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GRAIN QUALITY OF TETRAPLOID WHEAT *TRITICUM DURUM* DESF. VAR. *FALCATOMELANOPUS JAKUBZ. & FILAT*

Liana RELINA, Liudmyla VECHERSKA, Roman BOHUSLAVSKYI, Oleg GOLIK
Plant Production Institute nd. a. V. Ya. Yuriev of National Academy of Agrarian Sciences of Ukraine

Abstract. *Triticum durum* var. *falcatomelanopus* is tetraploid wheat with some valuable traits, though it is scarcely described in literature. Given that this convariety can be promising material for breeding, our objective was to evaluate the quality, performance and processing parameters of its grain. Grain harvested in 2015, 2016 and 2017 was analyzed. The protein content was determined by Kjeldahl digestion. The carotenoid level was spectrophotometrically assessed in acetone extracts. The antiradical activity was investigated by DPPH• assay in ethanol extracts. The contents of trace minerals were determined by atomic absorption spectrometry. The parameters under investigation were divergently affected by weather conditions. Our results have demonstrated that *T. durum* var. *falcatomelanopus i*s a source of large seeds; it can be used in wheat breeding as a source of high iron and sufficient zinc contents, balanced copper content, high vitreousness and grain hardness; it cannot be referred to high-carotenoid or high-antioxidant, or high-protein species.

Key words: *Triticum durum* var. *falcatomelanopus*; Antiradical activity; Carotenoids; Grain hardness; Protein content; Trace minerals; Vitreousness.

INTRODUCTION

Tetraploid wheat *Triticum durum* Desf. var. *falcatomelanopus* Jakubz. & Filat. was first described in 1932 (Udachin, R. 1973). It is a late-ripening form occurring in small locations in the countries of the Middle East (Syria, Israel, Jordan) and Central Asia. This wheat is relatively cold-tolerant (in comparison with other spring wheat from the same areal) (Udachin, R. 1973), and, despite considerable height, lodging-resistant due to thick straw. Plants require considerable water during the growing season. *T. durum var.falcatomelanopus* is sufficiently resistant to brown and yellow rusts. The caryopsis size is remarkable: up to 10 mm. The vitreousness is high: up to 75%. Another benefit of this convariety is easy threshing (Dorofeev, V. 1987).

This wheat is scarcely described in literature, and its grain quality has not been investigated at all. *T. durum var.falcatomelanopus* can be a promising, though little studied, source for breeding to expand biodiversity of tetraploid wheat species. From this perspective, our objective was to evaluate the grain quality parameters (protein content, carotenoid level, antioxidant activity and trace mineral contents) in *T. durum var.falcatomelanopus as well as* performance and processing parameters of *T. durum var. falcatomelanopus grain.*

MATERIALS AND METHODS

The analyses were carried out on *T. durum var. falcatomelanopus* (accession IR 00137) from a collection of the National Center for Plant Genetic Resources of Ukraine. The plants were grown on typical black soil. Grain harvested in 2015, 2016 and 2017 (years with various weather conditions) was used in analyses. All the accessions, including cultivars mentioned for comparison, durum wheat Spadshchyna and emmer Holikovska, were grown under identical conditions using conventional agro techniques.

The protein content was determined by Kjeldahl digestion (S´aez-Plaza, P. et al. 2013a; 2013b). The carotenoid level was spectrophotometrically assessed in acetone extracts as described in Luterotti, S.et al. (2010). The antiradical activity (ARA) was investigated in ethanol extracts by DPPH· assay (Sytar, O. et al. 2018; Żmijewski, M. et al. 2015). The contents of iron, zinc and copper were determined by atomic absorption spectrometry (Jorhem, L. et al. 2008). The test weight and vitreousness were evaluated in compliance with the State Standard of Ukraine (3768:2010). The grain hardness was determined on a YPD-300 hardness tester (Ltpm China) (measuring force applied to crush kernels) by the method developed by A.V. Yarosh et al (2014) and expressed in newtons. The data presented as mean ±SEM.

RESULTS AND DISCUTIONS

The protein content in T. durum var. falcatomelanopus grain was within 12.2 - 15.7% (see Table 1), with the average of 13.7%±0.9, so this variety cannot be considered a high-protein one, as good durum wheat grain contains 15-18 % of protein (grade I grain has ≥14.0% of protein (the State Standard of Ukraine 3768:2010). However, it is comparable with the average (2015-2017) protein content in grain of domestic emmer variety Holikovska bred at the Plant Production Institute named after V.Ya. Yuriev of NAAS - 13.3%±1.6. The lowest protein content (12.2±0.9%) was recorded in 2016, when the temperature during the 'green mass development' and 'grain filling' periods reached its peak (20.9°C and 23.2°C, respectively) over the study years, which is typical for wheat. There was no obvious relationship between the protein content and the precipitation amount. It is known that grain protein content negatively correlates with grain size (Abdipour, M. et al. 2016; Shewry, P. et al. 2013). The *Gpc-B1* locus was found to regulated senescence and grain protein content in wheat (Uauy, C. et al. 2006a). The common wild allele encodes a transcription factor that accelerates senescence and increases grain protein (as well as zinc and iron contents, see below). A nonfunctional *GPC-B1 (silenced by* RNAi*) allele* delayed senescence and significantly reduced protein content in grain (Uauy, C. et al. 2006b). This alteration is thought to result in larger seeds due to non-accelerated grain maturity (Nadolska-Orczyk, A. et al. 2017). It is possible that T. durum var. falcatomelanopus carries a nonfunctional *GPC-B1,* which accounts for relatively low protein content and large grain, since slower senescence is associated with larger seeds.

Table 1. *Biochemical parameters of Triticum durum var. falcatomelanopus grain*

Earing date	Green mass development		Grain filling		Protein content, %	Carotenoid content, mg/kg	ARA, CGAE/ g of seeds
	Σ_p, mm	t_{av}, °C	Σ_p, mm	t_{av}, °C			
06.12.2017	41	18.1	49	21.6	13.3±1.3	3.43±0.2	380±16
06.20.2016	157	20.9	107	23.2	12.2±0.9	3.85±0.5	440±19
06.18.2015	56	18.6	108	20.9	15.7±1.6	2.29±0.1**	384±15

Note (here and in Table 2): Σ_p = precipitation amount; t_{av} = average temperature.
** - 2016 - 2015 and 2017-2015 significant differences, p≤0.001.

Staples are not considered an important source of vitamins, antioxidants or minerals in the diet. However, there is an opinion (Garcia-Oliveira, A. et al. 2018; Kumar, S. et al. 2014) that because of high staple consumption, any increase in concentrations of these substances may have a significant effect on human nutrition and health. As of 2013, wheat products made up 179.26 g of food/capita/day, or 15.87 g of protein/capita/day, or 527 kcal/capita/day (Food supply quantity), in 2013 (FAOSTAT 2013).

Carotenoid content is a determinant of wheat nutritional value and affects end-product quality. Carotenoids have vitamin A activity and can also act as antioxidants. Carotenoids appear to be protective against cancer. Wheat grain generally contains very low carotenoid amounts and in order to enrich wheat grain with carotenoids, new high-carotenoid sources are searched for. The carotenoid content in *T. durum var. falcatomelanopus* grain was medium: 2.29 - 3.85 mg/kg (although significantly higher than the carotenoid content in grain of emmer Holikovska - 0.86±0.05) mg/kg). It is considered that high quality bright-yellow pasta can be made of grain containing not less than 5.5 mg/kg of carotenoids (Abdel-Aal, S. et al. 2012). Hence, this wheat cannot be referred to high-carotenoid ones. In this respect, *T. durum var. falcatomelanopus* is unlike to another tetraploid wheat, *Triticum timopheevii,* which had the lowest carotenoid content (2.72 mg/kg; range 2.72 - 3.54 mg/kg) in 2016 (Relina, L. et al. 2018). This can be due to an extremely large size of *T. durum var. falcatomelanopus* seeds, as metabolism and efflux of substances from vegetative tissues to grain in large-seeded forms may differ from those in small- and medium-seeded ones [see above].

Antioxidant content is another determinant of wheat nutritional value. Many authors believe that antioxidants could prevent chronic diseases (Zhang, Y. et al. 2015). The average ARA in *T. durum var. falcatomelanopus* grain amounted to 401±19 chlorogenic acid equivalents (CGAE) /g of seeds, which is lower than in durum wheat grain, cv. Spadshchyna bred at the Plant Production Institute named after V.Ya. Yuriev of NAAS (525 ± 39 CGAE /g of seeds) or in emmer Holikovska (569±19 CGAE /g of seeds) (Vecherska, L. et al. 2018). This parameter varied from 380 CGAE /g of seeds in 2017 to 440 CGAE /g of seeds in 2016. Thus, the highest ARA was associated with the highest air temperature during the periods of green mass development and grain filling (like the carotenoid content). This may be due to plants' response to increased temperatures as a stress factor. It is known that different stresses (including rising temperature) can activate antioxidant system (Uarrota, V. et al. 2018; Shamloo, M. et al. 2017), with carotenoids and radical scavenging compounds being its parts. Thus, *T. durum var. falcatomelanopus* responses to increased temperature by accumulation of these compounds. Similarly, in *T. timopheevii* grain, the ARA was the highest (628±25 CGAE /g of seeds) in 2017, which was characterized by the scantiest rainfall (drought stress) during the crucial periods (Relina, L. et al. 2018), and a positive correlation was observed between seed antioxidants and drought tolerance in other plant species (Lakshmi, S. et al. 2018). Relatively low antiradical activity of *T. durum var. falcatomelanopus* grain can also be attributed to its size, since a negative correlation was observed between contents of antioxidants and seed size (Shewry, P. et al. 2013).

Some minerals are essential in metabolism or for the synthesis of essential compounds. It is estimated that over three billion people experience micronutrient deficit worldwide (Bouis, H. et al. 2017; White, P. et al. 2009). Currently, mineral malnutrition is considered a very serious global challenge (Bailey, R. et al. 2015; Stein, A. et al. 2010). Iron deficiency is the most common nutritional deficiency in the world (Dlouhy, A. et al. 2013; Hider, R. et al. 2013). Bread and breakfast cereals are sometimes specifically fortified with iron (Gera, T. et al. 2012), therefore, there is a chance to breed high-iron wheat cultivars.

The iron content in commercial durum wheat varies within 25.7-40.5 mg/kg (Magallanes-López, A. et al. 2017), though S. Nadaf (2010) reported of unusually high iron content in durum wheat and emmer varieties: 76.0-84.0 mg/kg and 70.0-75.2 mg/kg, respectively. Other researchers published that the iron content in durum wheat grown in India ranged 29.1 to 51.0 mg/kg (Himani, M. et al. 2017). *T. durum var. falcatomelanopus* grain contains 35.15 - 44.42 mg/kg of iron (see Table 2), which is considerably higher than the iron levels in commercial durum wheat cultivars and comparable to the iron content in emmer grain (49 mg/kg in Polish emmer (Suchowilska, E. et al. 2012) and around 40±2,65 mg/kg in grain of variety Holikovska). This variation can be attributed to weather fluctuations during crucial periods in the plant development. The grain accumulated 44.42 mg/kg of iron, when the precipitations amounts were 56 mm and 108 mm during the periods of green mass development and grain filling, respectively. The plant massively intakes minerals from the soil during the green mass development. The increase in the precipitations amount in this period was associated with a reduction in the iron content, though the species prefers moist conditions, and a decrease in the precipitation to 41 mm was even accompanied by more drastic drop in the iron content. Here we can assume that too abundant rainfall dilutes minerals in the soil, and scanty rainfall does not allow the plant to intake them from the soil. Abundant precipitation during the grain filling period (108 mm) did not reduce the iron content, as the consumption of minerals was over by this time. There was no apparent relationship between the iron content and air temperatures during the crucial periods of the plant development. Thus, 2015 was the most favorable year for iron accumulation by *T. durum var. falcatomelanopus* grain. Despite this variation, high iron content in grain appears to be genetically intrinsic to T. durum var.falcatomelanopus, hence it can serve as a source of high iron content.

Table 2. *Mineral contents in Triticum durum Desf. var. falcatomelanopus grain in different years*

Earing date	Green mass development		Grain filling		Minerals		
	Σp, mm	tav, °C	Σp, mm	tav, °C	Zn	Fe	Cu
06.12.2017	41	18.1	49	21.6	34.8±1.2	35.2±1.0	3.72±0.10@@
06.20.2016	157	20.9	107	23.2	32.4±0.9	41.0±1.63#	0.83±0.04@@®®
06.18.2015	56	18.6	108	20.9	31.5±0.9	44.4±2.4##	0.17±0.02

Note: # - 2017 - 2016 significant differences, $p \leq 0.05$; ## - 2017 - 2015 significant differences, $p \leq 0.001$; @@ - 2017 - 2015 and 2016-2015 significant differences, $p \leq 0.001$; ®® - 2017 - 2016 significant differences, $p \leq 0.001$.

Zinc is also an essential trace element for humans. Wheat (especially germ and bran) is among the food plants that contain the most zinc (Deshpande, J. et al. 2013). For fortification of foods, cereals are recommended as a cheap, stable source of zinc that is as easily absorbed (Shah, D. et al. 2016). The zinc content in commercial durum wheat varies within 24.8-48.8 mg/kg (Magallanes-López, A. et al. 2017). The zinc content in durum wheat varieties grown in India was within 20.3-46.9 mg/kg (Himani, M. et al. 2017; Nadaf, S. 2010). The maximum allowable concentration of zinc in grain is 50.0 mg/kg

(Feschenko, V. 2014). *T. durum var. falcatomelanopus* grain contains 31.5 - 34.8 mg/kg of zinc (see Table 2), which is comparable to commercial durum wheat and our emmer, variety Holikovska (around 31.0 mg/kg), and somewhat less than in Polish emmer grain (54 mg/kg (Suchowilska, E. et al. 2012)) and much higher than in Indian emmer (13.5 - 15.2 mg/kg (Nadaf, S. 2010)). The zinc content-weather conditions relationship was opposite to the iron content-weather conditions relationship, which was unexpected, since the high grain protein content gene (*GPC-B1)* was also shown to confer higher concentrations of both Fe and Zn in grain (Distelfeld, A. et.al. 2007; Cakmak, I. et al. 2004). Correlations between Zn and Fe contents were positive for wild emmer and domesticated emmer (Chatzav, M. et al. 2010; Peleg, Z. et al. 2008; Distelfeld, A. et al. 2007; Cakmak, I et al. 2004). Lack of similar correlations in *T. durum var. falcatomelanopus* can be also due to its large grain (Nadolska-Orczyk, A. et al. 2017).

Copper is another essential trace element. The variations in this parameter can be wide: 3.12-12.2 mg/kg in durum wheat (Himani, M. et al. 2017; Nadaf, S. 2010). In Russian wheat grain the copper content ranged within 2.0-12.8 mg/kg, depending on the cultivation site (Pugaev, S. 2013). Other Russian researchers report that the copper level in spring wheat grain averaged 5.15 ± 0.40 mg/kg (throughout 10 year), with the maximum allowable concentration of 10 mg/kg (Feschenko, V. 2014). Suchowilska E. et al (2012) published that the grain of a related species, *Triticum dicoccum,* contained 4.4 mg/kg of copper. S. Nadaf (2010) reported a much higher copper content in *T. dicoccum* varieties: within 11.3-14.3 mg/kg. *T. durum var.falcatomelanopus* grain contained of 0.165 - 3.680 mg/kg of copper in different years (see Table 2). Thus, such levels can contribute to filling the need of human body for copper, on the one hand, and are far below the maximum allowable concentration, on the other hand. The variations copper content does not seem to have any relations to the weather fluctuations during the periods of green mass development and grain filling, since the minimal (0.17±0.02 mg/kg) and maximal (3.72±0.1 mg/kg) were both associated with similar air temperatures (18.6°C and 18.1°C, respectively) and precipitation amounts (56 mm and 41 mm, respectively) during green mass development. The rainfall during the grain filling period appears to have no influence on this parameter, either. The temperature of 23.2-23.5°C during the grain filling period is associated with lower values of the copper content, though this association remains unexplained at the moment. Divergent changes in the contents of iron, zinc and copper can be attributed to different pathways and roles of these ions in plant physiology.

Concurrently with biochemical characterization, we measured performance and processing parameters of T. durum var.falcatomelanopus*.* The average 1000-seed weight was 68.3±0.8 g (Table 3), meaning that T. durum var.falcatomelanopus has large-sized grain and is to b*e* involved in crossing as a source of large seeds. However, its ability to transfer this feature to other genomes (to become a donor) needs further verification. The test weight and vitreousness are criteria for determining grain grade. The test weight was around 836 g/L, which is sufficiently high, as the minimal test weight for durum wheat is set on 700 g/L, and grade I grain has ≥750 g/L (DSTU 3768:2010). The vitreousness was rather high - 72%, which is valuable for easy milling (grade I grain has ≥70% vitreousness (State Standard of Ukraine 3768:2010). The grain hardness is one of the key determinants of milling behavior and has a great influence on flour and dough quality. For example, the grain hardness was shown to correlate with bread-making quality (Fedotov, V 2011; Medvedev, P. et al. 2015). The T. durum var.falcatomelanopus grain hardness was on average 268 N. It is difficult to compare different researchers' data, as they use different techniques and devices to measure the grain hardness. However, Veha A. et al. (2011) cross-checked the Hardness Index (HI) produced Perten SKCS 4100 equipment against maximum breaking force in Newtons produced by Lloyd 1000R Testing Machines. Using their data, we can assume that 268 N correspond to HI» 66. Using Szabo B. et al's data (2007), we obtain a similar result. This means that T. durum var.falcatomelanopus is likely to belong to hard wheat varieties or to hard/medium hard bread wheat varieties according to Haraszi R. et al's (2016) classification.

Table 3. *Technological parameters of Triticum durum var. falcatomelanopus grain*

Year	Grain hardness, N	Test weight, g/L	Vitreousness, %	1000-grain weight, g
2017	266±11	836±7	74±0.8	68.6±0.9
2016	278±11	842±8	68±0.8	69.4±1.1
2015	259±10	830±7	84±1.1	66.8±0.8

CONCLUSIONS

Thus, our results have demonstrated that 1) *T. durum var. falcatomelanopus* is a source of large seeds. It should be verified if it can transfer this trait to offsprings in crossing with other accessions; 2) T. durum var. falcatomelanopus can be used in wheat breeding not only as a source of resistance to fungal diseases, but also as a source of high iron and sufficient zinc contents, balanced copper *content, high* vitreousness and grain hardness; 3) *T. durum var. falcatomelanopus* cannot be referred to high-carotenoid or high-antioxidant, or high-protein species.

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