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Chemical composition of wild and feral diploid wheats and their bearing on domesticated wheats

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ABSTRACT

The diploid wild wheats *Triticum monococcum* ssp. *thaoudar* and *Triticum urartu* are the direct ancestors of all domesticated wheats, but almost nothing is known about the chemical composition of their kernels. Aim of this research was to assess their content in several compositional traits and to compare it with that of domesticated wheats. To this end, fifteen diploid, tetraploid and hexaploid accessions belonging to different *Triticum* species were tested for 1000 kernel weight, protein, ash and starch content, β-amylase activity, carotenoid, tocol, anthocyanin and polyphenol concentration. The wild einkorns had high protein (21.7 ± 0.74 g/100 g), ash (3.0 ± 0.06 g/100 g), tocol (75.1 ± 3.95 mg/kg), carotenoid (8.0 ± 0.91 mg/kg) and anthocyanin (43.0 ± 4.66 mg/kg) content, and low β-amylase activity (20.2 ± 0.84 B3U/g). *T. urartu* instead coupled high protein (28.0 ± 0.07 g/100 g), ash (3.3 ± 0.03 g/100 g) and tocol (63.9 ± 2.91 g/100 g) content with low carotenoid (2.7 ± 0.02 g/100 g) and high β-amylase (57.7 ± 0.11 g/100 g) levels. These results fit well with those observed in the derived wheats, i.e. domesticated and feral einkorn on one side and emmer, durum, spelt and bread wheat on the other. Several positive nutritional traits present in the diploid wild species were not lost during the transition from wild to domesticated forms.

1. Introduction

Domesticated einkorn wheat (*Triticum monococcum* spp. *monococcum*) is a diploid species (2n = 2x = 14, genome A^mA^m) known for its unique kernel quality, rich in carotenoids, tocols, polyphenols, proteins and trace elements (Hidalgo and Brandolini, 2014). Today, einkorn, domesticated in the Near East about 10,000 years ago after the Younger Dryas (Haldorsen et al., 2011; Heun et al., 1997) and intensively used until the Bronze Age, is a relic crop but in recent years, interest has increased (Abdel-Aal et al., 2002; Hidalgo and Brandolini, 2014; Løje et al., 2003). However, the above-mentioned quality criteria have never been analysed in its wild relatives.

Wild einkorn can be distinguished into the true wild form *T. monococcum* ssp. *thaoudar*, which still thrives in the eastern part of the Fertile Crescent on 'fairly primary habitats' (Harlan and Zohary, 1966) and into the feral (escaped from farming during the spread of agriculture) form *T. monococcum* ssp. *aegilopoides*, which occurs as a weedy plant in the Balkans and Western Turkey on 'definitely segetal' habitats (Harlan and Zohary, 1966; Heun et al., 2008; Schiemann, 1948). The 'de-domesticated' brittleness of *Triticum aegilopoides* spikes is between that of truly wild *Triticum thaoudar* and of the domesticated form. Yet, *T. aegilopoides* is 1-grained like the domesticated form, whereas *T. thaoudar* is 2-grained (Heun et al., 1997, 2008).

Wild einkorn-like kernels are found in archaeological sites predating the birth of agriculture, e.g. in Syria (Willcox et al., 2008), but it is still unresolved if these kernels belong to *T. thaoudar* or *Triticum urartu* (Heun et al., 2008). Genetic data clearly indicate the mountains of Karacadağ (KD), in south-east Turkey, as the domestication hotspot for 1-grained Einkorn (Heun et al., 1997, 2008). However it is still unknown whether 'bridging' wild 2-

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grained Karacadağ lines (KD1) contain nutritional components making them more related to domesticate einkorn than less-related *T. thaoudar* lines from the Karacadağ (KD2) or wild einkorn from other locations (nKD). Theoretically, it could be possible that the humans who domesticated einkorn might have targeted unique characteristics beyond the prime domestication trait, i.e. brittleness (Abbo et al., 2014; Salamini et al., 2002; Zohary, 1969). Some compositional and/or nutritional features can be visually assessed and selected for (e.g. yellow pigments), while other are undetectable for ancient people, although they may influence food processing (e.g. a good β-amylase activity is essential for brewing/beer making, but not for bread making) or the general health of the population (e.g. proteins, antioxidants, vitamins, minerals). In this context, β-amylase activity is particularly interesting, as it is unsolved whether einkorn was initially domesticated for food consumption (e.g. in form of soups) or whether it was at first used to make alcoholic beverages (Dietrich et al., 2012). Of course the pre-historic ways of beer production are rather unknown, but can be in part deduced from vessels and other archaeological remains (Zarnkow et al., 2006).

Therefore, the aim of this study was to analyse thousand kernel weight, protein, ash, total starch, polyphenol and anthocyanin content as well as carotenoid and tocol composition, and β-amylase activity of well selected wild, feral and domesticated einkorns and compare the results with those of other domesticated *Triticum* species, i.e. emmer and durum wheat (*Triticum turgidum* ssp. *dicoccum* and ssp. *durum;* both are tetraploid: 2n = 4x = 28, genome BBA^uA^u) as well as spelt and bread wheat (*Triticum aestivum* ssp. *spelta* and ssp. *aestivum*; both are hexaploid: 2n = 6x = 42, genome BBA^uA^uDD). A line of *T. urartu* (diploid: 2n = 2x = 14, genome A^uA^u) is also considered, as this species is the donor of the A genome of the above mentioned polyploid wheats (Dvorak et al., 1993), and was never analysed for those traits either.

2. Materials and methods

2.1. Plant material

Fifteen wheat lines were selected (Table 1) and cropped in small plots (20 m^2) during the 2011–2012 season in Sant'Angelo Lodigiano (Italy). Domesticated einkorn, emmer, durum, spelt and bread wheat were used as controls; only one line for each polyploid wheat was tested, as these species are well characterised for all the traits analysed.

Planting date was 20 October 2011 and harvesting dates were 14 June 2012 (for the wild samples) and 25 June 2012 (for the feral and domesticated samples). Standard cultural practices included the use of 80 kg/ha of nitrogen fertilizer, applied half at planting and half at the end of tillering, and of a hormonic herbicide (Ariane II, Dow Agro Sciences Italia, Italy) to control weeds. The domesticated lines were hand-harvested at maturity, whereas the brittle lines were harvested as soon as the top spikelets started disassembling.

2.2. Experimental procedures

After harvesting, the kernels/spikelets were stored at +5 °C. Just before milling, the spikelets of the hulled wheats were de-hulled with a M3B micro-thresher (Co.Mi.L, Rome, Italy). The kernels were ground with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark), giving a whole meal flour with particle size < 200 μm. The whole meal flour was stored under vacuum at −20 °C for a maximum of 24 h.

The 1000 kernel weight (TKW) was assessed by weighing four independent replications of 50 naked seeds each, and sizing the results to 1000 kernels. The protein (N × 5.7), dry matter and ash content were determined following methods 46-10.01, 44-15.02 and 08-03.01 (AACC International). The total starch content and β-amylase activity were assessed using dedicated assay kits (Megazyme International Ireland Ltd., Bray, Ireland) and a DU-62 spectrophotometer (Beckman Coulters, Fullerton, CA, USA). These analyses were performed on two different whole meal samples per accession.

The total anthocyanins were measured by a spectrophotometric method (Abdel-Aal and Hucl, 1999) using a V650 specrophotometer (Jasco, Japan). The total polyphenols content (TPC) was assessed in saturated butanol extracts and their quantification was achieved with the Folin-Ciocalteu method as reported by Lavelli et al. (2009) with a DU-62 specrophotometer (Beckman Coulters, Fullerton, CA, USA). The TPC content, in mg ferulic acid equivalent (FAE)/kg on a dry matter basis (DM), was computed from a reference curve obtained from six ferulic acid (Sigma–Aldrich, St. Louis, MO, USA) concentrations in the range 0–100 mg/L. These analyses were performed on two different extracts per accession; each extract was tested two times, and its average value was used for statistical analyses.

Carotenoid extraction and quantification by NP-HPLC was carried out as described by Hidalgo et al. (2010). The following system and operating conditions were used: column Alltima Si column, 250 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); Alltima SI guard column 7.5 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); column oven at 20 °C L-2300 Elite LaChrom (VWR, Hitachi, Japan); mobile phase, hexane:isopropyl alcohol (5%); flow rate, 1.5 mL/min; pump L-2130 Elite LaChrom (VWR, Hitachi, Japan). The carotenoids were detected at 450 nm by Diode Array Detector L2450 Elite LaChrom (Merck, Hitachi, Japan) set in the range of 200–650 nm. The HPLC system was controlled by the

Table 1
Triticum accessions and their country of origin.

Species and subspecies	Abbreviation		Code/name	Country
T. monococcum ssp. *monococcum*	*T. monococcum*	domesticated	ID396	Romania
		domesticated	ID493	Turkey
T. monococcum ssp. *thaoudar*	*T. thaoudar*	wild	ID752	Turkey (KD1)
		wild	ID754	Turkey (KD1)
		wild	ID753	Turkey (KD2)
		wild	ID1280	Turkey (KD2)
		wild	ID870	Iraq (nKD)
		wild	ID1211	Iran (nKD)
T. monococcum ssp. *aegilopoides*	*T. aegilopoides*	feral	ID227	Balkans
		feral	ID228	Balkans
T. urartu	*T. urartu*	wild	ID1277	Turkey
T. turgidum ssp. *dicoccum*	*T. dicoccum*	domesticated	FAR262	Spain
T. turgidum ssp. *durum*	*T. durum*	domesticated	Dylan	Italy
T. aestivum ssp. *spelta*	*T. spelta*	domesticated	FAR62	Italy
T. aestivum ssp. *aestivum*	*T. aestivum*	domesticated	Blasco	Italy