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## THE INFLUENCE OF NITROGEN FERTILIZATION ON THE CONTENT OF STARCH, PROTEIN AND GLUTEN IN PERSPECTIVE GENOTYPES OF COLOURED WHEAT

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### Abstract

The content of starch, protein and wet gluten in the grain of 13 coloured wheat genotypes (*Triticum aestivum*) was analysed. The genotypes were grown at three levels of nitrogen nutrition and its effect on the observed parameters was statistically evaluated. The highest fertilisation level significantly increased protein/gluten content and decreased starch content, but certain differences were observed between genotypes.

### Introduction

Some cultivars of wheat (*Triticum aestivum* L.) have the ability to synthesize carotenoids (varieties with yellow endosperm) and anthocyanins (varieties with purple pericarp and blue aleurone) in the grain. The same carotenoids and anthocyanins are usually found in cereals as in vegetables and fruit, and their antioxidant effect has been shown in many papers to help prevent cardiovascular disease, diabetes, inflammation, cancer, obesity etc<sup>1</sup>. Most current wheat varieties have a grain color referred to as red, with white being less commonly seen in practice. The red color of the grain is determined by the presence of at least one of the three dominant alleles *R-A1b*, *R-B1b* and *R-D1b*. The white color of the grain is determined by the presence of the triplet recessive alleles *R-A1a*, *R-B1a* and *R-D1a*. In addition to these common colors, yellow endosperm, purple pericarp and blue grain aleurone may occur in wheat. The yellow endosperm is mainly due to the carotenoid, lutein or zeaxanthin. In poultry, their positive effect on yolk color has been demonstrated. In 2011, two varieties of wheat with yellow grain endosperm were registered in the Czech Republic. These are the winter variety Citrus and the spring variety Luteus. The surface of the grain with yellow endosperm is similar in color to that of the red grain. The purple color is due to the purple pericarp *Pp* genes, originally found in the tetraploid wheat *Triticum aethiopicum*, originating in Ethiopia. Donors for purple pericarp are now more widely available due to the orientation of some breeders towards this type of grain color. Known varieties include the spring wheat variety Konini (1981) from New Zealand, the winter varieties Indigo (2006), Rosso (2011) and Merlot (2015) registered in Austria, as well as the Slovak variety PS Karkulka (2014). In 2018, the variety AF Jumiko with purple grain was also registered. The blue coloration is caused by the genes for blue aleurone *Ba* (blue aleurone). The known donor is line UC66049, originating from California, which carries the *Ba1* gene. The blue color here comes from a wide hybridization with *Thinopyrum ponticum* Podp. and the genetic source Thatcher Blue carrying a gene *Ba2* from *Triticum boeoticum* is also used. The literature also indicates the existence of blue grain color in wheat from the estate of Erich von Tschermak-Seysenegg, which has been described as a separate taxon of *T. aestivum* var. *tschermakianum* Mansf. The winter wheat variety Skorpion (2012) was bred on the basis of this gene. In 2019, the AF Oxana variety with blue grain was registered. Three varieties of colored wheat were registered in Kroměříž between 2018 and 2021 – AF Jumiko (*Pp*), AF Oxana (*Ba*) and AF Zora (*Pp+Ba*).

The aim of this study was to assess the effect of nitrogen nutrition on grain composition and quality of colored wheat.

### Materials and methods

13 genotypes of colored wheat were grown on the experimental field in Cigliano (Italy) in the harvest year 2020. The crop management included three levels of nitrogen nutrition (0, 80 and 160 kg N/ha), individual treatments were grown in three field replications. The set of genotypes tested consisted of one control and four types of colored wheat.

Grain quality and chemical composition was evaluated using standard methods: crude protein content (CPC) (measured as the content of N-substances – N×5.75) according to EN 15948:2020 using Inframatic 9100, starch content according to Ewers polarimetric method (ISO 10520), the falling number (FN) was determined according to the ISO 3093:2009 using a Perten LM 3120 mill and 7 g of meal on 15% moisture basis, the sedimentation test (SEDI) was done according to the ISO 5529:2007 using a Brabender Sedimat mill, and the wet gluten content and gluten index was determined according to the ICC Standard No. 155.

Differences between genotypes and nitrogen levels were statistically evaluated using analysis of variance (ANOVA) followed with Tukey HSD's post hoc test. All calculations were performed using the software package Statistica 14 (TIBCO Software Inc.). The significance level was set at  $p < 0.05$  unless stated otherwise.

### Results

Genotype and fertilization level had significant effects on starch content, protein content, both wet gluten content and quality, and protein quality as expressed by the Zeleny sedimentation test (Table II). Wheat grain grown without added nitrogen (0 kg/ha) and with a nitrogen rate of 80 kg/ha had, on average across all varieties, all these monitored parameters statistically comparable, but a significant difference was found at a N rate of 160 kg/ha. Fertilization with N 160 kg/ha resulted in lower starch content, higher protein and gluten content with lower gluten index, higher SEDI and FN. Genotypes KM 72-18, AF Zora

and Skorpion had the highest average protein and gluten content, while Aubusson and AF Jumiko had the lowest protein content and Aubusson and Rosso had the lowest gluten content (Fig. 1). The gluten index values ranged from 65 to 97, separating the varieties with GI greater and less than 80. Aubusson (69.7%) and AF Zora (56.7%) had the highest and lowest starch content, respectively. Starch content was negatively correlated with both protein ( $r = -0.54$ ) and gluten ( $r = -0.48$ ) content and positively correlated with gluten index ( $r = 0.44$ ). The Zeleny test correlated positively with protein ( $r = 0.57$ ) and gluten ( $r = 0.40$ ) content, but no correlation was found with starch content. FN did not correlate with any parameter.

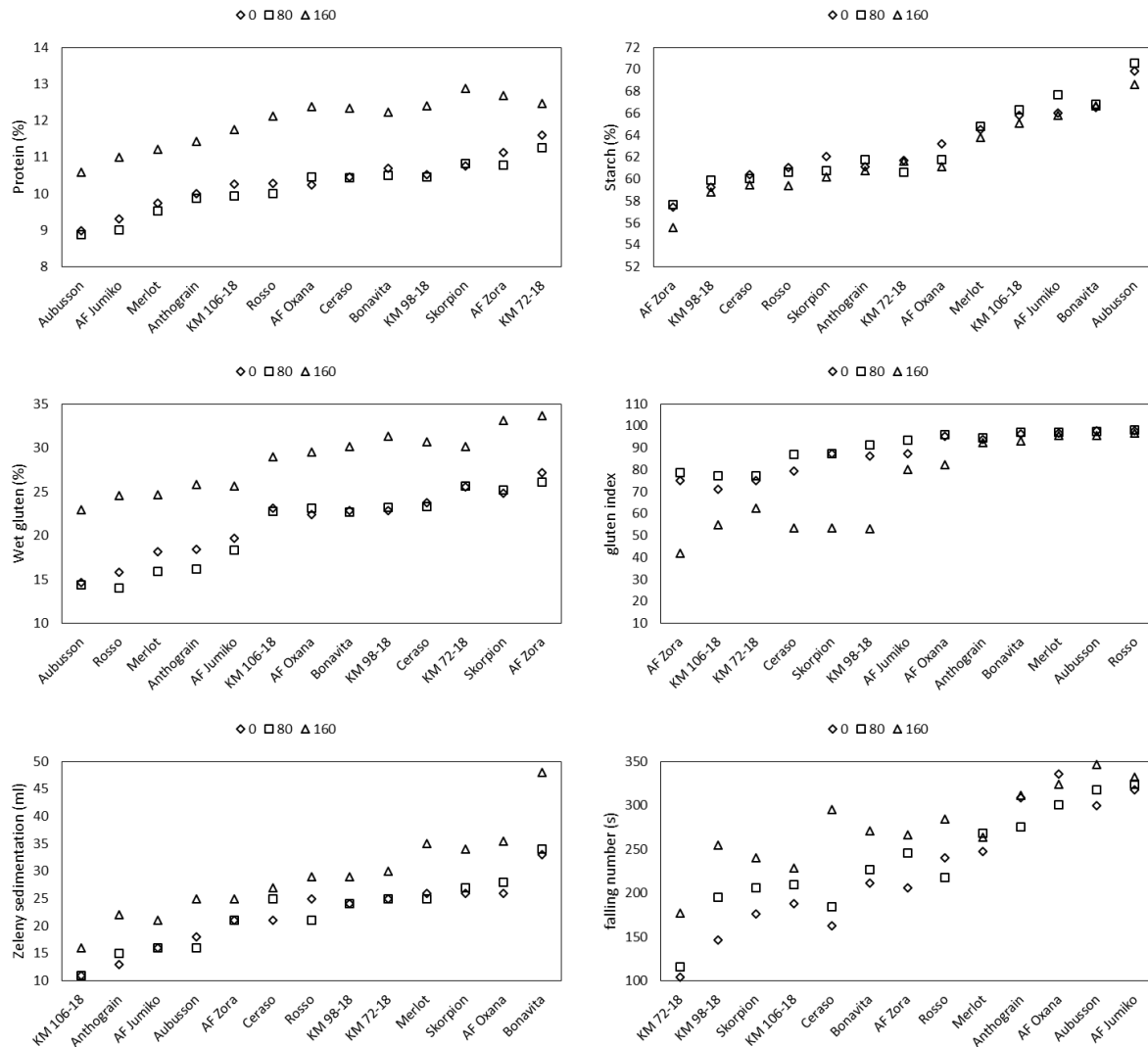


Fig. 1. Comparison of protein, starch, wet gluten, gluten index, Zeleny sedimentation and falling number of 13 genotypes at three levels of nitrogen nutrition, ordered by mean

## Discussion

Wheat quality is influenced by genotype, environment, and their interactions. The main environmental factor is the weather during the growing season, but the availability of individual nutrients also has a clear influence on grain quality. Of all the nutrients, grain quality is most sensitive to nitrogen, because nitrogen is the main component of protein and its availability is the main factor influencing protein concentration in grain. Increasing soil nitrogen will generally increase the protein content of the grain, but the timing of nitrogen application also has a significant effect on protein concentration. The earlier the nitrogen is applied, the higher the yield and the lower the protein concentration, and vice versa<sup>2</sup>. Wheat varieties differ in their ability to convert soil nitrogen into grain protein. Varieties targeting higher protein content tend to have lower yields, while varieties targeting higher yields tend to have lower protein content.

Table I  
List of genotypes with year of registration

Colour	Genotypes
Standard	Aubusson (2002)
Purple	Anthograin, Ceraso (2014), AF Jumiko (2018), KM 106-18 (elongated glumes), Merlot (2015), Rosso (2011)
Blue	Skorpion (2011), AF Oxana (2019), KM 72-18
Black	AF Zora (2021), KM 98-18
Yellow	Bona Vita (2011)

Table II  
Analysis of variance (*F* ratio) of quality parameters in 13 cultivars of wheat with coloured grain grown at different nitrogen fertilization levels

	Df	CPC	Starch	WG	GI	SEDI	FN
Nitrogen nutrition	2	65***	265***	71***	48***	36***	18***
Genotype	12	600***	27***	419***	118***	55***	17***

Df – degree of freedom, CPC – crude protein content, WG – wet gluten content, GI – gluten index, SEDI – sedimentation test (Zeleny), FN – falling number

The results obtained in this study for a set of colored wheat genotypes are consistent with the findings and patterns known for common wheat genotypes. A positive correlation was found between protein content, wet gluten and nitrogen nutrient doses. In addition to the N supplied as pure nutrients, N from soil reserves is also used for protein synthesis in the grain, which may account for the fact that the observed parameters were comparable when grown without N supply and at a rate of 80 kg/ha.

The Hagberg falling number is one of the most important quality characteristics of wheat. Nitrogen fertilization rate has been seen to both increase and decrease the HFN of wheat<sup>3</sup>. This is because the effect of nitrogen fertilization on FN is not direct and several factors play a role. Higher levels of nitrogen fertilization can cause lodging, which in itself leads to a reduction in FN. On the other hand, higher levels of nitrogen can increase the ripening period and it has been found that under optimum conditions, gradually longer ripening leads to higher FN. Some literature reports that higher nitrogen doses reduce alpha-amylase activity and therefore increase FN, which is consistent with our results. However, the influence of fertilization is regarded to be lower than the effects of cultivar and climatic conditions<sup>4</sup>. Gooding *et al.*<sup>5</sup> found that different wheat cultivars exhibited a significant difference in FN. This is also confirmed by our results, where, when grown under identical conditions, the difference between FN for the genotype with the lowest (132 s) and highest (325 s) value was almost 200 s. However, no correlation between grain protein content and FN was found in our experiment, in contrast to the work of Ayoub *et al.*<sup>6</sup>, who stated that a rise in the protein content in grain is associated with an increase in FN, which shows the strong influence of variety in our case. The starch content of the grain in the set of genotypes studied was in the range usual for wheat, given by e.g. Prugar and Hraška<sup>7</sup> from 50 to 70%.

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