

QUALITY PARAMETERS AND CHEMICAL COMPOSITION OF COLORED-GRAIN WHEAT AFTER FOLIAR FERTILIZATION

MACHALKOVA LENKA¹, HRIVNA LUDEK¹, JANECKOVA MARIE¹,
DOSTALOVA YVONA¹, MRKVICOVA EVA², VYHNANEK TOMAS³,
TROJAN VACLAV³

¹Department of Food Technology

²Department of Animal Nutrition and Forage Production

³Department of Plant Biology

Mendel University in Brno

Zemedelska 1, 613 00 Brno

CZECH REPUBLIC

lenka.machalkova@mendelu.cz

Abstract: Winter wheat varieties "Rosso" and "Skorpion" were cultivated in 2013–2014 in small-plot field trials. A half of variants were fertilized only with nitrogen as part of regeneration and production fertilization, while the other part was fertilized with sulfur and nitrogen. The total dosage amounting to 184 kg of nitrogen per hectare and 94 kg of sulfur per hectare. Accordingly, the qualitative fertilization involved NP solution, YARA Vita Thiotrac and combinations of both applied by foliar spraying. The application of sulfur combined with nitrogen or qualitative fertilization had no influence on grain yield. The qualitative fertilization increased the protein content by up to 0.8% for "Rosso" and only by 0.2% for "Skorpion", while the value of Zeleny sedimentation volume grew by 2.7 to 6.3 ml for "Rosso" and by 1 to 1.7 ml for the "Skorpion" variants fertilized with N₁S₁. The representation of individual protein fractions was significantly influenced only by the variety - no fertilization effect was demonstrated. For "Rosso", the content of selected cyanidins increased by 1.0%–81.0% after the application of qualitative fertilization, while the influence for "Skorpion" was less significant, ranging from -9.3% to 37.3%. The extremely favorable conditions in the given crop year significantly eliminated the effects of the qualitative fertilization applied.

Key Words: Colored wheat varieties, N fertilization, S fertilization, foliar nutrition, grain yield and quality, protein profile, anthocyan content

INTRODUCTION

Seeds of wheat varieties featuring a colored pericarp (blue or purple) are increasingly seen in the range available on the market. Colored-grain wheat is valued primarily for substances which their pericarp includes. The high content of anthocyanins and phenolic compounds represented mainly by ferulic acid, vanillic acid, p-cumaric acid and others is reflected in improved antioxidation capacity (Keguan et al. 2005, Liu 2007). As regards location, blue pigments are located in the aleurone layer of the grain, while purple pigments concentrate in the pericarp layers. Cyanidin-3-glucoside was found to be the major anthocyanine in purple wheat and the second most frequent such substance in blue wheat. Delphinidin-3-glucoside is predominant in blue wheat, where it covers about 41% of total anthocyanins (Abdel-Aal, Hucl 2003, Hosseinian et al. 2008). The concentration of anthocyanins rapidly increases during grain ripening while reducing when the grain becomes ripe (Knievel et al. 2009). The high content of anthocyanins reduces the risk of oxidative damage while increasing the ability to bind heavy metals, plus it has a preventive effect against cardiovascular disease, cancer, rheumatoid arthritis, neurodegenerative disease and diabetes mellitus type 2 (Fang et al. 2002, Lutsey et al. 2007). With the content and the effect of the substances, colored wheat grains find applications in the production of functional foods. Since envisaged in this context is namely applying bran parts of the grain, less attention is paid to the technological quality of endosperm from which we obtain flour and semolina. Any bigger chance for colored-pericarp wheat to become more widespread can be

however expected only when the newly bred varieties are profitable and are of a favorable bread-making quality. What is also important, the agrotechnology of growing such varieties should be comparable with conventional varieties of wheat. Yield and quality can be significantly influenced by fertilization. Above all, the application of nitrogen during the growing season is a crucial factor for not only the yield but also the quality of the grain (Zimolka et al. 2005). Subsequently, applying sulfur in addition to nitrogen in a suitable manner increases the protein content of the flour (Tea et al. 2007) and tends to ensure better quality of the gluten protein of the grain (Järvan et al. 2008). Fundamental can also be the distribution of application rates over time, where late fertilization after the earing stage may play an important part. Effective to this end may be not only N and S fertilization, but also one involving e.g. P-fertilizers. Late applications contribute to a higher nutrient content of the grain, which results, for instance, in sulfur, in higher volume of the bakery product (McGrath et al. 2002). Within our present experiments, we focused on evaluating the effect of the application of (i) nitrogen, (ii) nitrogen and sulfur, and (iii) qualitative fertilization in "Rosso", a purple wheat variety, and "Skorpion", a blue-pericarp wheat, on yield and technological quality of the varieties.

MATERIAL AND METHODS

A small-plot field experiment was set up in 2013–2014 to test the application of N- and NS-based fertilizers in the nutrition of colored-pericarp winter wheat varieties "Rosso" and "Skorpion". Checked was also the effect of foliar nutrition on grain yield and quality; this was carried out as part of qualitative fertilization. The wheat was grown in a small-plot trials. The development of weather in the most important months is shown in the following Table 1.

Table 1 Climate conditions during the growing period 2013–2014

| Month/ year | Average temperature (°C) | Average precipitation (mm) | Month/ year | Average temperature (°C) | Average precipitation (mm) | Month/ year | Average temperature (°C) | Average precipitation (mm) |
|----------------|--------------------------------|----------------------------------|----------------|--------------------------------|----------------------------------|----------------|--------------------------------|----------------------------------|
| 9/13 | 13.5 | 88 | 1/14 | 1.4 | 30.2 | 5/14 | 14.5 | 66.6 |
| 10/13 | 10.7 | 47.4 | 2/14 | 3.6 | 18 | 6/14 | 18.3 | 47.8 |
| 11/13 | 5.3 | 43 | 3/14 | 9.1 | 23.8 | 7/14 | 21.8 | 70.8 |
| 12/13 | 1.9 | 15.1 | 4/14 | 11.9 | 52 | 8/14 | 18.2 | 85.5 |

Both wheat varieties were sown after oilseed rape as the previous crop. This occurred on 4 October 2013 on a plot exhibiting agrochemical properties (Table 2) established by Zbíral (2002); the seed rate was 4 million of germinating seeds. As the growing season was underway, the vegetation was treated with growth regulators and pesticides along with applying fertilizers.

Table 2 The agrochemical characteristics of soil in $\text{mg}\cdot\text{kg}^{-1}$

| pH | K | P | Ca | Mg | S |
|------|-----|------|-------|-----|------|
| 6.44 | 289 | 94.6 | 1.870 | 153 | 11.9 |

Note: K,P,Ca,Mg according to Mehlich III, pH 0.01 mol CaCl_2 , S - aqueous extract

The fertilizer application pattern is shown in Table 3. Each of variants of 4 runs.

Table 3 The total applied dose of fertilizers ($\text{kg}\cdot\text{ha}^{-1}$)

| Variant | Regeneration Fertilization | | Production 1 (7 Apr 2014) | Production 2 (15 May 2014) | Qualitative (4 Jun 2014) |
|---------|----------------------------|--------------------|------------------------------|-------------------------------|-----------------------------|
| | A (20 Sep 2014) | B (21 Mar 2014) | | | |
| 1 | 52 LAV 27 | 52 LAV 27 | 40 MO | 40 MO | - |
| 2 | 52 LAV 27 | 52 LAV 27 | 40 MO | 40 MO | NP |
| 3 | 52 LAV 27 | 52 LAV 27 | 40 MO | 40 MO | Thio |
| 4 | 52 LAV 27 | 52 LAV 27 | 40 MO | 40 MO | Thio & NP |
| 5 | 52 LAV 27 | 52 LAV 27 | 40/47 SA | 40/47 SA | NP |
| 6 | 52 LAV 27 | 52 LAV 27 | 40/47 SA | 40/47 SA | Thio |
| 7 | 52 LAV 27 | 52 LAV 27 | 40/47 SA | 40/47 SA | Thio & NP |

Note: LAV 27: ammonium nitrate with limestone (27% N, 20% CaO); MO - Urea (46% N); SA: ammonium sulphate (20.3% N, 24% S); Thio: Thiotrac 5 $\text{l}\cdot\text{ha}^{-1}$ (300 g S, 200 g $\text{N}\cdot\text{l}^{-1}$), NP: solution 80 $\text{l}\cdot\text{ha}^{-1}$ (8 kg N and 24 kg P_2O_5 per 100 l).

The harvest within the experiment took place on 25 July 2014 using a small-plot threshing machine. Harvested grain was subjected to analysis. The specific weight was determined according to ISO 7971-2 (1995). Individual fractions of grain were also determined using grain sieves, mesh sizes 2.5 per 22 mm and 2.8 per 22 mm (ČSN 461011-7, 1988). To determine bread-making quality, wheat grains were ground to make a whole-grain meal using MILL 120 - a grinding mill of Perten Instr., and parameters were set as follows: protein content by Kjeldahl method (ISO 1871, 2009), Zeleny sedimentation value (ISO 5529, 2007), Hagberg falling number according to ISO 3093 (2004). The starch content was determined according to Ewers (ISO 10520, 1997).

The content was determined of individual protein fractions in the flour obtained by grinding grains using a laboratory mill CHOPIN. Extraction was done using a mixed solution (H₂O & Acetonitrile 3:1 w/v). The sample was subsequently shaken for 5 minutes using a vortex shaker. Then it was centrifuged for 10 minutes at 10,000 rpm. The content of protein fractions was measured by RP-HPLC (Bietz 1983, Wieser et al. 1987) using Vydac colon 218TP C18. The determination of selected anthocyanins for both wheat varieties was carried out according to the method of Abdell-Aal and Hucl (2003). The results were evaluated using STATISTICA 10.0 software (StatSoft, Inc.).

RESULTS AND DISCUSSION

The conditions during the crop year were highly benefitting for crop growth and development in both wheat varieties, which is reflected not only in grain yield, but also on the quality parameters of the grain. The purple wheat variety had statistically significantly lower yield than "Skorpion" variety (Table 4); grain in this wheat also features reduced mechanical properties, i.e. lower specific weight and smaller size, which is also reflected in significantly higher pass-through rates. Conversely, the starch and protein content in "Rosso" was higher compared to an average of all the variants, but not significantly. The quality of proteins was significantly lower than "Skorpion" on the basis of results of Zeleny test (Table 4).

Table 4 Average values of grain yield and quality parameters of both varieties

| Variety | Yield (t · ha ⁻¹) | Specific weight (kg · hl ⁻¹) | 2.8 mm (%) | 2.5 mm (%) | Pass-through (%) | Starch content (%) | Protein content (%) | Zeleny volume (ml) | Falling number (s) |
|----------|-------------------------------|--|--------------------|--------------------|-------------------|--------------------|---------------------|--------------------|--------------------|
| Rosso | 9.22 ^a | 73.48 ^a | 73.80 ^a | 21.10 ^b | 5.32 ^b | 68.20 ^a | 13.49 ^a | 38 ^a | 318 ^b |
| Skorpion | 10.40 ^b | 74.62 ^a | 88.60 ^b | 8.80 ^a | 2.84 ^a | 67.10 ^a | 13.38 ^a | 57 ^b | 296 ^a |

Note: values with different letters in the column differ significantly ($p < 0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

The grain yield achieved in most variants of fertilization was very high (Table 5). It was probably also the reason that neither the application of sulfur combined with nitrogen nor qualitative fertilization was manifest in grain yield. The highest yield observed was that of "Rosso", var. 3, where Yara Vita Thiotrac was applied by means of foliar nutrition, while for "Skorpion" the same was seen in the control variant (Table 6).

Table 5 Average values of grain yield and quality parameters of "Rosso" variety

| Variety | Yield (t · ha ⁻¹) | Specific weight (kg · hl ⁻¹) | 2.8 mm (%) | 2.5 mm (%) | Pass-through (%) | Starch content (%) | Protein content (%) | Zeleny volume (ml) | Falling number (s) |
|---------|-------------------------------|--|----------------------|---------------------|---------------------|--------------------|---------------------|--------------------|--------------------|
| 1 | 10.13 ^a | 73.97 ^a | 78.17 ^{bc} | 18.90 ^{ab} | 4.67 ^{ab} | 67.48 ^a | 13.13 ^a | 34 ^a | 308 ^a |
| 2 | 8.30 ^a | 73.95 ^a | 67.73 ^a | 24.90 ^b | 7.40 ^c | 64.58 ^a | 13.50 ^a | 37 ^{ab} | 323 ^a |
| 3 | 10.22 ^a | 76.00 ^a | 79.87 ^c | 16.43 ^a | 3.37 ^a | 68.20 ^a | 13.17 ^a | 37 ^{ab} | 325 ^a |
| 4 | 9.14 ^a | 74.73 ^a | 71.57 ^{abc} | 22.97 ^{ab} | 5.53 ^{abc} | 68.20 ^a | 13.53 ^a | 39 ^{ab} | 318 ^a |
| 5 | 8.25 ^a | 75.20 ^a | 68.57 ^{ab} | 24.47 ^{ab} | 6.90 ^{bc} | 67.48 ^a | 13.70 ^a | 40 ^b | 312 ^a |
| 6 | 9.45 ^a | 73.28 ^a | 77.03 ^{abc} | 18.70 ^{ab} | 4.33 ^a | 74.01 ^a | 13.43 ^a | 39 ^{ab} | 322 ^a |
| 7 | 9.07 ^a | 75.18 ^a | 73.50 ^{abc} | 21.20 ^{ab} | 5.03 ^{abc} | 67.48 ^a | 13.93 ^a | 38 ^{ab} | 319 ^a |

Note: values with different letters in the column differ significantly ($p < 0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

Specific weight was low in both variants, reaching 76 kg·hl⁻¹ only for "Rosso". There were not found significant differences between variants of both varieties. Foliar nutrition was not much reflected in the grain size save the treatment of spraying with the Yara Vita Thiotrac fertilizer which in the event variant 3 and variant 6 of the "Rosso" variety and variant 6 of the "Skorpion" variety was decreasing the percentage of small grains (pass-through rate). The highest starch content was determined for "Rosso", var. 6, where sulfur was applied during both the production and qualitative fertilization. For "Skorpion", the starch content was higher by about 3.64% to 5.82% compared to the control variant, i.e. var. 1, which however did not apply to variant 6. In "Rosso", the qualitative fertilization increased the protein content by 0.4% or even by 0.8% when combined with the production fertilizer using sulfur. For "Skorpion", any greater beneficial effect, i.e. an increase by 0.2%, was seen only for variant 6 treated with the combination of production and qualitative fertilization using sulfur (Yara Vita Thiotrac). The values of Zeleny test grew by 2.7–6.3 ml for "Rosso" and 1.0–1.7 ml for "Skorpion" (the variants fertilized with N₁S₁ only).

Table 6 Average values of grain yield and quality parameters of "Skorpion" variety

| Variety | Yield (t · ha ⁻¹) | Specific weight (kg · hl ⁻¹) | 2.8 mm (%) | 2.5 mm (%) | Pass-through (%) | Starch content (%) | Protein content (%) | Zeleny volume (ml) | Falling number (s) |
|---------|-------------------------------|--|--------------------|--------------------|-------------------|---------------------|---------------------|--------------------|--------------------|
| 1 | 10.95 ^c | 73.38 ^a | 89.67 ^a | 7.80 ^a | 2.67 ^a | 64.03 ^{ab} | 13.33 ^a | 57 ^a | 276 ^a |
| 2 | 10.29 ^{ab} | 73.30 ^a | 88.43 ^a | 8.77 ^a | 2.83 ^a | 69.12 ^{ab} | 13.30 ^a | 55 ^a | 325 ^a |
| 3 | 10.63 ^{bc} | 74.13 ^a | 89.93 ^a | 8.13 ^a | 4.03 ^a | 69.12 ^{ab} | 13.30 ^a | 55 ^a | 327 ^a |
| 4 | 9.93 ^a | 73.77 ^a | 87.50 ^a | 10.07 ^a | 2.40 ^a | 69.85 ^b | 13.33 ^a | 57 ^a | 299 ^a |
| 5 | 10.51 ^{abc} | 73.78 ^a | 87.47 ^a | 9.40 ^a | 2.97 ^a | 60.39 ^a | 13.27 ^a | 58 ^a | 287 ^a |
| 6 | 10.49 ^{abc} | 73.50 ^a | 89.53 ^a | 8.13 ^a | 2.10 ^a | 69.85 ^b | 13.53 ^a | 59 ^a | 284 ^a |
| 7 | 10.01 ^{ab} | 72.47 ^a | 87.43 ^a | 9.47 ^a | 2.90 ^a | 67.67 ^{ab} | 13.50 ^a | 59 ^a | 277 ^a |

Note: values with different letters in the column differ significantly ($p < 0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

The effect of different vegetation nutrition during the growing season on the representation of individual protein fractions was not very distinct; any considerable result was seen only for the "variety" factor (Table 7), where significant differences were observed.

"Skorpion" was the variety with significantly higher values of Zeleny sedimentation volume in comparison with "Rosso" (Table 4). The significantly higher content of HMW-GS was found in this variety as well (Table 7). The content of specific anthocyanins was also determined for additional information (Table 8, 9).

Table 7 Content of protein fractions (%)

| Variety | ∑Alb + Glo | ω-gliadins | LMW-GS + α, β-Gli | LMW-GS | HMW-GS | γ-gliadins |
|----------|--------------------|-------------------|--------------------|--------------------|--------------------|-------------------|
| Rosso | 10.36 ^b | 4.76 ^a | 50.75 ^a | 17.00 ^a | 10.35 ^a | 6.78 ^b |
| Skorpion | 8.44 ^a | 5.49 ^b | 52.20 ^b | 17.33 ^a | 11.16 ^b | 5.38 ^a |

Note: values with different letters in the column differ significantly ($p < 0.05$). ∑Alb + Glo: sum of albumins and globulins; LMW-GS + α, β-Gli: low-molecular weight subunits of glutenins and sum of α, β-gliadins; HMW-GS: high-molecular subunits of glutenins.

Table 8 Content of specific anthocyanins in the "Skorpion" variety (μg · g⁻¹)

| Cyanidin | Variant | | | | | | |
|------------|---------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Keracyanin | 9.91 | 13.37 | 12.08 | 11.31 | 10.38 | 10.59 | 9.98 |
| Kuromanin | 4.84 | 6.66 | 6.20 | 6.19 | 5.81 | 6.69 | 5.35 |
| Myrtilin | 4.90 | 6.98 | 6.31 | 6.22 | 5.83 | 6.15 | 5.28 |
| Peo 3-rut. | 1.18 | 1.75 | 1.52 | 1.51 | 1.41 | 1.38 | 1.24 |
| Tulipanin | 8.69 | 11.73 | 10.65 | 9.92 | 9.21 | 9.14 | 8.80 |
| ∑ | 29.54 | 40.55 | 26.78 | 35.15 | 32.64 | 34.01 | 30.66 |
| Rel. % | 100 | 137 | 91 | 119 | 111 | 116 | 104 |

Note: Peo 3-rut.: Peonidin 3-rutinosid

Table 9 Content of specific anthocyanins in the "Rosso" variety ($\mu\text{g} \cdot \text{g}^{-1}$)

| Cyanidin | Variant | | | | | | |
|-------------|---------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Calistephin | 0.18 | 0.14 | 0.27 | 0.23 | 0.22 | 0.21 | 0.19 |
| Keracyanin | 0.32 | 0.30 | 0.53 | 0.37 | 0.32 | 0.39 | 0.34 |
| Kuromanin | 1.51 | 1.52 | 2.61 | 2.01 | 1.87 | 2.04 | 2.11 |
| Peo 3-glu. | 0.71 | 0.75 | 1.36 | 1.08 | 0.97 | 1.00 | 1.08 |
| Σ | 2.72 | 2.75 | 4.94 | 3.69 | 3.39 | 3.68 | 3.72 |
| Rel. % | 100 | 101 | 182 | 136 | 124 | 135 | 137 |

Note: Peo 3-glu.: Peonidin 3-glucoside

The higher values of Zeleny sedimentation volume found for "Skorpion" can be interpreted as a higher bread-making quality of this variety (Dendy, Dobraszczyk 2001), the prerequisite being also confirmed with regard to the content of protein fractions in the flour obtained by milling the grain of this variety. Compared with "Rosso", "Skorpion" was found to have a significantly higher content of α -, β - and ω -gliadins as well as low-molecular weight subunits of glutenins and high-molecular weight subunits of glutenins, the fractions which are considered to be the carriers of bread-making quality of wheat (Gianibelli et al. 2001, Dendy, Dobraszczyk 2001).

"Skorpion" wheat variety had the highest content of keracyanin and tulipanin. Delphinidin-3-glucoside, however, was not determined although normally it is present to the greatest extent (Abdel-Aal et al. 2006). The highest level observed for "Rosso" was that of kuromanin and peonidin 3-glucoside. Similarly, however, cyanidin-3-glucoside the content of which tends to be the highest in this kind of wheat (Hosseinian et al. 2008) was not determined in this case. Nonetheless, we can conclude that the fertilization reflected in the content of anthocyanins, the content of which increased by up to 37.3% for "Skorpion" (var. 2) compared to the control variant (var. 1) within which no qualitative fertilization was carried out. Even a higher rate was determined for "Rosso", where the anthocyanin content increased by 1.0% to 81.5%. Based on the above the implementation of foliar nutrition can be expected to benefit higher antioxidant capacity of the harvested grain. Due to the fact that anthocyanins feature the capability of binding heavy metals while acting as inducers of hormones, i.e. glutathione-S-transferase and superoxide dismutase (Duthie et al. 2006, Manach et al. 2005), one can assume a higher resistance of plants to stress. The application of sulfur as a supplement and source for glutathione to form is expected to play its role as well.

CONCLUSION

The achieved results pointed to significant differences in the bread-making quality of the wheat types tested. The biggest problems, both of the varieties were shown to have involved the specific weight of grain; other quality parameters are acceptable in terms of bread-making application. Since the conditions of the crop year were highly favorable for grain yield, the differences between the fertilization variants were small. Positively evaluated can mainly be the effect of sulfur applied through Yara Vita Thiotrac as part of qualitative fertilization. Significantly higher contents of glutenins and gliadins were found for the "Skorpion" variety. The content of protein fractions and the values of the Zeleny sedimentation volume provide the grounds for expecting "Skorpion" to feature a higher bread-making quality compared with "Rosso".

ACKNOWLEDGEMENT

This work was supported by grant IGA FA MENDELU No. TP 4/2015.

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