Tritordeum

Subjects: Plant Sciences
Contributor: Sergio Atienza

Hexaploid tritordeum is the amphiploid derived from the cross between the wild barley Hordeum chilense and durum wheat.

Keywords: tritordeum ; Hordeum chilense ; pre-breeding

1. Introduction

Rice, maize and common wheat are the most important crops for human consumption in the world. Both rice and maize are diploids, but bread wheat is an allohexaploid (2n = 6x =42, AABBDD) derived from the cross between *Triticum turgidum* (AABB) and *Aegilops tauschii* (DD) [1]. The allohexaploid genome structure of bread wheat is, in part, responsible for the adaptability of this crop to a wide range of climatic conditions [1].

The wide adaptability of polyploids is an interesting feature for breeding, but alloploidy has not been generally exploited by breeders since it is usually associated with sterility. The first triticale was obtained by Rimpau in 1888, after spontaneous chromosome doubling of hybrids from crosses between bread wheat and rye Rimpau, 1891 (as cited in [2]). The development of triticale from the first cultivars released in the 60 s to our days, exemplifies the possibilities of alloploidy for the development of new crops (reviewed by [2]).

The success of triticale renewed the interest of developing new synthetic amphiploids between barley and wheat. Plant breeders had been interested in crossing both crops since the beginning of the 20th century (reviewed by [3]), but fertile amphiploids were only obtained when the wild barley *Hordeum chilense* Roem. et Schultz. was used. This new species was named tritordeum (× *Tritordeum martini* A. Pujadas) [4]. Octoploid [5] and hexaploid [6] tritordeums were obtained from the crosses between *H. chilense* (as mother) and common or durum wheat as pollen donors, respectively. Both tritordeums were initially considered for breeding but the hexaploid became the species of choice since octoploid tritordeums showed a high chromosome instability. A similar situation happens in triticale. Although different ploidy levels have been developed and studied, only hexaploid triticale (× *Triticosecale* Wittmack, 2n = 6x = 42) has commercial application (reviewed by [2]).

Antonio Martin is the father of tritordeum. After the development of the first amphiploid accessions at the University of Córdoba (Spain) he started a breeding program along with Juan Ballesteros at the Institute for Sustainable Agriculture (IAS-CSIC, Spain). After two decades of breeding, the potential of tritordeum was clear [3][7]. Hexaploid tritordeum was perceived as an interesting new crop with a similar role to bread wheat in the food industry and with potential as a bridge to transfer useful traits from *H. chilense* to wheat. However, tritordeum breeding still faced significant problems to become a new crop. The most important limitations were the persistence of traits from the wild progenitor, the lack of molecular tools for the effective study and utilization of traits of interests inherited from *H. chilense* and the competition in the food industry with bread wheat-derived products.

2. Progress in Quality and Potential for the Development of New Innovative Food Products

Although durum wheat is the male parent of hexaploid tritordeum, the grain texture of tritordeum is similar to that of bread wheat [8]. This quality parameter is controlled in wheat by the puroindoline genes (*Pina-D1* and *Pinb-D1*) located on chromosome 5D [9] and the homoeologue hordoindoline genes *Hina* and *Hinb* in barley. *Hina-Hch1* and *Hinb-Hch1* genes in *H. chilense* are very similar to *Pin* genes of bread wheat [10], which may explain the soft grain texture of tritordeum. Indeed, the addition of chromosome $5H^{ch}$ to bread wheat resulted in the enhancement of grain softness [11]. This makes tritordeum flour more adequate for the production of products similar to those obtained from bread wheat. Accordingly, High Molecular Weight (HMW) glutenin subunits were considered a primary target for tritordeum breeding due to their high influence on breadmaking quality [12]. Two alternative approaches were applied: transgenic and conventional breeding. The transgenic alternative allowed the development of tritordeum lines expressing HMW genes 1Ax1 and 1Dx5 [13][14]. On the other hand, chromosome substitution or translocation lines with the HMW glutenin subunits Dx5 + Dy10 were obtained by conventional methods [15]. The chromosome substitution lines obtained showed a similar agronomic performance than the euploid tritordeum, but they had a much higher gluten strength due to the addition of HMW glutenin subunits 1D [16].

The breadmaking quality of tritordeum is also influenced to a great extent by the *H. chilense* genome (reviewed by [3]). This promoted the study of the variability for endosperm storage proteins in the *H. chilense* accessions used to develop primary tritordeums [17][18][19], along with the diversity in the natural populations of the species [20]. Further studies focused on the effect of these proteins in breadmaking quality in tritordeums [21][22]. In summary, these studies revealed a wide diversity for storage proteins potentially useful for both tritordeum and wheat breeding, which could provide new functionalities not found in other cereals. Indeed, tritordeum has significantly lower levels of ω-gliadins in flour and levels of gluten around 50% lower than wheat [23]. Accordingly, tritordeum is considered an interesting choice to people wishing to reduce their gluten intake, although it is not suitable for patients suffering coeliac disease [23]. Furthermore, tritordeum bread has been recommended for a subset of non-celiac wheat sensitivity patients who do not need strict exclusion of gluten from their diet [24].

The increasing demand of healthier foods, including whole grain-derived foods, has promoted the investigation of other health related traits in tritordeum. Phenolic compounds are the main group of phytochemicals in barley grain and their main interest is due to their strong antioxidant power and their association to certain diseases prevention [25]. Considering that tritordeum expresses the properties of both barley and wheat, its phenolic content and profile was investigated [26]. A great variability for phenolic compounds content was reported, ferulic acid being the main one that happens in wheat [26]. However, comparative studies with wheat and barley showed that advanced lines of tritordeum have a similar total phenolic content to wheat but much lower than barley [27]. Phenolic content has not been a target in tritordeum breeding program and, thus, it might be possible that valuable diversity for this trait remains hidden in pre-breeding materials or in *H. chilense* accessions. However, total phenolic content in tritordeum was around half the reported in barley [27] and, thus, it is not likely that tritordeum could outperform barley as a natural source for these compounds.

Accumulation of compounds such as tocols [28] or polysaccharides (arabinoxylans and β-glucans) [29] has been also studied in tritordeum, along with the potential for accumulation of micronutrients as Selenium in grain [30]. Furthermore, the essential role of Selenium in animal and human nutrition promoted the evaluation of selenium fertilized tritordeum in relation to conventional dietary supplements of this micronutrient in laying hens [31]. The improvement of egg quality due to Se-enriched tritordeum suggests that selenium-fertilized tritordeum may be an interesting alternative for animal feeding [31].

Tritordeum can be also used for cake [32] and beer production [33]. Indeed, tritordeum and barley malts yielded comparable values for the majority of technological parameters including alcohol content, although tritordeum malts produced a slight acidification effect, a lower level of glucose and a higher amount of free amino nitrogen [33]. Besides, tritordeum malt did not cause any technological problem during the different stages of beer production and, thus, it is considered that it has a high potential for the brewing industry [33]. Furthermore, the utilization of brewers' spent grain from tritordeum, the major by-product of the brewing industry, may increase the nutritional potential of durum wheat pasta [34], by improving total antioxidant capacity, total dietary fibre and β-glucans and without compromising the sensory aspects of pasta [34]. All these findings show the potential of tritordeum for the development of food products with new functionalities.

Regarding health-related compounds, carotenoid content has been the most extensively studied due to its importance on the appearance of tritordeum products. The intensive yellow colour of tritordeum flour constitutes an important differential characteristic compared to bread wheat derived products [3]. This trait could be perceived as detrimental since white flour is usually preferred for breadmaking from bread wheat. As a consequence, initial studies confirmed the lack of effect of yellow colour in relation with the baking performance [3]. The high carotenoid content in the endosperm is responsible for the golden coloration of tritordeum products and it confers a clear differentiation from standard bread wheat products. Instead of a detrimental trait, the high carotenoid content was considered a potential commercial advantage. This motivated the study of the genetic bases of carotenoid content, which resulted in the identification of a QTL in chromosome $2H^{ch}$ [35], the selection of new genotypes with high carotenoid content such as HT621 [36] and the development of selection tools useful for the breeding program [37]. The genes responsible for carotenoid content in tritordeum and wheat were unknown at the time although they have been located in chromosomes $7H^{ch}$ [38] and 7B [39]. Thus, a candidate gene approach using rice as a model species and the gene *Phytoene synthase 1* was performed. Our results proved that *Psy1* was located in chromosome $7H^{ch}S$ in *H. chilense* and 7A and 7B in durum wheat [40]. Furthermore, the diagnostic marker developed for *Psy1_Hch* [40] was successfully used for marker assisted selection of

Psy1_Hch in bread wheat-*H. chilense* genetic stocks [41][42]. The cloning and heterologous expression in bacteria of *Psy1_Hch* confirmed the functionality of this gene [43] and its potential for the enhancement of carotenoids in wheat.

Further transcriptomic experiments showed that both *Psy1* and *e-Lcy* (Lycopene epsilon cyclase) were upregulated between 18 and 25 days after anthesis in tritordeum, while their homoeologue genes in durum wheat were downregulated [44]. The differences in the expression profile between tritordeum and durum wheat were associated with the differences in carotenoid content between both species [44]. The development of translocation lines of *H. chilense* $7H^{ch}S$ into bread wheat resulted, as expected, in the increase in endosperm carotenoid content due to the presence of *Psy1_Hch* [45].

In addition, tritordeums have a high proportion of carotenoid esters in contrast with durum wheat [46]. Esterification is a common way to accumulate carotenoids in plants [47]. Thus, it was hypothesized that the activation of the carotenoid pathway in tritordeum during grain development may be associated with the synthesis of carotenoid esters and the production of a metabolic sink. However, this hypothesis was not confirmed since no lutein esters are produced before 36 days after anthesis [48]. Nevertheless, carotenoid esterification can contribute to the accumulation of lutein in tritordeum endosperm by limiting carotenoid degradation in later stages [48].

The importance of esterified carotenoids goes beyond their role in carotenoid accumulation since they have a higher stability than free carotenoids [49][50]. Higher carotenoid retention has been observed during post-harvest storage due to esterification [49][51][52][53] and, thus, the increase in carotenoid esterification is a good target for the improvement of carotenoid retention in the food chain. This is relevant for tritordeum since this species has a high proportion of carotenoids in the esterified form in the endosperm [46][48][54], with a 3-fold higher content in the endosperm compared to the germ [55].

The potential of esterification for the improvement of carotenoid retention through the food chain has increased the interest on this trait despite these results are not confirmed at high-temperature regimes [56]. The identification of the xanthophyll acyl transferase (*XAT-7D*), responsible for carotenoid esterification in common wheat, opens new possibilities for marker assisted selection [57]. In fact, this gene is being transferred from common to durum wheat at present [58]. In tritordeum, carotenoid esterification is due to the *H. chilense* genome [59]. In particular, candidate genes at chromosome $7H^{ch}$ were identified by physical mapping and DArTSeq markers [60]. Recently, a GDSL esterase/lipase (XAT-7Hch), orthologue of XAT-7D, has been identified as the main responsible for lutein esterification in *H. chilense*/tritordeum [61]. As happens with XAT-7D, this gene can be used for wheat breeding through a marker assisted selection strategy with a diagnostic marker already available [61].

Table 1 summarizes the main quality attributes of tritordeum grain.

Table 1. Grain quality parameters in tritordeum.

Trait	Value	Relative Performance Over Control [1]	Reference
Carotenoid content (μg/g) (Primary tritordeums)	**5.8**	**4.8-fold increase (DW)**	[46]
Carotenoid content (μg/g) (Breeding lines)	**9.14**	**2.8-fold increase (DW)**	[44]
Carotenoid content (Bread) (μg/100 g)	**357.6**	**6.5-fold increase (BW)**	[62]
Carotenoid esterification (%)	**33.8**	**not detected (DW)**	[44]
Gluten content	**n.a.**	**51% reduction (BW)**	[23][24]
γ-gliadin epitopes	**n.a.**	**59% reduction (BW)**	[23][24]
α-gliadin epitopes	**n.a.**	**77% reduction (BW)**	[23][24]
Total tocols (μg/g)	**30.2**	**Similar to BW**	[28]
Beta-glucans (% dry matter)	**0.6**	**Similar to BW; 90% reduction (B)**	[29]

[1] DW = durum wheat; BW = Bread wheat; B = Barley.

The high carotenoid content of tritordeum has been used to widen the interest in this ‘Golden Cereal’ (https://www.tritordeum.com/?lang=en#whatis, accessed on 20 May 2021). Agrasys S.L., Barcelona, Spain, a spin-off of the Spanish High Council for Scientific Investigations (CSIC), has benefited from the commercial exploitation of tritordeum, as it has held the exclusive commercial rights of tritordeum since 2006. The registration of the tritordeum varieties ‘Aucan’ and ‘Bulel’ at the Community Plant Variety Office, along with the commercialization effort developed by

Agrasys, has made the expansion of tritordeum possible to many countries (https://www.tritordeum.com/ww/?lang=en, accessed on 20 May 2021); more importantly, it has made tritordeum products available to consumers. At present, there is a complete food chain comprised of farmers, millers, bakers and sellers for the development of tritordeum products.

References

1. International Wheat Genome Sequencing Consortium. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 2014, 345, 1251788.
2. Mergoum, M.; Singh, P.K.; Peña, R.J.; Lozano-del Río, A.J.; Cooper, K.V.; Salmon, D.F.; Gómez Macpherson, H. Triticale: A “New” Crop with Old Challenges. In Cereals; Springer: Berlin/Heidelberg, Germany, 2009; pp. 267–287.
3. Martín, A.; Alvarez, J.B.; Martín, L.M.; Barro, F.; Ballesteros, J. The development of tritordeum: A novel cereal for food processing. J. Cereal Sci. 1999, 30, 85–95.
4. Pujadas, A.J. ×Tritordeum martinii A. pujadas (Poaceae) Nothosp. Nov. Acta Bot. Malacit. 2016, 41, 325–338.
5. Martin, A.; Chapman, V. A hybrid between Hordeum chilense and Triticum aestivum. Cereal Res. Commun. 1977, 5, 365–368.
6. Martin, A.; Sánchez-Monge, E. Cytology and morphology of the amphiploid Hordeum chilense × Triticum turgidum conv durum. Euphytica 1982, 31, 261–267.
7. Martin, A.; Martínez, C.; Rubiales, D.; Ballesteros, J. Tritordeum: Triticale’s new brother cereal. In Triticale: Today and Tomorrow; Güedes-Pinto, H., Darvey, N., Carnide, V.P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp. 57–72.
8. Alvarez, J.B.; Ballesteros, J.; Sillero, J.A.; Martin, L.M. Tritordeum: A new crop of potential importance in the food industry. Hereditas 1992, 116, 193–197.
9. Bhave, M.; Morris, C.F. Molecular genetics of puroindolines and related genes: Allelic diversity in wheat and other grasses. Plant Mol. Biol. 2008, 66, 205–219.
10. Guzmán, C.; Alvarez, J.B. Molecular characterization of two novel alleles of Hordoindoline genes in Hordeum chilense Roem. et Schult. Genet. Resour. Crop Evol. 2014, 61, 307–312.
11. Yanaka, M.; Takata, K.; Terasawa, Y.; Ikeda, T.M. Chromosome 5H of Hordeum species involved in reduction in grain hardness in wheat genetic background. Theor. Appl. Genet. 2011, 123, 1013–1018.
12. Payne, P.I.; Nightingale, M.A.; Krattiger, A.F.; Holt, L.M. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J. Sci. Food Agric. 1987, 40, 51–65.
13. Barro, F.; Barceló, P.; Lazzeri, P.A.; Shewry, P.R.; Martín, A.; Ballesteros, J. Functional properties and agronomic performance of transgenic tritordeum expressing high molecular weight glutenin subunit genes 1Ax1 and 1Dx5. J. Cereal Sci. 2003, 37, 65–70.
14. Barro, F.; Barcelo, P.; Lazzeri, P.A.; Shewry, P.R.; Ballesteros, J.; Martin, A. Functional properties of flours from field grown transgenic wheat lines expressing the HMW glutenin subunit 1Ax1 and 1Dx5 genes. Mol. Breed. 2003, 12, 223–229.
15. Ballesteros, J.; Alvarez, J.B.; Gimenez, M.J.; Ramirez, M.C.; Cabrera, A.; Martin, A. Introgression of 1Dx5+1Dy10 into Tritordeum. Theor. Appl. Genet. 2003, 106, 644–648.
16. Ballesteros, J.; Ramírez, M.C.; Martínez, C.; Barro, F.; Martín, A. Bread-making quality in hexaploid tritordeum with substitutions involving chromosome 1D. Plant Breed. 2003, 122, 89–91.
17. Alvarez, J.B.; Martín, A.; Martín, L.M. Variation in the high-molecular-weight glutenin subunits coded at the Glu-Hch1 locus in Hordeum chilense. Theor. Appl. Genet. 2001, 102, 134–137.
18. Atienza, S.G.; Gimenez, M.J.; Martin, A.; Martin, L.M. Variability in monomeric prolamins in Hordeum chilense. Theor. Appl. Genet. 2000, 101, 970–976.
19. Atienza, S.G.; Alvarez, J.B.; Villegas, A.M.; Gimenez, M.J.; Ramirez, M.C.; Martin, A.; Martin, L.M. Variation for the low-molecular-weight glutenin subunits in a collection of Hordeum chilense. Euphytica 2002, 128, 269–277.
20. Alvarez, J.B.; Broccoli, A.; Martín, L.M. Variability and Genetic Diversity for Gliadins in Natural Populations of Hordeum chilense Roem. et Schult. Genet. Resour. Crop Evol. 2006, 53, 1419–1425.
21. Alvarez, J.B.; Moral, A.; Martín, L.M.; Martín, A. Linkage relationships between prolamin genes located on chromosome 1Hch in Hordeum chilense. Theor. Appl. Genet. 2004, 108, 891–895.

22. Caballero, L.; Alvarez, J.B.; Martin, L.M. Analysis of D-prolamins synthesized by the Hordeum chilense genome and their effects on gluten strength in hexaploid tritordeum. Plant Breed. 2001, 120, 185–187.

23. 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. 2018, 98, 2201–2209.

24. Sánchez-León, S.; Haro, C.; Villatoro, M.; Vaquero, L.; Comino, I.; González-Amigo, A.B.; Vivas, S.; Pastor, J.; Sousa, C.; Landa, B.B.; et al. Tritordeum breads are well tolerated with preference over gluten-free breads in non-celiac wheat-sensitive patients and its consumption induce changes in gut bacteria. J. Sci. Food Agric. 2020, jsfa.10982.

25. Idehen, E.; Tang, Y.; Sang, S. Bioactive phytochemicals in barley. J. Food Drug Anal. 2017, 25, 148–161.

26. Navas-Lopez, J.F.; Ostos-Garrido, F.J.; Castillo, A.; Martín, A.; Gimenez, M.J.; Pistón, F. Phenolic content variability and its chromosome location in tritordeum. Front. Plant Sci. 2014, 5.

27. Eliášová, M.; Paznocht, L. Total phenolic content and antioxidant activity of tritordeum wheat and barley. Agron. Res. 2017, 15, 1287–1294.

28. Lachman, J.; Hejtmánková, A.; Orsák, M.; Popov, M.; Martinek, P. Tocotrienols and tocopherols in colored-grain wheat, tritordeum and barley. Food Chem. 2018, 240, 725–735.

29. Rakha, A.; Saulnier, L.; Åman, P.; Andersson, R. Enzymatic fingerprinting of arabinoxylan and β-glucan in triticale, barley and tritordeum grains. Carbohydr. Polym. 2012, 90, 1226–1234.

30. Phuong, L.E.; Lachman, J.; Kotíková, Z.; Orsák, M.; Michlová, T.; Martinek, P. Selenium in colour-grained winter wheat and spring tritordeum. Plant Soil Environ. 2017, 63, 315–321.

31. Tufarelli, V.; Cazzato, E.; Ceci, E.; Laudadio, V. Selenium-Fertilized Tritordeum (×Tritordeum Ascherson et Graebner) as Dietary Selenium Supplement in Laying Hens: Effects on Egg Quality. Biol. Trace Elem. Res. 2016, 173, 219–224.

32. Gómez, M.; Manchón, L.; Oliete, B.; Ruiz, E.; Caballero, P.A. Adequacy of wholegrain non-wheat flours for layer cake elaboration. LWT Food Sci. Technol. 2010, 43, 507–513.

33. Zdaniewicz, M.; Pater, A.; Hrabia, O.; Duliński, R.; Cioch-Skoneczny, M. Tritordeum malt: An innovative raw material for beer production. J. Cereal Sci. 2020, 96, 103095.

34. Nocente, F.; Natale, C.; Galassi, E.; Taddei, F.; Gazza, L. Using Einkorn and Tritordeum Brewers' Spent Grain to Increase the Nutritional Potential of Durum Wheat Pasta. Foods 2021, 10, 502.

35. Vaz Patto, M.C.; Rubiales, D.; Martín, A.; Hernández, P.; Lindhout, P.; Niks, R.E.; Stam, P. QTL mapping provides evidence for lack of association of the avoidance of leaf rust in Hordeum chilense with stomata density. Theor. Appl. Genet. 2003, 106, 1283–1292.

36. 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.

37. Atienza, S.G.; Avila, C.M.; Ramírez, M.C.; Martín, A. Application of near infrared reflectance spectroscopy to the determination of carotenoid content in tritordeum for breeding purposes. Aust. J. Agric. Res. 2005, 56, 85–89.

38. Alvarez, J.B.; Martin, L.M.; Martin, A. Chromosomal localization of genes for carotenoid pigments using addition lines of Hordeum chilense in wheat. Plant Breed. 1998, 117, 287–289.

39. Elouafi, I.; Nachit, M.M.; Martin, L.M. Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat (Triticum turgidum L. var. durum). Hereditas 2001, 135, 255–261.

40. Atienza, S.G.; Avila, C.M.; Martín, A. The development of a PCR-based marker for PSY1 from Hordeum chilense, a candidate gene for carotenoid content accumulation in tritordeum seeds. Aust. J. Agric. Res. 2007, 58, 767–773.

41. Mattera, M.G.; Ávila, C.M.; Atienza, S.G.; Cabrera, A. Cytological and molecular characterization of wheat-Hordeum chilense chromosome 7Hch introgression lines. Euphytica 2015, 203, 165–176.

42. Mattera, M.G.; Cabrera, A.; Hornero-Méndez, D.; Atienza, S.G. Lutein esterification in wheat endosperm is controlled by the homoeologous group 7, and is increased by the simultaneous presence of chromosomes 7D and 7Hch from Hordeum chilense. Crop Pasture Sci. 2015, 66, 912–921.

43. Rodríguez-Suárez, C.; Atienza, S.G.; Pistón, F. Allelic variation, alternative splicing and expression analysis of Psy1 gene in Hordeum chilenseRoem. et Schult. PLoS ONE 2011, 6.

44. Rodríguez-Suárez, C.; Mellado-Ortega, E.; Hornero-Méndez, D.; Atienza, S.G. Increase in transcript accumulation of Psy1 and e-Lcy genes in grain development is associated with differences in seed carotenoid content between durum wheat and tritordeum. Plant Mol. Biol. 2014, 84, 659–673.

45. Rey, M.-D.; Calderón, M.-C.; Rodrigo, M.J.; Zacarías, L.; Alós, E.; Prieto, P. Novel bread wheat lines enriched in carotenoids carrying Hordeum chilense chromosome arms in the ph1b background. PLoS ONE 2015, 10.

46. Atienza, S.G.; Ballesteros, J.; Martin, A.; Hornero-Mendez, 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.

47. Berry, H.M.; Rickett, D.V.; Baxter, C.J.; Enfissi, E.M.A.; Fraser, P.D. Carotenoid biosynthesis and sequestration in red chilli pepper fruit and its impact on colour intensity traits. J. Exp. Bot. 2019, 70, 2637–2650.

48. Mattera, M.G.; Hornero-Méndez, D.; Atienza, S.G. Carotenoid content in tritordeum is not primarily associated with esterification during grain development. Food Chem. 2020, 310.

49. Ahmad, F.T.; Asenstorfer, R.E.; Soriano, I.R.; Mares, D.J. Effect of temperature on lutein esterification and lutein stability in wheat grain. J. Cereal Sci. 2013, 58, 408–413.

50. Subagio, A.; Wakaki, H.; Morita, N. Stability of lutein and its myristate esters. Biosci. Biotechnol. Biochem. 1999, 63, 1784–1786.

51. Mellado-Ortega, E.; Atienza, S.G.; Hornero-Méndez, D. Carotenoid evolution during postharvest storage of durum wheat (Triticum turgidum conv. durum) and tritordeum (×Tritordeum Ascherson et Graebner) grains. J. Cereal Sci. 2015, 62, 134–142.

52. Mellado-Ortega, E.; Hornero-Méndez, D. Carotenoid evolution during short-storage period of durum wheat (Triticum turgidum conv. durum) and tritordeum (×Tritordeum Ascherson et Graebner) whole-grain flours. Food Chem. 2016, 192, 714–723.

53. Mellado-Ortega, E.; Hornero-Méndez, D. Effect of long-term storage on the free and esterified carotenoids in durum wheat (Triticum turgidum conv. durum) and tritordeum (×Tritordeum Ascherson et Graebner) grains. Food Res. Int. 2017, 99, 877–890.

54. Mellado-Ortega, E.; Hornero-Mendez, D. Isolation and identification of lutein esters, including their regioisomers, in tritordeum (×Tritordeum Ascherson et Graebner) grains: Evidence for a preferential xanthophyll acyltransferase activity. Food Chem. 2012, 135, 1344–1352.

55. Mellado-Ortega, E.; Hornero-Méndez, D. Effect of lutein esterification on the differential distribution of carotenoids in germ and endosperm fractions from tritordeum grains. J. Cereal Sci. 2018, 79, 462–468.

56. Paznocht, L.; Burešová, B.; Kotíková, Z.; Martinek, P. Carotenoid content of extruded and puffed products made of colored-grain wheats. Food Chem. 2021, 340.

57. Watkins, J.L.; Li, M.; McQuinn, R.P.; Chan, K.X.; McFarlane, H.E.; Ermakova, M.; Furbank, R.T.; Mares, D.J.; Dong, C.; Chalmers, K.J.; et al. A GDSL Esterase/Lipase Catalyzes the Esterification of Lutein in Bread Wheat. Plant Cell 2019.

58. Watkins, J.; Pogson, B.; Mather, D. XAT Catalyzes Carotenoid Esterification in Wheat. Available online: (accessed on 20 May 2021).

59. 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.

60. Ávila, C.M.; Palomino, M.C.; Hornero-Méndez, D.; Atienza, S.G. Identification of candidate genes for lutein esterification in common wheat (Triticum aestivum) using physical mapping and genomics tools. Crop Pasture Sci. 2019, 70, 567–574.

61. Requena-Ramírez, M.D.; Atienza, S.G.; Hornero-Méndez, D.; Rodríguez-Suárez, C. Mediation of a GDSL Esterase/Lipase in Carotenoid Esterification in Tritordeum Suggests a Common Mechanism of Carotenoid Esterification in Triticeae Species. Front. Plant Sci. 2020, 11, 2032.

62. Barceló, P. Tritordeum, un nuevo cereal natural. In Jornadas Técnicas “Rotaciones y Diversidad en Agricultura”; Agrocon e Ibercide; Fundación Ibercaja: Zaragoza, Spain, 2014.