2.07 Minor Cereals and New Crops: Tritordeum

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Key Points

- The interest in tritordeum within today’s context of consumer demand;
- Description of its origin through breeding processes;
- Agronomic management of tritordeum based on its phenological, ecological and yield traits;
- An extensive comparison on several nutritional traits of tritordeum with bread and durum wheats;
- Rheological and technological properties of tritordeum flours;
- Gluten content and the related immunotoxic potential of tritordeum flours.

Abstract

Tritordeum is an amphiploid that originates from the backcrossing of a wild barley, *Hordeum chilense,* with durum wheat. Tritordeum thus represents a “bridge” between the desirable genetic traits of its parent lines, but its end-use is comparable with that of bread wheat. Commercial tritordeum cultivars are now available and have begun to be cultivated in different growing areas. This crop could easily be inserted into cereal cropping system, since it has a similar crop cycle and agronomic management to wheat. Tritordeum has a lower grain yield but higher protein content than wheat; it has inherited a good tolerance to abiotic stress and diseases from *H. chilense*, but it has a high susceptibility to *Fusarium* head blight and mycotoxin accumulation. Scientific works have so far mainly focused on the bioactive compound content: tritordeum in fact has higher levels of carotenoids and arabinoxylans than wheat, and these result in a greater total antioxidant activity. Moreover, a lower presence of immunotoxic peptides has been observed in tritordeum than in wheat, although the benefits for whose people who suffer from non-celiac gluten sensitivity still need to be studied in depth. If breeders can provide tritordeum cultivars with a lower yield gap and a better end-use value, it could represent an interesting alternative to wheat for the baked goods supply chain.

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2.07.1 Introduction

Food cereal-based products play a significant role in human diets throughout the world. Wheat is the second most important crop at the worldwide level, in terms of global production, after maize, but it is the first in terms of cultivated surface, and has shown a wide environmental adaptability, ranging from the tropics to the upper edges of temperate zones (FAO, 2021). Moreover, wheat is the most important staple crop for food in developed countries, and the increasing global demand for this commodity is a consequence of industrialization and westernization processes that are also now taking place in developing countries. The first element of success in the use of wheat is related to its ability to be used to make unique food products. According to the distinctive technological properties of gluten protein, wheat flour can be used for a wide range of food products with unique organoleptic and texture traits: bread, pizza, cookies and other baked goods, but also pasta, noodles, couscous, porridge, pearled grain, flakes and a variety of other foods and ingredients (Day et al., 2006).

As far as the nutritional intake is concerned, in addition to being a key source of starch and energy, wheat-based foods also provide an important amount of protein, with a higher intake and also slightly higher biological values than those derived from the consumption of other cereals, such as maize or rice (Bekes and Wrigley, 2004). Moreover, they also contain significant amounts of other important nutrients, particularly when wholegrain ingredients are used, including several non-digestible carbohydrates and such minor components as lipids, vitamins (particularly B group vitamins), minerals and phytochemicals, which may contribute to a healthy diet (Shewry and Hey, 2015). It has widely been reported that a diet containing cereals, and wheat in particular, improves the contents of dietary fiber, micronutrients and a wide range of bioactive compounds. Some of these phytochemicals, such as phenolic compounds (phenolic acids, lignans and flavonoids) and carotenoids, show a marked antioxidant activity (Van Hung, 2016) and their consumption has been associated with a reduced risk of degenerative and chronic diseases (Luna-Guevara et al., 2018).

Consumers' awareness about high-fiber diets and food naturally rich in beneficial components for the human diet is increasing, especially in developed countries (Ktenioudaki and Gallagher, 2012), as is the market demand for alternative and special food products. Improving the nutritional profile of baked foods, through supplementation with flour or ingredients of different origins, is therefore an important market requirement. Within this context, and according to a multigrain approach, the use of other minor cereals is a recent trend in the baking industry to obtain multiple functional benefits of bakery products (Torbica et al., 2021). The application of alternative cereal types for the production of special foods rich in bioactive compounds, obtained from innovative crops, has drawn the attention of both researchers and industrialists in the last few years (Donkor et al., 2012; Giordano et al., 2019; Zielinski et al., 2001).

These requests have again highlighted the need for the cultivation of minor cereals, such as barley, rye and oats, but also old types of cereals, such as einkorn, emmer, spelt, khorasan, old wheat cultivars and landraces, or pseudocereals, i.e., amaranth, quinoa, buckwheat and chia (Alvarez-Jubete et al., 2010). However, the use of flours and ingredients obtained from these crops in order to increase the nutritional value of baked products could be associated with some detrimental traits that could negatively impact their competitiveness on the market. First, these ingredients could cause a deterioration of the technological properties, due to both the absence or a different composition of gluten and the high fiber content, which interact with the gluten-starch matrix (Mihai et al., 2017). When developing functional bakery products, it is in fact important to realize a food which delivers the appropriate level of bioactive compounds, but which also meets the consumers' requirements, in terms of appearance, taste and texture (Bender and Schönlechner, 2021; Meral and Köse, 2019). In addition, the lower productivity and some difficulties connected to the agronomic and mechanical management of these alternative crops, make the production of these raw materials, with the exception of barley, less sustainable than that of wheat in several temperate growing areas (Fabio and Parraga, 2017).

Since the beginning of the 20th century, cereal breeders have focused their efforts on the development of interspecific wheat hybrids in order to obtain new cereals with increased phytochemical contents and improved agronomic performances and technological qualities. In this context, tritordeum (x *Tritordeum martinii* A. Pujadas, nothosp. nov.) is a new crop that is derived from the crossing of a South American wild barley with wheat, and it may be considered a promising alternative to wheat as a basic ingredient for a wide range of foods. At present, tritordeum is cultivated in the European Union over an area of approximately 600 ha, of which 70% is in Spain, 17% in North Italy and 12% in Greece, although its cultivation has also recently started in the Netherlands and Australia (Arcadia Spa, personal communication).

As previously reported, there is a progressive process of specialization in cereal production, with the aim of obtaining raw materials with a higher end-use value, which are therefore more remunerative on the market, and for the supply chain. Thus, a new crop, such as tritordeum, could be an interesting alternative for the preparation of baked products, because, if the yield and qualitative gap are not too wide, compared to wheat, there will be no particular constraints concerning the agronomic technique or its management along the supply chains. The aim of this chapter is to summarize the already available information on the cultivation of this new cereal, to make a comparison of its agronomic behaviors with those of wheat and other small cereals and to analyze the potential qualitative benefits of using tritordeum-based ingredients in the production of baked products and other foods. This framework may allow the strengths and weaknesses of this new crop, which is starting to spread in cereal cropping systems and on the market of several countries, to be outlined and the next objectives of genetic improvement to be addressed in a more effective way.

2.07.1.1 Origin and Breeding of Amphyploid Tritordeum

Tritordeum is an amphiploid cereal which is the result of a series of artificial hybridizations, starting from a wild barley species, *Hordeum chilense* Roem. et Schultz ($2n = 2x$, $H^{ch}H^{ch}$) and bread wheat (*Triticum aestivum* L. subsp. *aestivum*, which is also called common or soft wheat) ($2n = 6x$, AABBDD) or durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) ($4x$, AABB), whereby octoploid tritordeum ($2n = 8x$, $AABBDDH^{ch}H^{ch}$) and hexaploidy tritordeum ($2n = 6x$, $AABBH^{ch}H^{ch}$), respectively, are obtained (Martin and Chapman, 1977; Martin and Sanchez-Mongelaguna, 1982).

Apart from being used as a genetic bridge to transfer the useful traits of barley to wheat, tritordeum has also been subjected to a breeding program to become a new, hulless, small cereal crop (Martín et al., 1999). Interest in breeding experiments between the two genera, *Hordeum* and *Triticum*, has been of great interest since the beginning of the 20th century (Martín et al., 1999). This interest has led to the successful development of a hybrid between wheat (*Triticum* spp.) and rye (*Secale cereale* L.), that is, triticale (x *Triticosecale* Wittmack) (Cubero et al., 1986). The initial breeding aim of triticale was that of obtaining a promising wheat-rye from a crossing that would introgress the desirable genetic traits of the parents as a "bridge" to realize the typical high productivity and improved grain quality of the wheat genotype, together with a wider adaptation for a stronger resistance to disease and a tolerance to environmental stresses, as conferred by rye (Estrada-Campuzano et al., 2012). The first triticale was obtained almost one century ago, after the spontaneous chromosome doubling of hybrids from crosses between wheat and rye (Rimpau, 1891); a vigorous F1 was produced, but it presented sterile offspring (Guedes-Pinto et al., 2012). The triticale breeding process started to advance after the development of the embryo rescue and colchicine-induced chromosome doubling approach (Blakeslee and Avery, 1937; Laibach, 1925). The obtainment of the triticale that is currently on the market has been possible thanks to breeding studies that have been carried out over a century to optimize the agronomic and quality performance of this amphiploid. Since the mid-1970s, more than 200 cultivars have been released, and the reference center for major discoveries and productions is the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. Although octoploid (AABBDDRR, obtained from a chromosomal doubling of hybrids of hexaploidy wheat) and hexaploid ($2n = 42 =$ AABBRR, obtained from a crossing of durum wheat with rye) hybrids were the first products, there has mainly been an evolution and improvement of hexaploid genotypes over the years, because of their greater vigor and major reproductive stability (Mergoum et al., 2009). However, octoploid triticale cultivars have shown a higher incidence of decreased fertility (Fernández, 1989), meiotic instability and a strong aneuploid frequency (Guedes-Pinto et al., 2012). Thus, although different ploidy levels have been developed for triticale, only the hexaploid one (Mergoum et al., 2009) is commercialized.

The prodigious success of triticale breeding has led to interest in obtaining a valid and interesting crossing of barley and wheat, from both the agronomic and nutritional perspectives. Several preliminary attempts to cross two different cereal genera of *Hordeum* and *Triticum* were not successful, but then Kruse (1973) was able to achieve the first fertile success. This first important result was obtained using a wild relative of barley, *H. chilense*, as the female line for crop breeding, which permitted a good compatibility with the wheat cultivar to be obtained for the first time (Martin and Sanchez-Mongelaguna, 1982). *H. chilense* is a wild species that originated from South American. It is commonly found in Argentina and Chile, and is considered the *Hordeum* genus species with the highest breeding efficiency, thanks to its high crossability with the *Triticeae* species (Fernández, 1989). Furthermore, this plant has an interesting agronomical potential, due to its strong resistance to abiotic stress and its tolerance to diseases, such as brown rusts (*Puccinia hordei*, *P. recondite tritici*), powdery mildew (*Erysiphe graminis*), the Septoria leaf blotch complex (*Mycosphaerella graminicola*), common bunt (*Tilletia caries*), smuts (*Ustilago nuda* and *U. tritici*) and to root-knot nematodes (*Meloidogyne naasi*) (Martín et al., 2000). Other beneficial effects of transferring *H. chilense* genes include the qualitative traits of the grain, such as the high genetic variability of expression of the storage protein composition, and the high content of bioactive compounds, such as carotenoids, in the kernel (Martín and Cabrera, 2005; Palomino and Cabrera, 2019).

Numerous tritordeum hybrids have been obtained since 1970 with the aim of creating a new crop that would be of interest to food supply chains, but all of them have been accompanied by sterility problems and low seed fertility (Martín et al., 1999). The first obtained tritordeum cultivars were octoploids, which were obtained using a hexaploid bread wheat, as a pollen donor (Martin and Chapman, 1977). Furthermore, the thus obtained octoploid tritordeum showed a poor early growth, a high frequency of aneuploids and high chromosome instability, all of which are not optimal for the development of a potential and sustainable crop (Martín et al., 1996). A few years after having obtained the first fertile octoploid amphiploid, a durum wheat line was used instead of the bread one, and thus a hexaploid tritordeum was generated (Martin and Sanchez-Mongelaguna, 1982). Hexaploid tritordeum soon emerged because of its better initial growth, higher fertility and lower frequency of aneuploids than the octoploid ones, and it showed an overall better agronomic performance (Martín et al., 1996; Martín and Cubero, 1981; Martín et al., 1999), even though octoploid amphiploids remain of great interest, considering their good bread-making parameters, which are comparable with those of bread wheat (Alvarez and Martín, 1994; Alvarez et al., 1994). Although durum wheat is the male parent of hexaploid tritordeum, the grain texture and the rheological traits of tritordeum are similar to those of bread wheat (Martín et al., 1999).

Despite the first good results obtained from the amphiploid primary lines of tritordeum, some agronomic parameters, such as the grain yield, did not reach the same values as the parental lines, and breeding studies are therefore now under way using backcrossing techniques (Martín et al., 1999). The attention of the breeders was directed toward studying the role of different nuclear genomes and cytoplasms as well as their interaction in amphiploid lines (Ekiz and Konzak, 1991a,b,c). Although it has been shown that the male line chosen for the nucleus has a predominant effect on the transmission of the characteristics, the cytoplasm, which is inherited from the female parent, also has a considerable influence (Hernández et al., 2001a,b). Several studies have shown significant effects of the nucleus-cytoplasm interaction on agronomic and qualitative aspects, as for the amino-acid synthesis pathways

(Atienza et al., 2007a, 2008). However, even though very few data are available on the specific case of tritordeum, it seems that cytoplasm exploitation could be a source of variability for wheat breeding (Rodríguez-Suárez et al., 2011). Looking at the effects of using different cytoplasms as different sources of genetic variability to further improve agronomic performance (Millán and Martín, 1992) has led to studies targeted on obtaining a line with the best genetic stability, and the transfer and maintenance of the characteristics of interest from both parental lines, through different combinations of cytoplasm x nuclear variability (Atienza et al., 2007c; Hernández et al., 2001a,b; Millán and Martín, 1992; Rodríguez-Suárez et al., 2011). However, the alloplasmic lines obtained as result of several back-crossings with the same nucleus, but with cytoplasms from different species, such as *T. aestivum*, *T. turgidum* or *H. chilense*), did not show any clear differences, except for the grain yield, which resulted to be lower for the *T. turgidum* cytoplasm (Millán and Martín, 1992).

The genetic improvement of tritordeum is still in continuous evolution: according to Kakabouki et al. (2020), more than 250 tritordeum primary lines are available at present. Some lines that show a good yield performance, such as Aucan (2013) and Bulel (2015), have already been registered in the European Community Plant Variety Office (CPVO; www.cpvo.europa.eu) and have been commercialized in Europe as tritordeum Vivagrain since 2013 by Agrasys S.L., (Barcelona, Spain) and today by Arcadia Spa S.L.(Pamplona, Spain). Furthermore, recent studies have shown that the generation lines show even more interesting results for some agronomic aspects (Kakabouki et al., 2020) and new and better commercial cultivars are expected in the near future.

2.07.2 Agronomic Management

2.07.2.1 Phenological and Morphological Traits

Since tritordeum has a comparable crop cycle and agronomic management with that of wheat, it could easily be inserted into the cereal cropping system as an alternative to other small cereals, without the need of any specific adaptation for its cultivation (Martín et al., 1999; Millán et al., 1988). Both spring- and winter-type tritordeum genotypes exist, according to the form of the wheat genotypes used for crossing. The duration of the growth stages and the development of tritordeum are similar to those of wheat plants in different growing areas. Martinek et al. (2003) reported that spring tritordeum cultivars cultivated under Central-European conditions had a clear lateness and non-uniform maturation, as a consequence of a high level of rejuvenation of the tillers that characterize the perennial nature of *H. chilense*. Furthermore, these problems have successfully been avoided by means of a period of vernalization, together with an autumn sowing, which also result in a better development and higher yield of tritordeum in these environments. Moreover, Millán et al. (1988) and Villegas et al. (2010, 2008) reported that abiotic stresses during the vegetative stages in Mediterranean environments with frequent drought stress during ripening led to a longer delay in the anthesis moment for tritordeum than for wheat, and thus resulted in a shorter grain filling period.

The root system architecture of tritordeum is the typical one of small cereals. Furthermore, the root length, surface area and tips of tritordeum have been found to be lower than those of wheat (Visioli et al., 2020), although tritordeum has shown a greater root thickness. Furthermore, these authors highlighted that tritordeum had a root microbiome that was richer in species, particularly of *Bacterioides* species, than wheat cultivars.

The susceptibility of this crop to lodging has been debated in literature, in consideration of the production situations that could determine the manifestation of this agronomic problem. Several researches have reported a lower height for tritordeum, on average 20%, than for wheat, and thus a lower susceptibility to lodging (Barro et al., 1996; Martín et al., 1996; Visioli et al., 2020). However, Montesano et al. (2021) and Yousfi et al. (2010) reported a higher susceptibility of lodging for tritordeum than for wheat. Kakabouki et al. (2020) highlighted the key role of the genotype in influencing the plant height and showed clear intra-varietal differences within both the compared tritordeum and wheat. Visioli et al. (2020) also reported a clear variability of lodging for different tritordeum cultivars. The higher predisposition of tritordeum to lodging in high fertility growing areas may be linked to its greater tillering capacity than that of wheat (Pinto et al., 2002) and a higher culm weakness (Lima-Brito et al., 2006).

As far as the ear is concerned, tritordeum has a brittle rachis and harder glumes than wheat, which cause a higher physical impediment to threshing, which in turn leads to a slightly more difficult cleaning of the kernel and/or greater grain losses at harvest (Ávila et al., 2021). In addition to a possibly higher number of ears per plant, as a consequence of a superior tillering capacity, the number of spikelets per ear may also be higher than that of wheat, although a higher incidence of sterile spikelets has been recorded (Pinto et al., 2002).

The caryopsis of tritordeum has an ellipsoidal form of around 9 cm, with a diameter of 3 mm, and has thin hair on the top and a floury endosperm (Salvá, 2016).

2.07.2.2 Tolerance to Abiotic Stress

Tritordeum had shown a widespread adaptability to different environmental growth conditions. It is mainly cultivated in South Europe, but its cultivation has also been reported in several continental production situations in Central and North Europe.

Several researches, carried out in Mediterranean growing areas, have reported the good agronomic response of this crop in semiarid environments, thanks to its adaptability to drought stress conditions (Kakabouki et al., 2020; Martín et al., 2000, 1999; Villegas et al., 2010), and a general better drought tolerance than bread wheat (Martín et al., 1999), but sometimes also than durum wheat (Gallardo and Fereres, 1989; Simane, 1993). Lower stomatal conductance and transpiration under water-deficit conditions have been reported for tritordeum than for wheat during the pre-anthesis period (Villegas et al., 2010). Tritordeum has shown a high generation rate of molecular oxygen, under high irradiance conditions, but also a lower dark respiration rate and light compensation, which help to explain the lower grain yield of tritordeum than that of wheat (Barro et al., 1996).

Tolerance to salinity is another ecological trait of *H. chilense* that also seems to be expressed in tritordeum (Villegas et al., 2010; Martín et al., 2000) and it is probably connected to a lower stomatal conductance of tritordeum than that of wheat (Aranjuelo et al., 2009). Yousfi et al. (2010), studying a combination of water deficiency and salinity concentration in the cultivation of durum wheat, triticale and tritordeum, confirmed a higher tolerance of this crop to moderate salinity, due to a lower stomatal conductance, a lower leaf accumulation of Na+ and a higher K+/Na+ ratio, all of which delay plant senescence and help maintain a plant's capacity to assimilate nitrogen.

Martinek et al. (2003) highlighted that the winter-hardiness of tritordeum is clearly related to the freezing tolerance of the wheat genotypes used for the crossing with *H. chilense*. The tritordeum cultivars that are currently cultivated in central Europe in fact demonstrate a low risk to damage from winter frost.

2.07.2.3 Nutrient Absorption and Nitrogen Use Efficiency (NUE)

The possibility of limiting the use of fertilizers in modern cereal cropping systems, and nitrogen (N) in particular, could lead to a significant increase in environmental sustainability. Thus, the aim of researchers is to optimize the crop management practices and improve the Nitrogen use efficiency (NUE; Congreves et al., 2021) of the crop, without any excessive decrease in the cereal yields or quality (Lea and Azevedo, 2007; Lea and Miflin, 2010).

As tritordeum is a new cereal, it could be of particular interest to verify its capacity to maximize its nutrient use efficiency, in those areas that are the most suitable for its cultivation, through a correct management of such a cultivation and of its management, in fertilization terms. The first experiments in this direction, which were carried out in growth chambers (Aranjuelo et al., 2013; Barro et al., 1991, 1994, 2003), highlighted a higher NUE in tritordeum than in wheat. Barro et al. (1994) showed that tritordeum has a higher affinity to nitrate than durum wheat and, consequently, a greater capacity to absorb it from soil. Tritordeum has shown higher levels of nitrate reductase activity at the leaf level than durum (Barro et al., 1991) and bread wheat (Wallace, 1986), which could lead to a high remobilization and translocation capacity of this nutrient to the kernel during ripening. Aranjuelo et al. (2013) found that tritordeum resulted in a higher N uptake than wheat in a controlled growth chamber, albeit only under low N rate conditions.

The efficiency in the use of N by tritordeum, and by durum and bread wheat, has been compared, in field production situations, with ordinary N rate fertilizations of between 120 and 230 kg N ha^{-1} (**Fig. 1**). The NUE of tritordeum was found to be 40% and 30% lower than that of bread and durum wheat, respectively. The NUE values for tritordeum were generally higher in growing areas with temperate climatic conditions than in Mediterranean areas.

Unlike the positive physiological traits observed for tritordeum under controlled conditions, the field comparison of the tritordeum cultivars available on the market has highlighted a lower performance than wheat for the NUE, expressed as yield per unit of fertilization applied. According to the fertilized-based NUE reported in **Fig. 1**, the low efficiency shown by tritordeum is mainly a consequence of an already existing yield gap with wheat (see **Grain yield** section). Furthermore, since tritordeum has a higher N content in the grain at harvest than wheat (see **Grain Protein Content** section), the recovery efficiency of a fertilizer, which is used to indicate the apparent increase in plant N uptake in response to the N input (Congreves et al., 2021), is expected to be similar to that of wheat. In order to increase the nutrient efficiency of tritordeum and its sustainability, it will first be necessary to increase the yield potential of the next generation of commercial cultivars.

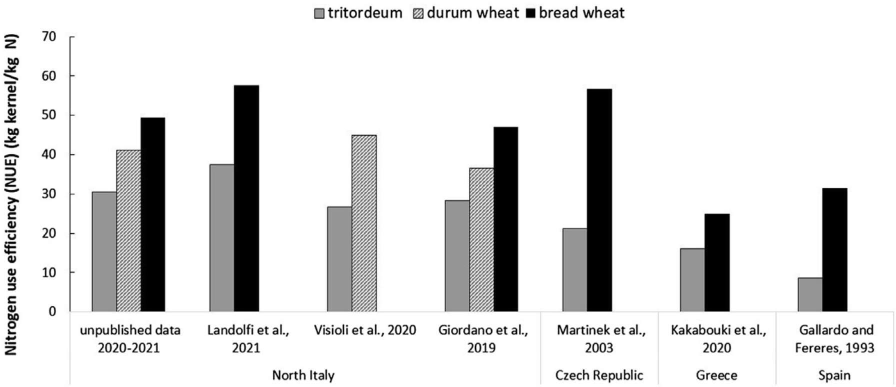

Fig. 1 Comparison of fertilized-based nitrogen use efficiency (NUE) between tritordeum, durum and bread wheat for different production situations. Fertilized-based nitrogen use efficiency (NUE) expressed as partially factor productivity, calculated according to Congreves et al. (2021) as the ratio between the grain yield, expressed in kg/ha, and the total nitrogen applied with fertilization expressed in kg N/ha. The data sources are reported in the figure.

2.07.2.4 Disease Tolerance and the Related Sanitary Traits

The *H. chilense* genome presents numerous types of resistance to many foliar diseases which could be transferred to tritordeum (Martín et al., 1996). According to the date available in the literature, tritordeum shows a good tolerance to barley brown rusts (*Puccinia hordei*), while its susceptibility to wheat brown rusts (*P. recondita* f.sp. *tritici*) is similar to that of wheat (Rubiales et al., 1991). Prats et al. (2006) observed that tritordeum genotypes are sometimes more resistant to powdery mildew (*Erysiphe graminis tritici*) than wheat, while Martinek et al. (2013) reported a good tolerance to Septoria leaf blotch (caused by *Mycosphaerella graminicola*). Rubiales et al. (2001) suggested that the better tolerance of tritordeum to Septoria leaf blotch is due to genes located on chromosome 4Hch and which are inherited by *H. chilense*. However, the typical avoidance mechanism of the *Hordeum* genome, that is, an appressorium formation to overstep the wax barrier on the stomata, has not been identified for tritordeum (Martín et al., 1996).

Hexaploid tritordeum is less susceptible to common bunt (*Tilletia caries*) than wheat, although the tolerance to this disease was observed to decrease in octoploid lines (Rubiales and Martın, 1999). On the other hand, a high susceptibility to *Fusarium* head blight (FHB), mainly caused by *Fusarium graminearum* and *F. culmorum*, has been reported for tritordeum cultivars when grown in temperate areas prone to this disease (Spaggiari et al., 2019). The occurrence of FHB in tritordeum, as in other small cereals, leads to a high yield loss (Duffeck et al., 2020), a reduction in the test weight and, consequently, in the milling rate, and the accumulation of mycotoxins, that is, toxic compounds for which legislative thresholds have been established in several countries (European Commission, 2006). Tritordeum grown in North Italy has shown a higher content of trichothecenes, the most frequent class of mycotoxins found in small cereals, than bread wheat, and a similar content to durum wheat, a species considered highly prone to this sanitary risk (Spaggiari et al., 2019).

Although the tolerance of durum wheat to FHB is very low and the variability for this trait in the tetraploid gene pool is very limited (Haile et al., 2019), the development of tritordeum cultivars that are less prone to the occurrence of mycotoxins for Central-Europe growing areas would require the use of durum parental lines specifically selected for this trait. Moreover, after screening some accessions of *H. chilense* and detecting a certain variability of reaction to FHB, Fedak (2017) crossed the best accessions with a durum cultivar that resulted in a clear variability to disease tolerance in the obtained tritordeum lines. In addition to FHB, Martinek et al. (2003) also reported a higher infection by ergot (*Claviceps purpurea*, another fungal specie able to produce mycotoxins) for several tritordeum genotypes.

With the exception of the clearly higher susceptibility of FHB, the tritordeum presented in the literature shows a similar or better tolerance to foliar diseases than wheat, thus generally making the disease control of this crop simple. Furthermore, the majority of field experiments reported in the literature have been carried out in growing areas with a low disease pressure, and the tolerance of tritordeum to these biotic adversities still needs to be verified in production situations more prone to the diseases. Field experiments carried out in North Italy have highlighted that an application of fungicide at heading results in a 10% increase in grain yield, which is similar to that recorded for bread wheat (unpublished results). Thus, in temperate growing areas, tritordeum, as well as wheat, could benefit from control strategies that are able to prevent the infection of fungal diseases, while the cropping system primarily needs to be designed to limit the contamination of kernels by mycotoxins.

2.07.2.5 Grain Yield

Tritordeum is a new species that has been the subject of limited breeding activities, compared to the reference small cereal species. Yield comparisons have been carried out between the first available genotypes of tritordeum and the conventional and widely cultivated bread and durum wheat cultivars over the last 35 years, and this crop has been confirmed to have an interesting yield potential. On average, the yield gaps with bread wheat and durum wheat fall between 43% and 40% respectively (**Fig. 2**). The reported tritordeum grain yields show averages of 2.6 t/ha and 3.6 t/ha for temperate Mediterranean and Central-Europe continental environments, respectively. As far as the interaction with the growing areas is concerned, the yield performance of tritordeum in such Mediterranean regions as South Spain, Tunisia, Lebanon, Greece and South Italy, is quite close to that of wheat and other species, such as triticale (Villegas et al., 2010). In such environments, the yield gap between tritordeum and durum and bread wheat is on average 46% and 39%, respectively.

The reported difference, in terms of grain yield, between tritordeum and wheat in continental and more fertile growing areas, such as the Czech Republic and North Italy, falls between 23% and 45%, respectively.

Although inferior to modern wheat cultivars, tritordeum has shown a 34% higher grain yield than the wheat landraces that were cultivated in the last century (Landolfi et al., 2021), and for which there has been a relaunching for the production of special baked goods in developed countries.

There is a consistent difference in the kernel productive traits between the thousand kernel weight (TKW) of tritordeum and that of wheat: tritordeum on average results in a lower TKW than durum (−48%) and bread wheat (−38%) (**Table 1**). The lower TKW reported in Mediterranean growing areas could explain the lower yield potential of the crops in these production situations. Villegas et al. (2008) demonstrated that the low TKW of tritordeum could be due to a delay in reaching the anthesis stage, compared to other small cereals, which would result in a shorter period for the accumulation of starch in the grains, thus lightning their weight.

The test weight (TW) is a representative index of the yield capacity of a crop, as a result of a correct ripening stage. The TW is important from a nutritional point of view, because of the direct relationship between this parameter and the energy content

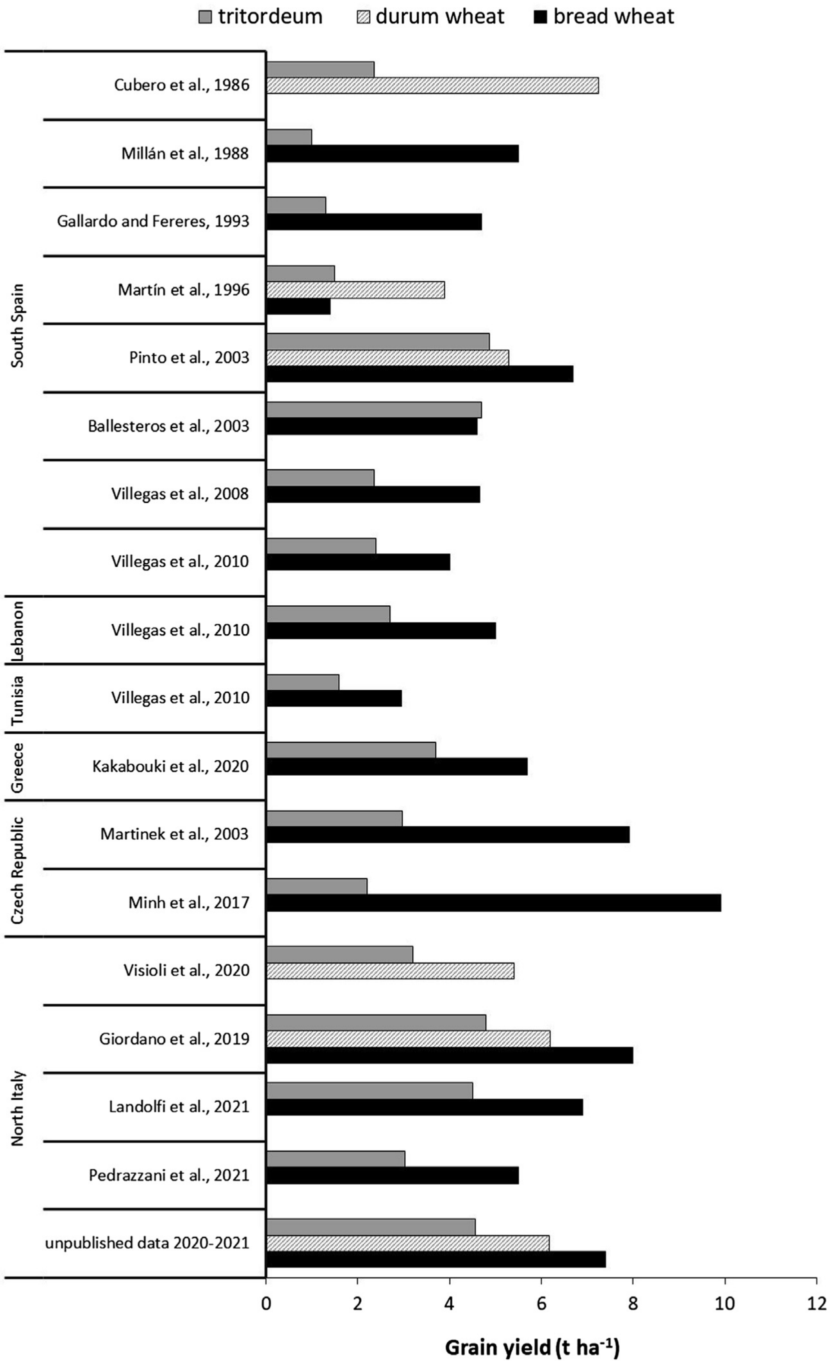

Fig. 2 Comparison of the grain yields of tritordeum, durum and bread in different environments. The data sources are reported in the figure.

and feeding value of the grain. Moreover, it is an important qualitative index, since it is directly related to the milling rate and thus to the profitability of the transformation of grains into refined flour for millers. Hulled barley is characterized by a much lower TW than hulled durum or bread wheat, as the hull, which is rich in fiber, remains attached to the seed at maturity. Although the hull of tritordeum is removed during the harvesting operations, the reported TW has always been much lower than that of wheat. In the reported production situations, the TW of tritordeum has always been lower than 76 kg/hL, a value that is often considered the minimum threshold for the purchasing of wheat by mills. No information is available in the literature on the milling rate of tritordeum, compared to wheat. However, an improvement in the ability of this crop to accumulate starch during ripening and increase the TW may be a desirable trait for future breeding programs, in order to increase its productivity and the potential milling use of this cereal.

Table 1 The thousand kernel weight (TKW) and test weight (TW) of tritordeum, and of durum and bread wheat

		TKW (g)			*TW (kg/hL)*		
Location	*References*	*Tritordeum*	*Durum wheat*	*Bread wheat*	*Tritordeum*	*Durum wheat*	*Bread wheat*
South Spain	Alvarez et al., 1992	35.4	54.3	44.6	75.3	81.2	80.34
South Spain	Gallardo and Fereres, 1993	28.3		48.7			
South Spain	Martín et al., 1996	33.0	56.0	39.0			
South Spain	Pinto et al., 2002	21.6	39.9	36.5			
South Spain	Pinto et al., 2003	21.0	39.9	36.5			
South Spain	Villegas et al., 2008	21.3		34.9			
South Spain	Villegas et al., 2010	33.4		51.7			
South Italy	Villegas et al., 2010	41.0	53.1				
Lebanon	Villegas et al., 2010	27.2		42.0			
Tunisia	Villegas et al., 2010	23.5		36.0			
South Spain	Ballesteros et al., 2003	46.5		49.7	74.0		81.0
South Spain	Atienza et al., 2007a,b,c	26.0		34.5			
Greece	Kakabouki et al., 2020	29.8		29.0	67.4		72.7
South Italy	Montesano et al., 2021	36.3	35.4		73.9	74.8	
North Italy	Giordano et al., 2019	39.4	47.9	46.8	72.7	72.9	81.2
North Italy	Landolfi et al., 2021	36.5		38.9	69.9		75.5
North Italy	Unpublished data 2020–2021	37.7	51.1	45.0	72.9	80.1	81.3

The data sources are reported in the table.

2.07.3 Nutritional Traits

A few works in the literature have dealt with the characterization of the nutritional composition of tritordeum and compared it with bread and/or durum wheat. The tritordeum grain is overall very similar in proximal composition to that of wheat (**Fig. 3**). The protein content and, albeit to a lesser degree, the fat concentration in the whole grain, which increase from bread to durum wheat to tritordeum, represent the main differences. Thus, tritordeum has a lower starch content than bread or durum wheat, while no difference has been observed in the kernel content of the total dietary fiber (TDF) or ash.

2.07.3.1 Carbohydrates

2.07.3.1.1 Starch

Cereals generally mainly consist of carbohydrates, mostly starch, which give the crop a particular source of energy, and this allows them to play a central role in human nutrition. Starch is the main component of grain and it is a digestible polysaccharide composed of amylose and amylopectin, whose properties are fundamental for the quality of cereal and, consequently, for numerous food formulations that are studied carefully by food processing industries (Khatkar et al., 2009).

Erlandsson (2010) and Mikulíková et al. (2011) reported slightly lower starch values in tritordeum (65% dry weight) than in bread and durum wheat (70%), thus confirming previously reported data obtained in other growing areas. However, there is a lack of studies on the starch content and above all on the starch properties of tritordeum.

As far as the starch composition is concerned, Alvarez et al. (2019) investigated the waxy (Wx) gene responsible for amylose synthesis, and demonstrated a greater similarity of the gene structure of tritordeum with the barley structure than with the wheat one. Any mutation of this gene determines a decrease in the amylose and amylopectin ratio, which results in a higher flour swelling

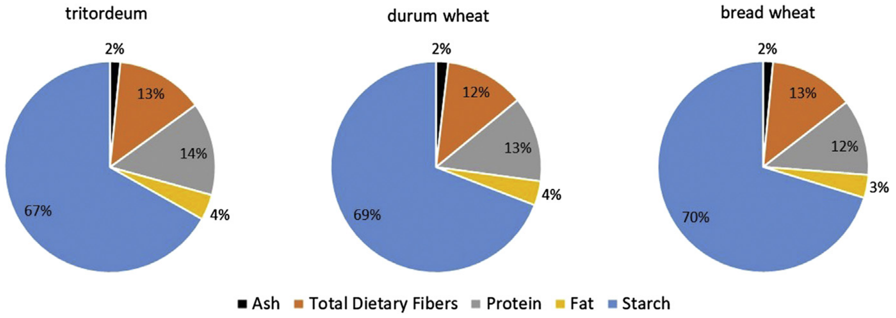

Fig. 3 Comparison of the proximal composition (%) of tritordeum, and of durum and bread wheat. Data sources: Cubero et al. (1986), Giordano et al. (2019), Mikulíková et al. (2011).

power and, consequently, a higher loaf volume (Martín et al., 2008). Although various differences have been observed in the position and presence of some amino acids in one transit-peptide of 70 amino acids belonging to the Wx gene, the enzymatic function seems to be almost the same (Alvarez et al., 2019). Further investigations are needed to select new tritordeum cultivars with different starch properties in order to satisfy the specific demands of the food industry.

2.07.3.1.2 Fibers

The TDF of grain includes insoluble fiber (cellulose, hemicelluloses as arabinoxylans, lignin) and soluble fiber (fructans, galactose, resistant starch, β-glucans). The intake of the dietary fiber of cereals has been shown to confer several beneficial effects to the health of individuals as it reduces the risk of developing numerous diseases, such as coronary heart disease, type II diabetes, obesity, several gastrointestinal disorders, and cancer, and it also promotes immunomodulatory activity, cholesterol lowering activity and several prebiotic effects (Anderson et al., 2009; Mendis and Simsek, 2014).

Although Giordano et al. (2019) did not observe any difference in the occurrence of the TDF of tritordeum, a few studies have reported that this crop could have a higher TDF (**Table 2**) than bread (+10%) or durum wheat (+10%), even though hulled barley still presents considerably higher amounts (on average +30%, compared to tritordeum) (Cubero et al., 1986; Erlandsson, 2010). This promising potential is stimulating a great deal of interest in developing food products rich in fiber, using tritordeum as a raw material (Dreher, 2001). An improvement in the TDF content of pasta has recently been verified, using not only brewers' spent grain, but also a portion of flour made from tritordeum (Nocente et al., 2021), without compromising the sensorial quality of the final product.

Although knowledge on the distribution of the fiber components in the tritordeum kernel has not yet been explored in any great detail, Giordano et al. (2019) reported that the content of the TDF can reach up to 80%, albeit only in the most external kernel layers, in analogy with wheat and barley.

Among the various types of soluble fiber, fructans are considered to be very important compounds that have a characteristic heathy prebiotic function which helps to preserve the correct functioning and balance of the intestinal bacterial flora. Åman (1988) observed their presence in higher concentrations in tritordeum (1.9% of dry matter) than in bread wheat (0.8%). At the same time, the presence of these soluble compounds may not always be a desired element in a flour or food, because they also constitute one of the major fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) reported in cereals (Pejcz et al., 2019). FODMAP has been shown to trigger carbohydrates and cause the characteristic symptoms of celiac disease and irritable bowel syndrome (IBS). Recent studies have recommend a low FODMAP diet to reduce these disorders (Fraberger et al., 2018).

Certain components of dietary fiber, such as arabinoxylans (AX), present numerous interesting structural and functional properties at both the cellular and technological levels, and these properties influence the chemical-physical properties of the derived dough and food. AX structurally strengthens plant cells (Saulnier et al., 2007) and acts as a storage element for phenolic acids, e.g., ferulic acid. From the technological point of view, these polysaccharides confer physic-chemical properties that are particularly interesting for the food industry, both for bread-making (Courtin and Delcour, 2002) and for brewing use (Nocente et al., 2021). In addition, important evidence has emerged of beneficial effects on human health, because of their role in the prebiotic function (Grootaert et al., 2007), immunomodulatory activity (Samuelsen et al., 2011), the control of postprandial glucose and the insulin level (Lu et al., 2000), as well as antitumor activity (Cao et al., 2011). The total AX amount (**Table 2**) in tritordeum whole grain has shown higher values (+26%) than bread wheat (Giordano et al., 2019), with the highest concentration having been quantified in the Aucan variety (2.15% dry weight). Rakha et al. (2012) also confirmed a higher content of AX in tritordeum whole grain than in bread wheat (+11%) in colder and continental environments. Giordano et al. (2019) demonstrated a homogeneous distribution of these hemicellulose polysaccharides in all the grain pearled fractions.

Another soluble fiber class with an interesting health claim is β-glucan, which contributes to preventing type 2 diabetes, by improving the postprandial glucose and lipid control (Andrade et al., 2015; Henrion et al., 2019), and cardiovascular diseases (Henrion et al., 2019). Its capability to alter the gut microbiota profile has been associated with a decreasing impact of cardiovascular risk markers (Wang et al., 2016). According to EFSA (2009), a regular consumption of this element, that is, at least 3 g per day, helps to maintain a normal blood cholesterol concentration and maintain or achieve a normal body weight. Although developed from a wild barley species, tritordeum does not result in an enrichment of β-glucan, compared to wheat. On average, barley results

Table 2 Comparison of the total dietary fibers, arabinoxylan and β-glucan for tritordeum, durum and bread wheat, and for barley

Dietary compound	*Study*	*Tritordeum*	*Durum wheat*	*Bread wheat*	*Barley*
Total dietary fiber (%) (TDF)	Cubero et al., 1986	3.9	3.5	–	–
	Erlandsson, 2010	14.3		12.8	17.6
	Giordano et al., 2019	13.5	12.2	11.7	25.2
Arabinoxylans (%) (AX)	Giordano et al., 2019	1.9	1.1	1.4	1.3
	Rakha et al., 2012	6.9		6.2	8.1
β-glucans (%)	Giordano et al., 2019	0.7	0.4	0.9	3.5
	Rakha et al., 2012	0.6		0.6	5.9

The data sources are reported in the table.

in 33% more β-glucan than tritordeum (Table 2). Furthermore, as can be seen in the table, the β-glucan content of tritordeum (0.65% of dry matter) is very similar to that of the reference wheat cultivar (0.75%) (Giordano et al., 2019; Rakha et al., 2012), while tritordeum has a larger portion of β-glucan (+43%) than durum wheat (Giordano et al., 2019). The β-glucan in tritordeum is principally distributed in the intermediate layers of the kernel (10%–25% of grain kernel), with a very similar distribution to that of durum wheat, while the bread wheat variety presents the largest content of β-glucan in the most external layer (5%–10% pearled fraction) (Giordano et al., 2019).

2.07.3.2 Grain Protein Content

Cereals play a major role in human nutrition, with the highest consumption of wheat products being observed in Mediterranean diets, and this consumption is increasing throughout the world (Shewry, 2009). For this reason, a cereal with a higher protein content and a good protein composition can be considered an advantage for a healthy diet, even though it remains a food source with a lower protein content than other ones. Since the first stages of tritordeum breeding, the grain protein content has been reported to be higher than that of durum or bread wheat (Alvarez et al., 1995a,b; Cubero et al., 1986; Folina et al., 2020).

Several field studies have compared the grain protein content (GPC) of tritordeum with that of durum and bread wheat in different climatic areas over the last two decades (Fig. 4). On average, tritordeum results in a GPC of between 11% and 17%,

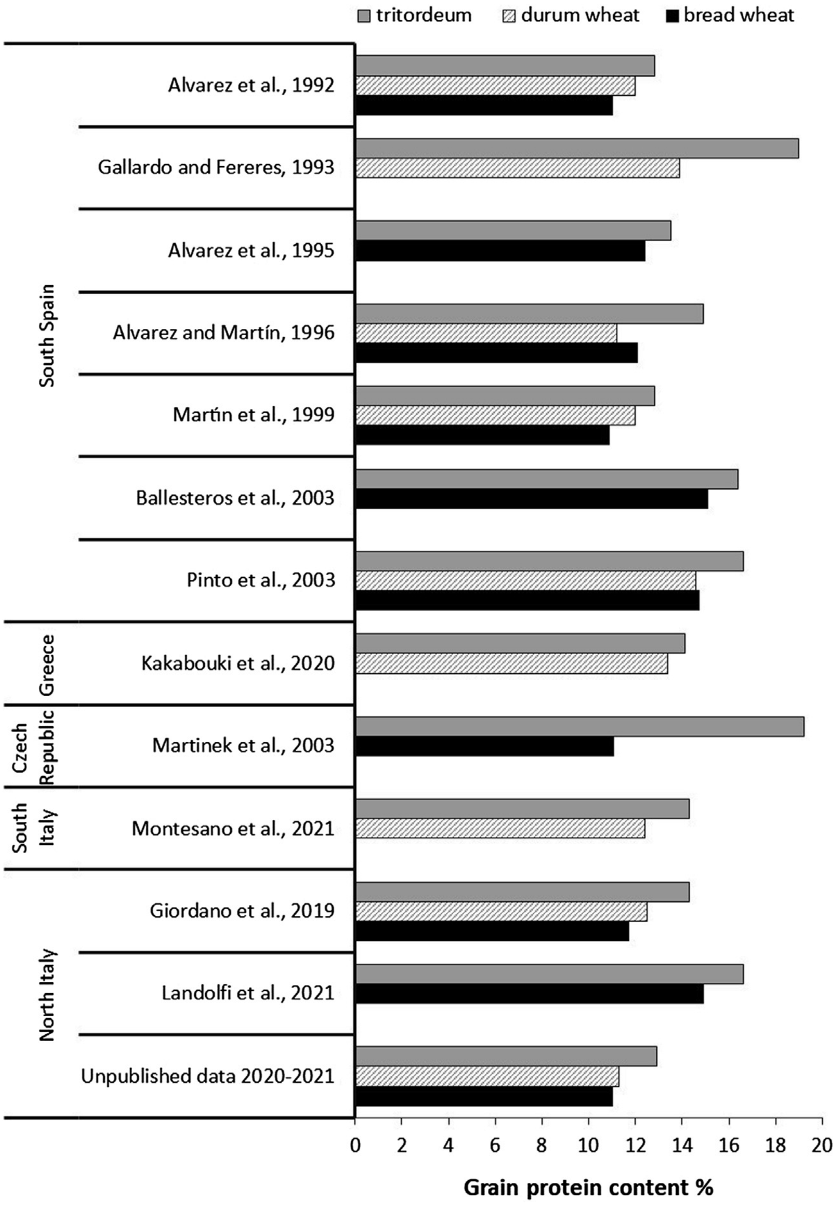

Fig. 4 Comparison of the grain protein content (GPC) in tritordeum, durum and bread wheat for different environments. The data sources are reported in the figure.

and it reaches higher values than bread (+22%) and durum (+16%) wheat. This advantage, in terms of the GPC of tritordeum, has been confirmed in both continental and Mediterranean environments.

The large amount of grain protein in the kernel could be related to the negative correlation with the typical grain yield of Mediterranean environments, where the short duration of the grain filling stages results in a lower dilution of protein with starch, which is the main grain component (Garrido-Lestache et al., 2004). Different tritordeum cultivars have been studied in Greece to investigate their adaptation to a dry and warm climate, and the negative correlation between grain yield and grain protein was confirmed (Kakabouki et al., 2020). Furthermore, the large difference in GPC between tritordeum (+42%) and bread wheat cultivars was also recorded under central European climatic conditions (Martinek et al., 2003), even though tritordeum presented the same growth and development timings as the wheat varieties. However, under certain conditions, such as those characterized by a warm and dry Mediterranean climate, higher levels of GPC than those of durum (+12.5%) and bread wheat (+27%) have been confirmed, without showing any significant differences in grain yield (−2% and −7%, respectively) (Ballesteros et al., 2003; Martín et al., 1996). Tritordeum seems to uptake N fertilization more efficiently during post-anthesis than wheat and triticale varieties (Aranjuelo et al., 2013).

It is well known that GPC, in addition to the genetic properties, is influenced to a great extent by the crop management practices. Visioli et al. (2020) observed that tritordeum showed a higher GPC than durum wheat under organic management, while they observed no differences between the species under a conventional system. Folina et al. (2020) verified that tritordeum played a major role in increasing GPC after inorganic fertilization, compared to an organic one. Landolfi et al. (2021) compared a tritordeum cultivar with a landrace and a modern wheat, and reported GPCs of 16.6%, 17.7% and 14.9%, respectively. They showed that the kernel gluten content of tritordeum and landrace, which showed the highest values of GPC, was influenced by increases in the total N fertilization rate.

Tritordeum seems to have a lower essential amino acid content of these compounds (66 g amino acid/16 g of N) than durum wheat (69.8 g amino acid/16 g of N) (Cubero et al., 1986). Furthermore, a slightly higher essential amino acid content in tritordeum has been reported in Central-Europe, and of phenylalanine, valine and cysteine in particular, than in bread wheat (Martinek et al., 2003).

2.07.3.3 Bioactive Compounds With Antioxidant Activity

Since the primary tritordeum lines were first developed, their phytochemical composition has shown its great potential as a raw ingredient for the production of healthy food (Alvarez et al., 1995a,b). In fact, *H. chilense* is an interesting source of phytochemicals that exert antioxidant activity (Eliášová and Paznocht, 2017). The total antioxidant capacity depends on the synergistic interaction of several phytochemical compounds that are present in cereals, whole grains and flours (Adom and Liu, 2002). Although phenolic acids are generally the main compounds of cereals with antioxidant activity, other grain phytochemicals could also contribute to their overall antioxidant potential (Cömert and Gökmen, 2017).

The tritordeum kernel has shown an overall higher content and activity of antioxidant compounds than bread or durum wheat (**Fig. 5**). Most of the available data, which have been obtained considering different methods to compare the antioxidant activity (DPPH, ABTS●+, FRAP), have highlighted better results for tritordeum than for bread or durum wheat. Only the study of Eliášová and Paznocht (2017) reported a clearly better total phenolic content and total antioxidant activity for bread wheat than for

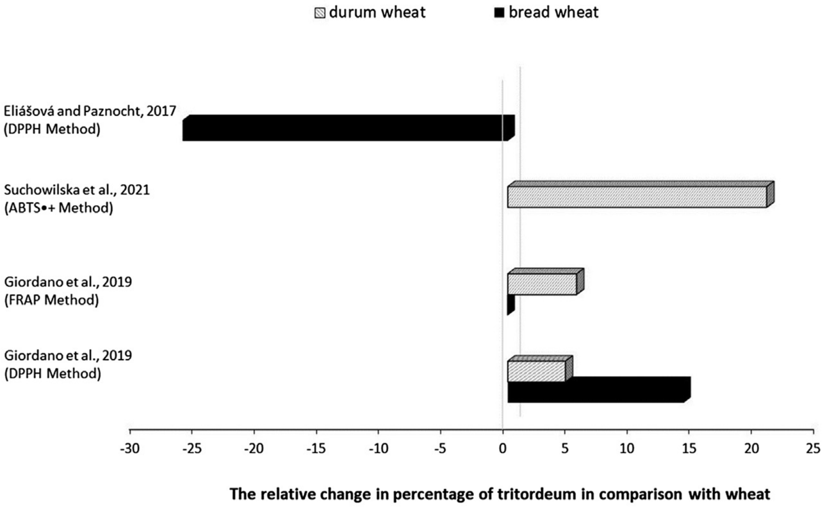

Fig. 5 Comparison of the percentage difference in the total antioxidant activity of tritordeum, durum and bread wheat. The data sources are reported in the figure.

tritordeum. Furthermore, Eliášová and Paznocht (2017) and Giordano et al. (2019) reported that barley kernels had an even higher total antioxidant activity than tritordeum and wheat.

Giordano et al. (2019) verified that the compounds that confer an antioxidant capacity are mainly concentrated in the outer layer (10%–20% external pearling layers) of tritordeum, durum and bread wheat, and of barley, thus also confirming the importance of the use of the pericarp fraction of tritordeum to obtain flour with a high health value. Furthermore, in their study on tritordeum, they identified a greater antioxidant activity (+50%) in the inner layers of the grain than for the other cereals. Thus, refined tritordeum flours represent a promising healthy raw food material and have a better photochemical profile than wheat. The bioactive compound content of tritordeum with antioxidant activity is analyzed in depth in the following sections.

2.07.3.3.1 Carotenoids

Carotenoids are isoprenoid compounds that can be divided into hydrocarbon carotenes (i.e., α and β-carotene and lycopene) and their oxygenated derivatives, which are called xanthophylls (i.e., lutein and zeaxanthin) (Paznocht et al., 2018). These elements are the characteristics that to give endosperm grains and, consequently, flour, their yellow color. The assumption of carotenoids in a diet confers several beneficial aspects because of their function of protecting individuals from photo-oxidative damage and limiting membrane damage (Atienza et al., 2007a), as well as of reducing the risk of developing certain types of cancer and other degenerative and/or chronic diseases (Al-Delaimy et al., 2005). Moreover, β-carotene presents provitamin A activity, which is responsible for maintaining certain properties against ocular degeneration, for protecting the skin from sunlight as well as being involved in healthy bone development and strengthening the immune system (Olson, 1989). Carotenoids have a great health potential, which is an important target for cereal bio-fortification (Farré et al., 2010). They are also of great interest because of their visual role in the consumers' choice of derived food products.

As far as the carotenoid profile is concerned, lutein is the most represented compound (>90%) in tritordeum (Mellado-Ortega and Hornero-Méndez, 2015). With an average of 7 mg/kg of lutein, tritordeum presents much higher values than durum (+53%) and bread wheat (+67%), or barley (+37%). Durum wheat does not contain any lutein in esterified form and this aspect weighs negatively on its final amount, compared to tritordeum, which, on the other hand, accumulates most of the luteins in this more stable form (Mellado-Ortega and Hornero-Méndez, 2018). Another important factor is related to the weather conditions during grain filling: it has been reported that high temperatures during ripening could lead to a decrease in the total carotenoid and free lutein contents of tritordeum (Mattera et al., 2017) (**Fig. 6**). Zeaxanthin compounds, even though in a smaller number than luteins, are the second xanthophyll in number in tritordeum. This compound is principally concentrated in the intermediate part of the kernel (Giordano et al., 2019), and it has a similar content to that of wheat, although even 100 times greater concentrations have been found in barley (**Table 3**).

Tritordeum and bread wheat show similar amounts of α- and β-carotenes (almost 0.1 ppm) to durum wheat, while no carotenes have been identified in barley (**Table 3**). An extremely low level of carotene compounds has been detected in all of the so far studied cereal cultivars, and this is probably due to the rapid hydroxylation and formation of xanthophylls followed by different esterification reactions (Paznocht et al., 2018). The esterification process in tritordeum is caused by the *Hordeum* genome (Mellado-Ortega and Hornero-Méndez, 2015). This process, which is clearly present in both tritordeum and durum wheat, permits the accumulation of carotenoids to be preserved at the endosperm grain level, thus transforming them into esterified forms during both the storage period and the flour baking process (Atienza et al., 2007a; Mattera et al., 2020; Mellado-Ortega and Hornero-Méndez, 2016), but also their bioavailability to be improved and their uptake and transport to be facilitated (Pérez-Gálvez and Mínguez-Mosquera, 2005). As far as the distribution of carotenoid compounds in the kernel is concerned, there are conflicting results in the literature: Mellado-Ortega and Hornero-Méndez (2018) observed a homogeneous distribution between the outermost and innermost layers, while Giordano et al. (2019) verified a marked decrease from the external to the inner pearled fractions, similar to the behavior of durum and bread wheat observed by Mellado-Ortega and Hornero-Méndez (2015).

2.07.3.3.2 Phenolic Compounds

Numerous studies have demonstrated the presence of a wide range of antioxidant compounds in cereal grains, mainly concentrated in the bran tissues, but among these, phenolic compounds certainly stand out because of their greater beneficial effect on human health (Beta et al., 2005). Phenolic compounds include all the molecules that have one (phenolic acids) or more (polyphenols) aromatic rings with hydroxyl groups. These elements are responsible for the distinctive antioxidant activity of these compounds, which are able to inactivate free radicals and prolong the quality and shelf-life of food (Minatel et al., 2017). Phenolic acids are important elements for a healthy diet because of their antioxidant and anti-inflammatory properties (Sosulski et al., 1982) as well as their antitumor preventive effect (Riahi-Chebbi et al., 2019). Furthermore, phenolic acids are known to give excellent organoleptic characteristics, such as the flavor, taste, and color of the raw flour material used for the final baking of foods, to dough and, consequently, to processed products.

The main grain antioxidant compounds in tritordeum are phenolic acids, as in wheat and barley (Navas-Lopez et al., 2014). A relationship between the presence of phenolic acids and antioxidant activity has been reported for both tritordeum and bread wheat (Eliášová and Paznocht, 2017).

Phenolic acids are divided into soluble (SPA) and cell-wall bound (CWBPA) types, according to their solubility. The total phenolic acids measured in a few studies (**Fig. 7**), which compared tritordeum with durum and bread wheat, as well as with barley, showed that tritordeum grains had a greater soluble phenolic acid content than bread wheat (+25%) and barley (+46%),

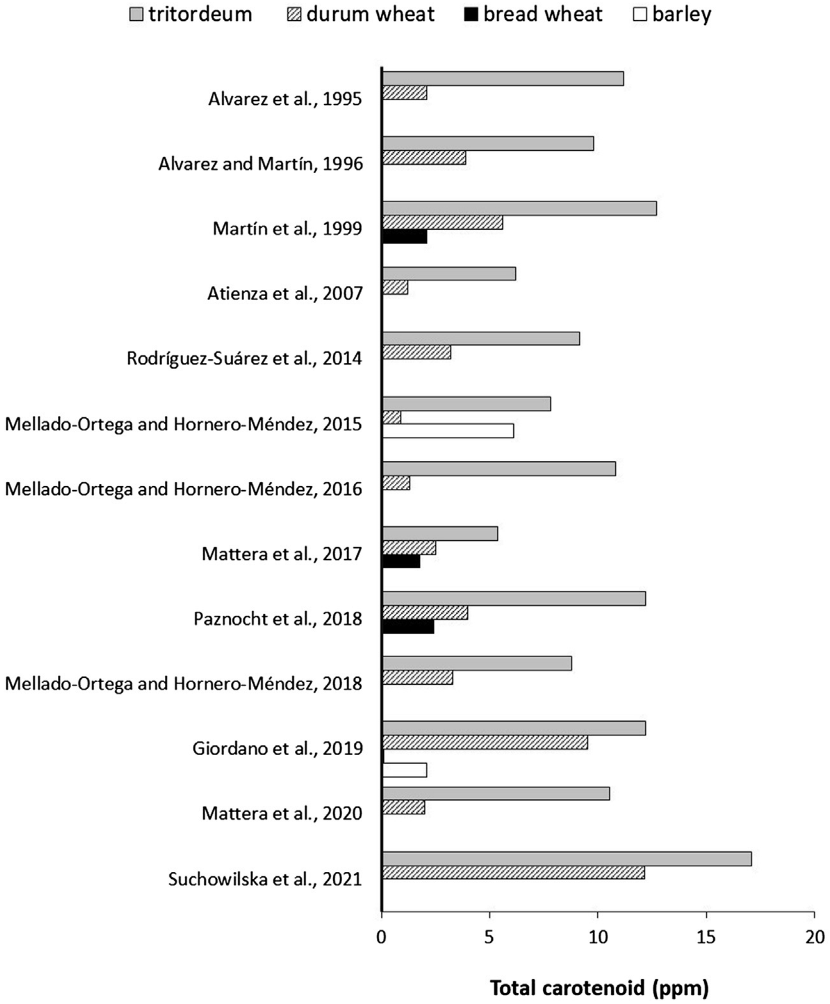

Fig. 6 The total carotenoid content in tritordeum, durum wheat, bread wheat and barley. The data sources are reported in the figure.

respectively. Giordano et al. (2019) confirmed the high content of SPA in tritordeum, compared to bread wheat, although it resulted in an 11% lower content than a durum wheat cultivar. Montesano et al. (2021), Suchowilska et al. (2021) and Giordano et al. (2019) also reported a higher CWBPA content in tritordeum than in durum wheat. Furthermore, Giordano et al. (2019) found that the content of these compounds was the highest in barley, followed by bread wheat. Giordano et al. (2019), studying the distribution of these bioactive compounds in grain pearled fractions, highlighted that the CWBPAs in whole grain were even lower in tritordeum than in bread wheat, and that tritordeum presented a 30% higher content at the inner level of the kernel than bread wheat.

Several differences in the phenolic acid composition in tritordeum have been pointed out in the available studies (**Fig. 8** and **Fig. 9**), mainly on SPAs. Montesano et al. (2021) have underlined a greater difference in the composition of tritordeum than that of durum wheat, while Giordano et al. (2019) observed a clearly different SPA profile of tritordeum, albeit only in barley.

Among the various CWBPAs, ferulic acid is always the most widely concentrated phenolic acid, and it is followed by sinapic acid (Giordano et al., 2019) or cinnamic acid (Montesano et al., 2021; Suchowilska et al., 2021). These studies reported a similar composition of CWBPAs in tritordeum and durum wheat.

2.07.3.3.3 Other Compounds

Among the other compounds that play an antioxidant role, selenium, which is essential for the antioxidant function of the glutathione peroxidase enzyme (Haug et al., 2008), has been receiving increasing attention because of the natural fortification of foods with this element. Tufarelli et al. (2016) demonstrated that an increased fertilization of selenium in tritordeum grain used for feeding hens can improve egg quality, without any collateral effects on their productive performance. Furthermore, Minh et al. (2017) found that in natural conditions the selenium content was lower in tritordeum kernels (0.08 mg Se/kg DM) than in wheat (1.6 mg Se/kg DM).

Table 3 Concentration (ppm) of the main carotenoid compounds in tritordeum, durum and bread wheat, and in barley

Carotenoid component		*Study*	*Tritordeum*	*Durum wheat*	*Bread wheat*	*Barley*
Xanthophyll	Lutein	Atienza et al., 2007a,b,c	2.75	0.9		
		Giordano et al., 2019	5.3	4.6	2.2	2.1
		Mattera et al., 2017	0.2	0.2	0.1	
		Mattera et al., 2020	7.6		1.0	
		Mellado-Ortega and Hornero-Méndez, 2018	7.9	1.8		
		Mellado-Ortega and Hornero-Méndez, 2015	7.7	0.7		5.4
		Mellado-Ortega and Hornero-Méndez, 2016	7.5	0.9		
		Paznocht et al., 2018	10.5		2.9	0.9
		Rodríguez-Suárez et al., 2014	9.1	2.9		
		Suchowilska et al., 2021	12.0	9.3		
	Zeaxanthin	Giordano et al., 2019	0.5	0.5	0.6	1.4
		Mattera et al., 2017	5.13	2.31	0.5	
		Mattera et al., 2020	0.19		0.14	
		Mellado-Ortega Hornero-Méndez, 2018	0.5	0.9		
		Paznocht et al., 2018	0.9		0.7	1.3
		Suchowilska et al., 2021	2.02	1.58		
Carotene	α-carotene	Paznocht et al., 2018	0.1		0.1	
	β-carotene	Mattera et al., 2017	0.1	0.02	0.03	
		Mattera et al., 2020	0.4		0.2	
		Mellado-Ortega and Hornero-Méndez, 2018	0.1	0.1		
		Mellado-Ortega and Hornero-Méndez, 2015	0.1	0.02		0.02
		Mellado-Ortega and Hornero-Méndez, 2016	0.1	0.03		
		Paznocht et al., 2018	0.04		0.1	0.03
		Rodríguez-Suárez et al., 2014	0.1	0.1		
		Suchowilska et al., 2021	2.5	1.0		

The data sources are reported in the table.

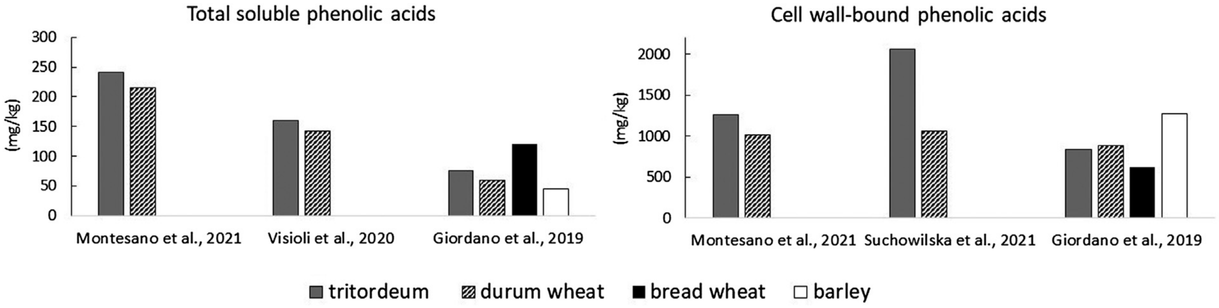

Fig. 7 The total soluble and cell-wall bound phenolic acids in tritordeum, durum wheat, bread wheat and barley. The data sources are reported in the figure.

Pedrazzani et al. (2021) quantified 5-*n*-alkylresorcinol (AR) for the first time, in saturated and unsaturated AR homologues, and in the oxidized forms (2′-oxo) of different small cereals. Although they reported a large between cultivar variability for bread wheat, tritordeum showed a higher AR content than bread wheat (+11%). ARs have a high antioxidant activity and play many different biological roles, such as that of a cholesterol-like effect (Zawilska and Cieślik-Boczula, 2017). Bordiga et al. (2016) found that these compounds are mainly located in the external layer of cereal kernels.

Tritordeum kernels have shown higher vitamin E and tocol contents than bread wheat (+5%), although they are lower than in barley (−4%). Lachman et al. (2018) reported that tritordeum contains fewer tocopherols (−36%) but more tocotrienols (+30%) than bread wheat, and these are mainly located in the endosperm.

An interesting oleic acid content (14% of the total fatty acids) is present in tritordeum, in particular in the outermost layers of the kernel, and this is one of the most important fats in the Mediterranean diet because of its characteristic beneficial effects on the cardiovascular system (Mellado-Ortega and Hornero-Méndez, 2012).

The wholemeal flours obtained from tritordeum provide several other important nutrients that are fundamental for metabolic processes, such as calcium, potassium, sulfur, iron and zinc, and in higher concentrations than in durum wheat (Visioli et al., 2020).

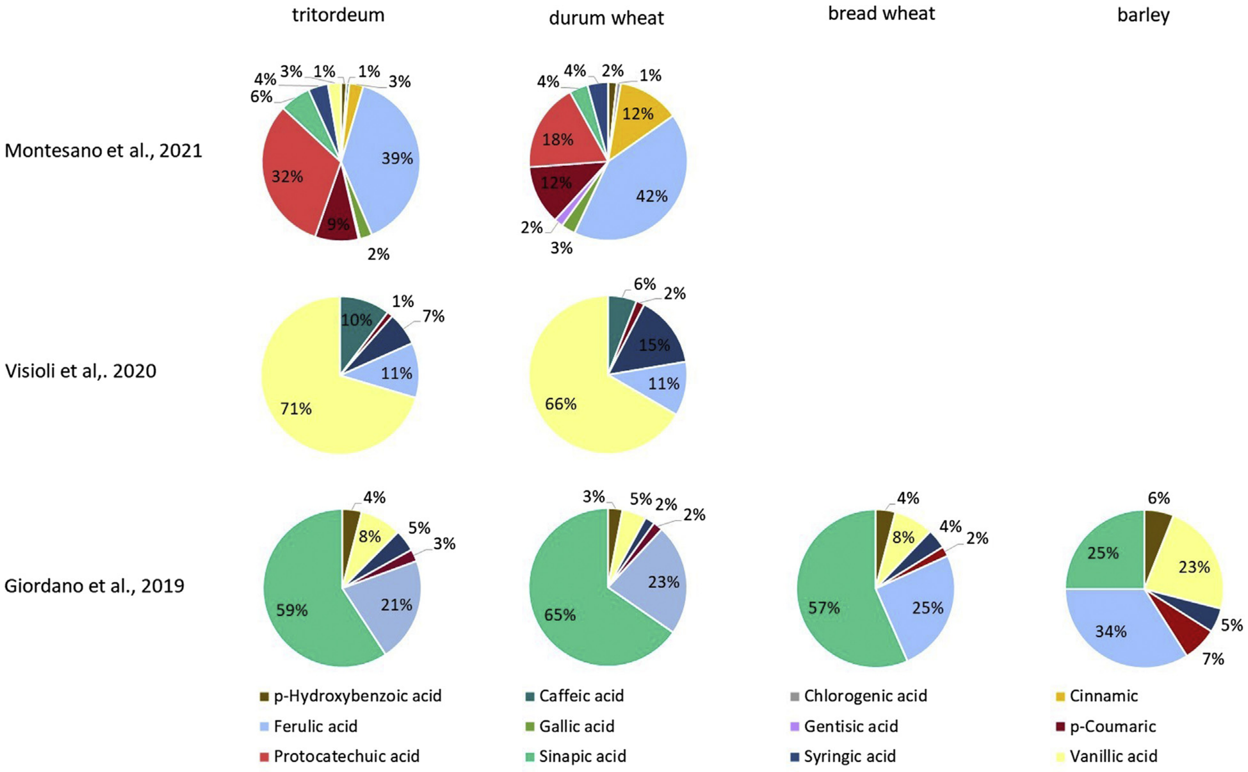

Fig. 8 Soluble phenolic acid composition in tritordeum, durum wheat, bread wheat and barley. The data sources are reported in the figure.

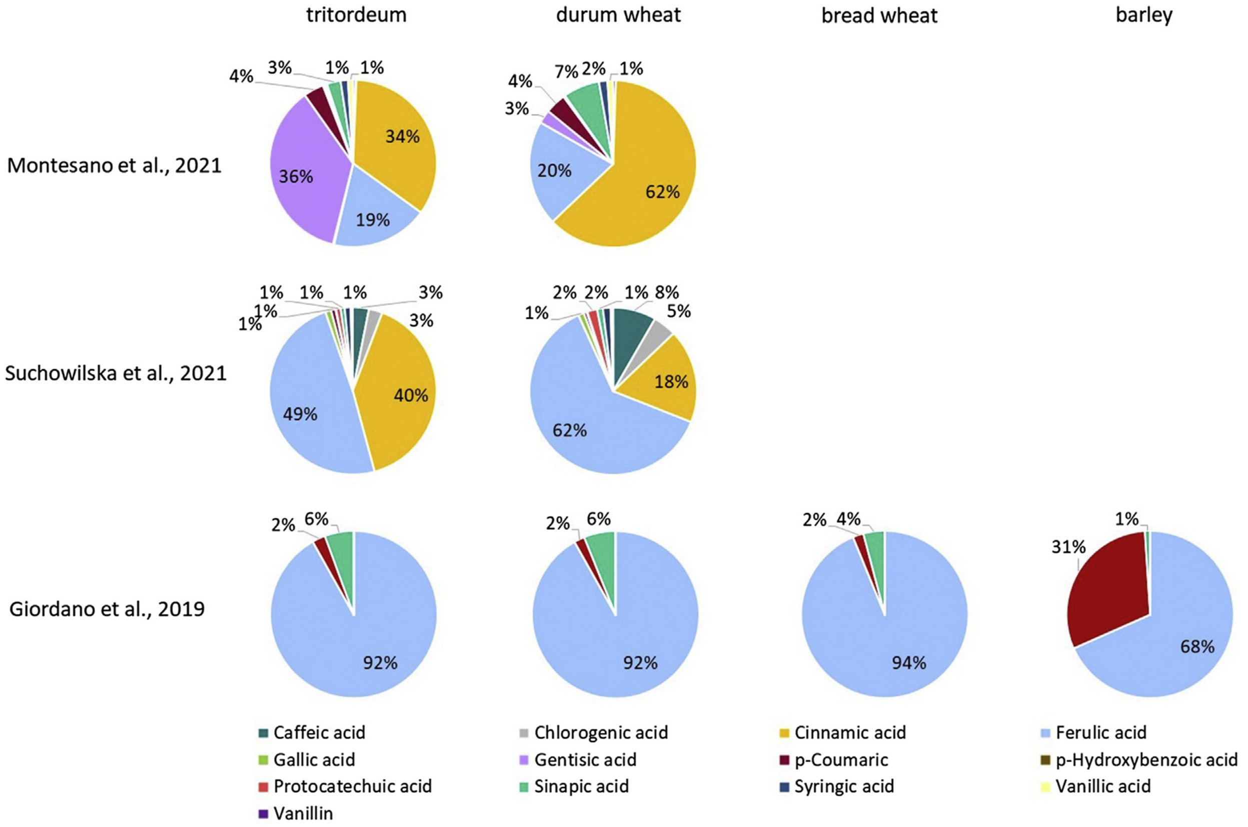

Fig. 9 Composition of the cell wall-bound phenolic acid in tritordeum, durum wheat, bread wheat and barley. The data sources are reported in the figure.

2.07.4 Technological Aspects

2.07.4.1 Rheological and Baking Quality

Gluten, which is a complex matrix composed of endosperm storage proteins (gliadins and glutenins), represents 60% of the grain proteome and has the function of supporting seedling growth (Shewry, 2009). From a food technology point of view, gluten plays a major role in the formation of the technological properties of dough and, consequently, the potential bread making use and dough rheological properties are based on the gliadin/glutenin ratio and the amount and composition of prolamins (Ronga et al., 2020). According to the information that is currently available, the bread-making aptitude of tritordeum seems to be closely related to the gluten composition inherited from *H. chilense* (Sillero et al., 1995). In fact, the high storage protein content of tritordeum is derived from both the *Hordeum* and *Triticum* genomes, since *H. chilense* is also characterized by a high aptitude to accumulate seed storage proteins (Alvarez et al., 2004; Martín et al., 1999).

The *Hordeum* genome is a good source of genetic variability of prolamin proteins, and it could be an excellent source of interesting technological and bread-making characteristics for tritordeum (Martín et al., 1999). These quality traits are linked to the puroindoline genes (Pina-D1 and Pinb-D1), which are located on wheat chromosome 5D, and the homologue hordoindoline genes Hina and Hinb in barley (Guzmán and Alvarez, 2014). The Hina-Hch1 and Hinb-Hch1 genes in *H. chilense* are very similar to the puroindoline genes of bread wheat, which may help to explain the soft grain texture of tritordeum. In fact, the addition of chromosome 5Hch to durum wheat resulted in the enhancement of grain softness (Yanaka et al., 2011). Thus, although one of the parental lines of tritordeum is a durum wheat cultivar, the similarity, in terms of derived flour quality, is much closer to that of bread wheat (Alvarez and Martín, 1994; Pinto et al., 2002) and it is interesting for bread-making and baking processes (Alvarez et al., 1994, 1995a,b; Alvarez and Martín, 1996) in addition to pasta-making use (Martín et al., 1999).

In order to compare the bread-making traits of tritordeum and bread wheat, the flour gluten content and composition, that is, the gliadin/glutenin ratio (glia/gs), the HMW-GS/LMW-GS (H/L), the alveographic parameters (dough strength, P/L ratio), the farinographic stability, the dough development time (DDT) and the loaf volume are reported in **Table 4**. It can be seen that the gluten content of tritordeum is higher than that of bread wheat (+14%), in agreement with the higher GPC. As far as the gluten composition is concerned, Landolfi et al. (2021) reported that tritordeum had a 10% higher glia/gs ratio than bread wheat. Moreover, tritordeum has a clearly lower occurrence of high-molecular-weight glutenin subunits (HMW-GS), which are closely related to the dough strength, than low-molecular-weight glutenin subunits (LMW-GS). Thus, the H/L ratio of tritordeum is clearly lower (−170%). Ballesteros et al. (2003) demonstrated how an artificial introgression of HMW glutenin subunits 1Dx5 + 1Dy10 from a bread wheat into a tritordeum cultivar can improve its bread-making quality, without significantly changing its agronomic traits.

Another particular difference in the gluten composition of tritordeum is its lower levels of ω-gliadins than wheat, which have been verified in both flour (Landolfi et al., 2021) and bread (Vaquero et al., 2018). Landolfi et al. (2021) investigated the effect of a higher N fertilization on the gluten composition of old and modern varieties of bread wheat, compared with tritordeum, but they did not observe any significant effect. These data also confirm that the crop management of this new species clearly

Table 4 Comparison of the rheological and technological properties of tritordeum and bread wheat

Rheological and technological parameter		*References*	*Tritordeum*	*Bread wheat*
Gluten	Gluten (% dry weight)	Gallardo and Fereres, 1993	11.2	8.9
		Alvarez et al., 1995a,b	12.5	11.0
		Martín et al., 1999	11.2	8.9
		Landolfi et al., 2021	16.6	16.3
Gluten composition[a]	Glia/GS	Landolfi et al., 2021	1.0	0.9
	H/L	Landolfi et al., 2021	0.3	0.8
Chopin alveographs[b]	W ($J*10^{-4}$)	Alvarez et al., 1992	121	330
		Alvarez et al., 1995a,b	111	290
		Pinto et al., 2003	77	283
		Ballesteros et al., 2003	84	267
	P/L	Alvarez et al., 1992	0.6	0.9
		Alvarez et al., 1995a,b	0.3	0.6
		Ballesteros et al., 2003	0.7	1.0
Brabender farinograph[c]	Stability (min)	Alvarez et al., 1995a,b	5.1	15
		Martinek et al., 2003	2.7	9.3
	DDT (min)	Alvarez et al., 1995a,b	2.5	7.3
		Martinek et al., 2003	2.7	1.0
Baking properties	Loaf volume (cm^3)	Alvarez et al., 1995a,b	441	579
		Martinek et al., 2003	260	397

[a] Relative quantification of the gluten fraction on the basis of an RP-HPLC analysis; Glia: gliadins, GS: glutenins; H: high molecular weight glutenin; L: low molecular weight glutenin.

[b] Alveographic parameters W: dough strength; P/L: ratio between tenacity (P) and extensibility (L).

[c] Farinographic parameter, dough stability and dough development time (DDT).

The data sources are reported in the table.

does not affect the composition, but instead affects the gluten content (Johansson et al., 2001), while the relative percentage of the gluten fraction is mainly a consequence of the genotype (Landolfi et al., 2021).

Thus, mainly as a consequence of a lower HMW-glutenin occurrence in gluten, tritordeum overall results in lower bread-making and baking qualities than bread wheat (Alvarez et al., 1995a,b; Alvarez and Martín, 1996; Martinek et al., 2003; Martín et al., 1999). Tritordeum has shown a lower W (−200%) than bread wheat, and also a lower or similar tenacity (P), but a generally higher extensibility (L), thus resulting in a decrease in the P/L ratio (−60%). The lower P/L ratio could make tritordeum dough rather too extensible and sticky for the bread-making process (Martinek et al., 2003). According to these authors, the high extensibility of tritordeum dough could be a consequence of the higher content of sulfur-containing amino acids, such as cysteine, which are involved in the formation of intra- and inter-disulfide bonds among individual groups of prolamin.

Overall, on the basis of the rheological quality of the flour, tritordeum can be used as a raw material for bread-making, although not for prolonged leavening, and appears to be more suitable for bread-making than for pasta-making, due to the lower HMW-GS content (Alvarez et al., 1995a,b; Martín et al., 1999). Furthermore, apart from the positive and distinctive characteristics of tritordeum flour, concerning its extensibility of the dough, the eye-catching color of the bread and other baked products, which is obtained thanks to the high carotenoid content of the flour, is of particular interest.

2.07.4.1.1 Gluten Related Diseases

In addition to its technological role, the complex network of gluten protein and peptide sequences has been investigated in depth in recent years, in relation to its role in gluten disorders. Gluten is in fact the primary cause of celiac disease (CD) but is also considered to be responsible for other gluten-related diseases, such as non-coeliac gluten sensitivity (NCGS), and this topic needs a greater understanding of how it acts as a trigger in these widespread diseases.

Tritordeum contains glutens, and it is therefore not suitable for people who suffer from celiac intolerances/allergies, just like bread and durum wheat, spelt, emmer, einkorn, barley and rye. Furthermore, the consumers and food supply chain are now demanding clearer information about the factors that could exert an effect on the peptides that are responsible for different gluten disorders from CD (Jouanin et al., 2018). Numerous fragments of peptides with CD-toxic effects, which are called epitopes, may also be responsible for other gluten-related diseases, such as NCWGS. CD-toxic epitopes can be quantified using an ELISA immune-enzymatic assay with antibody R5, which recognizes the QQPFP, LQPFP, QQQFP and QLPFP amino acid sequences in cereal flour or digested food samples (Kahlenberg et al., 2006). The R5 ELISA assay has demonstrated that tritordeum flour could have fewer highly celiac epitopes than bread wheat (Landolfi et al., 2021; Vaquero et al., 2018). Landolfi et al. (2021) verified this significant difference through a comparison with landrace (−51%) and modern wheat (−58%) cultivars and suggested that a link exists between the lack of the D genome in this amphiploid and a lower immunodominant toxicity. Sánchez-León et al. (2021) have also recently studied tritordeum bread, in terms of its potential triggering of celiac disease (CD) and non-celiac wheat-sensitivity (NCWS). They studied the resistance of immunogenic peptides to digestion and the structure of the intestinal microbiota after tritordeum bread ingestion. They also studied the response of NCWS patients, either non-celiac or affected by a wheat allergy, but with health symptoms related to the ingestion of gluten-containing foods, and they reported no significant changes between gluten-free bread and tritordeum bread. They analyzed the bacterial gut microbiota, showed that the ingestion of tritordeum bread does not modify the overall composition of the intestinal microbiota, and observed only a few changes of some butyrate-producing bacteria. This result suggests that some patients with NCWS might find tritordeum more tolerable than gluten-free bread, while the same good quality levels of bread could be maintained, in terms of the organoleptic and nutritional properties.

2.07.5 Conclusion

Tritordeum has been studied for more than 40 years, although its cultivation for commercialization in the baked goods supply chain is a recent achievement. This crop could be an interesting alternative to wheat for producers and consumers, since it shows a high adaptability to different growing areas, the possibility of being used in the production of a large number of baked and non-baked food products, and many notable and captivating features, from the health and organoleptic points of view.

This review has shown that genetic studies and breeding are crucial to increase the overall competitiveness of tritordeum, considering the possible agronomic, yield, sanitary, nutritional and technological improvements. The necessity of addressing the specific traits of new tritordeum cultivars, considering the environmental conditions of different growing areas, has been pointed out, as well as the need of a greater ability to adapt to climate change in order to increase the sustainability of this species. In addition to fundamental genetic improvements, it will be necessary to set up cropping system and agronomic practices for this species, in order to achieve a substantial increase in the yield and an improvement of the qualitative traits, as well as to minimize the sanitary risk related to contamination by mycotoxins, particularly in Central-European areas.

Considering the interest of consumers and industries in special baked products, obtained from different raw materials, and the interesting nutritional profile of both refined and whole-grain flours, tritordeum could successfully be used as an ingredient for food production purposes. In such a context, more information is necessary on the technological properties of flours derived from this cereal and the end-use values necessary to obtain food products, in order to allow the raw materials to be fully exploited and the management of the technological processes and the future breeding activities to be correctly addressed.

In short, the research activity that will be conducted in the coming years will be fundamental for the development of the cultivation of tritordeum and its supply chains, in order to obtain an agronomical and economical interesting alternative to the present cropping systems, as well as high innovative food products with health and organoleptic added value.

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