Journal of Life Sciences 5 (2011) 207-212

Evaluation of Emmer Wheat Genetic Resources Aimed at Dietary Food Production

Zdeněk Stehno[1], Ivana Paulíčková[2], Jana Bradová[1], Petr Konvalina[3], Ivana Capouchová[4], Eva Mašková[2], Dana Gabrovská[2], Marie Holasová[2], Vlasta Fiedlerová[2], Renáta Winterová[2], Jarmila Ouhrabková[2] and Ladislav Dotlačil[1]

1. Department of Gene Bank, Crop Research Institute, Praha 161 06, Czech Republic

2. Department of Nutritive Substances, Food Research Institute, Praha 102 31, Czech Republic

3. Faculty of Agriculture, University of South Bohemia, České Budějovice 370 05, Czech Republic

4. Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Praha 165 21, Czech Republic

Received: July 28, 2010 / Accepted: October 24, 2010 / Published: March 30, 2011.

Abstract: Emmer wheat cultivated by organic farmers is used as a component of some bio (organic) food products. Its positive influence on consumer health is caused by grain composition. With the aim of selecting varieties or landraces for their possible further use we tested selected emmer wheat accessions maintained in the Czech Gene Bank. In the set of 8 emmer wheat accessions, the main grain components, bread making characteristics and contents of health supporting chemical substances such as total dietary fibre content and its components, content of total polyphenols plus catechin and ferulic acid contents, vitamins of the B group and E plus total content of carotenoids were evaluated by standard methods. Tests of bread making quality confirmed a very well known fact that emmer wheat grain is much more suitable for other purposes as whole grain mixtures, cereal pure, etc. than for bread preparation. The results indicate the possibilities to select emmer wheat genotypes differing in grain composition and containing compounds with positive effects on human health. Among the tested emmer wheat accessions the Rudico variety had a complex of positive characteristics such as content of total dietary fibre, total polyphenol content with prevailing catechin and the highest amount of B group vitamins such as B1, B2, niacin, pantothenic acid and B6.

Key words: Emmer wheat, *Triticum diccocon*, grain quality, HMW glutenin subunits, polyphenols, vitamins, wheat genetic resources.

1. Introduction

Genus *Triticum* L. consists of over 30 different species with large interspecific variability. Some of them such as bread wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.) play an important role in human nutrition. Other species are used for human consumption rarely or not at all. For example, spelta wheat (*T. spelta* L.) and emmer (*T. dicoccon* Schrank) belong to the rarely used, so they were called as minor wheat species.

The number of accessions in wheat collections of genetic resources usually corresponds to crop importance. The wheat collection in the Czech Gene Bank at the Crop Research Institute is comprised of 30 wheat species (passport data accessible at EVIGEZ – The Czech National Database of Plant Genetic Resources [1]). Among 10,691 accessions hexaploid bread, wheat is clearly the prevailing species (86.4%). The sub collection of hulled tetraploid emmer wheat (*T. dicoccon* Schrank) contains 117 landraces and obsolete varieties. In addition to this set of cultivated emmer, there is a group of wild emmer *T. dicoccoides* (Körn. Ex Aschers. Graebn.) Schwienf. containing 26 accessions.

Interest in minor wheat species has been increasing in the Czech Republic (CR) recently as it has in certain European countries like: Austria, Germany, Belgium,

Corresponding author: Zdeněk Stehno, head of the Gene Bank Department, research field: wheat genetic resources collection. E-mail: stehno@vurv.cz.

Italy and others, owing to the developments in and their extending of their organic farming systems. In the CR there are 2 registered varieties of winter spelt and one emmer variety is legally protected.

The varieties of emmer wheat are less demanding and more adaptable [2, 3]. Therefore, they are suitable for low-input or organic farming systems [4-6]. When grown in marginal areas, they provide a lower, but more stable harvest level [7] and they may contribute, as organic products, to a higher economic profit than other conventional varieties of cereals [8].

Increased interest in emmer wheat has stimulated research aiming at evaluation of a pre-selected set of emmer accessions for their food and dietary quality. Emmer wheat cultivated by organic farmers is used as a component of some bio (organic) food products. Its positive influence on consumer health is caused by grain composition. With the aim of selecting varieties or landraces for their possible further use we tested selected emmer wheat accessions which were maintained in the Czech Gene Bank.

2. Materials and Methods

A pre-selected set of 10 emmer wheat accessions is characterized by high molecular weight glutenin subunits (HMW-GSs). The electrophoretic patterns of HMW-GSs were determined by the one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using the Laemmli buffer system [9]. The acrylamide/bisacrylamide concentration (T) and the cross linker (C) used were as follows: T = 10% and C = 2.60%. The particular alleles of HMW-GSs were identified according to a published catalogue [10].

With the aim of evaluating grain quality parameters, a set of 8 emmer landraces including a legally protected variety, Rudico, has been tested in field plot experiments in organic farming for two years. Average grain samples from 3 replications were analysed in the second year of evaluation to assess standard bread making quality characteristics as well as content of dietary valuable compounds. For the analyses, the following methods were used:

(1) Crude protein content: The Kjeldahl Method according to ČSN ISO 1871;

(2) Wet gluten content and Gluten index (GI): on Glutomatic 2200 according to ČSN ISO 5531;

(3) Sedimentation index – The Zeleny Test: according to ČSN ISO 5529;

(4) Total dietary fibre (TDF): The enzyme – gravimetric method according to AOAC 991.42 using the enzyme set Bioquant (Merck), and filter machinery FIBERTEC (Scan Tec);

(5) The total polyphenol content: the sample was extracted with 80% ethanol under a reverse cooler in a temperature of 90 ℃ for 120 min. The extract was filtrated through a folded filter. Ten millilitres of distilled water, 2.5 mL of Folin-Ciocalteu reagent (FCR) and 7.5 mL of 20% bicarbonate dilution were added to an aliquot part of the extract. The content was mixed and filled to 50 mL by adding distilled water. After 2 hrs (max. coloration) the sample was measured spectrophotometrically (wave length λ = 765 nm). The calibrating curve was put together of 0.1-0.4 $g \cdot l^{-1}$ concentrations of Gallic acid. The results are presented as Gallic acid equivalents (GAE) [$g\ GAE \cdot kg^{-1}$] [11];

(6) The content of selected phenolic substances (catechin, epicatechin, ferulic acid, chlorogenic acid): RP – HPLC method – Summit system (DIONEX);

(7) Vitamin B1 (thiamine): isolation by acid and enzyme hydrolysis, oxidation of thiamine by potassium hexacyanoferrate in an alkalic environment to thiochrom, The RP – HPLC method;

(8) The Vitamin B2 (riboflavin) lumiflavin method according to ČSN 65 0054: isolation by acid and enzyme hydrolysis, transformation of riboflavin to lumiflavin, fluorescence method λex -290 nm – λem -330 nm;

(9) The Niacin – *Lactobacillus plantarum* microbiological method ATCC 8014, ČSN 56 0051;

(10) The Pantothenic acid - *Lactobacillus plantarum* modified microbiological method ATCC 8014, ČSN

56 0051;

(11) The Vitamin B6 – *Saccharomyces uvarum* microbiological method ATCC 9080, ČSN 56 056;

(12) Vitamin E - sample saponification and extraction of unsaponifiable parts, HPLC method;

(13) Total carotenoids - sample saponification and extraction of unsaponifiable parts, spectrophotometric method, calculation based on absorbance of hexanol dilution under 445 nm using the extinction coefficient for β-carotene.

3. Results and Discussion

3.1 Polymorphism of HMW Glutenin Subunits

Selected emmer wheat accessions differed considerably in the polymorphisms of HMW-GSs. Out of the total of 10 studied emmer wheat landraces, 5 accessions [*T. dicoccon* (Ruzyně), Kahler Emmer, May-Emmer, Weisser Sommer and *T. dicoccon* (Tapioszele)] appeared to be homogeneous in the electrophoretic patterns of HMW-GSs; they were formed by a single glutenin line (Table 1).

3.2 Standard Bread Making Quality Characteristics Plus Content of Dietary Valuable Compounds

3.2.1 Crude Protein Content

All tested emmer accessions exceeded the check bread wheat variety Vlasta in crude protein and fat contents. The highest crude protein content (14.74%), measured by the Kjeldahl method was detected in the legally protected emmer variety Rudico. In total this parameter ranged from 14.39% to 11.99% (Table 2). A high content of crude protein in emmer wheat is described in Refs. [12, 13].

Also in the category of fat content, all emmer accessions (3.77-4.40%) overcame the Vlasta variety (by 2.76%). Fibre content was relatively variable with the check bread wheat variety nearly in the middle of the range of this parameter. The content of carbohydrates reflects reciprocally crude protein content.

Table 1 HMW Glutenin markers of emmer landraces and obsolete cultivars.

T. dicoccon landrace	Stat of origin	*Glu* lines	*HMW-GSs*+		
			Glu-A1	*Glu-B1*	*Glu-1D*
T. dicoccon (Kroměříž)	(CZE)	A	1	7+8	-
		B	1	21	-
		C	1	6+7	-
T. dicoccon (Ruzyně)	(CZE)	A	1	7+8	-
Kahler Emmer	GER	A	1	7+8	-
May-Emmer	CHE	A	1	7+8	-
Weisser Sommer	GER	A	1	(7+8)	-
T. dicoccon (Tapioszele)	(HUN)	A	1	7+8	-
Krajova-Podbranc (Toman)	CSK	A	2*	21	-
		B	2*	6+9	-
Poering Jaarma (Nachitchevan.)	AZE	A	1	22	-
		B	1	7+8	-
		C	0	7+8	-
		D	2*	7+8	-
T. dicoccon (Balkan)	(GER)	A	2*	14+15	-
		B	0	14+15	-
T. dicoccon (Brno)	CSK	A	2*	6+8	-
		B	2*	21	-
Sandra (*T. aestivum*) - control	CSK	A	2*	7+8	3+12
		B	2*	7+9	3+12
		C	1	7+9	3+12

+) alleles of HMW-GSs identified 0, 1, 2*, etc. according to catalogue (Payne & Lawrence 1983) [11].

3.2.2 Wet Gluten Content and Gluten Index

The quality of wet gluten, characterised by gluten index (GI) which is evaluated by Glutomatic 2200, was very low in all emmer accessions (Table 3). Gluten of 3 landraces - *T. dicoccon* (Dagestan ASSR); *T. dicoccon* (Palestine) and *T. dicoccon* (Brno) – was completely flowable.

3.2.3 Sedimentation Index – The Zeleny Test

Zeleny Sedimentation, an important parameter of bread making quality, was very low and ranged from 6 mL [*T. dicoccon* (Dagestan ASSR)] to 19 mL (Rudico). Based on such results, the emmer wheat landraces can be considered potentially more suitable for other purposes than for the preparation of bread (*e.g.* for different grain mixtures, pure, etc.). The emmer wheat may be used in pasta production, as the grains contain the necessary weak gluten and high level of protein [13].

Table 2 Basic emmer grain components in comparison to bread wheat variety Vlasta.

Genotype / variety	Dry matter %	Crude protein content %	Fat content %	Fibre %	Carbohydrates %	Crude ash %
Rudico	89.78	14.74	4.00	13.89	55.30	1.85
May-Emmer	89.23	13.35	3.77	9.18	61.03	1.90
Weisser Sommer	89.12	12.15	3.81	8.77	62.51	1.88
T. dicoccon (Dagestan)	89.25	11.99	3.83	8.68	62.99	1.76
T. dicoccon (Palestine)	89.37	13.91	4.10	8.90	60.44	2.02
T. dicoccon (Tapioszele)	89.27	13.10	4.40	10.59	59.20	1.98
T. dicoccon (Brno)	89.53	13.42	4.19	9.56	60.28	2.08
T. dicoccon (Tabor)	89.74	14.39	4.38	12.51	56.69	1.77
Vlasta (*T. aestivum* check)	89.09	11.59	2.76	11.13	62.23	1.38

Table 3 Bread making parameters of emmer accessions.

Genotype / variety	Wet gluten content (%)	Gluten Index	SDS-sedimentation (mL)	Zeleny sedimentation (mL)	Falling number (s)
Rudico	24.21	4	32	19	402
May-Emmer	28.06	6	37	10	337
Weisser Sommer	27.55	6	34	8	331
T. dicoccon (Dagestan)	*	*	11	6	386
T. dicoccon (Palestine)	*	*	17	9	379
T. dicoccon (Tapioszele)	30.2	9	18	11	421
T. dicoccon (Brno)	*	*	17	9	317
T. dicoccon (Tabor)	33.84	4	25	18	328
Vlasta (*T. aestivum* check)	26.69	65	-	36	-

*) totally flowable gluten.

On the other hand, emmer wheat is becoming a more and more popular crop in organic farming. With the aim of describing other compounds of emmer grain, we analysed them in greater detail.

3.2.4 Total Dietary Fibre Content and Its Components

The content of total dietary fibre (TDF) ranged from 8.68% to 13.89% in Rudico. The proportion between soluble and insoluble fractions of TDF in average was nearly 1 : 1 (5.3 : 4.8% soluble to insoluble fractions respectively), however there were differences among accessions (Table 4).

Differences among emmer accessions in the ratio between IDF and SDF reflect the opportunity to select suitable genotypes. The results obtained by D'Antuoni et al. [14] suggest that the native emmer genetic material may represent a source of high-value dietary fibre.

3.2.5 The Content of Total Polyphenols Plus Catechin and Feluric Acid Contents

Table 4 Total dietary fibre content (%) and its components in emmer grain.

Genotype / variety	TDF*	IDF**	SDF***
Rudico	13.89	7.01	5.88
May-Emmer	9.18	4.34	4.84
Weisser Sommer	8.77	5.02	3.75
T. dicoccon (Dagestan)	8.68	3.33	5.35
T. dicoccon (Palestine)	8.9	4.89	4.01
T. dicoccon (Tapioszele)	10.59	6.71	3.88
T. dicoccon (Brno)	9.56	4.85	4.71
T. dicoccon (Tabor)	12.51	6.56	5.95
Vlasta (*T. aestivum* check)	11.13	7.06	4.07

*) Total Dietary Fibre **) Insoluble Dietary Fibre ***) Soluble Dietary Fibre.

Differences in content of total polyphenols among emmer accessions were relatively deep; they ranged from 2.54 g GAE/kg DM (Weisser Sommer) to 3.55 g GAE/kg DM (Rudico) (Table 5). Five emmer accessions overcame the check bread wheat variety Vlasta in its content of total polyphenols. The highest content, 3.55 g GAE/kg DM, was detected in the Rudico variety. The

Table 5 Total polyphenols plus catechin and feluric acid contents.

Genotype / variety	Total polyphenols (g GAE/kg DM)	Catechin (mg/100g DM)	Ferulic acid (mg/100g DM)
Rudico	3.55	149.5	2.2
May-Emmer	2.72	66.0	2.3
Weisser Sommer	2.54	57.5	2.5
T. dicoccon (Dagestan)	2.85	109.6	1.5
T. dicoccon (Palestine)	3.22	112.5	1.2
T. dicoccon (Tapioszele)	3.31	43.6	2.3
T. dicoccon (Brno)	3.40	66.8	1.2
T. dicoccon (Tabor)	3.50	78.6	1.1
Vlasta (*T. aestivum* check)	2.81	97.1	1.3

antioxidant activity of polyphenols is described by Serpen et al. [15].

Catechin prevailed among polyphenolyic substances. Very low catechin content (57.5 mg/100 g DM) was detected in Weisser Sommer, on the other hand, Rudico contained 149.5 mg/100 g DM of catechin. Feluric acid was another measurable polyphenol; its content ranged from 1.1 mg/100 g DM in *T. dicoccon* (Tabor) to 2.5 mg/100 g DM in Weisser Sommer. Contents of chlorgenic acid and epicatechin were immeasurable.

3.2.6 Vitamins of B Group in Emmer Accessions

A higher content of vitamins B1 and B2 was ascertained in Rudico (0.44 mg/100 g DM, 0.135 mg/100 g DM respectively) in comparison to May Emmer (0.33 mg/100 g DM, 0.108 mg/100 g DM respectively).

Also the highest contents of other B vitamins (niacin, pantothenic acid and B6) were found in the Rudico variety (Table 6).

3.2.7 The Content of E Vitamin and Carotenoids in Emmer Accessions

The highest content of E vitamin was measured in *T. dicoccon* (Tapioszele) (1.30 mg/100 g DM). The total content of carotenoids ranged from 18.31 mg/100g DM in *T. dicoccon* (Tabor) to 26.58 mg/100g DM in *T. dicoccon* (Palestine).

4. Conclusions

In our experiments we have concluded that emmer wheat bread makes quality pure, because of the low swelling capacity of gluten. That is why emmer is suitable for other purposes as whole grain mixtures, cereal pure etc. than for classical yeast bread preparation.

The results described above indicate possibilities to select emmer wheat genotypes differing in grain composition, containing compounds with positive effects on human health.

Among tested emmer wheat accessions the Rudico variety had a set of positive characteristics such as: content of total dietary fibre, total polyphenol content with prevailing catechin and the highest amount of B group vitamins such as B1, B2, niacin, pantothenic acid and B6.

Table 6 Content of B group vitamins.

Genotype / variety	B1 (mg/100g DM)	B2 (mg/100g DM)	niacin (mg/100g DM)	pantothenic acid (mg/100g DM)	B6 (mg/100g DM)
Rudico	0.44	0.135	10.6	1.06	0.45
May-Emmer	0.33	0.108	8.4	0.95	0.27
Weisser Sommer	0.36	0.108	8.9	0.91	0.28
T. dicoccon (Dagestan)	0.29	0.111	9.7	0.78	0.27
T. dicoccon (Palestine)	0.36	0.113	9.7	1.04	0.38
T. dicoccon (Tapioszele)	0.36	0.115	10.2	0.92	0.39
T. dicoccon (Brno)	0.35	0.12	8.5	0.84	0.38
T. dicoccon (Tabor)	0.34	0.115	8.4	0.94	0.43
Vlasta (*T. aestivum* check)	0.36	0.071	6.8	0.95	0.37

Table 7 Content of E vitamin and carotenoids.

Genotype / variety	vitamin E (mg/100g DM)	total carotenoids (mg/100g DM)
Rudico	1.24	20.23
May-Emmer	1.21	20.65
Weisser Sommer	1.09	23.88
T. dicoccon (Dagestan)	1.09	24.86
T. dicoccon (Palestine)	0.83	26.58
T. dicoccon (Tapioszele)	1.30	21.56
T. dicoccon (Brno)	1.03	20.43
T. dicoccon (Tabor)	1.09	18.31
Vlasta (*T. aestivum* check)	1.22	20.44

Acknowledgment

This research was supported by the research projects of the Ministry of Agriculture QH82272 and QI91B095.

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