Breeding for improved gluten strength and yellow pigment content in winter durum wheat

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Abstract

The cultivation of winter durum wheat in Hungary dates back only a few decades. Under the climatic conditions of the country, mainly varieties of winter-types are grown, which are able to achieve higher yield than that of spring-types. It is also an important aspect for the players in the cereal sector to grow varieties that meet the quality requirements of the pasta industry. The strength of the gluten and the yellow pigment content are extremely important technological quality characteristics of durum wheat. In durum wheat breeding programs, the former is often determined by measuring the gluten index, while the latter one is estimated by the Minolta b* value. During the decades following the start of the breeding program, both testing methods were successfully introduced and used in selection aimed at improvement of technological quality at Martonvásár. In this publication, we present the results achieved by using genetically diverse set of varieties, as well as the results of the durum wheat breeding program. With our experiments, we could prove that, based on the repeatability (h^2), both traits are genetically well defined (gluten index: 0.949; Minolta b*: 0.978), and a large degree of genetic variability can be observed in the winter and facultative gene pool. As a result of successful selection, the technological quality of winter durum wheat varieties registered in recent years has become competitive with spring ones.

Key words: Gluten index; Minolta b*; technological quality; *Triticum turgidum* ssp. *durum*; selection; genetic improvement

INTRODUCTION

Durum wheat is the second-most-produced *Triticum* of the World, the amount of the crop varied between 26.2 and 41.0 million tons annually since 1991 (data from the International Grains Council; cit. Le Lamer & Rousselin, 2011; Bryant-Erdmann, 2017). Its grain is primarily processed by the pasta industry (Troccoli et al., 2000). In Hungary, durum wheat does not belong to the traditionally cultivated plant species. Although there have been attempts to introduce the species in Hungary since the 1920s (Odry, 1929; Veneny, 1930), this work has failed. At the beginning of the 1980s, breeders from Szeged achieved a breakthrough in this area (Beke & Barabás, 1981), and from 1996, winter durum wheat varieties bred in Martonvásár also appeared in domestic grain production (Szunics et al., 1998). Since then, the species has found its place in the domestic plant production. Its sown area increased continuously until 2018, when it was produced on 44.280 ha. Since then, its cultivated area has decreased, but it still exceeds 25,000 ha (Eurostat, 2021). The durum wheat produced in Hungary is a valuable raw material for domestic mills and companies involved in the pasta production, but it is also an important export goods. The primary trading partners are Italy, Germany and Austria (WITS, 2019).

Pasta and other products made from durum wheat are made as a result of a relatively simple processing steps, during which few other ingredients are used (only water in most cases) or none at all (e.g. bulgur), therefore the technological quality of the raw material has an extremely important effect on the quality of the final product. In modern durum wheat breeding programs, improving techno-

logical quality is a priority (Hare, 2017). There are several favorable properties of durum wheat, which have advantage during the industrial processing and use. The main product of milling is semolina, which contains a large amount of yellow pigments (carotenoids). These ingredients do not affect the pasta-making and cooking properties, or only to a very small extent, but at the same time it basically determines the aesthetic value, salmonella-free feature, storability and thus its marketability and exportability of pasta (Dexter et al., 1981). Its protein content is high, but in addition, even its gluten structure is special. The strong gluten network is able to retain starch molecules during cooking (Feillet, 1984), the surface of the pasta does not become sticky, and maintains its shape stably (Dexter & Matsuo, 1980). It can be concluded that when evaluating the technological quality of durum wheat, the gluten strength and the yellow pigment content are of outstanding importance.

The strength of gluten can be determined by several methods. The common laboratory devices used for rheological measurements can also be used for testing durum wheat samples (Fabriani & Lintas, 1988). In addition to Farinograph (Irvine et al., 1961; Matsuo & Irvine, 1975; Aalami et al., 2007), mixograph (Bendelow, 1967; Edwards et al., 2007) and alveograph (Matsuo & Irvine, 1970; Perego et al., 2002; Miravalles et al., 2007) several other instruments were used for this purpose. Visco-elastographic test (Damidaux & Feillet, 1978), SDS sedimentation test (Dexter et al., 1980) and its micro version developed for a sample size of 1 g (Dick & Quick, 1983), hand-made elasticity test (D'Edigio et al., 1990), Mixolab (Torbica et al., 2016) and the GlutoPeak measurement (Sissons, 2016; Sissons & Smit, 2018) can also be found among the methods suitable for determining gluten strength. Measuring the gluten index (GI), the methodological basis of which was developed by Harald Perten (1990), is also useful for durum wheat breeding programs. Cubbada et al. (1992) modified the gluten index method and they successfully used it to determine durum wheat quality from both whole meal and semolina. Based on their results, the gluten index correlated very closely with the data of the manual gluten strength test. Since the measurement can also be performed from whole meal, only 20 g of sample is required for the test, so the method is particularly suitable for testing samples from early generations in breeding programs. Compared to the SDS sedimentation test, the results depend less on the protein concentration, which enables a more objective selection for breeders (Clarke et al., 2010). Gluten index measurement is nowadays a generally accepted method for determining the strength of gluten (Oikonomou et al., 2015), the description of its method is included in international standards (AACC International 2010; ICC 1994 and 1995).

The gluten index is a highly heritable genetic trait, the h^2 value based on the examination of 120 offspring of three crossing combinations was between 0.84 and 0.93 (Clarke et al., 2000). Those results were later confirmed using a broader range of varieties (six combinations, 398 lines; h^2= 0.84–0.95; Clarke et al., 2009b). The first gluten index measurement in durum wheat took place almost 30 years ago, but there are still few published results to this day. This finding is especially true for winter durum wheat.

Both indirect and direct measurement methods have been developed to measure the yellow pigment content and the amount of the most important component, lutein. Indirect measurements make use of the property of carotenoid derivatives that pigments absorb or reflect electromagnetic waves in the visible or near-infrared range at different wavelengths (spectrophotometry), while direct methods mainly involve chromatographic measurements.

Nowadays, devices using the L*a*b* color system (CIE S 017-4/E:2007 standard) accepted by the CIE (Commission Internationale l'Eclairage, 2008) are the most widely used in the processing industry and breeding programs. In this color system, the L* value indicates the brightness of the sample (0 = black, 100 = white), the a* value on the green-magenta (negative value: green, positive value: magenta) axis, and the b* value on the blue-yellow (negative: blue, positive: yellow) gives information about the color specified. All visible colors can be clearly identified in the three-dimensional space formed by the coordinate axes (Brainard, 2003). Several research groups have proved the close correlation between chromametric color measurement and the yellow pigment content, and thus the effective applicability of the method. Wehrle et al. (1997; Minolta CR-300 Chroma Meter) calculated a correlation coefficient value of 0.88, Borrelli et al. (1999; Minolta CR-200) 0.95, Humphries et al. (2004; Minolta CR-100) 0.89,

Fratianni et al. (2005; Minolta CR 200) 0.72–0.94, Digesù et al. (2009; Minolta CR-300) 0.88–0.90, Blanco et al., (2011; Minolta CR-300) 0.87–0.93, Hung and Hatcher, (2011; Hunterlab Labscan XE) 0.98, N'Diaye et al. (2017; Minolta CR-200) 0.96 between the carotenoid content of the whole meal or semolina and the b* value.

Yellow pigment content is also a genetically well-determined trait (Martini et al., 2015). Braaten et al. (1962) reported heritability values between 72-96%, Lee et al. (1976) determined at 79%. In the experiment of Johnston et al. (1983) the same value was between 31 and 69%, for combinations created with more diverse parents based on the yellow pigment content, it was 66 and 69%. Santra et al. (2005) calculated a value between 0.67 and 0.93. Based on the results of Clark et al. (2006) in their studies with six populations, the h^2 value varied between 0.88 and 0.95, while in the case of one combination, the realized heritability of the yellow pigment concentration was as low as 0.34. All the above-mentioned authors agree that additive gene effects influence the yellow pigment content, and that the selection of transgressive individuals can be successfully carried out already in the early offspring generations. The high heritability value also shows that the trait is oligogenically determined and by only a few alleles. In winter durum wheat, Longin et al. (2013) analyzed the repeatability value of Minolta b* data of 105 winter durum wheat genotypes using data originated from four environments with REML (restricted maximum likelihood) analysis. Based on their results, the authors calculated an h^2 value of 0.9. This last observation proves that the yellow pigment content in winter durum wheat varieties and lines is primarily a trait determined by the genotype.

Over the past 25 years, we have conducted complex and detailed examinations into the genetic and environmental determination of the gluten index and the Minolta b* value. The broadness of available genetic variability in the autumn and facultative varieties was assessed. Last but not least, we have produced durum wheat varieties that, in addition to the needs of cereal growers, must also meet the requirements of the milling and pasta-making industry. In this publication, the some relevant results achieved in the field of durum wheat research and breeding over two and a half decades are presented.

MATERIAL AND METHODS

Our experiments set up to study the gluten index (GI) and the Minolta b* value (MB) were connected to two research areas:

1. Examination of a set of winter and facultative durum wheat genotypes of broad genetic base;

2. The results of the winter durum wheat breeding program in Martonvásár aimed at improving the gluten index and the Minolta b* value.

All experiments were set up on the same field (Lászlópuszta, 47°18'N/18°49'E). According to the laboratory analysis of standard soil samples taken from the cultivated layer (0-20 cm) of chernozem soil with forest residues. The soil close to the surface, which does not contain lime and harmful salts, has a neutral pH (pH = 6.99) and is loam in terms of its physical properties. Based on its humus content (2.4 m/m%), it has a moderate nitrogen supply, the phosphorus content is medium (120 mg kg^{-1}), while the potassium supply was uniformly good (> 300 mg kg^{-1}). In terms of microelements, the zinc content of the soil is less than optimal (1.1 mg kg^{-1}), while copper (2.7 mg kg^{-1}) and manganese (156 mg kg^{-1}) contents are sufficient.

The pre-crop of the experiments was oilseed radish. Soil was prepared by harrowing, and the seedbed was opened with Amazone or Synchrogerm seedbed preparation equipment. Nutrient supply in the experiments was carried out by applying 60:60:60 kg ha^{-1} N:P:K active ingredient in the fall, followed by a single top fertilization of 60 kg ha^{-1} nitrogen in early spring. HEGE-80 or HEGE-90 type plot-drill (Hans-Ulrich Hege GmbH und Co., Waldenburg, Germany) were used for sowing, the plant density in all experiments was 450-500 seeds/m^2 recommended in Hungary. During the growing season, we protected the plots against weeds (MCPA, clopyralid, triasulfuron, tribenuron-methyl + fluroxypyr, if necessary fenoxaprop-P-ethyl) and insect pests (lambda-cyhalothrin, esfenvalerate), but no fungicide treatment was carried out. After full ripening, the plots were harvested with a Wintersteiger plot combine (Wintersteiger AG, Reid, Austria). Our data are based on technological quality measurements of durum wheat samples harvested between 1996 and 2020. The meteorological characteristics of the vegetation periods are described in Table 1.

Table 1. Meteorological characteristics of the vegetation periods between 1995/1996 and 2019/2020 in Martonvásár, Hungary

Vegetation period	Precipitation (mm)		Mean temperature (°C)		No. of heat days[3]	Day of the year	
	Σ[1]	GFP[2]	Σ	GFP		sowing	harvest
1995/1996	480.7	103.6	8.13	19.85	13	278	194
1996/1997	196.2	74.2	8.23	18.81	8	276	178
1997/1998	440.2	154.0	10.30	18.42	12	287	201
1998/1999	492.6	175.8	7.09	18.51	5	282	184
1999/2000	364.0	24.4	7.85	19.58	22	283	179
2000/2001	450.2	93.2	9.23	18.76	5	269	188
2001/2002	188.8	45.0	9.14	19.91	11	271	179
2002/2003	231.0	39.0	7.00	21.37	27	283	178
2003/2004	484.4	130.6	7.35	18.46	8	282	194
2004/2005	458.4	49.2	6.96	18.69	10	280	177
2005/2006	421.6	118.4	7.30	19.01	17	283	191
2006/2007	167.8	86.6	10.64	20.71	30	285	173
2007/2008	361.4	88.8	7.97	19.91	14	285	184
2008/2009	320.0	85.5	8.32	18.06	6	283	183
2009/2010	629.5	186.5	8.06	19.96	14	281	195
2010/2011	238.1	54.5	7.26	19.29	3	287	192
2011/2012	210.2	78.2	7.39	18.73	9	284	180
2012/2013	381.8	68.2	7.63	17.96	7	279	183
2013/2014	304.9	87.7	9.22	17.78	6	276	183
2014/2015	320.9	83.4	8.44	18.39	9	283	183
2015/2016	365.1	125.6	8.65	19.01	7	302	186
2016/2017	236.4	50.5	7.52	20.69	11	288	184
2017/2018	463.6	117.3	8.67	20.10	6	285	176
2018/2019	349.4	117.0	8.92	20.27	13	277	183
2019/2020	355.0	111.3	8.80	18.17	6	288	183

Notes: [1]Σ = period from sowing to harvest; [2]GFP = Grain filling period; [3]Daily maximum temperature ≥ 30°C

Examination of a set of winter and facultative durum wheat genotypes of broad genetic base

In the experiment, 100 varieties and breeding lines were tested from the durum wheat breeding programs of 12 countries (Austria, Bulgaria, Germany, Croatia, Hungary, Italy, Romania, Russia, Serbia, Slovakia, Turkey and Ukraine). The number of tested genotypes varied from two (Croatia, Italy) to 18 (Hungary, Russia). The genotypes represent approximately the results of the last 50 years of autumn and facultative durum wheat breeding efforts. The experiments were set up in five consecutive years (2014–2018). The varieties were sown in small plots with an area of 2.0×0.9 m, with row spacing of 15 cm. The data were analyzed using the SPSS 16.0 program package (SPSS Inc., Chicago, IL, USA). The effect of genotype, year and the in-

teraction of the two main factors were calculated with analysis of variance (General Linear Model/ Univariate Analysis of Variance module). The Genotype factor was treated as a fixed factor and the Year as a random factor. According to Longin et al. (2013) variance components (σ^2_G = genotypic variance; σ^2_{GY} = Genotype × Year interaction variance; σ^2_e = residual variance; σ^2_P = phenotypic variance) were determined, and then repeatability values (genotypic/phenotypic variance = h^2) were calculated. Populations of durum wheat varieties by countries were characterized by descriptive statistics, then the distribution of gluten index and Minolta b* values of the genotypes were graphically presented by the violin plot method. For this latter purpose, we used the *ggplot2* package of the R (ver. x64 4.0.3.) programming environment (Wickham, 2016). Graphi-

cally, on a scatter-plot diagram (Microsoft Excel program, 2013), the correlation between the date of registration of the variety and the technological quality was illustrated. Next, we searched for biochemical and molecular markers associated with the gluten index and the Minolta b* value using storage protein subunits and DNA markers in 50 durum wheat genotypes. The presence of γ-gliadin subunit 42 or 45 was examined according to Jackson et al. (1996). RAPD, SSR and gene-specific primers were used for DNA-level molecular studies. The RAPD primers were identified in the OTKA T038044 project, and (OPK02) by Santra et al. (2000). The following Operon primers were used for the tests: OPA16 (800 bp), OPK02 (500 bp) OPT16 (1500 and 900 + 1500 bp), OPZ17 (300 and 900 bp). Amplification of the specific products was carried out on a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA; 94°C for 1 min, 36°C for 1 min, 72°C for 1 min for 36 cycles; 72°C for 6'). The amplified products were separated by electrophoresis on a 1.2% agarose gel containing ethidium bromide, and then the bands were visualized with the Bio-Rad Gel Documentation System (Bio-Rad, Hercules, CA, USA). For detecting the Xgwm344 locus linked with yellow pigment content (Elouafi et al., 2001) WMS344 microsatellite marker (Röder et al., 1998) was amplified, and then the products were detected using a LI-COR 4300 DNA Analyzer (LI_COR Biosciences, Lincoln, NE, USA) on a polyacrylamide gel according to the method recommended by the manufacturer (LI-COR 2009). Alleles of phytoene synthase (*Psy*) genes, which play a prominent role in the process of lutein synthesis, were detected with specific PCR-based markers. The primer pairs Psy1-A1_STS and YP7A-2 were used to identify the a, l, o (Singh et al., 2009) and e and d (He et al., 2009b) alleles of the Psy1-A1 gene located on chromosome arm 7AL. At the Psy1-B1 locus on chromosome arm 7BL, He et al. (2009a) identified three (e, f, g) and two additional alleles (n and o) identified by Zhang and Dubcovsky (2008).

The results of the winter durum wheat breeding program in Martonvásár in the field of improving the gluten index and the Minolta b* value

Based on the data originated from the years 1996-2020, the result of the introduction of the gluten index and the Minolta b* value measurement was investigated. The tested genotypes included winter durum wheat varieties registered earlier, as well as variety candidates and the most promising breeding on the Martonvásár breeding program. The number of genotypes ranged from 9 (1996) to 31 (2008–2011) in different years. In this series of experiments, the tests were also carried out in two replications. The characteristics of the tested durum wheat lines were compared to the data of the standard 'Martondur 1' and 'GK Bétadur' (year of recognition 1996 for both varieties). Genetic improvement in gluten index and Minolta b* was determined in two ways. On the one hand, we calculated the change in the average relative value of the lines compared to the check varieties and their average. On the other hand, we measured the absolute value of the released Martonvásár durum wheat varieties for five consecutive years (between 2014 and 2018) and fitted a regression line to the data based on the following formula:

$y_i = ĉ + bX_i$

where: $ĉ$ is a constant (the intercept on the y-axis), b is the slope of the regression line, and X_i is the gluten index, or Minolta b* value of the durum wheat variety recognized in the ith year. The value of b represents the degree of genetic progress (Khalil et al., 2002).

Technological quality measurements

The gluten index of winter durum wheat samples was determined from semolina based on the ICC158 (ICC, 1995) standard, using Perten Glutomatic 2200 gluten washer and Perten 2015 Centrifuge (Perten Instruments AB, Hägersten, Sweden). The Minolta b* value was measured with a Minolta CR-300 between 1996 and 2016, and from 2017 with a Minolta CR-400 chromameter (the data measured by the two devices were, the values matched). In all cases, the tests were performed from semolina samples.

Until 2010, semolina was produced at a Brabender Junior laboratory mill (Brabender GmbH & Co. KG, Duisburg, Germany), modifies according to the instructions of Vasiljevic et al. (1977). Then the coarse bran fraction was separated on a Retsch KS 1000 sieve line (Retsch GmbH, Haan, Germany). The 160–315 μm fraction was then purified using a Chopin Semolina Purifier (Chopin Technologies, Villeneuve-la-Garenne, France). Since 2010, the durum wheat samples have been milled on a Chopin CD2 laboratory mill. The semolina was purified on the previously used Chopin Semolina Purifier de-

vice, so the particle size was the same in case of both sample sets.

RESULTS AND DISCUSSION

Examination of a set of winter and facultative durum wheat genotypes of broad genetic base

In five consecutive years (2014–2018), the gluten index and Minolta b* value of 100 winter and facultative durum wheat varieties/breeding lines from 12 countries were examined. In the first step, the effect of varieties and lines was analyzed using analysis of variance (Table 2).

The two main factors and their interaction also proved to be significant. When calculating the F value of the main factors - since the interaction was also significant - we divided by the MSQ value of the Genotype × Year factor.

In the second step, we investigated the genetic determination of the two traits. To determine the repeatability (h^2), the variance components were calculated according to Longin et al. (2013) by REML analysis (Table 3). Based on the results of the examination of the broad genetic base of varieties, the values of both technological quality traits were ranged within a wide interval. Based on the high repeatability (h^2) values, the genetic determination of the gluten index and the Minolta b* value is extremely strong.

The h^2 value for gluten index was similar than that of calculated by Clarke et al. (2000 and 2009b) for spring durum wheat populations ($h^2 = 0.84–0.95$) in the winter and facultative durum wheat variety). For the Minolta b* value, the repeatability in Longin

et al. (2013) experiment ($h^2 = 0.90$) was close to our own data, but several research groups also made a similar observation for the yellow pigment content that determines the Minolta b* value (Braaten et al., 1962; Santra et al., 2005; Clarke et al., 2006; Taneva et al., 2019). Due to the high repeatability, the selection of transgressive offsprings can be effectively started already in early generations in the case of both technological quality traits.

The tested durum wheat varieties and strains represent approximately the breeding results of the last fifty years. The oldest variety in the collection was certified in 1967 (Kunduru 1149, Turkey; Palamarchuk 2005), but currently cultivated varieties

Table 3. Descriptive statistics and variance components of the gluten index and the Minolta b* value, based on the examination of 100 varieties of durum wheat (Martonvásár 2014–2018)

	Gluten index		Minolta b*	
Mean	39.277		21.721	
Minimum	0.656		15.195	
Maximum	97.693		29.963	
Standard deviation	30.254		2.791	
σ^2_G	679.769	***	6.685	***
σ^2_{GY}	165.138	***	0.670	***
σ^2_e	34.504		0.153	
σ^2_P	716.247		6.835	
Repeatability (h^2)	0.949		0.978	

Notes: σ^2_G = genotypic variance; σ^2_{GY} = Genotype × Year interaction variance; σ^2_e = error variance; σ^2_P = Phenotypic variance; $h^2 = \sigma^2_G/\sigma^2_P$; *** a variance components significant at $p < 0.001$ level.

Table 2. The effect of genotype, years and their interaction on the gluten index and Minolta b* value of durum wheat genotypes (Martonvásár, 100 varieties, 2014–2018)

Factor	Gluten index					Minolta b*				
	SSQ	df	MSQ	F		SSQ	df	MSQ	F	
Genotype	709084.554	99	7162.470	19.635	***	6766.418	99	68.348	45.755	***
Error (I)	144453.036	396	364.780	a		591.533	396	1.494	a	
Year	59950.392	4	14987.598	41.087	***	418.434	4	104.609	70.030	***
Error (II)	144453.036	396	364.780	a		591.533	396	1.494	a	
Genotype×Year	144453.036	396	364.780	10.572	***	591.533	396	1.494	9.754	***
Error	17252.171	500	34.504	b		76.572	500	.153	b	

Notes: [a] and [b] = the error term was used for the calculation: [a]MSQ $_{Genotype×Year}$; [b]Error; *** the effect of the factor was significant at $p < 0.001$ level.

Table 4. Gluten index and Minolta b* value of winter and facultative durum wheat varieties grouped by country of origin (Martonvásár, 2014–2018)

Country	Abbrev.[1]	Number of genotypes	Gluten index				Minolta b*			
			Mean	Min	Max	sd[2]	Mean	Min	Max	sd
Austria	AUT	14	60.24	29.29	89.05	20.97	24.49	22.00	26.67	1.55
Bulgaria	BGR	6	31.78	8.28	53.57	16.84	20.89	18.95	24.23	1.84
Germany	DEU	13	60.29	14.95	86.40	21.99	25.30	21.02	27.98	2.06
Croatia	HRV	2	36.93	6.60	67.26	42.89	19.11	17.88	20.34	1.74
Hungary	HUN	18	39.22	2.67	88.99	27.48	20.94	17.75	24.56	1.87
Italy	ITA	2	78.80	68.43	89.16	14.66	23.64	22.65	24.63	1.40
Romania	ROM	4	58.48	50.99	65.09	6.70	19.27	18.89	20.29	0.68
Russia	RUS	18	28.71	2.42	75.67	21.33	20.44	17.58	24.31	1.71
Serbia	SRB	4	19.48	2.40	29.19	12.31	20.71	19.43	21.34	0.87
Slovakia	SVK	4	24.47	2.17	47.67	23.68	20.98	17.57	25.52	3.36
Turkey	TUR	5	23.25	1.77	56.31	22.32	20.63	19.77	21.27	0.57
Ukraine	UKR	10	12.98	3.94	42.98	13.30	19.76	18.66	21.40	1.08

Notes: [1] ISO 3166 standard; [2] standard deviation

were also included in the experiment. In most of the genotypes from different countries, the gluten index and the Minolta b* value also varied within wide range based on the average values of the 5 years (Table 4).

Based solely on descriptive statistical data, it is almost impossible to assess the phenotypic variability observed in groups of genotypes from different countries. The violin plots (Figure 1) created from the data over the 5 year-period contain more information on the distribution of gluten index and Minolta b* values.

The gluten index data for all countries can be found within a wide interval. According to the mean data, the average gluten index of the Italian varieties was the highest (78.80; the gluten indices of the two breeds were 68.43 and 89.16), while the Ukrainian ones had the lowest (12.98). The variability observed in Austrian, German, Croatian, Hungarian and Russian genotypes is exceptionally high. In the first four listed countries, the distribution of the samples was even (the average value was in the middle), but at the same time, in the case of the Russian varieties, samples with lower success occurred in a higher proportion. A special shaped distribution can be seen in the Ukrainian pool. Among the average samples per year, there was at least one variety with a gluten index exceeding 70, but varieties with a low values predominated. This is in contrast with the distribution of the samples of the Italian

breeds, which shifted in the direction of higher gluten index values.

Analyzing the Minolta b* data, it can be concluded that the German and Austrian genotypes – based on their mean values – were ahead of the varieties from Italy. In the case of several countries, the mean Minolta b* value of durum wheat varieties was in the range of 19–21 (BGR, HRV, HUN, ROM, RUS, SRB, SVK, TUR and UKR. This group includes the countries in which the breeding of true winter-type durum wheat varieties is mainly taking place. However, it can be concluded from the figure that some of the samples from these countries (BGR, HUN, RUS, SVK) were ahead of the Italian varieties. This also means that, in terms of their technological quality, the real winter durum wheat varieties are now competitive with the facultative varieties based on their Minolta b* value.

Based on a detailed examination of the data, it can be concluded that older, genuine winter-type durum wheat varieties are primarily characterized by weaker yield (Figure 2), which can be assumed to be because improving yield strength during the selection of these varieties was not yet among the priorities of the breeding programs. The primary goal of breeders was to improve adaptability and, within that, cold tolerance, in addition, productivity had to be raised to an acceptable level, since winter durum wheat competes with winter wheat varieties in their growing areas (Szunics, 1986; Dorofejev et
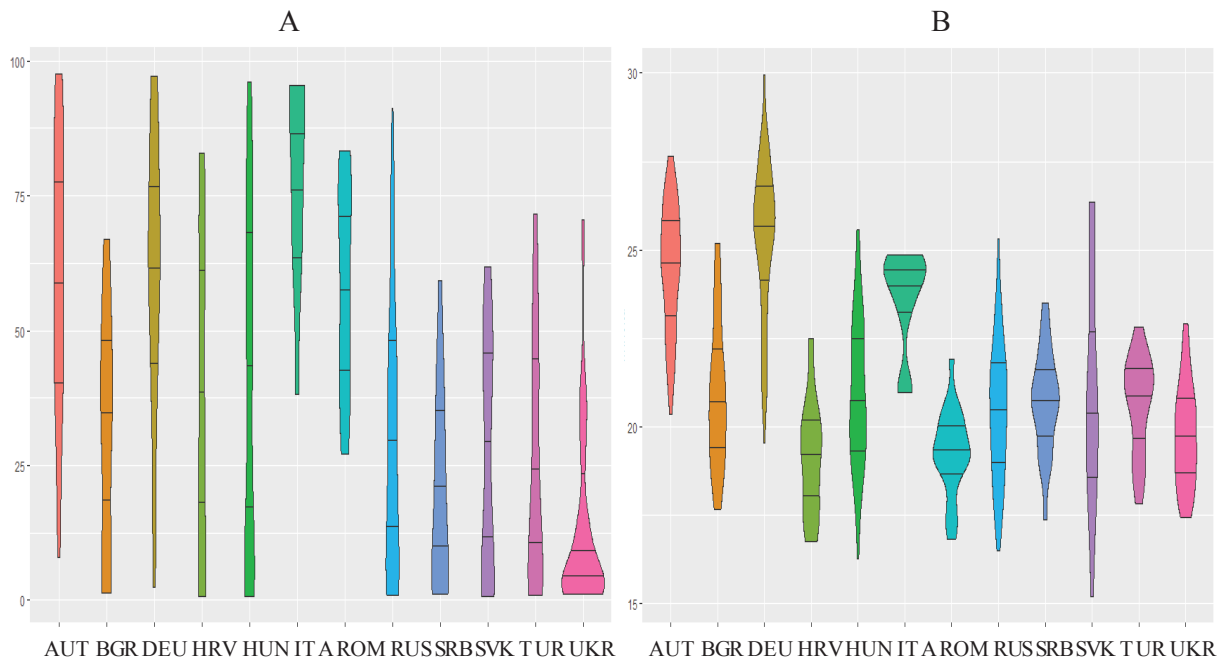
Figure 1. Gluten index (A) and Minolta b* value (B) of durum wheat varieties grouped by country (Martonvásár, 2014–2018)

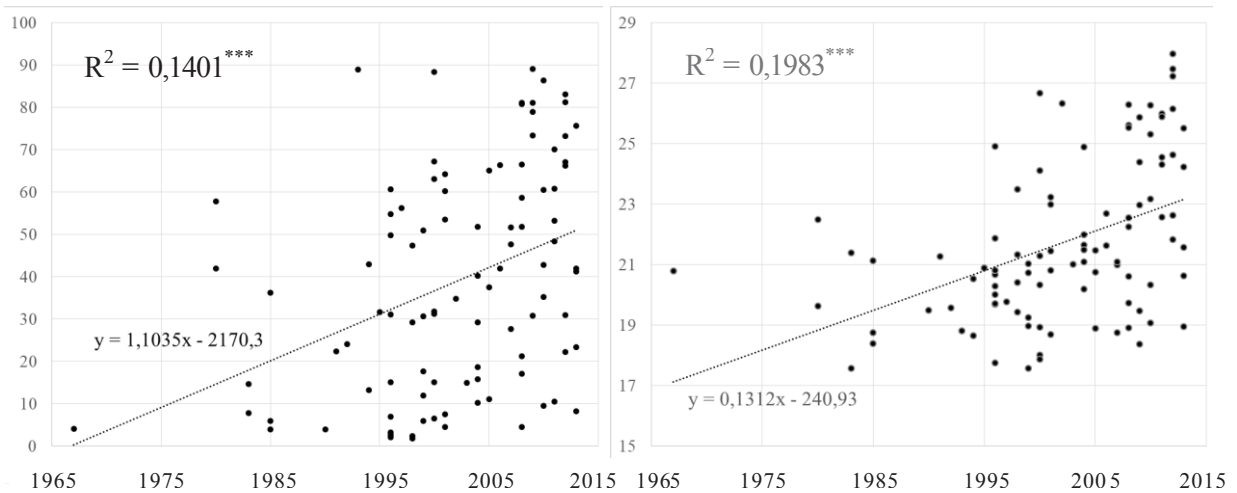Notes: The abbreviations of the countries are in Table 4. The horizontal black lines are the quartiles.

In panel A: $R^2 = 0{,}1401^{***}$; $y = 1{,}1035x - 2170{,}3$

In panel B: $R^2 = 0{,}1983^{***}$; $y = 0{,}1312x - 240{,}93$

Figure 2. Gluten index and Minolta b* values of durum wheat genotypes based on their year of registration (Martonvásár, 2014–2018)

al., 1987). Once this quest was completed, the improvement of technological quality characteristics could begin.

Among the durum wheat varieties included in the experiment, further tests were carried out on 50 genotypes with biochemical/molecular markers linked to the gluten strength or the yellow pigment content. Some of the markers were identified in our own earlier experiments, while others were selected based on results published by various research groups. After the detection of the polymorphism, the difference between the groups carrying different types of markers was analyzed with *t*-test in the case of two variants, in the case of three vari-

ants – after checking the equality of the variances– with the Kruskal-Wallis test (in the case of different variances) or analysis of variance (in the case of the same variances). Among the biochemical markers suitable for estimating the strength of the stem, the effect of γ-gliadin subunits 42 and 45 was already demonstrated in spring durum wheat in the 1970s (Damidaux et al., 1978). Subunit 45 was detected in the vast majority of winter and facultative durum wheat varieties. Of the 50 genotypes, 39 carried this subunit and subunit 42 was present in only 4. Less common spare protein subunits were also identified in some genotypes (51 and 55), however, the gluten index of the genotypes carrying these and subunit 45 did not differ based on the result of the Kruskal-Wallis test ($\chi^2 = 0.548^{ns}$). The "classic" 42/45 data pair in the winter and facultative pool was analyzed with t-test (Table 5).

Based on the results of the t-test, the two groups differed significantly. The average gluten index of durum wheat varieties carrying subunit 42 was 4.149, and in those with γ-gliadin subunit 45, this value was 44.878. However, the minimum value of 2.668 in group 45 draws attention to the fact that the gluten index of durum wheat is determined significantly, but not exclusively by the gliadin subunits 42 and 45 (basically the low molecular weight glutenin subunits LMW-1 and LMW-2 linked to them; Payne et al. al., 1984 and Pogna et al., 1988).

Molecular (DNA) markers were used to investigate the genetic background of the differences observed in the Minolta b* value. Table 6 contains the statistical characteristics of the groups with different patterns.

A statistically verifiable correlation with the Minolta b* value was demonstrated for several of the molecular markers included in the study. However, this finding is true only for the three markers previously identified by our research group. The durum wheat cultivars carrying OPA160, OPT16900+1500 or OPZ900 exceeded the group carrying the alternative allele by an average of 1.919, 1.715 and 2.145 Minolta b* values.

Most of the published markers were suitable for detecting the alleles of phytoene synthase loci on chromosome 7 (*Psy1*), and one on chromosome 5B (*Psy2*). However, based on our own results, it was not possible to separate varieties into high and low yellow pigment content groups. This can be attributed to two reasons. We could not detect polymorphism with the Psy-A1 marker. The marker we linked to the loci encoding phytoene synthase on chromosome arms 7AL. Phytoene synthase plays a prominent role in the formation of the amount of yellow pigment, as it converts geranylgeranyl pyrophosphate to prephytoene diphosphate, than to phytoene in the first steps of lutein synthesis (Kanehisha et al., 2012). The effect of the genes on chromosome group 7 has been proven by several groups (Elouafi et al., 2001; Cervigni et al., 2005; Pozniak et al., 2007; Patil et al., 2008; Zhang et al., 2008; Alsaleh, 2011; Blanco et al., 2011; Roncallo et al., 2012; Giraldo et al., 2016; Fiedler et al., 2017), however, the research confirming this was all conducted on spring durum wheat genotypes.

Testing the gwm344 microsatellite, four markers and two versions of each were identified. Based on durum wheat consensus map of Maccaferri et al. (2014), the marker is located on chromosome 7B right next to the Psy-B1-7B locus, so it could most likely be inherited with the gene encoding this phytoene synthase enzyme. However, its specific nature is questioned by the fact that its association with other traits was also proven in tetraploid wheat species. In durum wheat, Herrera-Foessel et al. (2008) with the leaf rust resistance gene Lr14a, Letta et al. (2014) with stem rust resistance, Ji et al. (2008) showed its connection with powdery mildew resistance in wild stock.

Table 5. Gluten indices of durum wheat varieties carrying γ-gliadin subunits 42 or 45 (Martonvásár, 2014–2018)

γ-gliadin subunit	No. of varieties	Mean	Minimum	Maximum	Standard deviation	Equality of variances[1]	t-value[2]	
42	4	4.149	1.772	6.986	2.209	12.097***	8.955	***
45	39	44.878	2.668	89.161	27.552			

Notes: [1]Levene test F-value; [2]unequal variances were taken into account.

Table 6. Characterization of groups divided by markers linked to yellow pigment content (Martonvásár, 2014–2018)

Alleles	Locus	No. of varieties	Mean	Minimum	Maximum	sd	Equality of variances[1]	t- or F-value[2]
[3]$OPA16_0$		9	23.252	21.871	24.891	1.122	5.204*	2.157 *
[3]$OPA16_{800}$		41	21.333	17.580	26.667	2.601		
[3]$OPK02_0$		18	22.084	18.700	26.295	0.504	1.802ns	0.852 ns
[3]$OPK02_{500}$		32	21.450	17.580	26.667	0.477		
[3]$OPT16_{1500}$		23	20.752	17.580	24.627	0.399	5.990*	2.614 *
[3]$OPT16_{900+1500}$		27	22.467	18.700	26.667	0.521		
[3]$OPZ17_{300}$		31	20.863	17.580	25882	1.951	3.42ns	3.202 **
[3]$OPZ17_{900}$		19	23.008	18.401	26.667	2.783		
[4]Psy-A1	7AL	50	Polymorphism couldn't be detected (all of them with *d* allele)					
[5]Psy1-B1_e		0	-	-	-	-		
[5]Psy1-B1_f		49	21.745	17.580	26.340	2.426		
[5]Psy1-B1_g	7BL	1	26.667	-	-	-		
[6]Psy1-B1_n		0	-	-	-	-		
[6]Psy1-B1_o		0	-	-	-	-		
[7]gwm344_1$_{144}$		26	21.233	18.401	26.295	2.290	0.802ns	1.318 ns
[7]gwm344_1$_{146}$		24	22.161	17.580	26.667	2.686		
[7]gwm344_2$_0$		12	21.926	17.580	26.340	3.049	2.090ns	0.389 ns
[7]gwm344_2$_{130}$	7AL or 7BL	38	21.600	17.748	26.667	2.352		
[7]gwm344_1+2$_{144+0}$		7	21.825	17.580	26.340	3.139	1.422ns	0.850 ns
[7]gwm344_1+2$_{146+0}$		5	22.067	18.401	26.295	3.276		
[7]gwm344_1+2$_{144+130}$		21	21.034	18.700	25.882	2.048		
[7]gwm344_1+2$_{146+130}$		17	22.299	17.748	26.667	2.570		

Notes: [1]Levene test F-value; [2]to compare data of one pair t-test (t-value), for multiple data analysis of variance (F-value) were used; [3]markers identified in OTKA K68127 project; [4]He et al. 2009b, [5]He et al. 2009a; Zhang & Dubcovsky, 2008; [7]Elouafi et al., 2001; *,**, ns the value is significant at $p < 0,05$, $< 0,01$ level, or not significant, respectively.

Unfortunately, Elouafi et al. (2001) did not provide a photo of the results of the gel electrophoresis and did not describe the size of the DNA fragments representing the polymorphism, so the linkage between the different alleles we identified with the chromosomal region encoding the phytoene synthase enzyme is uncertain.

The results of the winter durum wheat breeding program in Martonvásár in the field of improving the gluten index and the Minolta b* value

The durum wheat breeding program in Martonvásár was started in 1982 under the leadership of Dr. László Szunics, but until the mid-1990s selection was made solely on the basis of grain size and vitriousness. The instrumental tests began in 1996 with the measurement of the moisture content and the gluten index. Although the first gluten index measurements were already carried out in 1996, it became evident only after the year 2000 that the strength of the gluten is an essential component of the quality of winter durum wheat. The first four years of the tests were enough to realize that in the case of the gluten index, a broad range of genetic variability can be observed in the breeding material, and that this technological quality characteristic

is highly genotype-dependent, so during the breeding process, so it can be effectively used in selection process for a stronger gluten type.

The measurement of the Minolta b* value also began in the mid-1990s in the durum wheat breeding program. Based on the measurements of several research groups in spring durum wheat, the value of the correlation coefficient between the Minolta b* value and the yellow pigment content is in the range of $r = 0.87–0.96$ (Wehrle et al., 1997; Borelli et al., 1999; Humphries et al., 2004; Fratianni et al., 2005; Digesù et al., 2009; Blanco et al., 2011; N'Diaye et al., 2017). However, results of such measurements were not available for winter durum wheat genotypes. We compared the values of the yellow pigment content determined by the spectrophotometric method of the quality testing laboratory of the National Agricultural Certification Institute (currently NÉBiH) in Tordas (thanks to the laboratory manager Zsuzsanna Juhász for the data) with the results of our measurements with the Minolta CR-300 chromameter. In the case of semolina samples ground in our laboratory, we calculated an extremely close ($r = 0.99$) correlation between the two properties (Vida et al., 2002). With this, we proved that the yellow pigment content can also be effectively estimated in winter durum wheat genotypes by measuring the Minolta b* value.

In the years 1996–2020, we determined the gluten index and Minolta b* values of a total of 619 durum wheat breeding lines, in addition to samples of state-registered varieties. These were advanced breeding lines ($F_7–F_{10}$ generation), which included candidates that could potentially be entered or were currently being tested in the state registration experiment. The number of these lines varied between 9 and 31 depending on the year. Since the year affects both technological quality features, the analysis of absolute values has little information content. Two durum wheat varieties have been sown every year since the beginning of the tests, so by using them as checks, the genetic progress can be estimated based on the relative values. One of the check varieties was 'Martondur 1'. This is the first variety of the Martonvásár breeding program, which was registered in 1996. In comparison to domestic durum wheat varieties of a similar age, it was characterized by exceptionally good cold tolerance, but also average gluten strength and low yellow pigment content. The other winter durum wheat variety used for

comparison, 'GK Bétadur' originated from the Cereal Research Institute in Szeged. The latter variety is still cultivated to this day, and for many years, it was included as a check variety in the state registration experiment. At the time of its recognition, it stood out from its competitors based on both its gluten index and its yellow pigment content.

The percentage of the breeding lines with stronger gluten structure or higher Minolta b* value than that of the two checks is shown in Table 7.

Based on the gluten index data it can be declared that during the 25 years 'Martondur 1' reacted sensitively to changes in environmental factors (range 1.54–78.62), based on its average value (40.28) it belongs to the "promising" category belonged according to Cubbada's classification system (Cubbada et al., 1992). The gluten index of 'GK Bétadur' - although it also varied within a wide range (26.01-98.22) and based on its average value of 61.31, it was classified as "better than average", in five years it was rated as "good", in four as "very good", and in three years as "excellent". Based on the grand mean of all the years, the breeding lines from Martonvásár achieved a value between the two varieties (56.57), however, while the average of the strains in the period up to 2012 was lower than that of the 'GK Bétadur', from 2013 onwards, the mean gluten index was higher every year. Compared to the 'Martondur 1' variety, we achieved a significant improvement in the mean gluten index of the breeding lines. Based on the equation of the trend line, the genetic progress was 9.13%, which means an increase of 3.68 per year in gluten index value. Except for three years (2003, 2010 and 2012), the relative gluten index of the Martonvásár breeding lines was over 100%. 'GK Bétadur' is a variety with a better gluten structure than 'Martondur 1', however, since 2013, the average value of breeding lines has been over 100% every year, but the trend line is less steep. Expressed in numbers, this means an average growth of 4.25% and 2.61 per year. Compared to the average of the two check varieties, the progress is 5.60%, i.e. a gluten index value of 3.08. Based on the data in Table 7, from the year 2013, the vast majority of breeding lines were significantly more successful than 'Martondur 1', and from 2014 (except for 2016), this statement also holds in relation to the variety 'GK Bétadur'.

The Minolta b* value of 'Martondur 1' was stably small in all years, exceeding the value of 22 in only

Table 7. The number and proportion of breeding strains with a significantly higher gluten index or Minolta b* value than the standard varieties 'Martondur 1' and 'GK Bétadur' in the winter durum wheat breeding program in Martonvásár (1996–2020)

Year	Number of lines	*Gluten index*				*Minolta b**			
		Better than Martondur 1		Better than GK Bétadur		Better than Martondur 1		Better than GK Bétadur	
		no.	%	no.	%	no.	%	no.	%
1996	9	4	44.44	0	0.00	5	55.56	0	0.00
1997	15	6	40.00	1	6.67	15	100.00	1	6.67
1998	18	5	27.78	4	22.22	13	72.22	0	0.00
1999	18	3	16.67	0	0.00	14	77.78	0	0.00
2000	14	6	42.86	4	28.57	13	92.86	2	14.29
2001	23	10	43.48	7	30.43	16	69.57	0	0.00
2002	20	5	25.00	2	10.00	20	100.00	0	0.00
2003	19	2	10.53	0	0.00	19	100.00	1	5.26
2004	23	7	30.43	0	0.00	23	100.00	3	13.04
2005	22	14	63.64	7	31.82	22	100.00	6	27.27
2006	28	16	57.14	8	28.57	25	89.29	4	14.29
2007	29	15	51.72	4	13.79	29	100.00	5	17.24
2008	31	17	54.84	0	0.00	31	100.00	11	35.48
2009	31	28	90.32	0	0.00	31	100.00	12	38.71
2010	31	6	19.35	16	51.61	31	100.00	29	93.55
2011	31	29	93.55	9	29.03	31	100.00	24	77.42
2012	29	3	10.34	8	27.59	29	100.00	28	96.55
2013	30	21	70.00	15	50.00	30	100.00	30	100.00
2014	30	26	86.67	23	76.67	30	100.00	17	56.67
2015	30	28	93.33	25	83.33	30	100.00	29	96.67
2016	29	26	89.66	15	51.72	29	100.00	28	96.55
2017	29	27	93.10	27	93.10	29	100.00	29	100.00
2018	27	26	96.30	23	85.19	27	100.00	27	100.00
2019	27	25	92.59	23	85.19	27	100.00	25	92.59
2020	26	26	100.00	21	80.77	26	100.00	26	100.00

two years (2002 and 2004). The yellow pigment content of 'GK Bétadur' exceeded that of 'Martondur 1' in all 25 years, and based on the average value of the 25 years, we measured a 5.08 higher Minolta b* value in its samples than in the case of 'Martondur 1' ('Martondur 1' = 18.53, 'GK Betadur' = 23.61). However, the mean Minolta b* value of the Martonvásár winter durum wheat lines was higher than that of 'GK Bétadur' (24.29). The mean Minolta b* value of the breeding lines approached that of 'GK Bétadur' until 2008, in some years it exceeded it. Starting from 2009, however, we measured a higher average value every year than in the samples of 'GK Bétadur'. In contrast to what was observed for the gluten index the trend line calculated from the relative Minolta b* values is approximately parallel in relation to both standard varieties. Compared to the variety 'Martondur 1', the linear trend line starts above 100% already in the first year, while in the case of 'GK Bétadur' it exceeded 100% only in 2007. The progress compared to 'Martondur 1' is 1.33%, to the variety 'GK Bétadur' 1.52%, and the average of the two varieties is 1.46%. Calculated from the average of the two varieties over 25 years (0.0146 slope of the trend line × 21.07 the average Minolta b* value of the two standard varieties × 25 years =) 7.69 Minolta b* value means an increase in durum wheat breeding lines. In the Minolta b* value, only 6 years were needed after the start of targeted selection for all of the breeding lines (with

the exception of three lines in 2006) to have a statistically higher value than that of 'Martondur 1'. Compared to 'GK Bétadur' – due to the variety's high yellow pigment content – it took much longer to catch up. It was only in 2010 when the Minolta b* value of the majority of the breeding lines exceeded that of the 'GK Bétadur' variety. Since 2015, the proportion of these lines has varied between 92.59 and 100%.

The genetic improvement available to cereal producers can be determined based on the change in the technological quality of the state-registered Martonvásár winter durum wheat varieties (Table 8). It should be noted, however, that based on the gluten index and Minolta b* value of the durum wheat variety registered in the given year, it was selected from lines of better than average quality, but it is not at all certain that it was the best line. In durum wheat breeding, the two technological quality traits analyzed are of outstanding importance, but in addition, a favorable constellation of several other features is necessary for the birth of a successful variety and its spread in cultivation. It is not enough to have an excellent gluten strength and a high yellow pigment content. A successful candidate supposed to be high-yielder, resistant to abiotic and biotic stresses, and even based on other technological quality properties (in the state variety experiments,

the crude protein and wet starch content, semolina yield and vitriousness is also included among the examined traits) must also be better than average. It is difficult to meet all of the listed criteria, so breeding lines with an optimal combination of high yielding and agronomic and technological quality traits are usually submitted to the state registration experiments. The best of these will become registered plant varieties.

The average gluten index of the four durum wheat varieties certified in 1996 was 11.00, which increased to an average of 65.99 in registered varieties released in the second decade of the 21st century. This represents a genetic improvement of 2.638 per year in gluten index. The gluten index of two varieties is exceptionally high. The gluten quality of 'Mv Pennedur' based on the five-year average data is 'Excellent', and 'Mv Pelsodur' variety's is 'Good', but in two years (2014 and 2017) it belonged to the 'Very good' category based on the Cubbada classification system (Cubbada et al., 1992).

After the introduction of selection method based on chromametric measurement in the winter durum wheat varieties of Martonvásár, the Minolta b* value of the varieties was successfully increased. Compared to the value of 19.54 measured in the early varieties (registered in 1996), the Minolta b* value in the varieties released in 2011 and thereafter increased to an average of 24.16. This represents a genetic improvement of 0.189 Minolta b* per year in the breeding program. The newly registered (after 2010) durum wheat varieties all have a Minolta b* value above 23 based on five-year averages, but for 'Mv Hundur' (25.74) and 'Mv Masnidur' (25.40) this value was even higher.

The introduction of increasing the gluten strength can only be traced back to a short history in durum wheat breeding programs. The simple explanation for this is that only a few decades ago, the test methods (SDS sedimentation test, mixograph test and the gluten index) that made it possible to measure this property were incorporated into the selection process (Blum et al., 1987; Cubbada et al., 1992). With the introduction of the measurements, there was a significant improvement in spring durum wheat, for example, the gluten index value in modern Italian varieties more than doubled compared to the landraces and registered varieties collected between 1900 and 1970 (Motzo et al., 2004). According to Longin et al. (2013), the mean gluten index of cold-

Table 8. Gluten index and Minolta b* value of state-registered winter durum wheat varieties from Martonvásár (Mean of 2014–2018)

Variety	Year	Gluten index	Minolta b*
Odmadur 1	1996	2.67	20.68
Odmadur 2	1996	6.99	19.70
Martondur 1	1996	31.08	17.75
Martondur 2	1996	3.27	20.02
Martondur 3	1999	17.74	21.03
Mv Maxidur	2001	64.32	20.82
Mv Makaróni	2001	7.56	23.24
Mv Gyémánt	2004	10.30	20.19
Mv Pennedur	2011	87.35	23.40
Mv Hundur	2011	47.42	25.74
Mv Szuladur	2015	66.47	23.48
Mv Masnidur	2017	58.73	25.40
Mv Pelsodur	2017	73.51	23.20
Mv Vékadur	2019	62.45	23.75

tolerant durum wheat genotypes was somewhat lower than that of non-cold-tolerant ones (51.87 vs. 59.05). The difference in favor of the spring genotypes was also supported by our own experimental results. The mean gluten index of the Italian varieties was 78.80, while the in case of new Martonvásár varieties 65.99 was measured in the period of 2014–2018. years on average. However, the mean gluten index of 'Mv Pennedur' of 87.35 and 'Mv Pelsodur' of 73.51 does not differ from spring durum wheat varieties in terms of gluten quality.

In the case of yellow pigment content, no significant differences can be observed in spring durum wheat genotypes between landraces collected during the first decades of the 20th, older and modern varieties (De Vita et al., 2007). In modern durum wheat lines, Longin et al. (2013) also showed no difference between cold-tolerant and non-cold-tolerant genotypes. In our experiments, the average Minolta b* value of the Italian spring varieties was 23.64, and that of the winter varieties from Martonvásár, registered after 2011, was 24.02, so we did not observe a negative difference in this characteristic either.

Longin et al. (2013) mention in their article the opinion that, according to the breeders working in spring durum wheat breeding programs, the quality of the cold-tolerant varieties is inferior to that of the varieties with a spring seasonal type. As our data prove, this statement is acceptable in the case of real winter durum wheat varieties registered more than 20 years ago. However, the plant breeders working in the winter durum wheat breeding programs – and this was also the case at Martonvásár – made successful efforts to catch up in the field of technological quality. The current winter durum wheat varieties are now competitive with the spring varieties in terms of their gluten index and Minolta b* value.

CONCLUSIONS

We have proven that the gluten index and the Minolta b* value are extremely well heritable genetic traits in winter durum wheat. Valuable genetic resources have been identified that can be utilized in the breeding program. Although the average genetic progress can be estimated based on our results (gluten index = 1.104; Minolta b* = 0.131), however, according to our data, the range is extremely large

for the durum wheat varieties registered a few years ago for both traits examined. This suggests that the improvement of technological quality is still not considered a priority for some of the breeding programs, or that the technical background of the selection has not yet been established.

We investigated the correlation of biochemical and molecular markers in a broad genetic based set of durum wheat cultivars with gluten strength and yellow pigment content. The linkage already known in spring durum wheat with the 42/45 □-gliadin subunit was also proven in the case of winter durum wheat varieties. The linkage between random (RAPD), as well as published microsatellite and gene-specific molecular markers and Minolta b* value, was examined. The correlation with three RAPD markers was proven, but the usability of the published markers is limited in winter durum wheat, due to either the narrow range of alleles or the difference in the genetic background.

We have achieved significant genetic improvement both in terms of the gluten index of Martonvásár durum wheat varieties (2.638/year) and the Minolta b* value (0.189/year) compared to varieties registered in the mid-1990s. The technological quality of Martonvásár durum wheat varieties is now competitive with spring ones, with higher productivity and better adaptability.

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