($m\,s^{-1}$), and solar radiation (Phillip-Schenk 8102) ($MJ\,m^{-2}$). Each experimental site was located about 600.0 meters from the meteorological station.

Reference evapotranspiration (ET_0) (FAO Penman–Monteith method) and crop evapotranspiration (ETc) were calculated according to Allen (1998). Supporting information S3: Table 3 shows the average monthly climatic variables for each experimental site.

2.4 | Growing Degree Days (GDD)

At all experimental sites, the main phenological stages of durum wheat were recorded during the growing season, as defined according to the BBCH scale (Zadoks et al. 1974). The GDD (Growing Degree Days), calculated as the sum of the daily average temperatures starting from the sowing dates (McMaster 1997) (Equation 1), were determined at the following stages: 29 BBCH, 39 BBCH, 69 BBCH, and 99 BBCH.

$$\mathrm{GDD} = \frac{T_{\max} + T_{\min}}{2} - T_{base}$$

where $T_{\max}$ is the daily maximum temperature (°C), $T_{\min}$ is the daily minimum temperature (°C), and T_{base} is the temperature below which the photosynthetic process stops (°C). For the base temperature and critical temperature, values of 0.0°C and 37.0°C were chosen, respectively (Porter and Gawith 1999).

2.5 | Agronomic and Qualitative Traits

At physiological maturity, data were collected on 25 randomly selected plants per replication, harvested by hand, and the following morphological traits were recorded: number of tillers, number of nodes, stem height, spike length, number of spikes per m^2, total spikelets, and fertile spikelets per spike.

At harvest, which was carried out mechanically between late May and June when the grain moisture content was 12.0%, the yield [$q\,ha^{-1}$] and the harvest index (HI) were recorded.

For each replication, three 500.0 g seed samples were analyzed using the Infratec NOVA instrument (Foss Analytics, Italy), which utilizes near-infrared (NIR) transmittance technology, to determine the following qualitative, and commercial parameters: thousand kernel weight (TKW) [g], test weight (TW) [$kg\,hL^{-1}$], protein content (PC) [%], yellow index (YI) [%], and dry gluten (DG) [%]. The weight of 1000 seeds (TKW) [g] was calculated from the average weight of five sets of 100 seeds per replication.

2.6 | Plant Physiological Data

To monitor the physiological conditions of durum wheat, the following physiological indices were used: leaf chlorophyll content (SPAD), RWC, and normalized difference vegetation index (NDVI). Measurements were taken during BBCH stages 29, 39, and 69 (Zadoks et al. 1974) for SPAD, and during BBCH stages 39 and 69 (Zadoks et al. 1974) for RWC and NDVI.

For the determination of leaf chlorophyll content (SPAD), the Minolta 502 SPAD chlorophyll meter (Konica Minolta, Italy) was used. Measurements were made on 20 fully expanded leaves per replication. The values are expressed in SPAD units.

The RWC of the leaves was determined as described by Saghouri el idrissi et al. (2023) (Equation 2), on 20 fully expanded leaves. RWC is expressed as a percentage (%).

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight of the leaf.

NDVI was measured using the RapidScan CS-45 active canopy sensor (Holland Scientific, Lincoln, NE, USA). Measurements with the RapidScan CS-45 were taken while the sensor was passed over the crop surface at a height of 1 m from the ground, always maintaining a 90.0° angle with the crop and walking at a constant speed. For each replication, three 5.0-meter rows were scanned. NDVI values were automatically calculated by the instrument according to Equation 3:

$$\mathrm{NDVI} = \frac{\mathrm{RNIR} - \mathrm{RRED}}{\mathrm{RNIR} + \mathrm{RRED}}$$

where RNIR is the reflectance in the near-infrared, and RRED is the reflectance in the red.

2.7 | Statistical Analysis

A one-way ANOVA was conducted for the phenological stages (expressed in days), morphological, yield, and quality parameters. The experimental site was considered as a fixed factor. For RWC and the measurements of SPAD and NDVI, a repeated measures ANOVA was applied. The software MINITAB 19.1.1.0 (State College, PA, USA) was used for Windows. To ensure compliance with ANOVA assumptions, all data were tested for sphericity, normality and variance homogeneity through Mauchly's test ($\alpha = 0.05$), Ryan–Joiner test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$). The percentage data were subjected to arcsine transformation. The means were separated using Tukey's test at $p \leq 0.05$.

Pearson's correlation coefficients for the means of the genotype across the five experimental sites were estimated between yield and the physiological stress indices such as RWC, SPAD readings, and NDVI. For the analysis of Pearson's correlation coefficients, the Windows Excel application was used.

3 | Results

3.1 | Climatic Data

Figure 2 shows, for the five experimental stations, and with reference to the 2023/2024 growing season, the trends in monthly average maximum (T_{max}) and minimum (T_{min}) air temperature, monthly cumulative precipitation, and monthly crop evapotranspiration (ETc).

During the winter season, the coastal stations in the province of Trapani (Castelvetrano and Marsala) showed similar and generally higher values compared to the other stations, with average Tmin of 9.0°C and average Tmax of 17.0°C and 16.0°C, respectively for Castelvetrano and Marsala. The average Tmin and Tmax recorded at the other stations were 6.0°C and 14.0°C at Alia, 5.5°C and 16.0°C at Caltanissetta, and 3.5°C and 15.5°C at Sparacia. In March and April, during heading and flowering stages, the average Tmax values were similar across all the areas, ranging between 19.0°C and 21.0°C. However, the greatest differences were observed in the average Tmin values, with the highest values recorded at Castelvetrano and Marsala (10.0°C), while the lowest values were measured at Sparacia (5.0°C). In May, during the maturation of the caryopses, the highest temperatures of the season were recorded across all stations, peaking in the second half of the month. The inland stations reported the highest values, with an average Tmax of 27.0°C for Caltanissetta and Sparacia, peaking at 34.0°C, while an average Tmax of 25.0°C was observed for Castelvetrano, Alia, and Marsala, with peaks up to 30.0°C.

Precipitation was low during the entire growing season, with variable distribution across the experimental stations. The total rainfall values during the crop cycle ranged between 224.0 and 341.0 mm, recorded at Sparacia and Castelvetrano, respectively. Generally, the winter months were the wettest; in fact, at Castelvetrano, about 50.0% of the total rainfall amount occurred in January (89.3 mm) and February (83.8 mm), while May was the

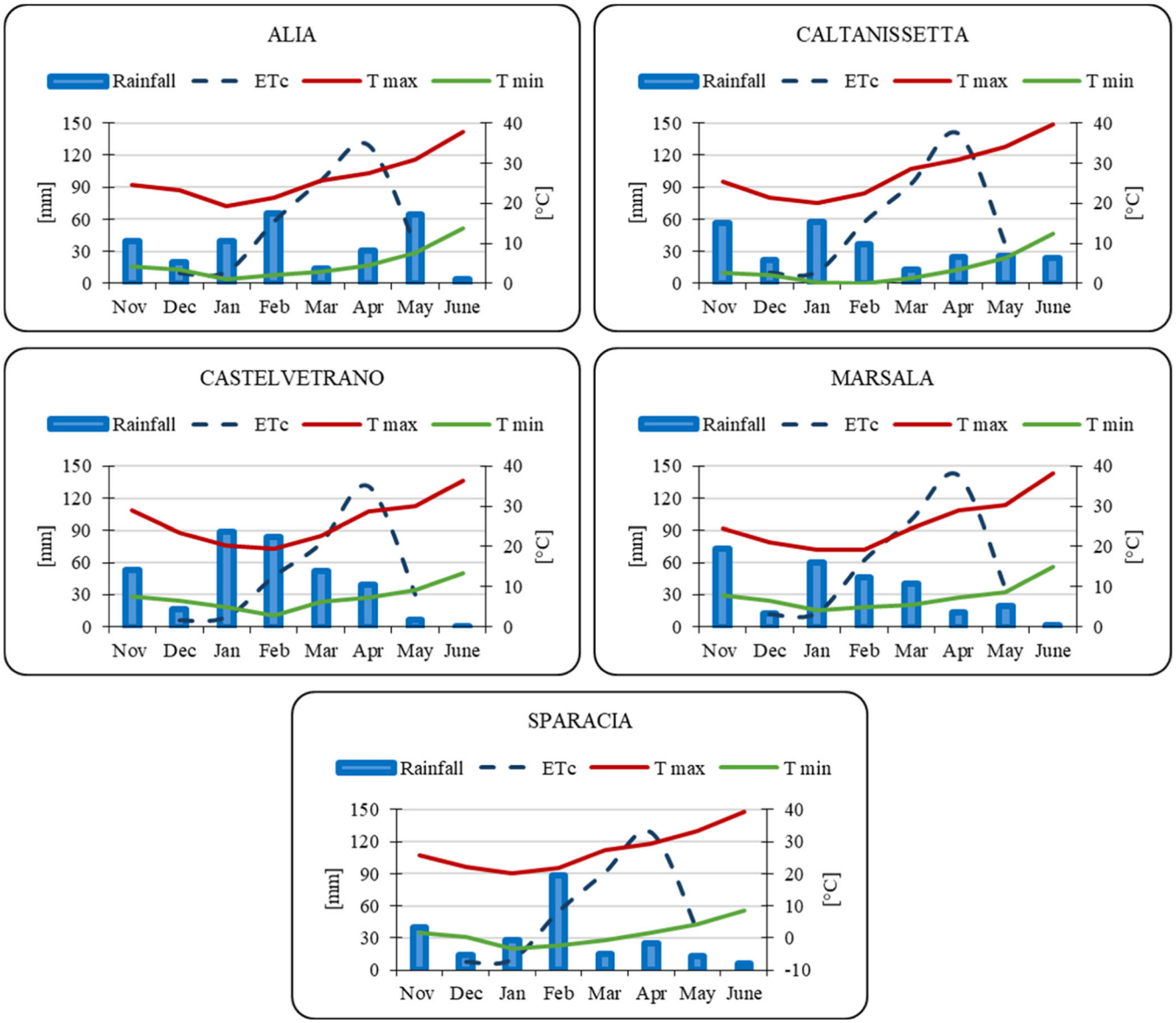


FIGURE 2 | Temperature, Rainfall and Crop Evapotranspiration Trends for each experimental site. *Note:* ETc = crop evapotranspiration; T_{max} = maximum temperature; T_{min} = minimum temperature.

driest month (6.8 mm). Rainfall was negligible starting from March at the Caltanissetta site and from April at the Marsala station. At Sparacia, 88.0 mm of rain was recorded during February, representing 40.0% of the total precipitation during the crop cycle, while the other months were dry. In contrast, at Alia, the heaviest rainfall occurred in February (65.2 mm) and May (64.6 mm).

Regarding crop evapotranspiration, a growing trend was observed from sowing until April, when the highest evapotranspirative demand was observed at all experimental sites. Afterward, the evapotranspirative demand decreased until it stopped in conjunction with crop maturity. In particular, the highest crop evapotranspirative demand occurred at the Caltanissetta (140.1 mm) and Marsala (141.7 mm) sites, while the lowest occurred at the Sparacia (128.6 mm) and Alia (129.9 mm) sites.

3.2 | Crop Phenology

Figure 3 shows the cumulative GDD and the duration in days of the phenological stages from sowing to maturity of durum wheat for each experimental site. Specifically, GDD and duration in days are reported for the phenological stages of tillering (29 BBCH), stem elongation (39 BBCH), flowering (69 BBCH), and maturity (99 BBCH).

In the different experimental sites, the growth cycle lasted between 164 and 190 days, with values recorded at Sparacia and Alia, respectively. The accumulation of GDD was highest at the Marsala site (2608.0 GDD) and lowest at the Sparacia site (2060.0 GDD) (Figure 3).

The duration in days of the individual phenological stages showed statistically significant differences between the experimental sites (Supporting information S4: Table 4). In particular, at the Sparacia site, the duration in days to reach the 29 BBCH stage was longest (64 days) compared to the Castelvetrano site, which was shorter (44 days). For the 39 BBCH phenological stage, the most days were required at the Alia experimental site (28 days), which did not differ statistically from the Caltanissetta (27 days) and Sparacia (26 days) sites, while the shortest number of days was recorded at the Castelvetrano site (22 days). To reach the 69 BBCH phenological stage, the greatest number of days was spent at the Alia (57 days), Castelvetrano (57 days), Caltanissetta (56 days), and Marsala (54 days) sites, showing no statistical differences among them. In contrast, the shortest duration was observed at the Sparacia site (35 days). Finally, the 99 BBCH phenological stage was reached in more days at the Castelvetrano (50 days), Marsala (50 days), and Alia (48 days) sites, with no statistical differences among them; whereas, the shortest number of days was spent at the Caltanissetta (38 days) and Sparacia (39 days) sites.

3.3 | Morphological Parameters

Table 1 shows the results of the analysis of variance (ANOVA) for the morphological parameters measured: tillers [n], number of nodes [n], plant height [cm], and spike length [cm]. The durum wheat variety, cultivated in the five experimental sites, exhibited variable vegetative behavior with statistically significant differences for all the parameters considered. In general, the plants cultivated at the Castelvetrano experimental site showed the highest values.

The number of tillers ranged from 2.0 to 5.2, with the lowest values recorded at the Caltanissetta experimental site. The highest number of nodes was observed at the Castelvetrano and Marsala sites (4.5–4.7), while the lowest was found at the Alia, Caltanissetta (3.0), and Sparacia (3.0) sites, with no statistical differences between them. The highest plant height was recorded at the Castelvetrano site (78.8 cm), while the lowest values were found at the Alia (40.1 cm), and Caltanissetta (39.1 cm) sites. Finally, the longest spike length was observed at the Marsala (18.1 cm) and Castelvetrano (17.5 cm) sites, while the shortest spike length was recorded at the Sparacia (7.3 cm) and Caltanissetta (6.9 cm) sites.

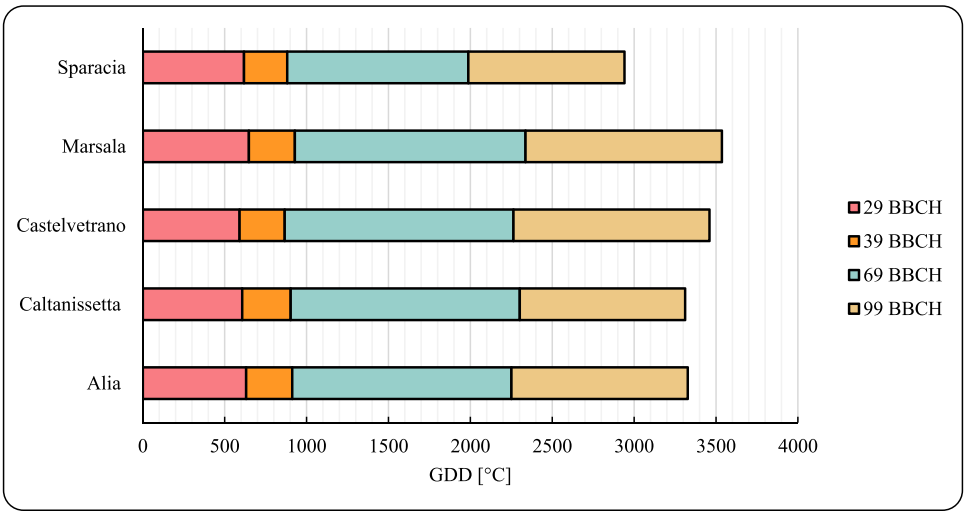


FIGURE 3 | Cumulative GDD (°C) from sowing to harvest for each phenological stage and experimental site.

TABLE 1 | Morphological traits for each experimental site.

Experimental site	Tillers (n.)	Nodes (n.)	Plant height (cm)	Spike length (cm)
Alia	3.3 ± 0.13 c	3.3 ± 0.1 b	40.1 ± 0.9 c	13.9 ± 0.1 b
Caltanissetta	2.0 ± 0.2 d	3.3 ± 0.1 b	39.1 ± 0.5 c	6.9 ± 0.2 c
Castelvetrano	5.2 ± 0.3 a	4.5 ± 0.2 a	78.8 ± 2.1 a	17.5 ± 0.2 a
Marsala	4.4 ± 0.1 ab	4.7 ± 0.1 a	46.4 ± 0.6 b	18.1 ± 0.5 a
Sparacia	3.5 ± 0.1 bc	3.0 ± 0.1 b	41.1 ± 1.6 bc	7.3 ± 0.1 c
Significance	0.000	0.000	0.000	0.000

Note: Means and standard errors are shown. Values followed by a different letter are significantly different at $p \leq 0.05$, under Tukey's test.

TABLE 2 | SPAD and RWC values for phenological stage and for each experimental site.

Experimental site	SPAD 29 BBCH	SPAD 39 BBCH	SPAD 69 BBCH	RWC 39 BBCH (%)	RWC 69 BBCH (%)
Alia	47.8 ± 0.5 b	52.4 ± 0.8 a	47.6 ± 1.0 bc	76.0 ± 0.9 b	60.5 ± 1.7 ab
Caltanissetta	42.5 ± 0.5 c	45.8 ± 1.3 b	34.2 ± 2.2 d	56.2 ± 1.5 c	54.6 ± 1.0 b
Castelvetrano	51.1 ± 0.1 a	54.0 ± 0.9 a	53.0 ± 0.4 ab	88.1 ± 1.4 a	64.1 ± 0.5 a
Marsala	51.9 ± 0.7 a	54.0 ± 1.2 a	54.3 ± 1.0 a	80.3 ± 2.5 b	62.6 ± 2.2 a
Sparacia	45.9 ± 0.8 b	52.2 ± 1.9 a	42.4 ± 0.5 c	65.1 ± 1.3 c	55.0 ± 1.3 b
Significance	0.000	0.007	0.000	0.000	0.003

Note: Means and standard errors are shown. Values followed by a different letter are significantly different at $p \leq 0.05$, in accordance with Tukey's test.

3.4 | Physiological Indicators

The SPAD readings acquired during the three phenological stages (29, 39, 69 BBCH) showed highly significant differences among the experimental sites (Table 2). In all measurement stages, the highest SPAD units were recorded at the Marsala experimental site (51.9; 54.0; 54.3 SPAD units), which, at stage 29 BBCH, did not show statistical differences compared to the values measured at the Castelvetrano site (54.0 SPAD units) and the Sparacia site (52.2 SPAD units), and at stage 69 BBCH, did not show differences with the values measured at the Castelvetrano site (53.0 SPAD units). The lowest SPAD values, however, were always measured at the Caltanissetta experimental site (42.5; 45.8; 34.2 SPAD units).

The RWC measured at stages 39 and 69 BBCH also showed statistically significant differences. The RWC values ranged from 56.2% to 88.1% during stage 39 BBCH and 55.0% to 64.1% during stage 69 BBCH. The highest value in both stages was recorded in plants cultivated at the Castelvetrano experimental site (88.1% at 39 BBCH; 64.1% at 69 BBCH), which, at stage 69 BBCH, did not differ from the values measured at the Marsala site (62.6%) and the Alia site (60.5%). The lowest average RWC values were consistently measured at the Caltanissetta (52.6% - BBCH 39; 54.6% - 69 BBCH) and Sparacia (52.6% - BBCH 39; 65.1% - 69 BBCH) sites.

During the two dates corresponding to the phenological stages 39 and 69 BBCH, the NDVI was measured (Figure 4), with values showing statistically significant differences among the experimental sites. The NDVI ranged from 0.5 to 0.8 for the first measurement date (39 BBCH) and from 0.3 to 0.8 for the second measurement date (69 BBCH). The highest values were recorded at the Castelvetrano and Marsala sites on both dates. Meanwhile, at stage 39 BBCH, the other sites showed statistically similar and lower NDVI values. At 69 BBCH stage, there was greater variability among the sites, with the lowest values recorded at the Caltanissetta site (0.3).

3.5 | Yield and Yield Components

The yield and its components showed highly significant differences among the experimental sites, except for the total number of spikelets per spike (TSS) and the harvest index (HI) (Table 3).

The highest number of spikes per m^2 (Spikes) was measured at Castelvetrano (55.7) and Marsala (202.3), with no statistical differences between them. The lowest number of spikes was recorded at Caltanissetta (55.7). The highest number of fertile spikelets (FSS) was obtained at Castelvetrano (18.5), Marsala (18.4), Alia (17.3), and Sparacia (17.0), with no statistical differences among them; while the lowest number was recorded at Caltanissetta (15.1). Regarding the yield (GY), the highest was obtained at Castelvetrano (27.0 q ha^{-1}) and Marsala (25.7 q ha^{-1}), showing a 37.0% lower yield than the one obtained at Caltanissetta (10.0 q ha^{-1}), which also stood out for its lower 1000 grain weight (TGW) (34.5 g). The highest TGW was recorded at Castelvetrano (50.2 g), Marsala (47.3 g), and Alia (44.1 g), with no statistical differences among them. The highest biomass yield (BY) was measured at Castelvetrano (97.0 q ha^{-1}), which did not differ statistically from the yield measured at

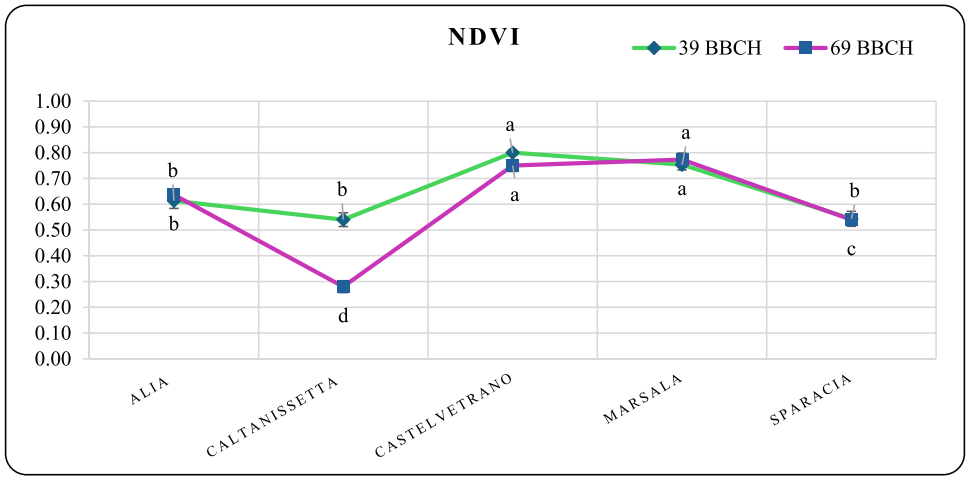


FIGURE 4 | NDVI values collected in two phases (39 e 69 BBCH) for each experimental site. Means and standard errors are shown. Values followed by a different letter are significantly different at $p \leq 0.05$, in accordance with Tukey's test.

TABLE 3 | Yield components for each experimental site.

Experimental site	Spikes (n. m^{-2}]	TSS (n)	FSS (n)	GY (q ha^{-1})	TGW (g)	BY (q ha^{-1})	HI (%)
Alia	110.7 ± 0.7 b	18.8 ± 0.4 a	17.3 ± 0.3 a	18.4 ± 0.5 b	44.1 ± 1.3 a	60.6 ± 4.8 bc	30.8 ± 2.9 a
Caltanissetta	55.7 ± 0.3 d	18.0 ± 0.4 a	15.1 ± 0.4 b	10.0 ± 0.1 c	34.5 ± 1.4 b	39.1 ± 2.8 d	25.8 ± 1.8 a
Castelvetrano	199.3 ± 4.6 a	19.0 ± 0.3 a	18.5 ± 0.2 a	27.0 ± 2.0 a	50.2 ± 0.5 a	97.0 ± 7.0 a	28.1 ± 2.8 a
Marsala	202.3 ± 4.8 a	18.9 ± 0.6 a	18.4 ± 0.5 a	25.7 ± 2.1 a	47.3 ± 4.1 a	80.7 ± 2.7 ab	32.0 ± 3.0 a
Sparacia	83.7 ± 1.9 c	18.0 ± 0.7 a	17.0 ± 0.2 a	16.0 ± 0.3 bc	41.3 ± 0.3 ab	42.4 ± 3.3 cd	38.2 ± 3.3 a
Significance	0.000	0.437	0.000	0.000	0.000	0.000	0.087

Note: Means and standard errors are shown. Values followed by a different letter are significantly different at $p \leq 0.05$, in accordance with Tukey's test.
Abbreviations: BY = biomass yield, FSS = fertile spikelets per spike, GY = grain yield, HI = harvest index, TGW = thousand grain weight, TSS = total spikelets per spike.

Marsala (80.7 q ha^{-1}). The lowest BY, as for the other parameters, was recorded at Caltanissetta (39.1 q ha^{-1}).

3.6 | Correlation Between Yield and Physiological Parameters

From the estimation of Pearson's correlation coefficients between yield and the SPAD, RWC, and NDVI measurements from the five experimental sites for the two observation times (39 BBCH and 69 BBCH), very strong positive correlations emerged in both growth stages between each calculated index and yield (Figure 5).

During the 39 BBCH phase, the highest correlation with yield was highlighted by RWC ($r = 0.96$), followed by NDVI ($r = 0.95$) and SPAD ($r = 0.89$). In contrast, during the 69 BBCH phase, the strongest correlation with yield was observed with the average SPAD value ($r = 0.98$), followed by NDVI ($r = 0.96$) and RWC ($r = 0.94$).

3.7 | Quality Parameters

Of the quality traits analyzed, only the test Weight did not show statistically significant differences (Table 4). The highest protein content was measured in the grain from the Caltanissetta site (15.8%), which, however, did not differ statistically from the grain from the Sparacia (14.3%) and Alia (14.2%) sites. The lowest protein content was measured in the grain from the Castelvetrano (13.1%) and Marsala (13.6%) sites. The highest yellow index was recorded in the grain from the Castelvetrano site (24.3%), which did not differ statistically from the grain from the Marsala (24.0%), Sparacia (23.9%), and Alia (23.8%) sites. The lowest value was recorded in the grain from the Caltanissetta site (22.9%). Finally, the highest gluten percentage was found in the grain from the Caltanissetta site (11.9%), while the lowest was in the grain from the Alia (9.0%), Castelvetrano (9.5%), and Marsala (8.8%) sites. Regarding the gluten percentage from Sparacia (10.0%), it did not differ statistically between the higher and lower values.

3.8 | Correlation Between Yield and Quality Parameters

The Pearson linear correlation analysis, performed between yield and each qualitative parameter that showed significance in the ANOVA (proteins, yellow index, and dry gluten), is presented in Figure 6. The calculated Pearson coefficient indicates a strong negative correlation ($r = -0.96$) between yield and proteins, and between yield and dry gluten ($r = -0.81$),

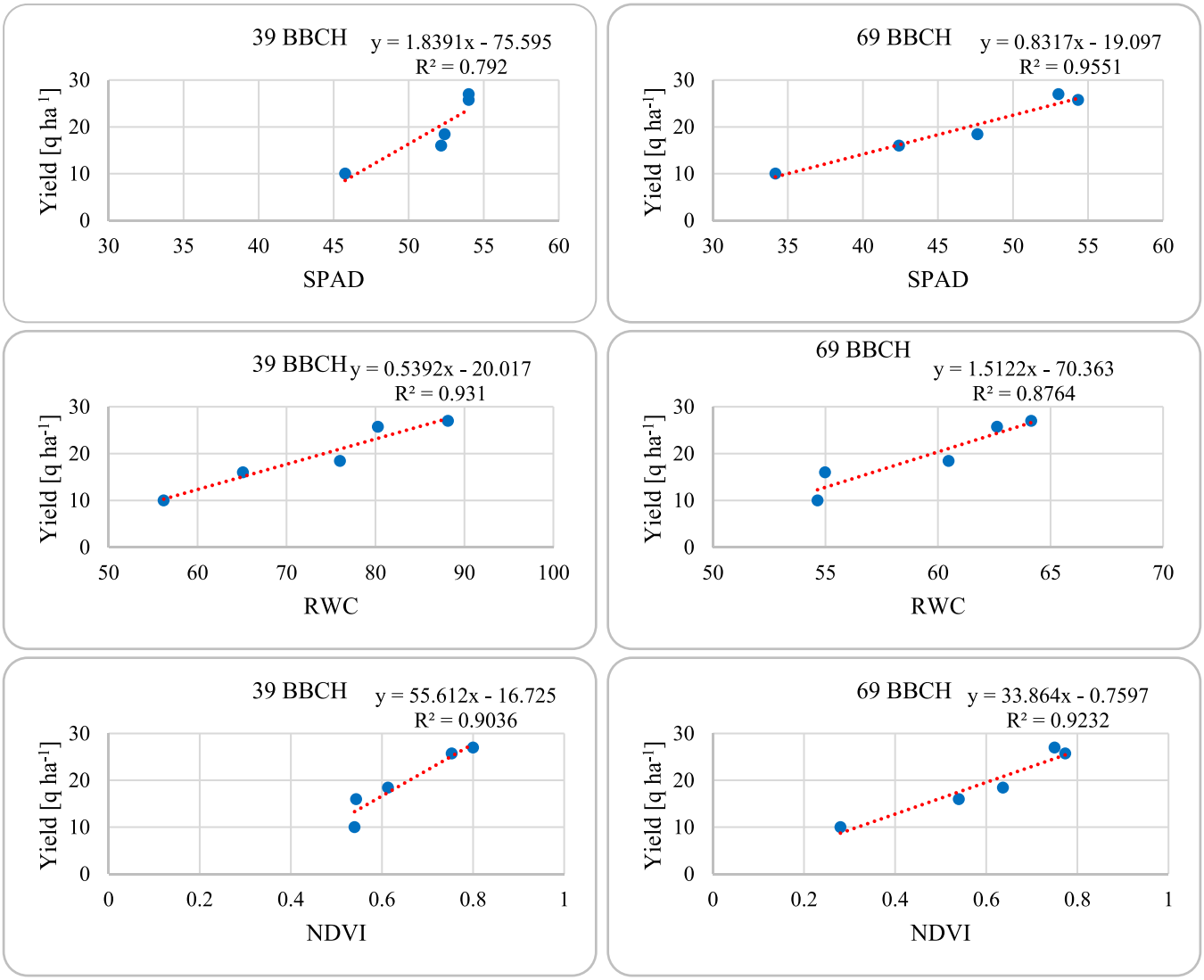


FIGURE 5 | Relationship between wheat grain yield (q ha^{-1}) and SPAD, RWC, NDVI in two phases (39 e 69 BBCH).

TABLE 4 | Quality traits for each experimental site.

Experimental site	Protein (%)	Yellow index (%)	Test weight (kg hL^{-1})	Dry gluten (%)
Alia	14.2 ± 0.6 ab	23.8 ± 0.0 ab	79.6 ± 1.3 a	9.0 ± 0.9 b
Caltanissetta	15.8 ± 0.2 a	22.9 ± 0.1 b	79.7 ± 0.2 a	11.9 ± 0.1 a
Castelvetrano	13.1 ± 0.4 b	24.3 ± 0.4 a	78.6 ± 1.7 a	9.5 ± 0.3 b
Marsala	13.6 ± 0.5 b	24.0 ± 0.1 ab	79.6 ± 0.5 a	8.8 ± 0.4 b
Sparacia	14.3 ± 0.4 ab	23.9 ± 0.4 ab	75.6 ± 1.1 a	10.0 ± 0.3 ab
Significance	0.016	0.028	0.102	0.009

Note: Means and standard errors are shown. Values followed by a different letter are significantly different at $p \leq 0.05$, in accordance with Tukey's test.

while a strong positive correlation is found between yield and the yellow index ($r = 0.87$).

4 | Discussion

Durum wheat in the Mediterranean grows under rainfed conditions, where unpredictable climatic conditions cause significant variability, both spatially and temporally, in wheat yield and quality (Kyratzis et al. 2022). It is well-established that, despite durum wheat being considered one of the most drought-tolerant cereal crops and therefore typically grown in hot-arid environments, it can suffer severe impacts due to water stress (De Vita and Taranto 2019; Kaddour and Fuller 2004), especially when water scarcity is accompanied by high temperatures (Cetin et al. 2022; De Vita and Taranto 2019; Bożek

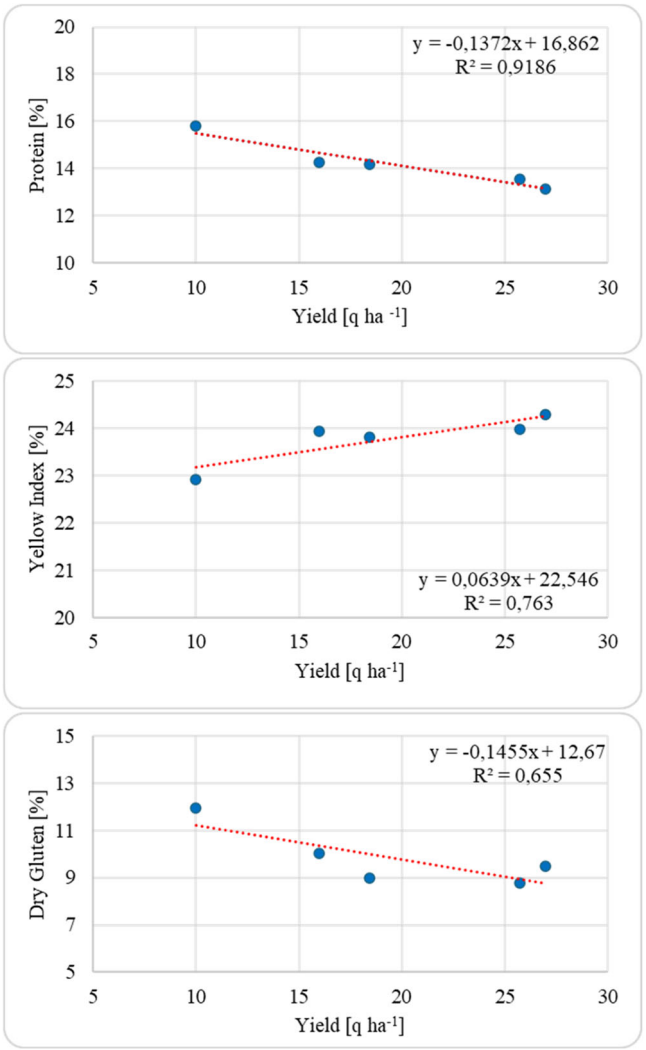


FIGURE 6 | Relationship between wheat grain yield (q ha^{-1}) and quality parameters (%).

et al. 2021; Chaparro-Encinas et al. 2021). Thus, temperature and water availability are the two dominant and often limiting factors in durum wheat growing areas, significantly influencing its morpho-physiological, biochemical, and agronomic parameters, as highlighted by many studies (Liu et al. 2015; El Idrissi et al. 2023; Habash et al. 2009; Pour-Aboughadareh et al. 2020; Amal et al. 2020; Prajapat et al. 2022). In line with these previous findings, the present experiment recorded statistically significant differences for almost all phenological, morphological, physiological, and productive parameters observed on the same genotype grown in five environments across Sicily, representing the pedo-climatic variability of the territory where durum wheat is typically cultivated.

The phenology of durum wheat is considered an important component in the crop's adaptation to local environmental conditions. A positive correlation between the length of the growing season and the duration of phenological stages with wheat yield is well-established (Kyratzis et al. 2022; Pour-Aboughadareh et al. 2020; Liu et al. 2016; Camargo et al. 2016). At the sites of Castelvetrano, Marsala, and Caltanissetta, the cycle was completed in 173 and 177 days from sowing, with a similar total GDD accumulation (2593.9 in Castelvetrano, 2608.0 in Marsala, and 2408.7 in Caltanissetta). In Caltanissetta and Sparacia, extreme conditions, such as high temperatures and low precipitation, led to a reduction in the flowering-maturation period (about 10 days). Additionally, at Sparacia, a reduction in the tillering-flowering period was also observed, contributing to the lowest GDD value (2059.6 GDD) compared to the other sites. The shortening of these stages observed at Sparacia can also be attributed to the late sowing (December 20), which shifted the timing of the most critical phenophases (from flowering to maturity) into periods characterized by higher temperatures (Sattar et al. 2023). In fact, it is well established that thermal stress caused by high temperatures during these stages accelerates phenology due to the early aging of leaves, resulting in a shortened growth period (Figueiredo et al. 2015; Aslam et al. 2017; Salazar-Gutierrez et al. 2013; Sattar et al. 2020). Additionally, limited water availability contributes to reducing the period in which the leaf area is physiologically active (Pal et al. 1996). As a result, the heat unit (GDD) requirement to reach different phenological stages is also reduced (Sikder 2009). On the other hand, due to lower average Tmin (3.5°C), Sparacia also had the longest vegetative period (90 days from sowing), but with a GDD accumulation similar to other sites. The Alia site, however, sown at a normal time but earlier than the others (November 20), completed the cycle in 190 days with a GDD accumulation of 2415.2, lower than sites like Castelvetrano and Marsala. Indeed, during the initial growth phase, when soil water content is high, the most limiting factor to a delayed start could be soil temperature, which likely contributed to reduced biomass and yield during this season (Magney et al. 2016). Similar results have been found in other studies where the growing degree days demand and thermal effects on growth and yield were evaluated in crops sown under different sowing and irrigation conditions (Prajapat et al. 2022; Khichar and Niwas 2007; Paul and Sarker 2000).

In line with the growth conditions, the morphological traits considered showed strong variations due to environmental influences, as also reported by other studies (Pour-Aboughadareh et al. 2020; Liu et al. 2016; Zhao et al. 2007; Prasad and Jagadish 2015). The meteorological conditions that occurred at Castelvetrano (average Tmin of 9.0°C and average Tmax of 17.0°C; precipitation about 189.0 mm) during the initial period allowed for optimal vegetative development, with an average number of tillers, an average number of nodes, average culm height, and average spike length of 5.0, 5.0, 79.0 cm, and 17.5 cm, respectively. In other sites, however, the plants showed reduced development. In Marsala, despite the number of nodes being similar to Castelvetrano, the internodes were poorly developed; in fact, the culm had an average length of 46.0 cm. The spike length, however, did not follow the same trend as the culm, with spike length values (18.1 cm) similar to those of Castelvetrano. This contrasts with what was reported by Yagdi and Sozen (2009), where there is a positive correlation between plant height and spike length. As shown in other studies (Aslam et al. 2017; Cetin et al. 2022; Prajapat et al. 2022), the different behavior observed during this growing year can be attributed to the water deficit recorded during the early stages of the cycle. In fact, in our environments, temperatures during the early growth phases are not a limiting factor, as it is generally considered that durum wheat benefits from an optimal temperature range of

17.0–23.0°C throughout the entire growing season, with T_{min} of 0.0°C and T_{max} of 37.0°C, beyond which growth stops (Porter and Gawith 1999). Conversely, water availability during this period has a positive correlation with the emission of tillers, the number of nodes, and the subsequent stem elongation (Grosse-Heilmann et al. 2024; Urbanavičiūtė et al. 2022; Zhao et al. 2020; Pour-Aboughadareh et al. 2020). Even short recurring drought events during the vegetative phase, such as those occurring at the sites considered in this study, lead to negative effects on durum wheat plant development (Urbanavičiūtė et al. 2022).

In this study, RWC was used to determine the level of leaf hydration compared to the fully turgid condition (Patanè et al. 2022). It is, in fact, an important indicator of the crop's water status and its stress response (Larbi and Mekliche 2004), and it also has a positive correlation with yield (El Jaafari 2000).

The water stress observed during the two sampling periods (39 BBCH and 69 BBCH) had a negative effect on RWC values at all the experimental sites studied, causing reductions ranging from 12.0% to 44.0% in the first sampling period and from 36.0% to 45.0% in the second. The highest RWC values recorded at Castelvetrano (88.1%) during the first sampling period could be attributed to the frequent rainfall recorded during this period (about 173.0 mm of rain between January and February). The lowest RWC values in this phase were recorded at Caltanissetta and Sparacia (56.2% and 65.1%, respectively). In the second sampling period, all sites had low values, with the highest values recorded at Marsala and Castelvetrano (62.6% and 64.1%, respectively). This is consistent with results from previous studies on the same crop and in similar environments, where the response to different water regimes was assessed (Saghouri el idrissi et al. 2023; Larbi and Mekliche 2004). These studies showed that reduced water availability is always accompanied by a reduction in the RWC of plant tissues, but the variation depends on the genotype (Larbi and Mekliche 2004) and its ability to limit water loss through the leaf epidermis when stomata have reached their minimum opening (El Jaafari 2000). The differences in RWC observed across sites may also be due to varying water absorption capacities from the soil (Keyvan 2010). Additionally, as observed in other crops, the reduction in RWC in plants under drought stress can be due to a reduction in plant vigor (Liu et al. 2002).

Similarly, there was a decrease in leaf chlorophyll content (SPAD) at sites characterized by inadequate rainfall, with variable results depending on the stress level. Chlorophyll content in leaves, measured indirectly through SPAD readings, is an indicator of the photosynthetic capacity of plant tissues (Majid Khayatnezhad 2012), which is also correlated with water availability. SPAD values ranged from 42.5 to 51.9 SPAD units at 29 BBCH, 45.8 to 54.0 SPAD units at 39 BBCH, and 42.4 to 54.3 SPAD units at 69 BBCH, with the highest values generally coming from Castelvetrano and Marsala, while the lowest came from Caltanissetta. Various plant species, including durum wheat, under drought stress conditions have shown similar changes, and the intensity of these changes depended on the stress rate and its duration (Gholamin and Khayatnezhad 2011; Shahryari et al. 2011; Condorelli et al. 2018; Saghouri el idrissi et al. 2023; Bányai et al. 2017). The decrease in chlorophyll content is also a consequence of stomatal closure to limit water loss through transpiration, but at the same time, the entry of atmospheric CO_2, which is necessary for photosynthesis, is reduced (Saghouri el idrissi et al. 2023).

Furthermore, the NDVI derived from the active proximal sensor Rapidscan, calculated at the five experimental sites at the 39 and 69 BBCH stages, showed values consistent with the previously calculated indices on the same dates. Previous studies have reported positive correlations between these, highlighting the relationship between hydration and photosynthetic activity (Kyratzis et al. 2017; Roy et al. 2023; Sarlikioti et al. 2010). NDVI is closely related to a range of biomass-related and interrelated variables such as Leaf Area Index (LAI), leaf coverage, chlorophyll per unit area, green biomass, or the Green Vegetation Index (Aguilar et al. 2012). It is also widely used in cereal crops to estimate yield under stress conditions (Kyratzis et al. 2017).

The very low NDVI values recorded reflected the stress levels the plants were subjected to at the different sites, as reported by other studies (Aranguren et al. 2020). Furthermore, in our study, the values reported for both sampling dates were always lower than the threshold values (0.7-0.8) that have been identified for these stages (late stem elongation and flowering) in durum wheat genotypes grown in Mediterranean climatic conditions (Aranguren et al. 2020). The exceptions were Castelvetrano and Marsala, where the computed values were similar to these threshold values; however, the yields were still low. As some studies suggest, high NDVI values during the stem elongation phase due to greater biomass accumulation do not always translate into higher yields (Magney et al. 2016). In fact, yield heavily depends on the remobilization of assimilates acquired during vegetative growth towards the grain (Villegas 2001) and nitrogen mobilized from leaves and stems to the seeds. These mobilizations are largely driven by meteorological conditions post-anthesis (Magney et al. 2016).

Since each phenological period influences the yield components of durum wheat, the stresses that occur have different effects depending on their intensity and duration at each growth stage (Aranguren et al. 2020), making the NDVI-yield relationships complex. Recent studies (Aranguren et al. 2020; Ravier et al. 2017) report that the dynamics of NDVI values during the wheat growth cycle vary to obtain a specific yield. In fact, Wang et al. (2014) suggest that NDVI values measured from early growth stages to anthesis represent the growth trend of the plants that then translate into yield in wheat.

As described in the previous results, the analysis of ANOVA showed that the different environmental conditions at the five experimental sites had a marked impact on durum wheat yield and the components that determine it. A maximum yield difference of 37.0% was observed, with the highest yields coming from the Castelvetrano and Marsala environments (25.7–27.0 q ha^{-1}), followed by Alia (18.4 q ha^{-1}), Sparacia (16.0 q ha^{-1}), and finally Caltanissetta (10.0 q ha^{-1}). The reproductive phase, which spans from anthesis to grain filling, represents a critical period during which high temperatures and drought can lead to significant yield losses (Porter and Gawith 1999; Zampieri et al. 2017; Beres et al. 2020; Pour-Aboughadareh et al. 2020; El Idrissi et al. 2023), as well as influence grain quality (Cetin et al. 2022). High temperatures and water

shortage during this period can trigger a series of physiological changes, such as photosynthetic efficiency, metabolism, nutrient transport, as well as cause oxidative damage, pollen sterility, and leaf senescence (De Santis et al. 2021; Liu et al. 2015; El Idrissi et al. 2023).

These phenomena result in accelerated crop development, particularly leading to a shortening of the grain filling period, which negatively impacts the number of spikes per m^2, number of kernels per spike, thousand-grain weight, and biomass, thus reducing the harvest index (HI) (Pour-Aboughadareh et al. 2020; Liu et al. 2015; Valizadeh et al. 2014; Aslam et al. 2017). The variability of the results depends on the complex interaction between the environment, its components, and the genotype (Pour-Aboughadareh et al. 2020), as well as the duration and intensity of stress (Gholamin and Khayatnezhad 2020; Habash et al. 2009; Balla et al. 2019). According to several authors (Cetin et al. 2022; Kheiri et al. 2021), even increases of 1.0°C in the average seasonal temperature would cause yield decreases between 2.5% and 7.0%, due to a shortening of the crop cycle and a reduction in the reproductive period. In our environments, in addition to the heat stress normally occurring from anthesis to grain maturation, the thermal shock experienced by the crops during May at the Sparacia and Caltanissetta sites drastically contributed to the reduction in yields. In fact, the average weight of kernels, a component that determines yield and could compensate for the smaller number of grains produced earlier, was the lowest (41.3-34.5 g). These results are confirmed by other studies (Li et al. 2013; Balla et al. 2019; Aslam et al. 2017), where sudden and extremely high temperatures, lasting 3–5 days and exceeding 32°C during the maturation period, significantly decreased most yield traits. Similarly, moderate to severe water stress has negative effects on the final seed size (Zhao et al. 2020, Gaile et al. 2017). Other authors, however, argue that the weight of 1000 grains (or the weight of a single grain) is a cultivar-dependent characteristic and remains relatively stable (Zwer et al. 1995; Abdoli and Saeidi 2012; Li et al. 2016). Regarding the number of spikelets per spike, several studies assessing different accessions in various Mediterranean environments have shown that this component was less affected by environmental conditions compared to the number of spikes per m^2 (Kilic and Yağbasanlar 2010; Subira et al. 2015). However, once flowering began, a temperature of 27.0°C also led to a high percentage of sterile spikelets, as reported by Wheeler et al. (1996).

The strong positive correlations between wheat yield and the physiological indices studied are in line with previous studies (Wang et al. 2014; Kyratzis et al. 2017; Roy et al. 2023). However, contrary to our results, some authors (Lopes et al. 2012; Yousfi et al. 2016) argue that vegetation indices (VI) are better predictors of wheat yield under stress conditions than SPAD measurements under heat and/or drought conditions. Nevertheless, supporting our results, other authors have stated that vegetation indices are likely more successful under moderate conditions rather than under severe stress (Babar et al. 2006). A strong association between indices measured at one site and the yield of the same genotypes at another site would suggest that these indices could be used to predict yield in different environments.

Finally, even the desirable quality parameters for the industry, such as yellow pigment concentration, protein content, and gluten content, varied under the influence of the environment, except for test weight. As also demonstrated by our results, yield and protein content are inversely correlated, and similarly to protein content, gluten content follows the same trend due to a higher concentration (De Santis et al. 2021; Gaile et al. 2017; Arduini et al. 2018; Guzmán et al. 2016). Water shortage during maturation reduces starch accumulation and, therefore, durum wheat yields, while simultaneously increasing protein concentration (Brown et al. 2005). In the present study, the highest protein and gluten content (15.8% and 11.9%, respectively) were recorded in the treatments with the lowest yields. Similarly, yellow pigment content tends to increase under water or heat stress due to a concentration effect of carotenoid pigments (Arduini et al. 2018; Li et al. 2013); however, severe heat stress, such as the thermal shocks occurring in May, can negatively affect carotenoid pigment synthesis (Guzmán et al. 2016), and this is in line with our results, in which the Pearson correlation analysis suggested a positive relationship between yield and the yellow index.

As for test weight, although some studies (Dexter et al. 1989; Wang and Fu 2020) have reported a gradual decrease in test weight as seed size decreases, in our case, the lack of a significant difference between sites for this parameter may be due to the higher density of smaller seeds, which were therefore more compact, as described by Wang and Fu (2020).

5 | Conclusion

Our study showed how climatic variability in the different experimental sites led to completely different productive and qualitative results. Low precipitation and increased air temperature, events more pronounced in the experimental sites located in the inland areas of Sicily (Alia, Caltanissetta, and Sparacia), resulted in shortened phenological phases with negative impacts on both yield and quality of durum wheat grain. Monitoring based on physiological stress indices allowed us to understand some physiological phenomena and how these are linked not only to productivity but also to the growth traits of the crop. As expected, the low yields led to increased protein and gluten content, but these gains cannot justify the low production.

In the context of sustainability and meeting the agricultural policies goals, the authors emphasize the need to develop sustainable and transferable agronomic solutions for farming systems, such as the use of tolerant varieties, irrigation methods and programming, innovative fertilizers and biostimulants, and the use of unconventional waters, capable of mitigating and addressing the ongoing crisis in areas most affected by climate change, such as Sicily. The development of such strategies requires a synergistic effort between research institutions, policymakers, and agricultural entrepreneurs.

Author Contributions

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analysis: Nicolò Iacuzzi, Noemi Tortorici and Teresa Tuttolomondo. Investigation: Noemi Tortorici, Antonella Iurato and F.A. Resources: Teresa Tuttolomondo and Mauro Sarno. Data curation: Noemi Tortorici and Nicolò Iacuzzi. Writing – original draft preparation: Noemi Tortorici and Nicolò Iacuzzi. Writing – review and editing: Nicolò Iacuzzi, Antonio Giovino, Teresa Tuttolomondo and Mauro Sarno. Visualization: Teresa Tuttolomondo, Antonella Iurato and Federica Alaimo. Supervision: Teresa Tuttolomondo and Mauro Sarno. Project administration: Teresa Tuttolomondo and Nicolò Iacuzzi. Funding acquisition: Antonio Giovino and Mauro Sarno. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.