**Performance of Six Genotypes of Tritordeum Compare to Bread Wheat under East Mediterranean Condition**

Ioanna Kakabouki 1,*, Dimitrios F. Beslemes 2, Evangelia L. Tigka 3, Antigolena Folina 1, Stella Karydogianni 1, Charikleia Zisi 1 and Panagiota Papastylianou 1

1 Laboratory of Agronomy, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; foldini@gmail.com (A.F); stella.karidogianni@hotmail.com (S.K); xarikleiazisi@gmail.com (C.Z); pppapastyl@aua.gr (P.P)

2 Research & Development Manager Alfa Seeds ICSA/Seed Production and Commerce/10th km Mesorachis-Ag. Georgiou, 41500 Larisa, Greece; dbeslemes@alfa-seeds.gr

3 HAO Demeter, Institute of Industrial and Fodder Crops, 1, 41335 Larissa, Greece; evitiga@yahoo.gr

* Correspondence: i.kakabouki@gmail.com

Received: 9 October 2020; Accepted: 18 November 2020; Published: 20 November 2020

**Abstract:** Four advanced tritordeum lines were studied and compared to two commercial varieties of tritordeum and wheat cultivars in yield and quality features, in Greece. For this purpose, a two-year experiment was established in the Greek territory. The field experiment was set up in Randomized Complete Block Design (RCBD) with blocks, with different lines and varieties. Head emergence was calculated based on Growing Degree Days (GDDs), which was significantly affected by the year. The significance of differences between treatments was estimated by using Fisher’s least significant difference (LSD) test with significance level $p = 0.05$. The weight of 1000 seeds of the two commercial varieties differed from the equivalent weight of 1000 seeds of tritordeum lines, approximately 1 g. Yield was significantly affected by lines/varieties and year. Compared to tritordeum yield, wheat yield marked the highest values. This difference was almost 2 kg ha$^{-1}$. Gluten content was significantly affected by lines/varieties. The highest gluten content was firstly noticed at wheat GENESIS (34.2%) variety and secondly at tritordeum Aucan (33.2%) variety. Protein was higher in tritordeum lines HT-1704 (15.5%), HT-1707 (15.1%) and Aucan variety (15.2%) during the first experimental year. Tritordeum seems to have significant adaptability to dry conditions in Greece and significant yields compared to the existing commercial varieties and bread wheat.

**Keywords:** tritordeum; GDDs; relative production efficiency; gluten content

---

**1. Introduction**

Cereals are considered an important source of basic and energy-promoting ingredients [1]. Tritordeum (*Tritordeum Ascherson et Graebner*) is a man-made cereal deriving from the cross of wheat and barley. Particularly, tritordeum, is a fertile amphipliod resulting from the cross between wild relatives of *Hordeum vulgare* (*Hordeum chilense* Roem. Et Schultz) and cultivated durum wheat (*Triticum turgidum* ssp. Durum Desf.), the latter used as the maternal parent [2,3]. *Hordeum chilense* Roem. Et Schultz is a wild diploid barley originating from South America, that is included in the Anisolepis section [3]. More than 250 tritordeum primary lines exist today [4].

Tritordeum is considered as a genetic bridge transferring the useful properties of barley and wheat, such as storage proteins, carotene content [5,6], as well as drought and salinity tolerance [7]. Erlandsson et al. (2010) stated that through the cross of barley and wheat, humans sought to preserve the nutritional and technological characteristics of each species respectively [4]. According to several
studies, tritordeum is considered as a novel crop due to high levels of protein and carotene [6,8–10]. Additionally, tritordeum marks high adaptability in the Mediterranean basin [7].

In European-type diets, wheat breads occupy an important place, while nowadays in Asia and Africa, wheat products are increasingly being replaced [11]. Furthermore, protein content of cereals is a determining factor affecting the end product’s quality [5]. It is reported that tritordeum has a higher protein and gluten content compared to mother plant–durum wheat [5]. Thus, if gluten–protein ratio is increased, grain quality will be reduced as the end product will contain more gluten than protein. In performing a voluntary test to compare tritordeum-based diets with wheat-based diets, a remarkably significant lower secretion of gluten peptides in tritordeum based diets was indicated. However, it must be underlined that tritordeum-based diets are not suitable for people with celiac diseases [11]. In summary, tritordeum was eventually created to replace wheat due to its higher nutritional value as well as its lower gluten levels.

Villegas et al. reported that there is still room to improve yields by changing crop growth under Mediterranean conditions [7]. In addition, the combination of climate change and low genetic variability in crops such as wheat, rice, and maize leads to the vulnerability of agriculture [12,13]. The main effect of climate change in the Mediterranean region is reported to be drought, the main abiotic stress factor affecting the cereal crops [14,15]. More specifically, during the grain filling stage, warm and dry conditions lead to reducing the photosynthetic rate following the flowering stage. As a result, the contribution of current assimilates to the grain is limited [16].

In addition, tritordeum revealed higher stomatal conductance and photosynthesis under water-deficit conditions [17]. The first lines of tritordeum indicated better-quality characteristics such as long grain and high protein content but also lower agronomic yield than durum wheat [4]. According to Barro et al. (1996), tritordeum indicates a point of compensation of low light and a rate of dark breathing [18]. Therefore, it is feasible to cultivate tritordeum in the Mediterranean region, due to its drought resistance.

In 1730, growing degree days (GDDs) were used as an ecosystem indicator to study the daily temperature fluctuations and their effect on plant growth stages. This way, GDDs formed the basis for developing the future crops in various climates [19] and are considered a way of attributing to the daily heat values [20]. GDDs index play a key role in predicting the phenophasic duration for each crop, and therefore crop productivity [21]. According to Liu et al. (2012), GDDs represent the amount of heat that is required by the crops in order to develop [22]. In addition, GDDs index is used to quantify the effects of temperature as well as to describe the duration of biological processes [23]. Therefore, GDDs are used for determining the o sown date, growth period, and the crops’ physiological characteristics [19,24,25]. In several studies, GDDs have been used to also define crop development models for local areas [22]. Regarding the relationship between head appearance and temperature in cereal cultivation, flowering has a critical threshold. If this critical point is exceeded, it will lead to decreasing yield [26]. For instance, wheat is reported to have a strongly correlated phenology with temperature [27].

Complete and successful comparison of crops requires the use of certain economic indicators. One such indicator is the Relative Production Efficiency (RPE), which expresses the dynamics of the crop or the production system compared to the existing one [28]. Moreover, RPE provides information on the potential gains that each improvement can bring [29]. It is mainly used for comparisons between crops or crop systems and is mainly influenced by yields. Furthermore, there is general consensus that farmer’s income is a critical factor to choose a suitable crop for a region, based on yield.

The present study was designed to compare lines and two varieties of tritordeum with two varieties of wheat under Greece’s dry conditions. In spite of the fact that there is enough literature concerning the quality of tritordeum products, there is no similar data in relation to wheat. This study initially presents the comparison of quality among wheat, tritordeum cultivars, and advanced lines of tritordeum.
International literature provides information on the relationship between GDDs and development of cereal cultivation. In addition, most researches focus on their effect on yield and finding the suitable sowing season. Hence, there is a lack of data regarding GDDs’ tritordeum until head emergence stage. This study set out to compare tritordeum lines and varieties with two varieties of bread wheat, as well as to correlate growth characteristics with GDDs in Mediterranean dry conditions.

2. Materials and Methods

2.1. Experimental Design

Advanced lines of hexaploid tritordeum (HT-460, HT-1704, HT-1707 and HT-15-54-32), two tritordeum varieties (Aucan and Bulel), and wheat varieties (Genesis and Falado) were evaluated for two growing periods through 2018–2020. The experimental area was located 20 km west of Larisa (Latitude: 39°29′17.2″ N, Longitude: 22°21′22.8″ E, Altitude: 120 m) in Central Greece. The soil was clay (C), and the mean annual precipitation at the site was approximately 500 mm. The mean soil characteristics (at 0–25 cm sampling depth) of the experimental plot are illustrated in Table 1. Weather data (rainfall and average temperature) of the experimental site is presented in Figure 1. Former crop cultivation, before the first experimental year, was cotton.

Table 1. Soil Properties.

| Physical Composition (%) | Exchangeable Bases (cmol kg$^{-1}$) |
|--------------------------|-------------------------------------|
| sand                     | 23                                  | Na 0.37 |
| Silt                     | 24                                  | K 1 |
| Clay                     | 53                                  | Ca 28 |
| Textural class           | Clay                                |

| Chemical characteristics |
|--------------------------|
| pH (H$_2$O 1:1) (25 °C)  | 7.4 | Total N (Kjeldahl) (g/100g) | 0.11 |
| Organic Matter (%)       | 1.4 | CaCO$_3$                      | 1 |
| ($P_{Olsen}$) (mg/kg)    | 15  | EC 524                         |

Figure 1. Meteorological Data at Experimental Area for Growing Seasons 2018–2019 and 2019–2020. The Total Precipitation Was 399 MM and 343 MM, Respectively, for Two Seasons.

The field experiment was set up in RCBD where blocks were lines and varieties. The range of experimental area was 320 m$^2$, which was devised in 4 blocks that consisted of 8 plots (100 m$^2$) each. Each plot had 10 rows. Sowing took place in 12/12/2018 and 19/12/2019 using a HALDRUP SP-25 plot seeder. Seed rate was adjusted to 420 viable seeds m$^{-2}$. The row spacing was 15 cm. Soil tillage encompasses agronomic chisel plow at a depth of 40 cm, followed by secondary tillage with a disc...
harrow. The applied basic fertilization was 230 kg ha$^{-1}$ of di-ammonium phosphate (18-46-0) and the applied supplementary fertilization was 250 kg ha$^{-1}$ of urea and ammonium sulfate (40-0-0). Additional irrigation did not apply. Weed control was achieved through 1 application of Brominal Nuevo/Bayer (2,4-D 28% + bromoxynil 28%) in both experimental years. Head emergence in Days After Sowing (DAS) are presented in Table 2. Harvesting was held at 215 (1785.7 GDD) and at 201 DAS (2227.55 GDD) by using a HEGE 140 plot harvester.

| Lines/Variety | DAS |
|---------------|-----|
| HT-460        | 143 |
| HT-1704       | 134 |
| HT-1707       | 146 |
| HT-15-54-32   | 144 |
| AUCAN         | 145 |
| BULEL         | 140 |
| GENESIS       | 139 |
| FALADO        | 136 |

2.2. Plant Materials

Genetic material comprised four genotypes of hexaploid tritordeum (HT-460, HT-1704, HT-1707, HT-15-54-32). Two of them were already registered as commercial tritordeum varieties (Aucan and Bulel) and were obtained from Agrasys SA, Spain and two bread wheat varieties, Genesis and Falado. Aucan was the first tritordeum variety registered in 2011 and was selected for its robust performance and Bulel was the second tritordeum variety registered in 2013 and was also selected for its improved bread-making quality and higher protein content. The rest of the advanced lines were derived from crosses between tritordeum and bread wheat or back-crosses as a result of an extensive breeding program by Agrasys SA that makes over 150 crosses per year with selections for yield, threshing ability, disease resistance, bread-making quality, digestive gluten, and other health-related components. Field trials also included two varieties of soft wheat (Genesis and Falado), commercialized and grown in Greece. Genesis is a popular mid-early variety, owned by Syngenta, with high yielding, disease resistance, and of excellent quality. The genealogy is Colfiorito/Hereward and was registered in 2008. Falado is also owned by Syngenta and is a relatively new variety, early maturing, with stable production and high value in mill processing.

2.3. Sampling and Analytical Methods

Threshing efficiency is evaluated by the percentage of the threshed grains calculated on the basis of the total grains entering the threshing mechanism. Threshing efficiency was estimated as the weight ratio between the free kernels and the threshed sub-sample, expressed in percentage terms. The procedure included the following: 20 random spikes from each plot were manually cut and sorted on open trays. Integrity was examined and dispersal units (spikelets) and spike fragments were discarded. A 10-spike sub-sample was taken from the material harvested from each plot and scissors were used to remove the awns from the individual spikes and/or spikelets. Since both germplasm collection and control varieties hold a wide array of awn lengths and toughnesses, this procedure served to minimize any possible effects on the threshing procedures. Thereafter, the awnless spikes were weighed, placed on a threshing steel sieve with a 5 mm opening, and manually threshed (soft rubbing by hand wearing a heavy-duty worker glove) for 5 s, and then measured using a stopwatch. Following the threshing, the material was winnowed to separate the kernels from the chaff, and the grain fraction was weighed.

Total protein (%), moisture content (%), grain specific weight, and gluten content (%) were determined using the Infratec™ 1241 Grain Analyzer (FOSS, Eden Prairie, MN, USA, Serial no.:12417239) after harvest. Scanning temperature range was controlled between 21°–25 °C. The absorption
wavelength range of the samples was 850–1050 nm. The 1000-grain weight was determined by taking a subsample of the grain harvested from each plot.

Height was measured at 150 DAS from 5 plants per plot. Tillering, fungi infection, and vigor were recorded based on visual ratings and the range was: 1 = low, 5 = high. These first-level assessments were based on comparing the color distribution, size, and shape between healthy and poor plants. The rating was made by a researcher with experience in cultivation. Tillering was measured at 60 DAS, fungi infection and vigor were measured at 60, 75, and 90, respectively, and the values indicate the average value of all three observations.

2.4. Calculations and Statistics

Head emergence was recorded based on the growth degree day (GDD) during tritordeum’s phenological stages. Growing degree days (GDD) were calculated by using Equation (1) daily [30]:

\[
GDD = \frac{(T_{\text{max}} + T_{\text{min}})}{2} - T_b
\]

\(T_{\text{max}}\) and \(T_{\text{min}}\) = Maximum and minimum daily air temperature respectively in °C.

\(T_b\) = Minimum base temperature (threshold temperature) for a crop (°C). For tritordeum and lines, \(T_b = 5.0\) ºC; bread wheat \(T_b = 5.0\) ºC [31]; *Hordeum vulgare* \(T_b = 5.0\) ºC [32].

The indicator (Relative Production Efficiency, RPE) below is a comparative measure of the production between the new crop and the existing crop and is described by Equation (2):

\[
\text{Relative Prod. Efficiency \%} = \left(\frac{\text{Yield}_{\text{tritordeum}} - \text{Yield}_{\text{wheat}}}{\text{Yield}_{\text{wheat}}}\right) \times 100
\]

\(\text{Yield}_{\text{tritordeum}}\) = yield for each line and tritordeum variety.

\(\text{Yield}_{\text{wheat}}\) = mean average wheat yield for both varieties (first year was 7336 kg ha\(^{-1}\) and second year was 4056.75 kg ha\(^{-1}\)).

Analysis of variance was carried out on data using the Stat Soft (2011) logistic package as a RCBD. The significance of differences between treatments was estimated by using Fisher’s least significant difference test where probabilities equal to or less than 0.05 considered significant. The tests of correlation coefficients and linear regression by Statistica software were set at two levels with significance (\(p = 0.05\)) and remarkable significance (\(p = 0.01\)).

3. Results

3.1. Agronomic Characteristics

In 2019, GDDs until head emergence had a statistically significant difference in the Bulel variety compared to all other varieties. Additionally, HT-1704 line had a statistically significant difference with all the other varieties during 2020 (Table 3). Additionally, there was no statistically significant difference between Genesis, Falado, and Aucan varieties. The highest value was marked 622.4 GDDs in the Aucan variety during the second year and the lowest was 402.7 GDDs (HT-1704; first year). It was observed that in the second year, the values of GDDs were higher compared to the equivalents in the first year (Table 3). Concerning the period from head emergence to harvest, the highest value was 1355.6 GDDs (Falado variety; 2019), and the lowest was 1019 GDDs (Aucan variety; second year). HT-1704 line, Bulel, Genesis, and Falado varieties had no statistically significant differences between them as shown in Table 3 during 2019. However, in 2020, only HT-1704 line had no statistically significant difference with the Falado variety.
Concerning the agronomic characteristics, Falado variety had statistically differed with all the rest of the varieties and lines. Moreover, in the 1000-grain weight during the first year, HT-460, HT-1704 lines, and Bulel variety had no statistically significant difference among them. In addition, HT-15-54-32 line had statistically significant differences with all varieties and lines (Table 3). In 2020, HT-15-54-32 line had no statistically significant difference with the Aucan variety, whereas the Falado variety had statistically differed with the rest of varieties and lines. Regarding plant height, the highest value was 77 cm in the Falado variety (2020), and the lowest was 60 cm in HT-1704 line (first year).

With regard to the weight of 1000 grains, the Falado variety statistically differed to the rest varieties and lines, during 2019. In the first year, yield was higher than the second year owing to climatological data (Figure 1); in the first year, precipitation was reported higher than in the second year. As a result, the precipitation was higher and the yield was lower (Table 3). The highest value of 1000-grains weight was 33.4 g (Genesis) and the lowest was 23.7 g (Falado) during 2019. Furthermore, yield of bread wheat Aucan variety, in 2020. The highest value was 75.3 kg hl⁻¹ in Aucan (2019 and Falado) was reported as the highest values in both experimental years (6706 kg ha⁻¹).

Referring to protein yield, there were no statistically significant differences in both experimental years. The highest value was 2 kg ha⁻¹ in HT-1704, HT-1707, and Aucan while the lowest value was 1.5 kg ha⁻¹ in Falado, in 2019.

Moreover, grain specific weight of HT-460 line had a statistically significant difference with the other varieties and lines, in 2019. Although, the same line had no statistically significant differences with the Aucan variety, in 2020. The highest value was 75.3 kg hl⁻¹ in bread wheat varieties (Genesis and Falado) during 2019 and the lowest was 61.8 kg hl⁻¹ in HT-460 line during 2020 (Table 3).

3.2. Quality Characteristics

In Table 4, quality characteristics such as moisture content are presented. During the second year, HT-460, HT-1704 lines, Aucan, and Bulel varieties had no statistically significant difference among them. The highest value was 12.4% (Falado) and the lowest was 9.7% (Genesis) during the first year of experiment. Concerning the gluten content, in the first experimental year, HT-460 line had statistically significant differed with the rest of varieties and lines. The highest value was estimated at 37.1% in HT-1704 line and the lowest was 26.2% in HT-460 line during the first year (Table 4).
Values of relative production were lower in the second year compared to the first year. The lowest value was 1 in HT-15-54-32 for both years. In HT-460, the highest values (2.5) were observed for the Aucan variety, equal to 4.8, in 2019. The lowest tillering was observed up to 2.5 in the Falado variety, in 2020.

Moreover, fungi infection factor in Genesis significantly differed among (2019, and 2020). The highest value was marked as 94.3% in Genesis (2019) and the lowest was 11.7% in Falado variety during 2019 (Table 4). Concerning the gluten/protein ratio, HT-460 line did not statistically significant differ with Bulel, HT-15-54-32, and HT-1707. The highest value was estimated at 15.5% in HT-460 line during 2019.

With regard to threshing efficiency, HT-460 line had a statistically significant difference with the rest of the varieties and lines, in 2019.

As for the protein content, the Falado variety statistically significantly differed to the rest of the varieties and lines, in 2019. During the second year, HT-460, HT-15-54-32 lines, Bulel, and Falado varieties had no statistically significant difference among them. The highest value was estimated at 15.5% in HT-1704 line and the lowest was calculated as 6% in HT-460 line during 2019 (Table 4). Concerning the gluten/protein ratio, HT-460 line did not statistically significantly differ with Bulel, HT-15-54-32, and HT-1707. The highest value was estimated at 2.5 in HT-1704 (2020) and the lowest was 2 in HT-460 (2019 and 2020) as shown in Table 4.

With regard to threshing efficiency, HT-460 line had a statistically significant difference with the rest of the varieties and lines, in 2019.

Furthermore, in 2020, HT-460 line had a statistically significant difference with the other varieties and lines (Table 4). The highest value was estimated at 94.3% in Genesis (2019) and the lowest was 81.5% in HT-1707 (2020).

### 3.3. Tillering, Fungi Infection, Vigor, and Relative Production Efficiency

According to Table 5, HT-460 presented a significant difference with the other varieties and lines, in 2019. In 2020, HT-460 also presented significant differences with almost all of the other varieties and lines, except the Falado variety. The highest tillering was observed in HT-15-54-32 line and Bulel variety, equal to 4.8, in 2019. The lowest tillering was observed up to 2.5 in the Falado variety, in 2020. Moreover, fungi infection factor in Genesis significantly differed with the rest of varieties, in 2019. The lowest value was 1 in HT-15-54-32 for both years. In HT-460, the highest values (2.5) were observed for the first year. Concerning the vigor, the Aucan and Falado varieties did not differ during the first year, in contrast to the second year. In 2020, HT-1704 line significantly differed to the other varieties and lines in 2020. The highest value of vigor was reported in HT-15-54-32 line in both experimental years and the lowest in HT-460 (2019) and Falado (2020). For the relative production efficiency, HT-460 and HT-15-54-32 did not significantly differ. Additionally, HT-1704 lines, HT-1707 line, Aucan, and Bulel varieties had no difference among (2019, and 2020). The highest value was marked as −56.2% in the Bulel variety during the first year and the lowest was calculated as −6% in HT-460 line during 2019. Values of relative production were lower in the second year compared to the first year.
Table 5. Tillering, Fungi Infection, Vigor, and Relative Production Efficiency in Each Variety and Line.

| Varieties/Lines          | Tillering (1 = Low, 5 = High) | Fungi Infection (1 = Low, 5 = High) | Vigour (1 = Low, 5 = High) | Relative Production Efficiency (%) |
|--------------------------|--------------------------------|-------------------------------------|---------------------------|----------------------------------|
|                          | 2019                           |                                     |                           |                                  |
| HT-460                   | 3ᵇ                             | 2.5ᵃ                                | 3ᵃ                        | −36ᵃ                             |
| HT-1704                  | 4.5ᵇ                           | 2ᵃ                                  | 3.5ᵃ                      | −44.7ᵇ                          |
| HT-1707                  | 4ᶜ                             | 1.3ᵇ                                | 4ᵇ                        | −48.1ᵇ                          |
| HT-15-54-32              | 4.8ᵈ                           | 1ᵇ                                  | 4.8ᶜ                      | −35.2ᵃ                          |
| Aucan                    | 4ᶜ                             | 1.3ᵇ                                | 4ᵇ                        | −50ᵇ                            |
| Bulel                    | 4.8ᵈ                           | 1.3ᵇ                                | 4.5ᶜ                      | −56.2ᵇ                          |
| Genesis (bread wheat)    | 4.3ᵇ                           | 1.8ᶜ                                | 3.5ᵃ                      |                                  |
| Falado (bread wheat)     | 4ᶜ                             | 2ᵃ                                  | 4ᵇ                        |                                  |
|                          | 2020                           |                                     |                           |                                  |
| HT-460                   | 3ᵃ                             | 2.3ᵃ                                | 3.3ᵃ                      | −6ᵃ                             |
| HT-1704                  | 4ᵇ                             | 1.5ᵇ                                | 3.8ᵇ                      | −21.8ᵇ                          |
| HT-1707                  | 3.8ᵇ                           | 1.3ᵇ                                | 4.3ᶜ                      | −25.3ᵇ                          |
| HT-15-54-32              | 4.3ᶜ                           | 1ᶜ                                  | 4.8ᶜ                      | −3.8ᵃ                           |
| Aucan                    | 4.3ᶜ                           | 1.3ᵇ                                | 4.3ᶜ                      | −21.4ᵇ                          |
| Bulel                    | 4.5ᵈ                           | 1ᶜ                                  | 4.3ᶜ                      | −21.2ᵇ                          |
| Genesis (bread wheat)    | 4.5ᵈ                           | 1.3ᵇ                                | 4.5ᶜ                      |                                  |
| Falado (bread wheat)     | 2.5ᵃ                           | 1.3ᵇ                                | 3ᵃ                        |                                  |

F-test ratios are from ANOVA. Different letters within a column indicate significant differences according to Tukey’s test. Significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant (p > 0.05).

4. Discussion

Concerning the plant height, Karagon et al. mentioned that the mean plant height in wheat was 100 cm [33]. In our study, plant height marked a lower value, and differed in both environmental conditions and varieties. The plant height had a positive correlation with the 1000-grain weight ($r = 0.51, p < 0.001$) but had a negative correlation with the GDDs ($r = −0.40, p < 0.01$) as shown in Table 6. The increasing of GDDs to the head emergence negatively affected the late plant growth stage of tritordeum.
| Crop Properties                  | Height (cm) | 1000 Grain Weight (g) | Yield (kg ha$^{-1}$) | Protein Content (%) | Moisture Content (%) | Grain Specific Weight (kg hl$^{-1}$) | Gluten (%) | Threshing Efficiency (%) | Tillering (1 = Low, 5 = High) | Fungi Infection (1 = Low, 5 = High) | Vigour (1 = Low, 5 = High) | Head Emergence (GDDs) | Period from Head Emergence to Harvest (GDDs) | Protein Yield (kg ha$^{-1}$) | Gluten/Protein | Relative Production Efficiency (%) |
|----------------------------------|-------------|-----------------------|----------------------|---------------------|---------------------|-------------------------------------|------------|-------------------------|---------------------------------|--------------------------------|--------------------------|------------------------|--------------------------------|-----------------------------|--------------------------|--------------------------|
| Height (cm)                      | 1.0         | 0.51 ***              | 0.0                  | 0.62 ***            | 0.63 ***            | 0.51 ***                            | 0.52 ***   | 0.53 ***                | 0.60 ***                        | 0.61 ***                      | 0.61 ***                 | 0.61 ***               | 0.61 ***                        | -0.40 ***                  | 0.21 ***                 |                      |
| 1000 Grain weight (g)            | 0.51 ***    | 1.0                   | -0.41 ***            | 0.10 **             | 0.10 **             | -0.41 **                            | -0.41 **   | -0.30 **                | 1.0 **                           | 1.0 **                        | -0.52 ***                | -0.30 ***            | -0.20 ***                        | -0.30 ***                  | -0.10 ***                |                      |
| Yield (kg ha$^{-1}$)             | 0.0         | -0.41 ***             | 0.61***              | -0.44 **            | 0.10 ***            | -0.41 **                            | -0.30 **   | -0.30 **                | -0.42 **                          | -0.30 **                      | -0.19 **                | 0.41 **               | 0.30 ***                        | -0.22 ***                  | 0.12 ***                 |                      |
| Protein content (%)              | 0.62 ***    | 1.0                   | -0.41 **             | 1.0                 | 0.72 ***            | 0.97 **                             | 0.35 **    | 1.0 **                  | 1.0 **                           | 0.55 **                       | 0.35 **                | 0.46 **               | -0.28 ***                        |                      |                      |                      |
| Moisture Content (%)             | 0.63 ***    | 1.0                   | -0.44 **             | 1.0                 | 0.74 ***            | 0.95 **                             | 0.33 **    | 1.0 **                  | 1.0 **                           | 0.55 **                       | 0.35 **                | 0.46 **               | -0.28 ***                        |                      |                      |                      |
| Grain specific weight (kg hl$^{-1}$) | 0.51 ***   | 0.72 ***              | 0.10 **              | 0.72 ***            | 0.74 ***            | 1.0                                 | 0.61 ***   | 0.35 **                 | 0.88 ***                          | 0.78 ***                      | 0.34 **                | 0.21 **               | -0.02 ***                        |                      | -0.02 ***                | -0.02 ***                |
| Gluten (%)                       | 0.52 ***    | 0.91 **               | -0.41 **             | 0.97 **             | 0.91 **             | 0.63 **                             | 1.0        | 0.21 **                 | 0.92 **                          | 0.94 **                       | 0.97 **                | 0.81 **               | 0.81 ***                        |                      |                      |                      |
| Threshing efficiency (%)         | 0.53 ***    | 0.32 **               | 0.47 ***             | 0.35 **             | 0.33 **             | 0.35 **                             | 0.21 **    | 1.0                     | 0.31 **                          | 0.33 **                       | 0.37 **                | 0.34 **               | 0.45 ***                        |                      |                      |                      |
| Tillering (1 = Low, 5 = High)    | 0.60 ***    | 1.0                   | -0.30 **             | 1.0 **              | 1.0 **              | 0.78 **                             | 0.92 **    | 0.31 **                 | 1.0                              | 1.0 **                        | 1.0 **                 | 0.35 **               | -0.28 ***                        |                      |                      |                      |
| Fungi Infection (1 = Low, 5 = High) | 0.61 ***  | 1.0                   | -0.30 **             | 1.0 **              | 1.0 **              | 1.0 **                              | 0.94 **    | 0.33 **                 | 1.0 **                           | 1.0 **                        | 0.81 **                | 0.21 **               | -0.02 ***                        |                      |                      |                      |
| Vigour (1 = Low, 5 = High)       | 0.61 ***    | 1.0                   | -0.42 **             | 1.0 **              | 1.0 **              | 0.74 **                             | 0.97 **    | 0.37 **                 | 1.0 **                           | 1.0 **                        | 0.81 **                | 0.45 **               | 0.23 ***                        |                      |                      |                      |
| Head emergence (GDDs)            | -0.40 ***   | -0.52 **              | -0.21 **             | -0.65 **            | -0.63 **            | -0.88 **                            | -0.61 **   | -0.61 **                | -0.77 **                          | -0.65 **                      | 1.0                   | -0.97 **             | 0.65 ***                        |                      |                      |                      |
| Period from head emergence to harvest (GDDs) | -0.20 ***  | -0.30 **              | 0.41 **              | 0.35 **             | 0.12 **             | 0.55 **                             | 0.18 **    | -0.11 **                | 0.24 **                          | 0.22 **                       | -0.01 **               | 0.12 **               | -0.87 ***                        |                      |                      |                      |
| Protein yield (kg ha$^{-1}$)     | -0.03 **    | 0.20 **               | -0.10 **             | -0.11 **            | -0.10 **            | 0.80 **                             | 0.80 **    | 0.32 **                 | 0.01 **                          | -0.03 **                      | 0.02 **                | 1.0                  | 0.23 ***                        |                      |                      |                      |
| Gluten/Protein                   | -0.21 **    | -0.30 **              | -0.22 **             | 0.46 **             | 0.21 **             | 0.19 **                             | 0.81 **    | -0.28 **                | 0.35 **                          | -0.38 **                      | 0.14 **                | 0.45 **               | 0.10 ***                        |                      |                      |                      |
| Relative Production Efficiency (%) | 0.21 **    | -0.10 **              | 0.12 **              | -0.28 **            | 0.02 **             | -0.65 **                            | 0.01 **    | 0.45 **                 | -0.20 **                         | 0.01 **                       | 0.02 **                | 0.23 **               | 0.23 ***                        |                      |                      |                      |

Significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant (p > 0.05).
Erlandsson et al. (2010) stated that 1000 grain weight of various tritordeum lines was 40 g [4], whereas in our study, it was estimated at 30 ± 2.86 g. The highest value was indicated in the Genesis variety. This difference derives from the fact that the seed index consists a genetic trait of each variety and in our study were used nonidentical lines, and commercial varieties. In the 1000-grain weight, a negative correlation was noticed with yield ($r = -0.41, p < 0.001$) and a positive correlation with the grain specific weight ($r = 0.72, p < 0.001$). The rise of yield caused a higher number of seeds per spike but smaller seeds [34].

Villegas et al. (2010) reported that wheat marked a higher yield than tritordeum varieties and lines [7]. This is a common result since bread varieties (Falado and Genesis) marked the highest yield in both years.

Giordano et al. (2019) noted that the Aucan and Bulel variety grains yielded 4500–5000 t ha$^{-1}$ [2].

Our results revealed lower values. Yield had a negative correlation with protein content ($r = -0.41, p < 0.01$) and moisture content ($r = -0.44, p < 0.01$) as shown in Table 6. As a result, a negative correlation was noticed.

Due to the fact that there was negative correlation with capacity, high precipitation had a negative effect, resulting in reduced yields. Hence, we concluded that tritordeum crops could be established in south Greek territory. Tritordeum, as a new type of cereal, has been breeding over the years in arid climates thus it could adapted to climate change. As a result, greater precipitation reduces tritordeum’s yield.

Miller et al. (2001) noted that wheat GDDs, at the stage of head emergence, ranged from 592 to 659; higher values of wheat varieties were recorded in our study [20]. Daily temperature is a crucial factor for GDDs; that means different environmental conditions resulted in different GDDs. This is illustrated in our results in both experimental years.

It was observed that climate had a negative effect in the GDDs, which negatively affect the agronomic and quality characteristics of wheat, tritordeum varieties, and lines. Nevertheless, GDDs up to the head emergence stage were a key factor for the development of wheat, tritordeum varieties and lines in the Mediterranean conditions. Besides GDDs could be utilized as a tool to assess adaptability. Hence, present study shows that southern Mediterranean conditions are ideal for growing tritordeum.

In terms of grain moisture content, statistically significant differences have been observed between the lines and varieties. Moreover, moisture content of cereals presented significant differences between two experimental years; this difference is owing to higher precipitation in the second year (Figure 1). According to Table 6, a strong negative correlation was noticed between grain moisture content and GDDs ($r = -0.63, p < 0.001$). The specific weight of grain is considered as a significant quality characteristic of cereals [35], which measures the grain density, i.e., the weight of the grains per unit volume [36]. The present study showed that bread wheat grains had the highest specific weight in both experimental years. As shown in Table 6, specific weight had a negative correlation with GDDs until head emergence ($r = -0.88, p < 0.001$) and relative production efficiency ($r = -0.65, p < 0.001$). For gluten content and protein levels, higher values were marked in HT-1704.

Many studies have observed that protein deposition is influenced by environmental conditions, such as high temperatures, i.e., during the grain filling stage high temperatures cause thermal shock [37]. However, protein yield was not affected by weather conditions in both experimental years (Table 3). In addition, GDDs concerning the period from head emergence to harvest stage appear to be affected by years and lines/varieties. Specifically, during the second experimental year where rainfall levels were the highest values during March and April, the period starting from head emergence to harvest stage was shorter (Table 3). According to Table 6, this period was positively correlated with yield ($r = 0.41, p < 0.01$) and the grain-specific weight factor ($r = 0.55, p < 0.001$).

With reference to gluten, this research indicated that tritordeum varieties and bread wheat marked similar levels of gluten. On the contrary, many studies were observed that gluten levels in tritordeum were lower to bread wheat. Despite the fact that, in HT-460 line was noticed the lowest gluten level in both years. Both gluten and protein levels are negatively correlated with GDDs on head emergence.
stage (r = −0.0.61, p < 0.001; r = −0.0.65, p < 0.001 respectively); rising of GDDs, decreasing protein and, gluten levels. Despite the fact that protein varies among the lines/varieties, protein yield was not affected by year or lines/varieties. In addition, protein yield was negatively correlated with GDDs in head appearance stage (r = −0.34, p < 0.05; Table 6). Similar results were reported by Jolankai et al. (2018) [38], where protein yield was positively correlated with precipitation (r = 0.886), and negatively with temperature (r = −0.0.317).

Regarding the ratio between gluten and protein, our results revealed that this ratio was significantly affected by the lines/varieties; the lowest values was observed in HT-460 in both years. In addition, this ratio was correlated with protein yield (r = 0.45, p < 0.05) as well as with tillering (r = 0.35, p < 0.05; Table 6). In terms of threshing yield, bread wheat had the highest rate for both years. Threshing performance was negatively correlated with GDDs in head emergence stage (r = −0.0.22, p < 0.05; Table 6).

Prats et al. (2006) indicated that tritordeum could be a source of resistance for some fungal diseases [39]. Indeed, tritordeum generally presents low fungi inflection, although HT-460 line presented double infection compared to two commercial varieties of tritordeum. In addition, HT-460 line, under Greece’s dry crop conditions, showed a great deal of inflection: 2 and 2.3 in 5, respectively, for two years. The resistance of lines needs to be further explored. Prats et al. (2006) observed that commercial varieties and amphiploids are sometimes more resistant than wheat in pathogens such as powdery mildew. Vigor was high for two out of four lines, two tritordeum varieties and one wheat variety. Botwright et al. (2002) reported that rainfall is an important factor for wheat vigor [40]. In point of fact, during our experiment, there was a sufficient rainfall percentage until the flowering stage, resulting in high vigor in HT-460 line (3 and 3.3, respectively, during the first and second years), in the Genesis variety (3.5) during the first year, and in the Falado variety (3) during the second year.

Sharma et al. (2010) noted that relative production efficiency was one of the most important indices for predicting crop performance [41]. They also reported that there was a positive relationship between yield and relative production efficiency in wheat cultivation. On the contrary, relative production efficiency had a negative correlation with grain specific weight (r = −0.0.65, p < 0.001; Table 6). In our study, the values were negative in view of control yield (bread wheat) was higher than yield of tritordeum lines and varieties.

5. Conclusions

Tritordeum, an alternative cereal crop, is considered a promising crop for the Mediterranean basin. According to the present study, GDDs are negatively correlated with almost all the agronomic and quality characteristics. The increasing of GDDs to the head emergence stage, negatively affect the late plant growth stage of tritordeum. Additionally, gluten levels in tritordeum grains appear to be lower than in bread wheat, due to higher protein content. Besides, both gluten and protein levels are negatively correlated with GDDs on head emergence stage. It is worth noting that high precipitation levels did not significantly affect yield but did increase the GDDs, compared to lower rainfall levels. However, GDDs from head emergence to harvest stage positively affected yield. Tritordeum, as a new type of cereal, has been breeding over the years in arid climates, hence it could have adapted to climate change. As a result, greater precipitation causes reduced yield of tritordeum. Given the fact that GDDs can be utilized as a tool to assess adaptability, the present study shows that southern Mediterranean conditions are ideal for growing tritordeum. In summary, tritordeum could replace the cultivation of bread wheat in Mediterranean dry conditions, as well as other uses of bread wheat.

Author Contributions: The authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

RCBD  Randomized Complete Block Design
C    Clay
DAS  Days After Sowing
GDDs Growing Degree Days
RPE Relative Production Efficiency

References

1. Tayyem, R.F.; Bawadi, H.A.; Shehadah, I.; Agraib, L.M.; Al-Awwad, N.J.; Heath, D.D.; Bani-Hani, K.E. Consumption of whole grains, refined cereals, and legumes and its association with colorectal cancer among jordanians. *Integr. Cancer Ther.* **2016**, *15*, 318–325. [CrossRef] [PubMed]

2. Giordano, D.; Reyneri, A.; Locatelli, M.; Coïsson, J.D.; Blandino, M. Distribution of bioactive compounds in pearled fractions of tritordeum. *Food Chem.* **2019**, *301*, 125228. [CrossRef] [PubMed]

3. Bothmer, R.; Jacobsen, N.; Baden, C.; Jorgensen, R.B.; Linde-Laursen, I. *An Ecogeographical Study of the Genus Hordeum*, 2nd ed.; International Board for Plant Genetic Resources: Svalov, Sweden, 1995; p. 129. ISBN 978-92-9043-229-6.

4. Erlandsson, A. Tritordeum Evaluation of a New Food Cereal. Master’s Thesis, Agricultural Program Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2010.

5. Martin, A.; Cabrera, A.; Hernández, P.; Ramirez, M.C.; Rubiales, D.; Ballesteros, J. Prospect for the use of *Hordeum chilense* in durum wheat breeding. In *Durum Wheat Improvement in the Mediterranean Region: New Challenges*; CIHEAM: Zaragoza, Spain, 2000; pp. 111–115.

6. Ballesteros, J.; Ramirez, M.C.; Martinez, C.; Atienza, S.G.; Martin, A. Registration of HT621, a high carotenoid content Tritordeum germplasm line. *Crop Sci.* **2005**, *45*, 2662–2663. [CrossRef]

7. Villegas, D.; Casasús, J.; Atienza, S.G.; Martos, V.; Maalouf, F.; Karam, F.; Aranjuelo, I.; Nogués, S. Tritordeum, wheat and triticale yield components under multi-local mediterranean drought conditions. *Field Crops Res.* **2010**, *116*, 68–74. [CrossRef]

8. Millan, T.; Martin, A.; de Haro, A. Field trial of *Tritordeum*. *Cereal Res. Commun.* **1988**, *16*, 31–38.

9. Atienza, S.G.; Ballesteros, J.; Martín, A.; Hornero-Méndez, D. Genetic variability of carotenoid concentration and degree of esterification among tritordeum (*Tritordeum Ascherson et Graebner*) and durum wheat accessions. *J. Agric. Food Chem.* **2007**, *55*, 4244–4251. [CrossRef]

10. Mellado-Ortega, E.; Hornero-Méndez, D. Carotenoid profiling of *Hordeum chilense* grains: The parental proof for the origin of the high carotenoid content and esterification pattern of tritordeum. *J. Cereal Sci.* **2015**, *62*, 15–21. [CrossRef]

11. Vaquero, L.; Comino, I.; Vivas, S.; Rodríguez-Martín, L.; Giménez, M.J.; Pastor, J.; Sousa, C.; Barro, F. Tritordeum: A novel cereal for food processing with good acceptability and significant reduction in gluten immunogenic peptides in comparison with wheat. *J. Sci. Food Agric.* **2017**, *98*, 2201–2209. [CrossRef]

12. IPCC. Climate change: The physical science basis. In *Contribution of Working Group I to the Fourth Annual Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., Eds.; Cambridge University Press: Cambridge, UK, 2007; p. 996.

13. Kotschi, J. Agricultural biodiversity is essential for adapting to climate change. *GAIA Ecol. Perspect. Sci. Soc.* **2007**, *16*, 98–101. [CrossRef]

14. Mannion, A.M. Biotechnology and environmental quality. *Prog. Phys. Geogr.* **1995**, *19*, 192–215. [CrossRef]

15. Araus, J.L.; Slafer, G.A.; Reynolds, M.P.; Royo, C. Plant breeding and water relations in C3 cereals: What to breed for? *Ann. Bot.* **2002**, *89*, 925–940. [CrossRef] [PubMed]

16. Alvaro, F.; Isidro, J.; Villegas, D.; Garcia del Moral, F.L.; Royo, C. Breeding effect on grain filling, biomass partitioning, and remobilization in Mediterranean durum wheat. *Agron. J.* **2008**, *100*, 361–370. [CrossRef]

17. Gallardo, M.; Fereres, E. Resistencia a la sequia del triticale. tritordeo (*Hordeum chilense* × *Triticum aestivum*) en relacion a la del trigo, cebada y triticale. *Investig. Agrar. Prod. Prot. Veg.* **1989**, *4*, 361–375.

18. Barro, F.; Gonzalez-Fontes, A.; Maldonado, J.M. Relation between photosynthesis and dark respiration in cereal leaves. *J. Plant Physiol.* **1996**, *149*, 64–68. [CrossRef]
19. Anandhi, A. Growing degree days—Ecosystem indicator for changing diurnal temperatures and their impact on corn growth stages in Kansas. *Ecol. Indic.* 2016, 61, 149–158. [CrossRef]

20. Miller, P.; Lanier, W.; Brandt, S. *Using Growing Degree Days to Predict Plant Stages*. Ag/Extension Communications Coordinator, Communications Services; Montana State University-Bozeman: Bozeman, MO, USA, 2001; pp. 1–7.

21. Ram, H.; Gupta, N.; Saini, J.S. Growing degree day requirements and yield ability of irrigated durum wheat as influenced by sowing time. *Agric. Res. J.* 2016, 53, 303–306. [CrossRef]

22. Liu, Y.; Su, L.; Wang, Q.; Zhang, J.; Shan, Y.; Deng, M. Comprehensive and quantitative analysis of growth characteristics of winter wheat in China based on growing degree days. *Adv. Agron.* 2020, 237–273. [CrossRef]

23. Li, Q.; Yin, J.; Liu, W.; Zhou, S.; Li, L.; Niu, J.; Niu, H.; Ma, Y. Determination of Optimum Growing Degree-Days (GDD) Range before Winter for Wheat Cultivars with Different Growth Characteristics in North China Plain. *J. Integr. Agric.* 2012, 11, 405–415. [CrossRef]

24. Bonhomme, R. Bases and limits to using “degree.day” units. *Eur. J. Agron.* 2000, 13, 1–10. [CrossRef]

25. Haggard, G.B.; Weindorf, D.; Cacovean, H.; Rusu, T.; Lofton, J. Analysis of Growing Degree Days in the Transylvanian Plain, Romania. *Geographia* 2010, 2, 13–20.

26. Prasad, P.V.V.; Bheemanahalli, R.; Jagadish, S.V.K. Field crops and the fear of heat stress—opportunities, challenges and future directions. *Field Crop. Res.* 2017, 200, 114–121. [CrossRef]

27. Sadras, V.O.; Monzon, J.P. Modelled wheat phenology captures rising temperature trends: Shortened time to flowering and maturity in Australia and Argentina. *Field Crop. Res.* 2006, 99, 136–146. [CrossRef]

28. Bilalis, D.; Papastylianou, P.; Travlos, H. *Agronomy*; Pedio: Athens, Greece, 2019; p. 227. ISBN 978-960-546-039-6.

29. Gavian, S.; Ehui, S. Measuring the production efficiency of alternative land tenure contracts in a mixed crop–livestock system in Ethiopia. *Agric. Econ.* 1999, 20, 37–49.

30. Peterson, R. *Wheat Crop Series*; Polunin, N., Ed.; Inter Science Publication Inc.: New York, NY, USA, 1965; p. 422.

31. Kimball, B.A.; White, J.W.; Wall, G.W.; Ottman, M.J. Infrared-Warmed and Unwarmed Wheat Vegetation Indices Coalesce Using Canopy-Temperature–Based Growing Degree Days. *Agron. J.* 2012, 104, 114–118. [CrossRef]

32. Stewart, D.W.; Dwyer, L.M. Analysis of phenological observations on barley (*Hordeum vulgare*) using the feekes scale. *Agric. For. Meteorol.* 1987, 39, 37–48. [CrossRef]

33. Karagoz, A.; Zencirci, N. Variation in wheat (*Triticum spp.*) landraces from different altitudes of three regions of Turkey. *Gen. Resour. Crop Evol.* 2004, 52, 775–785. [CrossRef]

34. Preece, C.; Livarda, A.; Christin, P.A.; Wallace, M.; Martin, G.; Charles, M.; Jones, G.; Rees, M.; Osborne, C.P. How did the domestication of Fertile Crescent grain crops increase their yields? *Funct. Ecol.* 2017, 31, 387–397. [CrossRef]

35. Hoyle, A.; Brennan, M.; Jackson, G.E.; Hoad, S. 2019 Increased grain density of spring barley (*Hordeum vulgare* L.) is associated with an increase in grain nitrogen. *J. Cereal Sci.* 2019, 89. [CrossRef]

36. Jenner, C.F. Starch synthesis in the kernel of wheat under high temperature conditions. *Aust. J. Plant Physiol.* 1994, 21, 791–806. [CrossRef]

37. Fernando, N.; Panozzo, J.; Tausz, M.; Norton, R.; Fitzgerald, G.; Seneweera, S. Rising atmospheric CO$_2$ concentration affects mineral nutrient and protein concentration of wheat grain. *Food Chem.* 2012, 133, 1307–1311. [CrossRef]

38. Jolankai, M.; Kassai, K.M.; Tarnawa, A.; Posa, B.; Birkas, M. Impact of precipitation and temperature on the grain and protein yield of wheat (*Triticum aestivum* L) varieties. *Quart. J. Hung. Meteorol. Serv.* 2018, 122, 31–40. [CrossRef]

39. Prats, E.; Fondevilla, S.; Rubiales, D.; Carver, T.L. Cellular basis of resistance to different formae speciales of *Blumeria graminis* in Hordeum chilense, wheat, and tritordeum and agroticum amphiloids. *Can. J. Plant Pathol.* 2006, 28, 577–587. [CrossRef]

40. Botwright, T.L.; Condon, A.G.; Rebetzke, G.J.; Richards, R.A. Field evaluation of early vigour for genetic improvement of grain yield in wheat. *Aust. J. Agric. Res.* 2002, 53, 1137–1145. [CrossRef]
41. Sharma, A.; Arora, S. Soil Quality Indices and Relative Production Efficiency for Maize and Wheat Crops in Agroclimates of Northwest India. *Soil Sci.* **2010**, *175*, 44–49. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).