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Protein content and composition in organically grown wheat: influence of genotype

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Abstract: Protein composition and content play a critical role in bread quality and are governed by genetic factors. Organically grown primitive wheats, *Triticum monococcum, T. dicoccum, T. spelta,* old landraces and early Swedish breeding lines from the period 1900 to 1960 were tested for their protein composition and the amount and size distribution of polymeric proteins. Protein composition was determined by SDS-PAGE. The amount and size distribution of polymeric protein was carried out using SE-HPLC. The studies showed that genotype had an influence on the amount and size distribution of polymeric proteins in organically grown wheat. The variation in storage protein composition and the amount and size distribution of polymeric proteins in the organically grown wheat genotypes indicated significant variation among the genotypes. Also, variation in protein concentration was found between the investigated organically grown spring wheat varieties. This study showed that the primitive wheat varieties, and some breeding selections of wheat, have high protein contents and might be used as breeding materials for high-gluten strength organically grown wheat varieties.

Key words: genotypes; protein composition; protein concentration; organic farming; wheat

INTRODUCTION

Wheat is a staple food in many countries of the world. It is a source of different nutritional components i.e. proteins, carbohydrates, minerals and antioxidants. Wheat flour is used to make variety of products such as bread, pasta, biscuits, cake and pastries. Naturally consumers prefer to buy breads that are produced from organically grown wheat. The reasons for buying organic food are related to health security and environmental safety (Woodward & Meier-Ploeger, 1999). In conventionally grown wheat, the use of chemicals and fertilizers influence the quality, and also cause a negative impact on the environment. Instead of using synthetic fertilizers and pesticides, organic farmers utilize crop rotations, cover crops and naturally-based products to maintain or enhance soil fertility.

Evaluation of the quality of wheat includes the functional properties of wheat flour for bread making and the nutritional composition of wheat flour. Protein composition and contents play a critical role in bread quality and are governed by a combination of genetic and environmental factors (Johansson et al., 2001). Whereas the genotype determines the type and amount of proteins, growing conditions have a quantitative influence on the proteins (Payne et al., 1981, 1983; Wieser & Seilmeier,

1998). There have been many studies carried out to study the variation in content and composition of different wheat genotypes (Peterson et al., 1992; Johansson & Svensson, 1998; Johansson et al., 2000). Investigations into protein composition and content in organically grown wheat genotypes are much more limited.

The aim of the present study was to investigate the effect of genotypes on protein content and composition in organically grown wheat. In order to evaluate the possible amount of variation among genotypes, the primitive wheat, *Triticum monococcum, T. dicoccum , T.spelta*, old landraces and early Swedish breeding lines from the period 1900 to 1960 were used in this investigation.

MATERIALS AND METHODS

Sample collection and preparation

Grain samples of 29 organically grown winter wheat genotypes from different groups i.e. spelts, spelt selections, cultivars, primitive wheats and old landraces (name, year of cultivation and genotype are listed in Table 1.) were selected from a project dealing with old varieties from the Nordic Gene Bank. The collection of genotypes were grown during the years 2001–2003 in Alnarp, Sweden. Environmental data from 2001–2003 is presented in Table 2.

About 10g of each grain sample was milled into flour for one minute with a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany). After milling the flour samples were stored at -20°C.

Protein composition

Total protein from each grain sample was extracted in the presence of SDS and separated on polyacrylamide gels according to Johansson (1996). The gels were stained using Coomassie Brilliant Blue solution containing 10% ethanol and 8% trichloroacetic acid (TCA) according to Johansson et al. (1993). The samples were distained with 4% TCA water solution for 24 hours in order to produce clear bands.

SE-HPLC

Proteins from all flour samples were fractionated through Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC). Proteins from the threshed grain samples (16.5 mg) were extracted using a two-step extraction procedure developed by Gupta et al. (1993). In the first extraction 16.5 mg of the whole grain flour was suspended in 1.5 ml 0.5% SDS-phosphate buffer (pH 6.9) and centrifuged for 10 s. Samples were stirred with a shaker (IKA, VIBRAX, VxR basic) for 5 min at 2,000 rpm and centrifuged for 30 min at 1, 000 × g to obtain the supernatant protein. The supernatant was poured into HPLC vials and the pellet was subsequently resuspended in the SDS- buffer above for the second extraction and treated in an ultrasonic disintegrator (Soniprep 150, Tamro, Mölndal, Sweden) for 45 s, amplitude 5 microns, fitted with a 3 mm exponential microtip. The samples were centrifuged for 30 min at 10,000 × g to obtain a protein supernatant. The extracts were submitted to SE-HPLC and three replicates of each sample were fractionated. SE-HPLC analyses were performed on a Waters (Milford, MA, USA) HPLC system using a BIOSEP SEC-4000 Phenomenex column. Separation was achieved in 30 min by loading 20 µl of sample into an eluant of acetonitrile and water (1:1, v/v) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.2 ml/min. Proteins were detected by UV absorbance at 210 nm.

Table 1. Organically grown winter wheat genotypes.

Number	Genotype	Year	Genotype information
1	Ax/7	2001	Selection Spelt
2	Vama	2001	Cultivar
3	T. Polonicum	2003	Primitive wheat
4	Eroica 1026/4	2003	Cultivar
5	Hansa Ax	2002	Cultivar
6	Ax 14 1027	2003	Selection
7	Hartmut spiess	2001	Cultivar
8	SOL lv 1026/11	2003	Cultivar
9	Virtus 1026/14	2003	Cultivar
10	Schwabenkorn	2003	Spelt
11	Mirakel Ax	2002	Primitive wheat
12	Kippenhauser	2002	Spelt
13	6357 Klyvn	2002	Selection
14	Banco	2003	Cultivar
15	1345(43)	2001	Spelt
16	15 Schweiz	2002	Spelt
17	15 Schweiz	2003	Spelt
18	22 Klyvn	2002	Selection Spelt
19	22 klyvning	2001	Selection Spelt
20	3 schweiz	2002	Selection Spelt
21	32 comp Ax	2002	Selection Spelt
22	32 schweiz	2003	Selection Spelt
23	33 Schweiz	2002	Selection Spelt
24	4496 comp	2001	Landrace
25	4496 Lv.Got ax10	2003	Landrace
26	4496 spelt	2001	Landrace
27	4496-10	2001	Landrace
28	5113 (rad 4)	2003	Selection
29	5113 rad / Brun	2002	Selection

The chromatograms were divided into four sections with decreasing molecular size: large polymeric proteins (LPP), smaller polymeric proteins (SPP), large monomeric proteins (LMP) and smaller monomeric proteins (SMP). Protein parameters were calculated according to Gupta et al. (1993) with modifications according to Johansson et al. (2008) as follows:

total extractable proteins (TOTE) = LPP+SPP+LMP+SMP; total unextractable proteins (TOTU) = uLPP+uSPP+uLMP+uSMP; the percentage of large unextractable polymeric protein in the total large polymeric protein (%Large UPP) = uLPP/(uLPP+ LPP) x 100; the percentage of total unextractable polymeric protein in the total polymeric protein (%Total UPP) = uLPP+uSPP/(LPP+SPP+ uLPP+uSPP) x 100; the percentage of large unextractable monomeric protein in the total large monomeric protein (%Large UMP) = uLMP/(uLMP+LMP) x100; the amount of monomeric protein in the total amount of polymeric protein

(MON/POL) = uLMP+uSMP+ LMP+SMP/ uLPP+uSPP+LPP+SPP.

Table 2. Average monthly temperature and humidity data from 2001-2003 obtained from the Swedish Meteorological and Hydrological Institute (SMHI).

	Temperature (^{o}C)			Humidity %		
	2001	**2002**	**2003**	**2001**	**2002**	**2003**
JAN	1,84	2,03	-0,38	89,33	89,49	85,73
FEB	0,51	4,01	-2,23	82,30	85,46	85,12
MAR	1,21	4,44	3,00	81,14	77,79	75,86
APR	6,00	7,22	6,86	78,62	75,76	65,18
MAY	2,57	13,26	12,57	67,43	76,83	74,07
JUN	14,17	16,52	16,51	73,18	72,39	73,90
JUL	18,94	18,28	18,76	70,26	75,72	80,46
AUG	17,89	20,21	19,85	74,43	76,73	76,91
SEP	12,88	14,51	14,00	82,81	75,15	78,20
OCT	6,42	7,11	7,80	79,35	78,5	81,20
NOV	5,25	4,33	6,15	82,52	85,33	88,00
DEC	1,55	-0,30	1,20	72,16	83,96	81,35

Statistical analysis

The data from different years was analyzed collectively and separately for each year, to check the genotypes' (from 2001–2003) similarity. Cluster analysis was used, using Minitab Statistical Software (Minitab Inc., USA).

RESULTS AND DISCUSSION

Protein Composition

A small difference in the High Molecular Weight Glutenin Subunit (HMW-GS) composition among the organically grown winter wheat genotypes was found in the present investigation. The studied genotypes containing no subunit were, subunit 1 and 2* encoded on the locus Glu-A1, subunits 6+8, 7, 7+8, 7+9 and 14+15 which are encoded on the locus Glu-B, and subunits 2+12 and 5+10 encoded on the locus Glu-D1. No differences in specific protein composition was detected among specific years.

Most of the genotypes showed the subunits 2* and 1 encoded on the Glu-A1 and only 2 genotypes were with no subunit as shown in Table 3. Glu-B1 subunit 6+8 was present in 23 genotypes. The subunit 2+12 of Glu-D1 was dominant as it was found in 27 genotypes, while 5+10 subunit was present in only 2 genotypes (Table 3).

HMW glutenin subunit composition varied (Johansson, 1996; Johansson et al., 1993; Payne et al., 1983) among genotypes.

Protein Content

When protein content was analyzed separately for each year, no consistent relationships among cultivars were detected. Therefore, the results below are based on analyses of all the data from the three investigated years.

A dendrogram for similarity among different varieties on the basis of the amount and size distribution of protein fractions determined by SE-HPLC is shown in Figure 1. Clear differences among the studied genotypes of wheat on the basis of the amount and size distribution of proteins have been found.

Table 3. HMW glutenin subunit composition of the studied Swedish wheat genotypes.

Location of glutenin genes	HMW glutenin subunits	Genotypes
Glu-A1	0	2
Glu-A1	1	12
Glu-A1	2*	15
Glu-B1	6+8	23
Glu-B1	7	1
Glu-B1	7+8	1
Glu-B1	7+9	3
Glu-B1	14+15	1
Glu-D1	2+12	27
Glu-D1	5+10	2

The genotypes in different clusters are indicated in Table 4. According to differences in the amount and size distribution of proteins, the genotypes can be divided into three clusters. The first cluster mainly consists of spelt wheat and spelt breeding line selections. The second cluster is dominated by different varieties. The third cluster consists of primitive wheat and some selections of normal wheat (Table 4).

There is a small variation among the genotypes in the cluster 2 compared to the cultivars in clusters 1 and 3. Selection Ax 14 and primitive wheat, *Triticum Polonicum*, followed by other selections in the cluster 3, showed a higher quantity of different protein fractions compared with the genotypes of the other two clusters. The cultivar Hansa Ax showed a smaller content of proteins in cluster 3 and ,combined with landrace 4496 of cluster 2 and spelt wheat Kippen Hauser of cluster 1, had a similarity level of 73.37. Protein fractions (LPP, SPP, TUPP and LUPP) contributed to gluten protein content (Andrews et al., 2007; Gupta et al., 1993; MacRitchie, 1999) with all genotypes.

Most of the spelt wheat and the spelt selections showed similarity among themselves with little variation shown in cluster 1 (Fig. 1). The Swedish cultivars with small variation in the protein fractions are grouped in cluster 2, which combines with cluster1 at a similarity level of 75.

Table 4. Genotypes divided into groups according to protein fractions LPP, SPP, LMP, SMP, uLPP, uSPP, uLMP, uSMP,TOTE, TOTU, TUPP, LUPP and MON/POL. Numbers (1–29) indicating genotype position in Fig. 1.

Cluster 1		Cluster 2		Cluster 3	
Genotype	**Year**	**Genotype**	**Year**	**Genotype**	**Year**
1. Ax/7	2001	**2.** Vama	2001	**3.** T. Polonicum	2003
10. Schwaben korn	2003	**4.** Eroica 1026/4	2003	**5.** Hansa Ax	2002
12. Kippen Hauser	2002	**7.** Hartmut spiess	2001	**6.** Ax 14 1027	2003
16.15 Schweiz	2002	**8.** SOL lv 1026/11	2003	**13.** 6357 Klyon	2002
17. 15 scweiz	2003	**9.** Virtus 1026/14	2003	**28.** 5113 (rod 4)	2003
19. 22 klyoning	2001	**14.** Bamco	2003	**29.** 5113 rad / Brun	2002
20. 3 schweiz	2002	**15.** 1345(43)	2001	**11.** Mirakel Ax	2002
21. 32 comp Ax	2002	**18.** 22 Klyon	2002		
22. 32 schweiz	2003	**24.** 4496 comp	2001		
23. 33 Schweiz	2002	**25.** 4496 Lv.Got ax10	2003		
27. 4496-10	2001	**26.** 4496 spelt	2001		

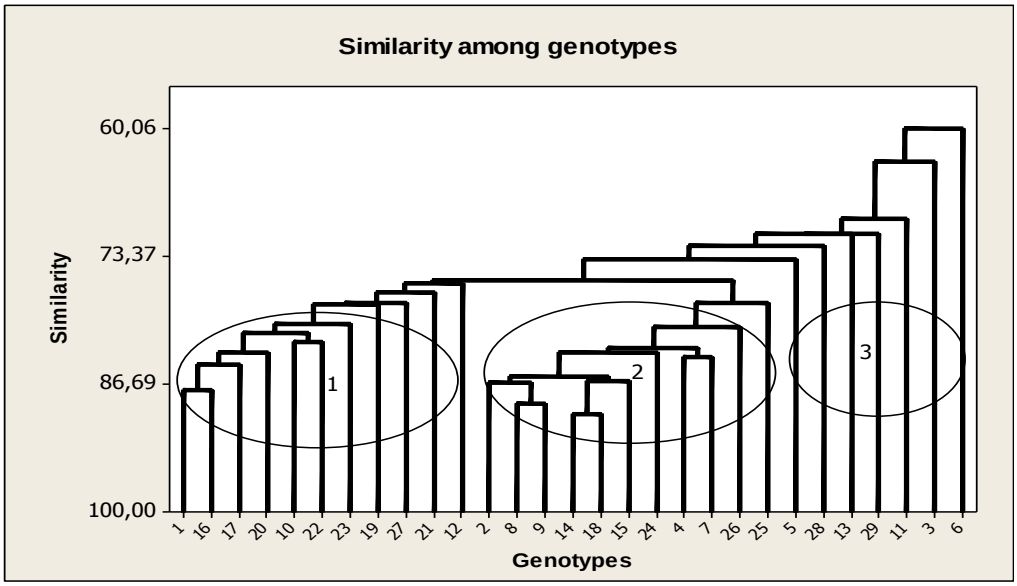

Fig. 1. Similarity among studied genotypes of wheat on the basis of different protein fractions.

CONCLUSIONS

Organically grown primitive wheat and some of the wheat selections in this study had higher protein concentrations compared to the spelt wheat and other wheat varieties studied. Therefore, breeding selection for higher concentration of wheat proteins can be used in breeding programs for improving better bread-making quality of organically grown wheat varieties.

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