

Antioxidant activity of grain of einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* Schuebl [Schrack]) and spring wheat (*Triticum aestivum* L.) varieties

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ABSTRACT

Wheat and cereals generally are largely consumed worldwide and contribute significantly to antioxidant intake with beneficial health effects. In the precise two-year field experiments, two varieties of wheat einkorn, two varieties of emmer wheat and three varieties of spring wheat in 2008 and moreover further two spring wheat varieties, three einkorn varieties and three emmer wheat varieties in 2009, were evaluated for antioxidant activity (AOA) using 2,2-diphenyl-1-picrylhydrazyl assay (DPPH). The higher grain AOA was observed in emmer (215.4–257.6 mg Trolox/kg DM) and einkorn (149.8–255.8 mg Trolox/kg DM) varieties, while in spring varieties the AOA ranged between 195.8 and 210.0 mg Trolox/kg DM. A linear correlation between total polyphenols and AOA was determined ($r = 0.739$, $P \leq 0.05$). Emmer and einkorn wheat varieties showed high AOA and can be promising sources of these nutritionally appreciated grain constituents.

Keywords: minority wheat varieties; radical scavenging activity

Cereals could be important sources of antioxidant vitamins and enzymes and other health-beneficial substances in human nutrition (Ehrenbergerová et al. 2009, Březinová-Belcredi et al. 2010). Wheat, together with maize and rice, supplies most of the dietary saccharides and proteins for human nutrition, but is also a relevant source of antioxidants (Liu 2007) together with other particular sources such as tartary buckwheat-enriched bread (Bojňanská et al. 2009, Vogrinčič et al. 2010). Einkorn (*Triticum monococcum* L. subsp. *monococcum*), a diploid wheat related to *T. turgidum* L. and *T. aestivum* L., shows higher protein, carotenoid and tocol contents (Hidalgo et al. 2006, Hejtmánková et al. 2010) than durum and bread wheats, being therefore a potential food source with high nutritional properties (Lavelli et al. 2009). The antioxidant activity of wheat is caused by antioxidants, which belong to different groups of hydrophilic and lipophilic compounds such as polyphenols, carotenoids, phytosterols, and selenium.

Nowadays, the global food supply has become increasingly dependent on only a few crops. Three cereals meet more than 50% of the worldwide requirement for plant-derived proteins and energetic needs: maize, rice and wheat (638, 589, and 556 million tonnes, respectively) (Knudsen et al. 2008). Wheat is the most widely grown food crop with a global production of about 600 million tonnes annually. Wheat was traditionally selected for functionality, for instance, baking or biscuit values, resulting in the selection of hard bread wheat (*Triticum aestivum* L.) varieties with a high level of strong gluten proteins, or of durum wheat (*T. turgidum* L. subsp. *durum* Desf. [Husn.], which enables to produce yellow-coloured pasta (Guarda et al. 2004). Einkorn wholemeal flour is rich in protein, lipid (mostly unsaturated fatty acids), microelements, and antioxidants such as carotenoids and tocols; the white flour still retains most of the protein and antioxidant compounds (Brandolini and Hidalgo 2011). Also crop diversi-

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fication, sustainable agriculture, and the demand for healthy food are among the factors that gave rise to a growing interest during the past few decades in emmer wheat (*Triticum turgidum* L. spp. *dicoccum*) to be maximally exploited to improve grain protein, fibre, minerals, and phytochemicals (Arzani 2011).

In order to enhance the existing knowledge of antioxidant activity (AOA) of einkorn and emmer, we focused in this study on the determination of AOA with the purpose to evaluate new selected wheat varieties. The objective was to explore the genetic variability of AOA among the selected cultivated wheat species, i.e. einkorn (*T. monococcum* L.), *T. dicoccum* durum wheat and modern bread wheat varieties in order to improve the nutritional value of bread and other wheat products.

MATERIAL AND METHODS

Plant material. Grains of spring einkorn, emmer and common bread wheat landraces were obtained from the Czech Gene Bank of the Crop Research Institute in Prague from the 2008 and 2009 harvests. In the precise two-year field experiments in 2008 and 2009 two varieties of wheat einkorn (Escana and Schwedisches Einkorn), two varieties of emmer (Kahler Emmer and Rudico) and three varieties of spring wheat (SW Kadrilj, Kärtner Früher and Granny) were grown. In 2009, the range was extended to spring wheat varieties Jara and Postoloprtská přesívka 6, three einkorn varieties (*T. monococcum* ECN 01C0204039, ECN 01C0204040 and ECN 01C0204044) and three emmer wheat varieties (Horny Tisovnik (Malov), *T. dicoccon* (Tapioszele) and *T. dicoccum* No. 8909; their major characteristics are described in detail in our previous study (Lachman et al. 2011).

Chemicals. Methanol was purchased from Merck KGaA (Germany, Darmstadt), DPPH (2,2-diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Sigma-Aldrich (Germany, Steinheim). All chemicals and reagent were at least of G.R. purity.

Sample preparation. Finely ground wheat samples (ca 5.0 g) were weighed into 100 mL volumetric flasks and dissolved in methanol. The flasks were filled up with methanol to volume of 100 mL. For AOA determination, 100 µL aliquots of sample solutions were pipetted.

Determination of AOA with DPPH assay. Indirect method described by Roginsky and Lissi (2005) was used. Sample containing antioxidants

reacts with a solution of stable synthetic radical being converted to a colourless product (DPPH assay). Methanolic DPPH solution [absorbance (t_0) 0.600 ± 0.01] was prepared and 100 µL of the sample were added. Reaction time was 20 min. Absorbency was measured at wavelength $\lambda = 515$ nm. AOA was calculated as the decrease of absorbency according to the equation (1):

$$\text{AOA (\%)} = 100 - [(A_{t20}/A_{t0}) \times 100] \quad (1)$$

Where: A_{t20} – absorbency in time 20 min; A_{t0} – absorbency in time 0 min.

Calculated AOA was expressed in mg Trolox/kg DM. A_{t0} and A_{t20} were determined from the standard calibration curve ($r^2 \geq 0.9945$). Calibration curves were prepared using working solutions of Trolox in methanol between 5–25 µg Trolox/mL (LOD = $0.601 \mu\text{g Trolox/mL}$, LOQ = $2.000 \mu\text{g Trolox/mL}$, RSD = 1.83%). All samples were analysed in triplicates.

Statistical analysis. Statistical analyses were performed using the software Statistica 7.0 (StatSoft) on the basis of parametrical and non-parametrical tests at the level of significance $\alpha = 0.05$. Further, ANOVA multiple factorial analysis, Tukey's HSD test and *t*-test were used for statistical evaluation.

RESULTS AND DISCUSSION

AOA activity. As promising varieties emmer varieties Rudico and Kahler Emmer, einkorn varieties Escana and Schwedisches Einkorn, and Kadrilj SW spring wheat were chosen with the following values in average of 2008 and 2009: 197.5 ± 12.7 , 177.4 ± 2.9 , 168.0 ± 0.7 , 162.9 ± 0.5 , and 163.9 ± 4.7 mg Trolox/kg DM, respectively (Figure 1). In 2009, within an extended range of 15 varieties, high levels of AOA were found (Figure 2). The high values were observed almost exclusively in emmer wheat varieties Rudico, Krajova-Horny Tisovnik (Malov) and Kahler Emmer (257.6 ± 25.0 , 242.3 ± 1.9 , 217.3 ± 5.2 mg Trolox/kg DM, respectively) and in einkorn wheat varieties *T. monococcum* ECN 01C0204044, *T. monococcum* ECN 01C0204039, *T. monococcum* ECN 01C0204040 and Escana with values 255.8 ± 28.8 , 233.7 ± 4.0 , 231.5 ± 2.7 , and 218.5 ± 1.3 mg Trolox/kg DM, respectively.

The mean AOA in 2009 (205.8 ± 7.72 mg Trolox/kg DM) was higher in comparison with 2008 average value (110.0 ± 0.29 mg Trolox/kg DM). It could be due to lower precipitation in June 2008 as well as above-average temperature during the growing season as compared to the long-term average (Table 1). Antioxidant activity and the content of

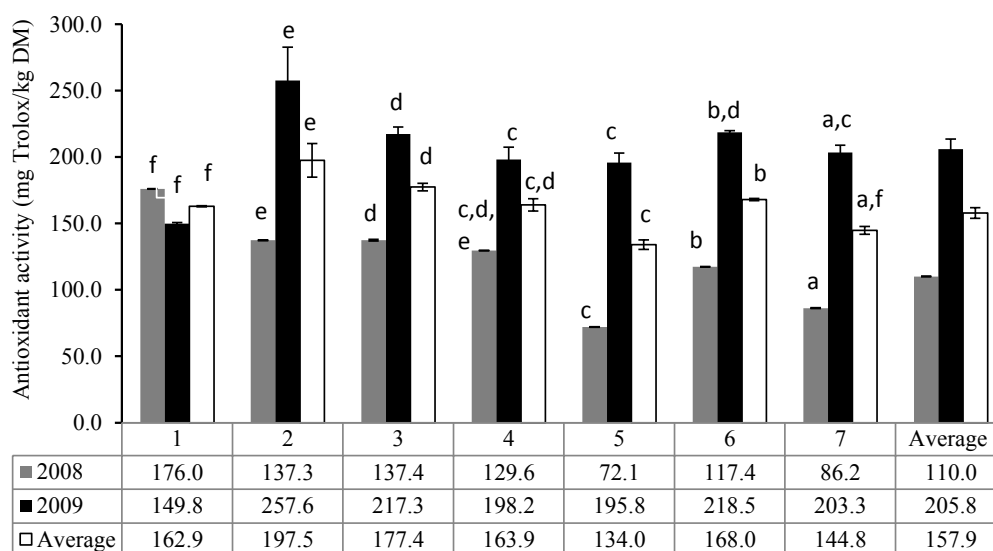


Figure 1. Antioxidant activity (average $\pm$ standard deviation – in mg Trolox/kg DM in einkorn, emmer and spring wheat varieties. Values from 2008 and 2009 harvests. (1 – Schwedisches Einkorn (einkorn); 2 – Rudico (emmer); 3 – Kahler Emmer (emmer); 4 – SW Kadrilj*; 5 – Kärtner Früher*; 6 – Escana (einkorn); 7 – Granny*; 8 – average content of analysed varieties); *marked varieties are those of spring wheat. Different small letters indicate significant differences ($P < 0.05$) among analysed wheat varieties in the same column

antioxidants could be related to stress factors of the weather conditions during the vegetation period and sunshine duration in the given year (Kalinová and Vrchotová 2011). Antioxidant activity of wheat should be also significantly affected by the content

of lipophilic antioxidants, mainly tocopherols and carotenoids (Hejtmánková et al. 2010).

Wheat varieties with high AOA were represented mainly by einkorn (four varieties) and emmer (three varieties).

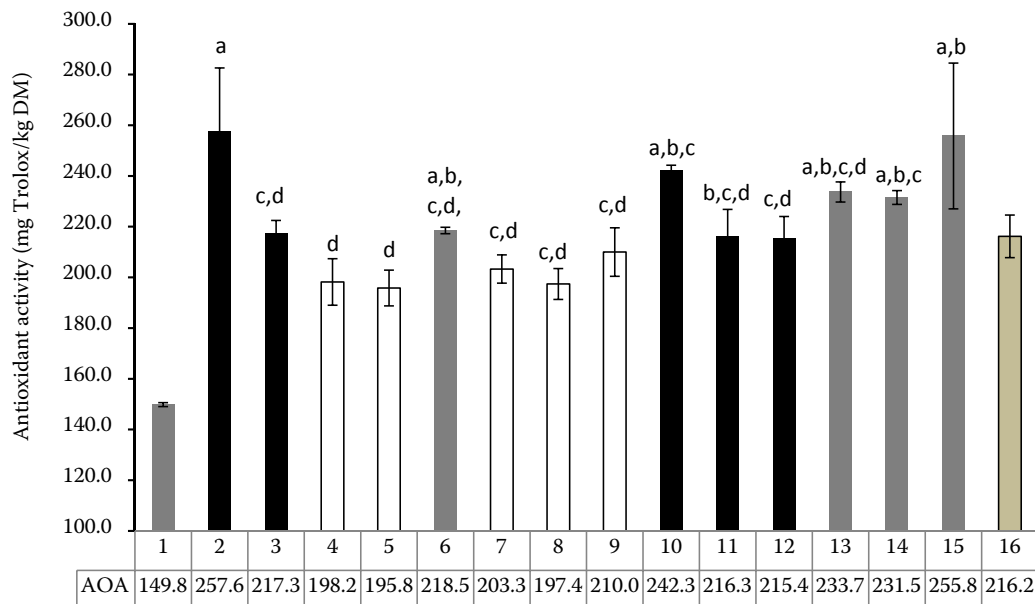


Figure 2. Antioxidant activity (AOA; average $\pm$ standard deviation – in mg Trolox/kg DM) in einkorn, emmer and spring wheat varieties in 2009 (1 – Schwedisches Einkorn (einkorn); 2 – Rudico (emmer); 3 – Kahler Emmer (emmer); 4 – SW Kadrilj*; 5 – Kärtner Früher*; 6 – Escana (einkorn); 7 – Granny*; 8 – Jara*; 9 – Postoloprtská přesívka 61*; 10 – Krajova-Horný Tisovnik (Malov) (emmer); 11 – *T. dicoccon* (Tapioszele) (emmer); 12 – *T. dicoccon* No. 8909 (emmer); 13 – *T. monococcum* 01C0204039 (einkorn); 14 – *T. monococcum* 01C0204040 (einkorn); 15 – *T. monococcum* 01C0204044 (einkorn); 16 – average content of analysed varieties); *marked varieties are those of spring wheat. Different small letters indicate significant differences ($P < 0.05$) among analysed wheat varieties in the same column

Table 1. Weather conditions during the vegetation period in 2008 and 2009 and comparison with long term period 1971–2001

Year		February	March	April	May	June	July	August	Vegetation period
2008	R	6.8	31.4	57.6	70.6	42.8	88.4	72.2	369.8
	T	3.9	4.4	8.7	14.7	18.8	19.1	19.0	12.7
2009	R	19.8	27.7	29.0	63.4	66.9	67.8	61.8	336.4
	T	0.1	4.6	13.6	14.4	15.7	19.3	20.3	12.6
Mean 1971–2001	R	16.8	37.6	24.2	109.2	69.0	79.0	20.8	356.6
	T	−0.2	3.8	7.9	13.3	16.2	18.1	18.1	11.0

R – sum of rainfalls (mm); T – average temperature (°C)

Correlation between total polyphenols (TP) and AOA. Correlation was found between the TP content (data reported by Lachman et al. 2011) and AOA ($r = 0.739$, $r^2 = 0.547$, $r^2_{\text{corr.}} = 0.529$, $P \leq 0.05$, Figure 3). Our results are in a good agreement with findings that the antioxidant activity of extracts from cereal products correlated with the content of phenols occurring in these cereals (Zieliński and Kozłowska 2000).

Evaluation of AOA of emmer, einkorn and spring varieties. Einkorn, emmer and common bread wheat species were grown in the Czech territory till the 6th century A.D., being then replaced by bread wheat. At present, only landraces and wild forms of these species are available in collections of genetic resources. With the aim to extend the spectra of grown crops, a collection of emmer and einkorn genetic resources in the Czech Gene Bank was studied. Accessions later in ripeness, with a

good level of resistance to fungal diseases and with a high yield potential were selected from the collection to determine their antioxidant activities (their characteristics are described in our previous article (Lachman et al. 2011)).

In term of the polyphenolic antioxidants content (Lachman et al. 2011) and AOA, considerably higher values were found in einkorn and emmer accessions as compared to the tested spring wheat cultivars. It is evident from the statistical evaluation of the varieties tested in 2009 by the method of the least squares (Figure 4) and Anova Tukey's HSD test (Table 2) that the Schwedisches Einkorn variety differs statistically from all other analysed varieties at $P \leq 0.05$. Relatively similar are spring wheat varieties SW Kadrilj, Granny, Jara, Kärtner Früher, and Postoloprtská přesívka. High AOA showed emmer varieties Rudico and Krajova-Horný Tisovník (Malov) and einkorn *T. monococcum* 01C0204044. Antioxidant activities in 2008 and 2009 in average of all analysed varieties were also statistically different.

The cultivated diploid (einkorn), tetraploid (durum wheat) and hexaploid (bread wheat) species and varieties possess antioxidant activity due to their content of hydrophilic (phenolics, selenium) and lipophilic (carotenoids, tocopherols) antioxidants. Einkorn is an underutilised wheat species with high protein, lutein and tocopherols content, particularly suited for infant and specialty foods (Hidalgo et al. 2008). Genetic variability of the content of antioxidants (Leenhardt et al. 2006), primary processing, namely milling (Liyana-Pathirana and Shahidi 2007), distribution of antioxidants in seed fractions (Hidalgo and Brandolini 2008) are together with the effect of different locations where wheat species are grown (Yu and Zhou 2004) the main important factors influencing antioxidant properties of wheat (caryopses, bran, germ and endosperm) and wheat products. As was proved

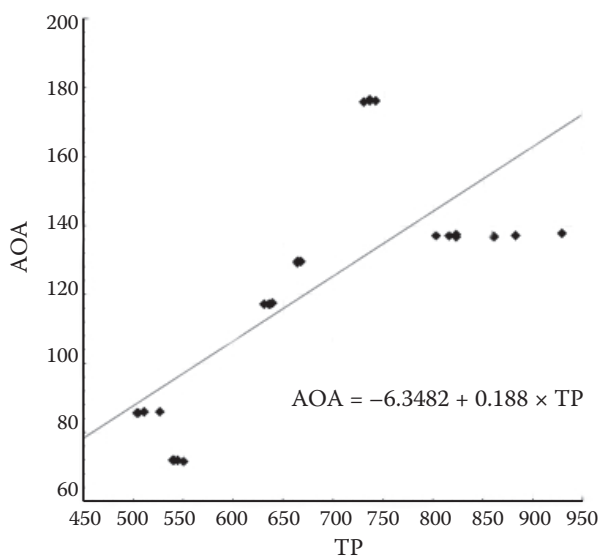


Figure 3. Linear correlation between total phenols (TP) and antioxidant activity (AOA) ($r = 0.739$, $r^2 = 0.547$, $r^2_{\text{corr.}} = 0.529$, $P \leq 0.05$)

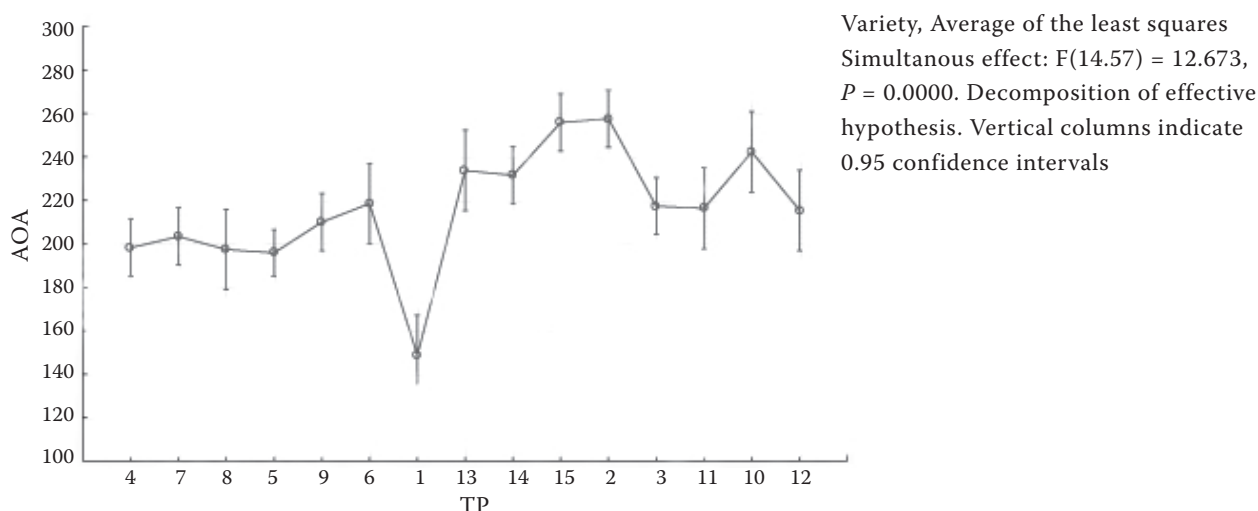


Figure 4. Statistical differences of analysed wheat varieties on the basis of the method of the least squares regression (1 – Schwedisches Einkorn (einkorn); 2 – Rudico (emmer); 3 – Kahler Emmer (emmer); 4 – SW Kadrilj*; 5 – Kärtner Früher*; 6 – Escana (einkorn); 7 – Granny*; 8 – Jara*; 9 – Postoloprtská přesívka 6l*; 10 – Krajova-Horný Tisovník (Malov) (emmer); 11 – *T. dicoccon* (Tapioszele) (emmer); 12 – *T. dicoccon* No. 8909 (emmer); 13 – *T. monococcum* 01C0204039 (einkorn); 14 – *T. monococum* 01C0204040 (einkorn); 15 – *T. monococcum* 01C0204044 (einkorn); *marked varieties are those of spring wheat

in hard winter wheat extracts (Yu et al. 2002), the chelating activities and free radical scavenging capacity against DPPH• are dependent mainly on varieties and accessions. Phenolic compounds may contribute to both the activities, while other chemical constituents may account for the total antioxidant activities of the extracts. Our results are similar to those of Liyana-Pathirana and Shahidi (2007), who compared antioxidant capacity of two wheat cultivars – *Triticum turgidum* L. var. *durum* Canada Western Amber Durum and *Triticum aestivum* L. Canada Western Red Spring. Oxygen Radical Absorbance Capacity (ORAC) values of *T. durum* fractions reported by Liyana-Pathirana and Shahidi (2007) were ranked in the order of bran > shorts > feed flour > whole grain > flour.

In our results, AOA values obtained for three wheat species, i.e. bread wheat, durum wheat and einkorn decreased in the order einkorn > durum wheat > bread wheat. The order varied from those for lipoxygenase activity (LOX) of wholemeal flour obtained from these three wheat species (Leenhardt et al. 2006), where LOX decreased in the order bread wheat durum wheat > einkorn. The LOX activity was negatively correlated to the carotenoid concentration ($r^2 = 0.9256$, $P < 0.0001$, $df = 8$) and consequently, in a perspective of bread-making the ratio between total carotenoid concentration and LOX activity would be a suitable criterion for wheat breeding programs (De Simone et al. 2010).

Although environmental factors play an important role in antioxidants concentration in cereals,

the genetic component is predominant with high heritability values. Wheat is a staple food supplying significant portion of antioxidant compounds intake (Baublis et al. 2000, Miller et al. 2000) and einkorn and durum could play an important role together with new accessions of bread in breeding of new genotypes with high carotenoids and tocopherols that may have synergistic antioxidant effects (Hidalgo et al. 2006, Brandolini et al. 2008, Hejtmánková et al. 2010).

Emmer wheat accessions showed 1.43 times higher AOA than the bread wheat cultivars, Schwedisches Einkorn even 1.84 times higher. Wheat varieties of the high AOA are represented mainly by einkorn and emmer (four and three varieties, respectively). The high levels of AOA were found almost exclusively in emmer wheat – Rudico, Krajova-Horný Tisovník (Malov) and Kahler Emmer and in einkorn wheat cultivars ECN 01C0204044, ECN 01C0204039, ECN 01C0204040 and Escana, respectively.

In conclusion, this study proved a genotypical variation in the antioxidant activity of einkorn, emmer and spring bread wheat accessions based on the high tocopherol and carotenoid content (Digesù et al. 2009, Hejtmánková et al. 2010) and the content of soluble phenolics (Lachman et al. 2011). The relevant antioxidant activity, along with superior contents of proteins, tocopherols, carotenoids and polyphenols, reinforce the prospect of einkorn and emmer as nutritionally superior cereal sources.

Table 2. Anova Tukey's Honestly significant difference test of antioxidant activity (AOA) of analysed wheat varieties in 2009

Variety	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
1 SW Kadrlj	198.18	203.34	197.37	195.83	209.98	218.49	149.75	233.70	231.51	255.78	257.56	217.29	216.31	242.30	215.44
2 Granny	1.000000	1.000000	1.000000	1.000000	0.993346	0.962287	0.026522	0.324209	0.043153	0.000151	0.000149	0.752089	0.985614	0.078774	0.990846
3 Jara	1.000000	1.000000	1.000000	0.999953	0.999990	0.997476	0.008257	0.581827	0.169226	0.000185	0.000165	0.970175	0.999529	0.195332	0.999786
4 Kärtner Früher	1.000000	1.000000	1.000000	1.000000	0.999657	0.948827	0.031561	0.290063	0.387357	0.003316	0.002157	0.967752	0.978877	0.067365	0.986049
5 Postoloprtská přesívka 6	1.000000	0.999953	1.000000	1.000000	0.966434	0.914402	0.043640	0.231332	0.021181	0.000149	0.000148	0.582395	0.959651	0.049474	0.971633
6 Escana**	0.993346	0.999990	0.999657	0.966434	0.999997	0.999997	0.001672	0.883702	0.577244	0.000740	0.000439	0.999967	1.000000	0.478622	1.000000
7 Schwedisches Einkorn**	0.962287	0.997476	0.948827	0.914402	0.999997	0.999997	0.000289	0.997362	0.999508	0.252392	0.191704	1.000000	1.000000	0.880850	1.000000
8 T. monococcum 01C0204039**	0.026522	0.008257	0.031561	0.043640	0.001672	0.000289	0.000149	0.000149	0.000150	0.000148	0.000148	0.000353	0.000418	0.000148	0.000498
9 T. monococcum 01C0204040**	0.324209	0.581827	0.290063	0.231332	0.883702	0.997362	0.000149	1.000000	1.000000	0.928769	0.879046	0.994336	0.990158	0.999997	0.984629
10 T. monococcum 01C0204044**	0.043153	0.169226	0.387357	0.021181	0.577244	0.999508	0.000150	1.000000	0.378657	1.000000	0.269494	0.965099	0.997379	0.999944	0.995378
11 Rudico*	0.000151	0.000185	0.003316	0.000149	0.000740	0.000439	0.000148	0.879046	0.378657	1.000000	0.000000	0.004706	0.133011	0.997262	0.113964
12 Kahler Emmer*	0.000149	0.000165	0.002157	0.000148	0.000439	0.999967	1.000000	0.994336	0.965099	0.008585	0.004706	1.000000	0.838447	0.798613	1.000000
13 T. dicoccon (Tapioszele*)	0.752089	0.970175	0.967752	0.582395	0.999967	1.000000	0.000353	0.994336	0.965099	0.179825	0.133011	1.000000	0.798613	0.798613	1.000000
14 Krajova-Horny Tisovník (Malov)*	0.985614	0.999529	0.978877	0.959651	1.000000	1.000000	0.000418	0.990158	0.997379	0.999279	0.997262	0.838447	0.798613	0.798613	0.759692
15 T. dicoccon No. 8909*	0.078774	0.195332	0.067365	0.049474	0.478622	0.880850	0.000148	0.999997	0.999944	0.155626	0.113964	1.000000	1.000000	0.759692	0.759692

*emmer varieties; **einkorn varieties

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