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DIFFERENCES BETWEEN WHEAT CULTIVARS IN GRAIN PARAMETERS RELATED TO ETHANOL PRODUCTION

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SUMMARY

Wheat grain samples from sixteen winter cultivars originated from four localities were evaluated and compared in traits related to ethanol production as grain yield, grain hardness, content of protein, starch and amylose, and α-amylase activity. Results obtained indicate significant differences between cultivars in amylose content, α-amylase activity, and grain hardness compared to grain yield, protein content, and starch content where differences were not significant. The amylose content, α-amylase activity, and grain hardness were affected by cultivar. Both testing methods for starch fermentation - separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) revealed difference between cultivars in ethanol yield.

Key-words: wheat, cultivars, starch, α-amylase, amylase, ethanol

INTRODUCTION

Requirement for replacement of energy and fuels from fossil sources by renewable sources also leads to utilization of domestic and local sources of biomass convertible to biofuel. Moreover former and actual troubles in nuclear energy generation could intensify this trend too. Renewable source for biofuel production in countries of temperate zone is also wheat as crop with relatively simply growing technology commonly used in variable growing conditions. Moreover, less favourable conditions and choice of cultivars with lower bread-making quality prefer higher yields of grains, starch, and finally fermented ethanol (Kindred et al., 2008; Burešová and Hřivna, 2011). Generally, several factors influence ethanol yield from seeds of wheat and other cereal (Rosenberger et al., 2002; Wu et al., 2007) such as content and composition of starch, liquefaction efficiency, final viscosity of starch suspension, high content of fermentable sugars. An ultimate relevance to ethanol production from wheat grains relates to starch content and activity of natural α-amylase. Reduced amylose content enhance starch swelling, efficient water binding, lower gelatinisation temperature (Sasaki et al., 2002), and improve starch conversion to ethanol (Rendleman, 2000; Wu et al., 2006, 2007; Zhao et al., 2009). Other factors as protein content, grain hardness, and grain yield also influence ethanol production from wheat grains (Wu et al., 2006, 2007; Swanston et al., 2007; Kindred et al., 2008, Agu et al., 2009).

The aim of this study was to compare locally adapted wheat cultivars in parameters of grain related to ethanol yield (content of starch, amylose, and proteins, α-amylase activity, grain hardness, grain yield) with expectation that differences within them allow to estimate the most advanced within them.

MATERIAL AND METHODS

The differences between cultivars were studied within the set of 16 registered winter wheat cultivars cultivated in Slovakia at present (Table 1). Analysed grain samples originated from the year 2006 and were harvested in four different locations of different productive regions: Vígľaš-Pstruša (potato growing region, altitude 375 m, average air temperature 7.8 °C, annual precipitation 610 mm), Malý Šariš (potato growing region, altitude 310 m, average air temperature 8.0 °C, annual precipitation 603 mm), Borovce (maize-sugar growing region, altitude 167 m, average air temperature 9.2 °C,

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annual precipitation 595 mm), and Milhostov (maize growing region, altitude 105 m, average air temperature 9.0 °C, annual precipitation 560 mm).

Field trials were set out in four replications by a randomized block design. The phosphorus (50 kg ha^{-1}) and potassium (130 kg ha^{-1}) were used in the pre-sowing, nitrogen (30 kg ha^{-1}) in the post-sowing fertilization. Herbicides were applied according to actual situation in weed occurrence at different locations, whereas fungicide treatment was not applied.

The starch content was measured by the standard Ewers´s polarimetric method (STN 461011-37, ISO 10520:1997), amylose content and α-amylase activity using the Amylose/Amylopectin Assay Kit (Megazyme, Ireland) and Alpha-Amylase Assay Procedure (Megazyme, Ireland), respectively, grain protein content and grain hardness by the near infrared reflectance spectrometry (NIR Systems 6500).

Samples from 11 wheats cultivated in location Vígľaš-Pstruša was assessed for ethanol yield. The starch suspensions (20 %, w/w) were fermented by two approaches: separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The commercial enzyme products Termamyl®SC (termostable α-amylase), Promozyme®D (termostable pullulanase), and AMG 300L (glucoamylase) (all from Novozymes, Denmark) in concentration 0.3, 0.8, and 0.8 ml kg^{-1} of starch and the *Saccharomyces cerevisiae* CCY 11-3 strain were used for starch hydrolysis and fermentation. The dextrose equivalent (DE) indicates degree of starch hydrolysis to glucose syrup, i.e. percentage of total solids converted to reducing sugars (Ballesteros et al., 2004), degree of conversion of glucose to ethanol relates to theoretical yield of ethanol by glucose fermentation.

The statistical software package Statgraphics Plus for Windows (StatPoint Technologies, Inc., USA) was used for correlation analysis and analysis of variance.

RESULTS AND DISCUSSION

Analysis of variance revealed influence of wheat cultivar to some of grain traits associated with the ethanol production as amylose content, α-amylase activity, and grain hardness ($P<0.01$). As for others, especially not to starch content and grain yield effect of cultivar was nonsignificant. The highest average starch content was found in wheat grains cultivated in both locations in the potato production region (Vígľaš-Pstruša and Malý Šariš), the lowest in the maize-sugar beet production region ($P<0.05$). On the contrary, wheat grains from the maize-sugar beet production region had improved parameters related to food and feed quality such as protein content ($P<0.05$) and grain hardness. Several studies reported effects of genotype, environment, and their interaction to wheat grains parameters related to

Table 1. Mean values of grain parameters of wheat cultivars grown in four localities

Tablica 1. Srednje vrijednosti parametara zrna kultivara pšenice na četiri lokacije

Cultivars	Starch (%)	Amylose (% of starch)	α-amylase (U.g^{-1})	Grain protein (%)	Grain hardness (%)	Grain yield (t.ha^{-1})
Torysa (C)	65.03	25.73^{abcd}	156.97^{bcd}	11.96	72.65^{bcd}	7.28
Malvina (C)	63.43	24.77^{defgh}	157.36^{bc}	11.45	63.83^{fgh}	7.22
Malyska (C)	65.28	25.20^{bcdef}	146.84^{de}	11.09	66.83^{defg}	7.17
Markola (B)	65.56	26.40^{a}	148.78^{cde}	11.57	60.76^{gh}	7.10
Pavlina (C)	66.83	26.23^{ab}	142.93^{e}	11.32	57.82^{h}	7.29
Veldava (C)	64.82	25.93^{abc}	177.92^{a}	11.74	74.65^{bc}	6.83
Mladka (C)	64.23	24.62^{efgh}	167.18^{b}	11.56	64.77^{efg}	7.08
Venistar (C)	64.74	26.23^{ab}	149.08^{cde}	11.16	61.91^{gh}	7.36
Mean 1-8	64.99	25.64	155.89	11.48	65.40	7.16
Akteur (E)	64.01	25.93^{abc}	116.09^{f}	11.60	70.23^{cde}	6.77
Vanda (A)	65.15	25.76^{abcd}	118.77^{f}	11.52	65.10^{efg}	6.78
Sulamit (E)	60.59	25.60^{abcde}	119.85^{f}	11.81	78.39^{ab}	6.69
Ilona (A)	63.46	25.02^{cdefg}	114.86^{fg}	11.26	68.60^{cdef}	6.98
Ilias (A)	65.36	24.29^{fgh}	103.70^{g}	11.60	82.01^{a}	7.33
Cubus (A)	63.35	23.74^{h}	112.41^{fg}	11.64	78.70^{ab}	6.96
Barroko (A)	62.67	23.99^{gh}	118.84^{f}	12.05	81.57^{a}	6.95
Petrana (A)	63.67	24.90^{cdefg}	111.89^{fg}	11.59	65.54^{efg}	6.64
Mean 9-16	63.53	24.90	114.67	11.63	73.76	6.89
Mean 1-16	64.26	25.27	135.22	11.56	69.58	7.03
$LSD_{0.05}$ for cultivars	NS	1.075	10.432	NS	6.332	NS
$LSD_{0.05}$ for groups	1.120	0.498	5.430	NS	4.014	NS

The means in columns followed by the same letter are not significantly different from each other at $P=0.05$; NS – not significant ($P>0.05$); Cultivars 1-8 are of poor (C) or complementary quality (B) bread-making quality; 9-16 are of elite (E) or high (A) bread-making quality technological quality (DuPont and Altenbach 2003, Kim et al., 2003, Hudec et al., 2006, Williams et al., 2008) or ethanol yield (Tester and Karkalas 2001, Labuschagne et al., 2007, Massaux et al., 2008).

Eight cultivars officially classified as poor or complementary for bread-making quality (categories C, B) had significantly higher starch and amylose content, and α-amylase activity ($P<0.05$), but lower grain hardness in comparison to other eight cultivars ranked as excellent or good for bread-making quality (E, A) (Table 1). The highest average starch content (66.8 %) in all testing localities had the samples of Pavlina but also others had grain starch content above 65 % (Markola, Ilias, Malyska, Vanda, Torysa). Amylose content ranged from 23.7 % (Cubus) to 26.4 % (Markola). The highest α-amylase activity had the cultivar Veldava (177.9 $U.g^{-1}$), the lowest Ilias (103.7 $U.g^{-1}$). Means of grain hardness ranged from 57.8 % in medium hard (Pavlina) to 82.0 % in hard (Ilias). Both groups of cultivars did not significantly differ in protein content and grain yield (Table 1).

In the second part of the experiment grains from eleven cultivars (Table 2) originated from locality Vígľaš-Pstruša were assessed for efficiency of starch suspension fermentation and ethanol yield, including seven more or less feeding cultivars and four the most cultivated at present time with high technological quality. The location Vígľaš-Pstruša was selected as the coldest and the most humid testing location during vegetation season where wheats could express their potential for ethanol production related traits.

Some of analysed grain parameters in four of them predict higher potential for ethanol production. The cultivar Pavlina was the best in starch content, had high yield, low grain hardness, Veldava was the best in α-amylase activity and had also high starch content, Venistar had high starch content and grain yield, low grain protein and grain hardness, and Ilias had high starch content and grain yield, low amylose. Both approaches applied for ethanol fermentation from grain starch (i.e. SHF and SSF) revealed differences between cultivars (Table 2). Cultivar Pavlina had the highest dextrose equivalent, degree of conversion, and ethanol yield using the SHF method. The second in ethanol production by SHF method were high bread-making quality cultivars Veldava and Illias. The mean ethanol yield and differences between cultivars were higher in SSF method and the best was again the cultivar Pavlina.

Pejin et al. (2009) observed that the highest ethanol yield was in wheat cultivar with the highest autoamylolytical quotient (percentage yield of ethanol obtained without the addition of saccharifying enzymes compared with the ethanol yield with the addition of optimal combination of technical enzymes) and the autoamylolytical quotient related to the Falling number. None of analysed grain parameters in our study had the predominant effect on ethanol production. Nevertheless the ethanol yield can be positively affected by choice of cultivar and growing locality (McLeod et al., 2010). Another factors, as production intensity and growing conditions, also influence wheat grain composition and subsequent ethanol yield (Rosenberger et al., 2001, 2002; Loyce et al., 2002; Swanston et al., 2007; Kindred et al., 2008), moreover biotechnological and molecular breeding approaches will also play role in improvement and modification of wheat starch and ethanol production (Bagå et al., 1999; McLauchlan et al., 2001). Dvořáček et al. (2010) in study of wheat cultivation for starch and ethanol observed significant relationship between starch and ethanol production and genotype on one side and growing locality, and crop management on the other side. Generally the cultivars with higher starch content produced more ethanol. Lower input of fertilizers (nitrogen and plant protection) as well as tillage minimisation increased starch ratio in grain and ethanol yield. The highest starch content and ethanol production were found in cultivars with low baking quality. According to Sedláček (2010) wheat varieties possessing high yields of low protein grains cultivated in localities with higher rainfalls would be ideal for the production of ethanol.

Table 2. Ethanol yield from wheat cultivars grown at the locality Vígľaš-Pstruša

Tablica 2. Prinos etanola iz kultivara pšenice na Vigľaš-Pstruša

Cultivars	Dextrose equivalent (%)		Degree of conversion (%)		Ethanol yield (w/w %)	
	SHF	SSF	SHF	SSF	SHF	SSF
Pavlina (C)	90.67[a]	20.33[abc]	73.67[a]	82.67[a]	8.36[a]	9.41[a]
Veldava (C)	90.67[a]	20.67[ab]	72.67[ab]	74.33[b]	8.22[ab]	8.41[bc]
Mladka (C)	88.67[bc]	19.00[cde]	66.33[e]	69.67[e]	7.89[cd]	7.79[f]
Venistar (C)	88.33[c]	16.33[f]	68.33[d]	74.67[b]	7.75[ef]	8.49[b]
Malvína (C)	90.67[a]	21.67[a]	71.33[bc]	73.67[bc]	8.09[bc]	8.39[c]
Malyska (C)	88.67[bc]	20.00[bcd]	70.67[c]	71.67[d]	8.01[cd]	8.13[e]
Markola (B)	90.33[a]	18.67[de]	64.67[f]	66.00[g]	7.35[h]	7.52[g]
Petrana (A)	89.67[ab]	20.67[ab]	70.67[c]	72.67[cd]	8.10[bc]	8.29[d]
Illias (A)	86.67[d]	20.67[ab]	72.67[ab]	73.67[bc]	8.22[ab]	8.40[bc]
Cubus (A)	88.00[c]	17.67[ef]	64.67[f]	67.33[fg]	7.48[gh]	7.61[g]
Barroko (A)	90.33[a]	19.67[bcd]	66.67[e]	68.67[ef]	7.61[fg]	7.80[f]
Mean	89.33	19.58	69.30	72.27	7.91	8.20
$LSD_{0.05}$	1.332	1.511	1.648	1.546	0.150	0.092

SHF – separate hydrolysis and fermentation; SSF – simultaneous saccharification and fermentation; The means in columns followed by the same letter are not significantly different from each other at $P=0.05$; A – good bread-making quality cultivars; C, B – poor bread-making quality cultivars

CONCLUSION

There were differences between wheat cultivars in grain parameters related to efficiency of ethanol fermentation from grains. Starch content varied from 60.59 to 66.93 %, amylose content 23.74-26.40 %, α-amylase activity 103.7-177.92 $U.g^{-1}$ and differences were also in grain yield, grain hardness, and protein content. These parameters affected ethanol yield measured by both methods (SHF and SSF) nevertheless the cultivar Pavlina generated the highest amount of ethanol by both of them.

Our results show that both the cultivar and environmental conditions in growing location affect content of starch and its characteristics, respectively, i.e. parameters relevant to ethanol production from grains. The cultivar Pavlina could be considered as favourable for ethanol production related traits in agro-climatic conditions of the Slovakia due to the highest starch content, very high amylase content, and low grain hardness, moreover very high average grain yield.

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RAZLIKE MEĐU KULTURAMA PŠENICE U PARAMETRIMA ZRNA U ODNOSU NA PROIZVODNJU ETANOLA

SAŽETAK

Vrijednosti uzoraka zrna pšenice (prinos zrna, tvrdoća, sadržaj proteina, škrob, amilaza i aktivnost a-amilaze) uspoređivali su se u odnosu na proizvodnju etanola. Dobiveni rezultati ukazuju na signifikantne razlike između kultivara u sadržaju amilaze, aktivnosti a-amilaze i tvrdoći zrna u odnosu na prinos zrna, sadržaj proteina i škroba, gdje vrijednosti nisu bile signifikantne. Kultivar je imao utjecaja na sadržaj amilaze, aktivnost a-amilaze i tvrdoću zrna.

Obje metode ispitivanja na fermentaciju škroba – odvojena hidroliza i fermentacija (SHF), kao i istovremena saharizacija i fermentacija (SSF), pokazale su razliku među kultivarima u prinosu etanola.

Ključne riječi: pšenica, kultivari, škrob, a-amilaza, amilaza, etanol

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