Grain quality of spring barley genotypes grown at agro-ecological conditions of the Slovak Republic and the Republic of Bulgaria

Kvalita zrna genotypov jarného jačmeňa pestovaných v agroekologických podmienkach Slovenskej republiky a Bulharskej republiky

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ABSTRACT

On two locations (Borovce, Slovak Republic and Karnobat, Republic of Bulgaria) 21 barley genotypes (*Hordeum vulgare* L.) were grown for two consecutive years (2017, 2018). Selected malting qualitative parameters were analysed in mature grains; the content of total starch, crude proteins, and β-D-glucan. The aim of the work was to analyse agro-ecological conditions affecting the content of selected quality parameters in the barley grain and to evaluate, select and exchange barley genotypes created with an added value in order to obtain suitable biological material usable in the changing environmental conditions of a country. The content of total starch in analysed samples was in the range 53.62% and 63.30%, crude proteins were between 11.50% and 15.46% and β-D-glucan was from 3.26% to 5.87%. Year, locality, genotype, and their interactions were factors affecting the content of all analysed parameters in the barley grain, however the content of proteins and β-D-glucan was relatively stable. In Slovak barley genotypes, higher levels of starch and lower levels of proteins and β-D-glucan were observed compared to Bulgarian genotypes. Higher amounts of starch were detected in the conditions of the Slovak Republic. In warmer conditions of the Republic of Bulgaria, higher amounts of proteins and β-D-glucan were observed. Statistically significant correlations ($P \leq 0.05$) were observed between total starch and crude proteins ($r=-0.59$) and between total starch and β-D-glucan ($r=-0.40$). The correlation between the content of proteins and β-D-glucan was positive ($r=0.05$), but in present experiment not statistically significant.

Keywords: barley, *Hordeum vulgare*, grain quality, β-D-glucan, starch, proteins

ABSTRAKT

Na dvoch lokalitách (Borovce, Slovenská republika a Karnobat, Bulharská republika) bolo pestovaných 21 genotypov jačmeňa siateho (*Hordeum vulgare* L.) počas dvoch po sebe nasledujúcich rokov (2017, 2018). Vybrané kvalitatívne parametre boli analyzované v zrelých zrnách: obsah celkového škrobu, proteínov a β-D-glukánu. Cieľom práce bolo analyzovať agroekologické podmienky ovplyvňujúce obsah vybraných parametrov kvality v zrne jačmeňa a vyhodnotiť, selektovať a uskutočniť výmenu genotypov jačmeňa vytvorených s pridanou hodnotou s cieľom získať vhodný biologický materiál použiteľný v meniacich sa podmienkach prostredia. Obsah celkového škrobu v analyzovaných vzorkách bol v rozsahu 53,62 % až 63,30 %, proteíny boli v rozmedzí 11,50 % a 15,46 % a β-D-glukán bol od 3,26 % do 5,87 %.

Rok, lokalita, genotyp a ich interakcie boli faktory ovplyvňujúce obsah všetkých analyzovaných parametrov v zrne jačmeňa, obsah proteínov a β-D-glukánu bol však relatívne stabilný. V slovenských odrodách jačmeňa boli v porovnaní s bulharskými genotypmi pozorované vyššie hladiny škrobu a nižšie hladiny proteínov a β-D-glukánu. V podmienkach Slovenskej republiky boli zistené vyššie obsahy škrobu. V teplejších podmienkach Bulharskej republiky boli pozorované vyššie hladiny proteínov a β-D-glukánu. Štatisticky významné korelácie (P<0,05) boli pozorované medzi celkovým škrobom a proteínmi (r=-0,59) a medzi celkovým škrobom a βP-D-glukánom (r=-0,40). Korelácia medzi obsahom proteínov a β-D-glukánu bola pozitívna (r=0,05), ale v našom experimente nebola štatisticky významná.

Kľúčové slová: jačmeň, *Hordeum vulgare*, kvalita zrna, β-D-glukán, škrob, proteíny

INTRODUCTION

Barley is one of the world's oldest and most versatile cereal crops. It ranks fifth among crops in grain production in the world after maize, wheat, rice, and soybean (Soleymani and Shahrajabian, 2011). The long history and wide spread of barley cultivation are responsible for a broad range of barley utilization. The adaptability of barley to various growing conditions and the diversity of end uses, malting, feed and food, emphasize the need for broad quality assessment. The desired and required quality characteristics for malting barley have been defined to a much greater extent than those for food and feed barley. The future of barley looks promising with beer consumption expected to continue to rise while health benefits are expected to increase consumers' demand for high β-D-glucan food barley. Barley's potential to grow under adverse conditions should be an asset in dealing with climate change but wild swings in the weather will further emphasize the need for quality assessment (Wrigley et al., 2017).

Barley grain contains starch (61.8%), proteins (13.1%), insoluble fiber (10.8%), moisture (7.55%), soluble fiber (4.85%), pentosan (4.28%), β-D-glucan (4.26%), lipids (2.92%) and ash (1.89%) (Helam et al., 1999). Starch, in the levels between approximately 60-64% (Izydorczyk and Dexter, 2008), is the major energy storage polysaccharide in higher plants and after cellulose, the second most abundant biopolymer on earth (Geigenberg, 2011). Structurally, starch is composed of a mixture of two major glucose polymers, i.e. amylose and amylopectin (Jiang et al., 2011), which represent approximately 98–99% of the dry weight (Tester et al., 2004). In linear amylose, the glucose units are joined through α-(1,4)-glycosidic linkages while amylopectin mainly consists of long chains of α-(1,4)-linked D-glucopyranosyl units with occasional branching α-(1,6)-linkages forming its branched structure (Zhang et al., 2017). The ratio of the two starch polysaccharides depends on the botanical origin of the starch and in general, starches from most food crops contain approximately 70–80% amylopectin and roughly 20–30% amylose (Hofman et al., 2016). The 'waxy' starches contain less than 15% amylose, and its content in 'high' amylose starches is greater than 40% (Tester et al., 2004). Song and Jane (2000) confirmed, that barley starches consist of different proportions of amylose contents (9.1% to 44.7%). Different is also total phosphorus content (0.022% to 0.068%) in barley starch that is mainly from phospholipids (0.36% to 0.97%). The barley starch has also short branch chain lengths. Unlike high-amylose maize starch, high-amylose barley starches have low gelatinization temperatures (Song and Jane, 2000). The content of starch is in the cereal grain influenced by the environment (Brooks et al., 1982; Zorovski et al., 2013).

The amount of proteins in barley mature grain is in the range 9–12% (Holm et al., 2018), where barley suitable for malting should have grain proteins content below 11.5%, as higher proteins content will not only reduce malt extract, but also reduce final beer quality (Wang et al., 2007). Proteins in barley grain can be divided into albumin, globulin, prolamin (hordein), and gluten fractions. The albumin, globulin, and gluten fractions consist predominantly of structural and metabolic proteins, while hordein is the major storage protein (Wang et al., 2007). Proteins content in barley grains is quite variable between environments (Holm et al., 2018), sensitive to the growing conditions like sowing date (Wang et al., 2007) and varying with available soil N level and climatic factors, although there is a distinct response between cultivars (Wang et al., 2007; Soleymani and Shahrajabian, 2011; Wrigley et al., 2017). The sandy soil, enough precipitation and relatively low soil temperature were the most beneficial for protein based quality of the malting barley. Fertilization, especially N and P which influences proteins and nucleic acid synthesis, while K influences protein synthesis are also limiting factors for proteins content and their quality in barley grain (Holm et al., 2018).

The polysaccharide (1→3)(1→4)-β-D-glucan, usually referred to as β-D-glucan, is a component of cell walls of *Gramineae* and a major component of the endospermic cell walls in oats and barley mature grains (Havrlentová and Kraic, 2006; Zorovski et al., 2013). High β-D-glucan content is reported in hull-less cultivars (Havrlentová and Kraic, 2006; Ehrenbergerová et al., 2008) and in cultivars having 100% amylopectin (waxy) starch (Xue et al., 1991). The mean value of β-D-glucan content in cereals is (41.6±0.6) g/kg for spring barley, (34.9±0.8) g/kg for oat, and (4.8±0.2) g/kg for wheat mature grains (Havrlentová and Kraic, 2006). Levels of β-D-glucan in production wort and laboratory wort can be as high as 1,290 mg/L, although 100–300 mg/L is considered a normal range (Jin et al., 2004). Genotype by environment (GxE) interaction for this trait has been reported in barley and oat genotypes (Ames et al., 2006). Güller (2003) has observed the effect of nitrogen and irrigation on barley grain β-D-glucan content. The low input conditions safeguarded the accumulation of dietary fiber functional components, a significant effect of chemical weed control, particularly on β-D-glucan content, was also published (Sgrulleta et al., 2004). The mechanism behind altered content of β-D-glucan was investigated in developing endosperm of barley mutants and all of them were primarily affected in polysaccharide biosynthesis, and hence effects on β-D-glucan are likely to be pleiotropic (Christensen and Scheller, 2012).

Cereals, as well as barley food products have positive effects on human health. Foods prepared from barley are useful for diabetic and high blood pressure patients. It is suggested that barley could be therapeutic diet for diabetic patients, a good diet for kidney patients and the referred diet after convalescence (Ames and Rhymer, 2008). β-D-glucan from barley has also been shown to be hypocholesterolemic (Bourdon et al., 1999) and this effect is related to the soluble nature of this dietary fibre and its ability to raise the viscosity of intestinal contents (Mälkki, 2004). Primarily, the function of β-D-glucan is related to its structure, molecular weight, and interaction with other components. On the other hand, the viscous nature of barley β-D-glucan may cause problems in the brewing process. It contributes considerable viscosity to the mash and may cause slow worth filtration and haze formation in the beer (Gomez et al., 1997).

The main reason to grow barley is the brewing industry, although its application in the food industry is increasing. The improvement of barley grain quality depends on the presence of genetic variation for grain quality traits. Therefore, it is essential to ensure the introgression of novel alleles from wild relatives, exotic germplasm, and elite germplasm from other breeding programs. Hence, the aim of the experiment was to evaluate grain quality of spring barley genotypes developed in Slovak Republic and Republic of Bulgaria grown at two locations in both countries.

MATERIAL AND METHODS

Plant material

Ten barley genotypes (*Hordeum vulgare* L.) of the Bulgarian origin and eleven barley genotypes of the Slovak provenience were used in this experiment. Grains of Bulgarian genotypes originated from the breeding program of the Institute of Agriculture - Karnobat (Republic of Bulgaria). Grains of the Slovak barley genotypes originated from the Gene bank of the Slovak Republic. All the materials were simultaneously grown on two locations (one in the Republic of Bulgaria, one in the Slovak Republic) in two consecutive years until the ripeness. Among barley genotypes, the variety Venera (high-yielding 2-rowed feed barley) and nine advanced breeding lines with high and stable grain yield were used. Eleven Slovak barley genotypes (Donaris, Expres, Ezer, Jubilant, Kompakt, Levan, Ludan, Nadir, Nitran, Sladar, and Sladko) were used in this experiment. All of them are 2-rowed spring barley genotypes of different year of origin. Donaris, Expres, Ludan, Nitran, Sladar and Sladko have excellent malting quality and Ezer, Jubilant, Kompakt, Levan and Nadir are of malting quality. Genotypes Jubilant and Kompakt belong to one of first Slovak malting barley genotypes because of low content of β-D-glucan in malt. Donaris, Ezer, Levan, Ludan, Nadir, Nitran and Sladar contain *mlo* genes in their DNA.

Field conditions

In two consecutive years, 2017 and 2018, the study was carried out in the experimental field of the National Agricultural and Food Centre – Research Institute of Plant Production on the Experimental Station in Borovce (Slovak Republic, 48°58' N, 17°72' E) and in the experimental field of the Institute of Agriculture - Karnobat, Southeast Bulgaria (42°39' N, 26°59' E), concurrently. Borovce is situated in the maize-barley producing area and the experimental fields are located at elevation of 167 m a.s.l. The region is characterized by continental climate with average annual rainfall of 593 mm (from it 358 mm during the vegetation period), and long-term average annual temperature of 9.2 °C (15.5 °C during the vegetation season). The weather conditions in Borovce are presented in the Table 1. The soil characteristics of field plots were as follows: Luvi-Haplic Chernozem. The depth of topsoil layer was 24–28 cm. The fertilizer NPK 15-15-15 was applied in the dose rates 260 kg per hectare. Conventional ploughing to depth 22–25 cm, sowing, fertilization and plant treatments were performed according to the recommendations for the given area (the term of sowing: at least 15th of April). The experimental field of the Institute of Agriculture - Karnobat is situated in the region, which is characterized by the transitional continental climate with average annual rainfall of 588 mm (253 mm during the vegetation period) and long-term average annual temperature of 12.8 °C (14.6 °C during the vegetation season). The weather conditions in 2017 and 2018 for Karnobat are presented in the Table 2. The soil of the experimental field is leached Chernozem, slightly acid (pH=6.2). In both years, the preceding crop of spring barley was a pea and sunflower mix. The experiments were organized in a complete block design with 3 replications on plots of 5 m^2. In the experimental years 2017–2018, 10 Bulgarian and 11 Slovak barley genotypes were sown on both locations.

In 2017 and 2019 the fertilization of barley carried out by the Methodology of plant fertilization and nutrition (Bizík et al., 1998). The methodology come out from the content of nutrient in the soil and from requests of crop to achievement the required yield. In both of years, 25 kg per hectare of phosphorus and 30 kg per hectare of potassium were applied in autumn. Nitrogen in dose 50 kg per hectare was applied in spring before sowing. Machinery for management of small units were used. The small-scale machine Oyord for the sowing of barley was used. The small-scale combine harvester Wintersteiger was used for the harvest of barley. In 2017 the herbicide Mustang Forte (substance active: 2.4 D 271 g/l; florasulam 5.0 g/l; aminopyralid 10 g/l) in dose 0.8 liter per hectare and Lontrel 300 (substance active: clopyralid 300 g/l) in dose 0.3 liter per hectare were used. The fungicide Falcon 460 EC (substance active: tebuconazole 167 g/l; triadimenol 43 g/l; spiroxamine 250 g/l) in dose 0.6 liter per hectare was applied. The protection against to pest *Oulema melanopa* was carried out by the insecticide Bulldock 25 EC (substance active: beta-cyfluthrin 25.8 g/l) in dose 0.3 liter per hectare. In 2018 the fungicide Archer Turbo (substance active: fenpropidin 450 g/l; propiconazole 125 g/l) in dose 0.8 l per hectare was used. The protection against to pest *Oulema melanopa* was carried out by the insecticide Decis EW 50 (substance active: deltamethrin 50 g/l) in dose 0.15 liter per hectare. In Karnobat, in both years, sowing and harvesting of barley was done by hand. Nitrogen in the dose 60 kg per hectare was applied in spring before sowing. No pesticides were used during growing season of barley.

Table 1. Weather conditions in the years 2017–2018 in Borovce

Month	n (1931-2018)		2017		2018	
	x_{td}	∑	x_{td}	∑	x_{td}	∑
	[C°]	[mm]	[C°]	[mm]	[C°]	[mm]
January	-1.8	32.0	-8.6	19.8	0.3	30.0
February	0.2	33.2	-0.5	16.0	-3.7	20.8
March	4.2	32.0	5.5	20.1	0.4	32.0
April	9.4	43.1	7.5	42.8	13.6	17.5
May	14.1	53.9	14.8	30.4	17.8	27.2
June	17.7	80.3	20.4	23.9	19.6	67.3
July	18.9	76.0	20.3	28.6	21.1	22.0
August	18.4	67.9	20.9	38.9	22.2	31.8
September	14.5	38.8	12.5	75.9	14.9	126.5
October	9.6	42.1	8.1	55.0	10.0	18.0
November	4.6	51.0	2.3	53.8	4.2	17.6
December	0.3	46.0	-1.0	52.6	-1.5	64.5
x_{td} [°C]	9.2		8.5		9.9	
$∑_z$ [mm]		596.3		458.0		475.0

n – long-term average air temperature and sum of precipitation; x_{td} – average air temperature; ∑ – sum of precipitation

Table 2. Weather conditions in the years 2017–2018 in Karnobat

Month	n (1931-2018)		2017		2018	
	x_{td}	∑	x_{td}	∑	x_{td}	∑
	[C°]	[mm]	[C°]	[mm]	[C°]	[mm]
January	0.6	36.5	-2.5	28.9	2.5	49.0
February	2.2	35.8	3.7	32.9	3.5	81.1
March	5.3	34.1	8.3	24.1	6.4	121.2
April	10.5	45.3	10.0	35.4	19.3	6.0
May	15.6	58.5	16.1	36.6	17.9	68.6
June	19.6	65.2	21.7	55.0	20.8	98.6
July	22.0	49.9	23.4	40.7	23.1	134.0
August	21.6	33.7	23.9	21.3	23.6	0
September	17.6	40.8	19.9	32.8	18.7	29.7
October	12.5	44.3	12.1	67.0	12.5	270.0
November	7.1	53.7	7.5	36.9	8.4	38.8
December	2.6	51.2	0.6	5.7	5.2	93.1
x_{td} [°C]	11.4		12.1		13.5	
$∑_z$ [mm]		549.0		417.3		990.1

n – long-term average air temperature and sum of precipitation; x_{td} – average air temperature; ∑ – sum of precipitation

Sowing of samples was on both localities in both years in March (in Karnobat in the beginning of March, till the 10th; in Borovce in the second half of the month). The heading dates of barley genotypes were between the 22nd and the 30th of May and the grain filling was in June. Harvesting of all materials was on both localities in both analysed years in July (in Karnobat in the middle of the July, in Borovce in the end of the month).

Sample preparation

Mature and dry grains of all barley genotypes were milled to pass a 0.5 mm screen using Ultracentrifugal Mill (ZM 100, Retsch GmbH&Co.KG, Haan/Germany). In the form of flour, they were kept dry in a closed plastic boxes in a storage room by the temperature 20 °C, humidity 68% and permanent darkness. Immediately in the day of analyses, the dry mass was measured using the automatic analyser Sartorius MA 45 (Sartorius, Germany).

Determination of starch content

The starch content was determined by Ewers polarimetric method (STN EN ISO 10520; 1997) based on the partial acid hydrolysis of starch followed by measurement of the optical rotation of the resulting solution. The optical rotations of all samples were measured at 20 °C by using a sample cell of 200 mm optical path length. The content of starch was then recalculated for the dry weight of the sample.

Determination of proteins content

Proteins determination was performed on a TruMac CNS analyser (LECO Corporation, St. Joseph, MI, USA) operating on the Dumas method. This method (AACC 46-30.01, AOAC 992.23, ICC 167) is based on the combustion of nitrogenous components of a homogenized plant sample at 1100 °C and in the presence of oxygen and helium, reducing the resulting nitrogen oxides by adding copper to nitrogen gas and thermal conductometry. The nitrogen level was then translated using the transformation factor (5.7) as the desired proteins content (McAuley and McLean, 1998) and calculated on the dry matter.

Determination of β-D-glucan content

The β-D-glucan content was determined using Mixed-linkage Beta-glucan assay procedure (Megazyme, Ireland). This method is based on the method published by McCleary and Codd (1991) and accepted by the AOAC (Method 995.16) and the AACC (Method 32-23). Samples were suspended and dissolved in a 0.02 M sodium phosphate buffer (pH 6.5), incubated with purified lichenase enzyme, and an aliquot of filtrate was reacted with purified β-glucosidase enzyme. The glucose product was assayed using an oxidase/peroxidase reagent. The polysaccharide's evaluation was calculated on a dry-weight basis.

Statistical determination

All measured data were statistically evaluated by analysis of variance using Statgraphics Centurion X64.

RESULTS AND DISCUSSION

Ten barley genotypes of Bulgarian origin and eleven barley genotypes of the Slovak provenience were analysed for selected quality traits in this study. All the materials were grown on two localities (Slovak Republic and Republic of Bulgaria) in two consecutive years (Table 3 for the year 2017, Table 4 for 2018).

The agronomical importance of cereal grains is based on their accumulation of storage products, mainly starch and proteins. Generally, barley grains consist of up to 65% of starch, with an approximate range 60–64% (Izydorczyk and Dexter, 2008). It has also been reported that barley starches from different genotypes vary in chemical compositions and properties (Tester, 1997). The content of total starch in analysed samples was in the range 53.62% and 63.30%, with an average 59.63%. Holtekjølen et al. (2006) reported from 51.31% to 64.20% variation in starch content of barley genotypes of different origin. Similar values for starch content in barley grain to those found in this study were reported from Helam et al. (1999) and Izydorczyk and Dexter (2008). High variability in the starch content observed in present study indicates an influence of many factors affecting this quality trait in the grain. The content of total starch was significantly (P≤0.05) influenced by year, locality, genotype, and their interactions, respectively (Table 5). The content of starch is in the cereal grain influenced by the environment (Zorovski et al., 2013) and especially stress conditions can increase the amount of the dry matter in the grain as well as the level of starch as a storage material (Brooks et al., 1982).

Crude proteins were in analysed samples between 11.50% and 15.46%, where the average amount was 13.54% (Table 3, Table 4). The level of proteins was in analysed grains rather balanced what shows relatively stable biosynthesis of this metabolite in the grain and importance of proteins during plant development. The amount of proteins in barley mature grain is in the literature described as 13.1% (Helam et al., 1999) or in the range 9–12% (Holm et al., 2018), where barley genotypes suitable for malting should have grain proteins content below 11.5% (Wang et al., 2007). Obtained results are similar to those described in the literature, however small differences can be caused by the influence of genotype and the growing conditions (Wang et al.,

Table 3. Content of selected qualitative parameters of the barley grains grown on two localities in the year 2017

Cultivar	Growing locality	Slovak Republic (SVK)			Republic of Bulgaria (BGR)		
	Origin	Total starch content [%]	Crude proteins content [%]	β-D-glucan content [%]	Total starch content [%]	Crude proteins content [%]	β-D-glucan content [%]
Venera	BGR	62.05 ± 0.49	13.45 ± 0.07	4.18 ± 0.31	58.65 ± 0.66	13.45 ± 0.07	4.25 ± 0.15
KT 338	BGR	61.00 ± 0.14	13.30 ± 0.00	4.56 ± 0.26	59.37 ± 0.00	13.95 ± 0.07	4.54 ± 0.07
KT 339	BGR	61.80 ± 0.00	12.60 ± 0.14	4.73 ± 0.07	57.24 ± 0.12	13.50 ± 0.00	5.43 ± 0.03
KT 340	BGR	62.75 ± 0.07	12.95 ± 0.07	4.00 ± 0.08	58.89 ± 0.00	13.00 ± 0.00	4.97 ± 0.16
KT 341	BGR	61.80 ± 0.00	13.00 ± 0.00	4.21 ± 0.13	57.89 ± 0.07	13.80 ± 0.00	4.46 ± 0.14
KT 1247	BGR	60.80 ± 0.00	12.33 ± 0.04	5.19 ± 0.13	55.74 ± 0.03	13.80 ± 0.00	5.47 ± 0.00
4511M-16	BGR	60.10 ± 0.00	13.10 ± 0.14	5.41 ± 0.28	56.34 ± 0.00	13.75 ± 0.07	5.85 ± 0.01
4580M-2	BGR	60.35 ± 0.07	13.45 ± 0.07	5.60 ± 0.35	57.64 ± 0.00	13.90 ± 0.00	5.47 ± 0.02
4580M-8	BGR	60.35 ± 0.07	13.30 ± 0.00	4.93 ± 0.04	59.48 ± 0.00	14.20 ± 0.00	5.21 ± 0.23
4620M-7	BGR	59.70 ± 0.00	13.40 ± 0.00	5.52 ± 0.07	59.74 ± 0.05	14.80 ± 0.00	5.47 ± 0.17
Donaris	SVK	63.40 ± 0.00	11.60 ± 0.00	4.96 ± 0.19	62.32 ± 0.00	13.70 ± 0.00	4.72 ± 0.01
Expres	SVK	62.00 ± 0.00	12.00 ± 0.14	4.58 ± 0.45	59.17 ± 0.00	13.90 ± 0.00	4.18 ± 0.23
Ezer	SVK	62.85 ± 0.07	11.60 ± 0.00	4.58 ± 0.23	60.83 ± 0.24	13.40 ± 0.00	4.19 ± 0.04
Jubilant	SVK	62.60 ± 0.28	11.45 ± 0.07	4.65 ± 0.11	59.80 ± 0.45	12.70 ± 0.00	4.06 ± 0.09
Kompakt	SVK	62.00 ± 0.00	12.20 ± 0.00	4.45 ± 0.01	59.50 ± 0.04	13.40 ± 0.00	4.02 ± 0.11
Levan	SVK	63.30 ± 0.14	12.20 ± 0.00	4.20 ± 0.20	62.24 ± 0.00	12.95 ± 0.07	4.15 ± 0.01
Ludan	SVK	61.30 ± 0.00	11.85 ± 0.07	5.50 ± 0.41	61.23 ± 0.03	13.50 ± 0.00	5.63 ± 0.48
Nadir	SVK	62.40 ± 0.00	11.50 ± 0.00	5.06 ± 0.15	62.07 ± 0.00	12.40 ± 0.00	4.42 ± 0.10
Nitran	SVK	62.70 ± 0.00	11.65 ± 0.07	4.35 ± 0.19	61.32 ± 0.00	13.05 ± 0.07	4.12 ± 0.10
Sladar	SVK	62.20 ± 0.28	11.50 ± 0.00	4.63 ± 0.27	56.94 ± 0.00	13.30 ± 0.00	5.47 ± 0.01
Sladko	SVK	62.55 ± 0.07	11.60 ± 0.14	4.82 ± 0.18	58.76 ± 0.00	12.85 ± 0.07	4.90 ± 0.06

Table 4. Content of selected qualitative parameters of the barley grains grown on two localities in the year 2018

Cultivar	Growing locality	Slovak Republic (SVK)			Republic of Bulgaria (BGR)		
	Origin	Total starch content [%]	Crude proteins content [%]	β-D-glucan content [%]	Total starch content [%]	Crude proteins content [%]	β-D-glucan content [%]
Venera	BGR	60.57 ± 0.00	15.01 ± 0.13	4.61 ± 0.04	55.87 ± 0.49	14.14 ± 0.02	4.36 ± 0.16
KT 338	BGR	59.88 ± 0.18	14.72 ± 0.09	4.65 ± 0.06	53.63 ± 0.25	15.24 ± 0.30	4.76 ± 0.03
KT 339	BGR	60.00 ± 0.00	13.78 ± 0.00	4.93 ± 0.06	53.82 ± 0.07	14.23 ± 0.03	5.30 ± 0.08
KT 340	BGR	62.44 ± 0.01	14.09 ± 0.09	3.74 ± 0.16	55.15 ± 0.00	14.33 ± 0.00	5.07 ± 0.07
KT 341	BGR	60.88 ± 0.34	13.83 ± 0.04	4.08 ± 0.04	57.57 ± 0.00	15.51 ± 0.01	4.57 ± 0.01
KT 1247	BGR	59.28 ± 0.00	13.54 ± 0.02	4.74 ± 0.05	59.03 ± 0.00	14.45 ± 0.06	5.52 ± 0.07
4511M-16	BGR	59.05 ± 0.06	13.81 ± 0.04	4.69 ± 0.04	58.63 ± 0.23	1510 ± 0.03	5.91 ± 0.05
4580M-2	BGR	58.80 ± 0.08	14.17 ± 0.03	4.67 ± 0.06	53.62 ± 0.22	15.02 ± 0.03	5.50 ± 0.01
4580M-8	BGR	59.54 ± 0.00	15.22 ± 0.00	4.24 ± 0.02	57.48 ± 0.00	15.21 ± 0.37	5.32 ± 0.07
4620M-7	BGR	59.29 ± 0.21	13.59 ± 0.14	4.74 ± 0.04	57.31 ± 0.23	15.46 ± 0.33	5.57 ± 0.04
Donaris	SVK	62.03 ± 0.04	13.65 ± 0.01	4.24 ± 0.08	58.49 ± 0.00	13.46 ± 0.08	4.73 ± 0.00
Expres	SVK	60.43 ± 0.00	13.74 ± 0.00	3.95 ± 0.05	56.37 ± 0.11	13.82 ± 0.11	4.29 ± 0.01
Ezer	SVK	62.30 ± 0.25	13.83 ± 0.00	4.05 ± 0.03	58.03 ± 0.00	14.15 ± 0.01	4.21 ± 0.01
Jubilant	SVK	61.80 ± 0.29	13.91 ± 0.00	3.56 ± 0.05	56.98 ± 0.00	13.96 ± 0.00	4.10 ± 0.07
Kompakt	SVK	60.69 ± 0.00	14.94 ± 0.00	3.77 ± 0.04	56.17 ± 0.24	15.19 ± 0.00	4.07 ± 0.06
Levan	SVK	61.53 ± 0.55	13.63 ± 0.00	3.60 ± 0.01	57.72 ± 0.00	14.30 ± 0.37	4.15 ± 0.00
Ludan	SVK	60.52 ± 0.00	13.29 ± 0.00	4.39 ± 0.01	56.93 ± 0.13	12.68 ± 0.00	5.87 ± 0.12
Nadir	SVK	61.44 ± 0.02	14.11 ± 0.00	3.65 ± 0.11	57.83 ± 0.00	12.72 ± 0.37	4.47 ± 0.00
Nitran	SVK	62.73 ± 0.36	12.76 ± 0.03	3.26 ± 0.06	57.61 ± 0.06	14.22 ± 0.04	4.17 ± 0.06
Sladar	SVK	62.07 ± 0.11	13.34 ± 0.02	3.96 ± 0.02	54.58 ± 0.10	14.10 ± 0.00	5.47 ± 0.01
Sladko	SVK	60.94 ± 0.08	14.00 ± 0.02	4.17 ± 0.09	56.49 ± 0.03	13.79 ± 0.32	4.53 ± 0.06

2007; Soleymani and Shahrajabian, 2011; Wrigley et al., 2017). In this experiment, the influences of year, locality, genotype, and their interactions were observed as factors affecting statistically significant (P≤0.05) the content of total proteins in the barley grain (Table 6).

The content of β-D-glucan was in the average 4.65%, where the lowest amount was 3.26% and the highest 5.87% (Table 3, Table 4). Relatively balanced levels in the content of this polysaccharide is seen in this results, where the average standard deviation was only 0.62. Among cereals, the highest content of β-D-glucan is in barley and oat mature grains (Havrlentová and Kraic, 2006). In the literature, the content of this polysaccharide in barley grains is in the range 3.00–4.40% (Kuusela et al., 2004), eventually in the average 4.16% (Havrlentová and Kraic, 2006) or 43.8 g/kg (Izydorczyk et al., 2000). Present study results are in the accordance with the literature, however factors as genotype and environment influence the content of this quality parameter in the grain (Ames et al., 2006; Dickin et al., 2011). The effect of year, locality, and genotype, and their interactions to influence statistically significant (P≤0.05) the content of β-D-glucan was also observed in present experiment (Table 7).

Table 5. Analysis of variance for the content of total starch in analysed barley samples

	d.f.	MS	P	LSD $_{0.05}$
Year [A]	1	143.77300	*	1.85018
Locality [B]	1	464.56100	*	3.325800
Genotype [C]	20	9.27917	*	0.705078
A x B	1	27.56860	*	
A x C	20	2.35233	*	
B x C	20	3.77441	*	
Error	103	0.50556		
Total	167			

d.f. – degrees of freedom, MS – mean squares, P – effect of the factor significant at the level 0.05, LSD $_{0.05}$ - least significant difference at the level α=0.05

Table 6. Analysis of variance for the content of crude proteins in analysed barley samples

	d.f.	MS	P	LSD $_{0.05}$
Year [A]	1	61.05740	*	-1.205710
Locality [B]	1	23.46030	*	-0.747381
Genotype [C]	20	2.384410	*	0.319136
A x B	1	5.47204	*	
A x C	20	0.39573	*	
B x C	20	0.52316	*	
Error	103	0.50556		
Total	167			

d.f. – degrees of freedom, MS – mean squares, P – effect of the factor significant at the level 0.05, LSD $_{0.05}$ - least significant difference at the level α=0.05

Table 7. Analysis of variance for the content of β-D-glucan in analysed barley samples

	d.f.	MS	P	LSD $_{0.05}$
Year [A]	1	3.15429	*	0.274058
Locality [B]	1	5.47204	*	-0.360952
Genotype [C]	20	1.92243	*	0.191012
A x B	1	4.29440	*	
A x C	20	0.11128	*	
B x C	20	0.30347	*	
Error	103	0.03710		
Total	167			

d.f. – degrees of freedom, MS – mean squares, P – effect of the factor significant at the level 0.05, LSD $_{0.05}$ - least significant difference at the level α=0.05

The comparison of the starch content in Bulgarian and Slovak barley samples on both analysed years and on both localities is shown in the Figure 1 for the year 2017 and in the Figure 2 for the year 2018, respectively. According to these results it can be summarizing that the content of starch was higher in Slovak barley genotypes. For Slovak barleys, the content of total starch was in the range from (56.87 ± 0.61)% to (62.39 ± 0.55)%. On the other side, for Bulgarian ones it was between (56.21 ± 1.06)% and (61.07 ± 0.97)%. The differences show that the Slovak mature barley germplasm is richer in starch, a storage polysaccharide in cereal grain. The highest levels of this polysaccharide was observed for both, Slovak and Bulgarian grains in the year 2017 in the locality of the Slovak Republic. In this year, significantly lower temperature was observed in April and May on the locality Borovce (Table 1) and also on the locality Karnobat (Table 2). The lowest levels of starch were detected in grains grown and maturated in Karnobat in the year 2018.

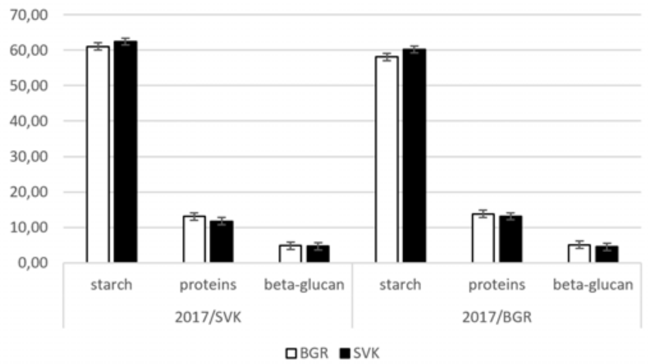

Figure 1. An average content of analysed parameters [%] in barley grains according the plant origin grown on two localities (SVK – Slovak Republic, BGR – Republic of Bulgaria) in the year 2017. BGR – Bulgarian genotypes, SVK – Slovak genotypes. Vertical bars indicate to standard deviation (± SD)

To compare two next analysed qualitative parameters, higher levels of crude proteins and β-D-glucan were detected in grains of Bulgarian origin (Figure 1, Figure 2). In Bulgarian genotypes, the content of proteins was in both analysed years on both localities between 13.09% and 14.87%. For Slovak genotypes it was in the range from 11.76% to 13.89%. Proteins content in barley grains is relatively variable among genotypes (Holm et al., 2018). Environmental conditions like for example available soil N level or climatic factors as well as the sowing date (Wang et al., 2007) can influence its level in barley grain. The highest content of proteins was observed in the year 2018 on the locality Karnobat (BGR) and the lowest, contrarily, was detected in Borovce (SVK) in the year 2017.

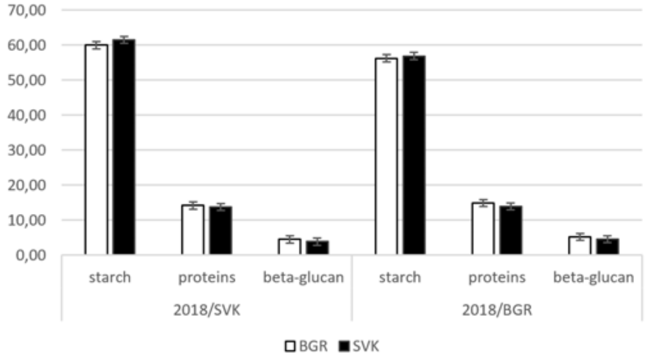

Figure 2. An average content of analysed parameters in [%] in barley grains according the plant origin grown on two localities (SVK – Slovak Republic, BGR – Republic of Bulgaria) in the year 2018. BGR – Bulgarian genotypes, SVK – Slovak genotypes. Vertical bars indicate to standard deviation (± SD)

The content of β-D-glucan was higher in both analysed years and on both growing localities in barley samples of Bulgarian origin, where was this parameter in the range from 4.51% to 5.19%. For Slovak genotypes, the content of β-D-glucan was between 3.83% and 4.68%. Genotype by environment interaction for this trait has been reported in barley and oat genotypes as a significant source of variability (Ames et al., 2006). In Bulgarian genotypes, the highest level of β-D-glucan was observed in the year 2018 and on the locality of Karnobat. For Slovak genotypes, results with higher content of β-D-glucan in the grain were observed in 2017 in Borovce. The lowest levels of this cell wall polysaccharide influencing the malting quality of barley (Gomez et al., 1997) were detected for both, Bulgarian and Slovak barleys in 2018 on the locality Borovce.

To analyse the quality of barley grains between two monitored localities, it is important to say that in the case of total starch content, higher levels were observed in grains grow in the locality Borovce in the Slovak Republic. In the year 2017, the level of total starch was in the average (61.81 ± 1.07)% in Borovce and this was the highest level. In the year 2018, the level of total starch was in Borovce (60.77 ± 1.20)%, whereby in Karnobat it was (59.29 ± 1.91)% and (56.63 ± 1.63)% in 2017 and 2018, respectively (Figure 3). In the Slovak Republic in both analysed years was observed not only higher content of total starch in analysed barley grains of two origins, but also the variability in the content of this parameter was lower, what indicate lower standard deviations in both analysed years. The content of starch is in the cereal grain influenced by the environment and especially stress conditions can increase the amount of the dry matter in the grain as well as the level of starch as a storage material (Brooks et al., 1982). This was shown in our experiment, when the sum of precipitation was in June lower in both analysed years on the locality Borovce compared to Karnobat (Table 1, Table 2). Drought could be a stress factor for growing barleys (Brooks et al., 1982) especially in the period of grain filling, whereby in Borovce the sum of precipitation was in 2017 and 2018 almost in one third lower compared to Karnobat and the temperature in Borovce was higher compared the second locality. The temperature during grain filling is also a key factor for the malting quality (Wallwork et al., 1998; Malik, 2012). The reduction in starch accumulation represent the most significant detrimental effect of high temperature and make the greatest contribution to the reduction in final grain weight (Wallwork et al., 1998). Higher temperature was observed in Karnobat during grain ripening compared to Borovce, so probably the temperature can be another limiting factor influencing higher amount of total starch in Borovce compared to Karnobat. Not only the climate conditions, but also the quality of the soil is different between two analysed localities, Slovakia and Bulgaria, whereby it was reported also by Holm et al. (2018).

The content of crude proteins was higher in the locality of the Republic of Bulgaria, in Karnobat. In the year 2017 it was in the average 13.49% and in 2018 the average level of proteins was 14.34%, what was the highest measured level. In Borovce, the average levels of this quality trait were 12.38% in the year 2017 and 13.95% in the year 2018, respectively. In 2018, the highest levels of this metabolite were observed on both localities (Figure 3).

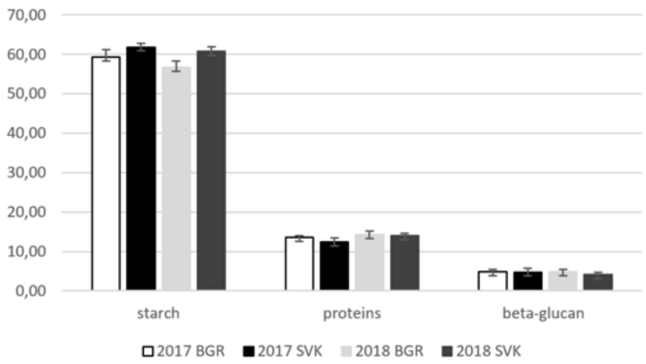

Figure 3. An average content of analysed parameters in [%] in barley grains grown in two consecutive years according the locality (BGR – Republic of Bulgaria, SVK – Slovak Republic). Vertical bars indicate to standard deviation (± SD)

In the literature, the influence of growing conditions on the content of proteins in barley grains is very well described (Brooks et al., 1982; Wang et al., 2007; Soleymani and Shahrajabian, 2011; Wrigley et al., 2017). Grain proteins percentages were increased by both nitrogen fertilization and heat stress (Passarella et al., 2008; Holm et al., 2018) and proteins as a proportion of grain dry weight were higher in stressed plants than in controls, however proteins and amino acid contents per grain were unaffected by water deficit (Brooks et al., 1982). Important is also the timing of the stress factor, which can be crucial (Malik, 2012). The two primary storage proteins fractions in barley grain, hordein and glutelin, were significantly affected by sowing date and N level, but were less influenced by N application time (Wang et al., 2007). The effects of brief periods of high temperature during post-anthesis have been studied to reduce grain weight, yield, and quality of barley (Passarella et al., 2008). In our work, the temperature in months April, May and June was in Karnobat more stable compared to the average temperature in these three months in Borovce. It is possible, that in our experiment the stability in the temperature resulted in higher levels of proteins. Also the sowing date as an important factor influencing the amount of proteins (Wang et al., 2007) was different between Borovce and Karnobat, whereby in Karnobat it was about two week earlier.

Generally, the content of β-D-glucan was higher in the locality of Karnobat compared to Borovce. In the year 2017, the average content of β-D-glucan was in Karnobat in analysed grains (4.81 ± 0.62)% and in the year 2018 it was (4.85 ± 0.63)%. In Borovce, the level of β-D-glucan was (4.77 ± 0.48)% and (4.17 ± 0.47)% in years 2017 and 2018, respectively. Seen from these results is also the fact that in Karnobat not only higher levels of β-D-glucan were detected, but also higher variability in this quality trait represented by higher standard deviations (Figure 3). On the other hand, lower levels of β-D-glucan and also lower variability in the content of this polysaccharide were detected in Borovce. Compared these two localities, the highest average level of β-D-glucan (4.85%) was in the locality of the Republic of Bulgaria in 2018 and the lowest (4.17%) was in the Slovak Republic in 2018. Genotype by environment interaction for this trait has been reported in barley and oat genotypes (Ames et al., 2006; Ehrenbergerová et al., 2008) and also the variability in β-D-glucan concentration can be attributed to year, locality, year x locality, and their interactions with genotype (Hang et al., 2007). Güller (2003) observed the effect of nitrogen and irrigation on barley grain β-D-glucan content and also the low input conditions safeguarded the accumulation of β-D-glucan (Sgrulleta et al., 2004). Malt β-D-glucan was lower in heat-treated grains than in control grains (Wallwork et al., 1998), although the effect of temperature on the β-D-glucan levels is controversial. There is also an observation in the literature, that higher temperature can increase the level of this metabolite (Havrlentová et al., 2016) or mild stress results in higher biosynthesis of β-D-glucan (Hoson, 1998). In the work of Dickin et al. (2011), the β-D-glucan was lower in the wet summer and slightly less in the dry summer and its role as an assimilate buffer add complexity to interpreting the effect of environment during grain filling. Also Ehrenbergerová et al. (2008) reported, that higher precipitation during the flowering time and grain filling period and lower temperatures during the flowering time had negative effects on concentration of β-D-glucan and conversely, drier and warmer weather enhanced the content of this metabolite. The timing of the heat or drought stress is important and drought stress occurring late in the grain-filling period had no effect on β-D-glucan content (MacNicol et al., 1993). With increased grain filling period a decrease in the concentration of this metabolite was observed (Dickin et al., 2011). In our work, the grain filling period was shorter in Karnobat, as well as the harvesting was earlier, so these could result in higher levels of β-D-glucan in Karnobat compared to Borovce. There is a positive correlation between proteins and β-D-glucan content observed (Passerella et al., 2008), so factors affecting the content of proteins can influence also the content of β-D-glucan in barley grain. In our work, the same behavior of proteins and β-D-glucan on two localities in two years was observed, so probably the same factors influencing higher levels of proteins can also influence higher levels of β-D-glucan in Karnobat.

Correlation coefficients among analysed quality parameters in analysed barley samples are described in the Table 8. It is evident, that statistically significant correlations ($P \leq 0.05$) were between total starch and crude proteins and between total starch and β-D-glucan. In both these occurrences, the correlation coefficient was negative (-0.59 for crude proteins and -0.40 for β-D-glucan, respectively). The correlation coefficient between the content of proteins and β-D-glucan was positive ($r=0.05$), but not statistically significant in our experiment.

Table 8. Correlation coefficients among analysed quality parameters in barley samples

	Crude proteins	Total starch	β-D-glucan
Crude proteins		-0.59*	0.05
Total starch			-0.40*

The main goal of the presented project was to evaluate and select barley genotypes with added value for various breeding targets in agro-ecological conditions of two countries, the Slovak Republic and the Republic of Bulgaria in order to obtain a suitable biological material in the form of barley genetic resources useful in changing environmental conditions. It is known and widely accepted, that human activities are projected to lead to substantial increases in temperature that will impact northern Europe during winter and southern Europe during summer. Moreover, it is also expected that these changes will cause increasing water shortages within Europe, which will influence the European agricultural ecosystems. In northern Europe, increases in yield and expansion of climatically suitable areas are expected to dominate, whereas disadvantages from increases in water shortage and extreme weather events (heat, drought, storms) will dominate in southern Europe. Among the adaptation options (i.e. autonomous or planned adaptation strategies) that may be explored to minimize the negative impacts of climate change and to take advantage of positive impacts, changes in crop species, cultivar, sowing date, fertilization, irrigation, drainage, land allocation and farming system seem to be the most appropriate (Bindi and Olesen, 2011). Phenotypic plasticity refers to some of the changes in an organism's behavior, morphology and physiology in response to a unique environment (Price et al., 2003) and is more important for immobile organisms (e.g. plants) than mobile organisms. Phenotypic plasticity in plants includes the timing of transition from vegetative to reproductive growth stage, the allocation of more resources to the roots in soils that contain low concentrations of nutrients, as well as the size and composition of the grains an individual produces depending on the environment (Sultan, 2000) by affecting the photosynthetic and biosynthetic pathways.

The productivity and grain quality of spring malting barley have been found to be affected by cultivar choice and cultivation practices and by the weather conditions during the growing season (Holm et al., 2018). For producing malting barley, it is important to use management practices which support good early growth in order to attain both high grain yield and the target quality traits (Le Bail and Meynard, 2003; Holm et al., 2018). A number of parameters are known to influence grain quality in malting barley including genotype, environmental conditions (year, soil parameters, and temperature) especially during grain filling (Malik, 2012), where enough water during grain filling is a limiting factor (Holm et al., 2018).

CONCLUSIONS

The content of total starch in analysed barley genotypes of the Slovak and Bulgarian origin was in the range 53.62% and 63.30%, crude proteins were between 11.50% and 15.46% and β-D-glucan was from 3.26% to 5.87%. Year, locality, genotype, and their interactions were factors affecting the content of all analysed parameters in barley grain. In Slovak barley genotypes, higher levels of starch were observed compared to Bulgarian ones. Bulgarian genotypes were characterized by higher levels of proteins and β-D-glucan. Higher amounts of starch were detected in the conditions of the Slovak Republic. In warmer conditions of the Republic of Bulgaria, higher amounts of proteins and β-D-glucan were observed. Statistically significant correlations ($P \leq 0.05$) were between total starch and crude proteins and between total starch and β-D-glucan (-0.59 and -0.40, respectively). The correlation coefficient between the content of proteins and β-D-glucan was positive ($r=0.05$), but not statistically significant. Lower sum of precipitation, higher temperature during grain filling, as well as lower temperature during ripening resulted in an increase in starch content in the barley grain. Enhanced levels of proteins were observed when plants were grown under stabile temperature in April, May and June and the sowing was earlier. Shorter grain filling period and earlier sowing caused higher amount of β-D-glucan. Significant effects of environmental conditions and their interactions with the genotype indicate the necessity to assess standard qualities of barley as a raw material.

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