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Effects of Elevated Atmospheric CO_2 Concentrations on the Quantitative Protein Composition of Wheat Grain

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The continuing increase in atmospheric CO_2 concentration is predicted to enhance biomass production and to alter biochemical composition of plant tissues. In the present study, winter wheat (*Triticum aestivum* L. cv. 'Batis') was grown under ambient air (BLOW, CO_2 concentration: 385 μL L^{-1}) and free-air CO_2 enrichment (FACE, CO_2 concentration: 550 μL l^{-1}) and two different nitrogen (N) fertilization levels (normal N supply: N100, 50% of normal N supply: N50). Mature kernels were milled into white flour and analyzed for the contents of crude protein, Osborne fractions, single gluten protein types and glutenin macropolymer. Elevated CO_2 caused significant reductions in crude protein and all protein fractions and types ($p < 0.001$) except albumins and globulins. Effects were more pronounced in wheat samples supplied with normal amounts of N fertilizer. Crude protein was reduced by 14% (N100) and 9% (N50), gliadins by 20% and 13%, glutenins by 15% and 15% and glutenin macropolymer by 19% and 16%, respectively. Within gliadins, ω5-gliadins (−35/−22%) and ω1,2-gliadins (−27/−14%) were more affected than α-gliadins (−21/−13%) and γ-gliadins (−16/−12%). Within glutenins, HMW subunits (−23/−18%) were more affected than LMW subunits (−12/−15%). According to these results, flour from high CO_2 grown grain will have a diminished baking quality. To our knowledge, these are the first results of elevated CO_2 concentrations impacts on wheat grain protein composition gained under relevant growing conditions at least for Central Europe.

KEYWORDS: Wheat; flour proteins; atmospheric CO_2; FACE; nitrogen fertilization; *Triticum aestivum*

INTRODUCTION

Under the prospects of climate change and the growing world population a major challenge for global nutrition in the next decades is to increase food yield in a sustainable manner, while maintaining its end use value. Of particular importance is the rapid rise of the atmospheric carbon dioxide concentration, which is expected to reach 550 μL L^{-1} from currently 385 μL L^{-1} by the middle of the century (*1*). Elevated CO_2 concentrations have been found to have direct effects on photosynthesis and to increase growth and biomass of crop plants (*2, 3*). Concomitantly to an increased biomass production, a frequently observed phenomenon is that plants grown in CO_2 enriched air exhibit significant changes of their chemical composition (*4–6*). One of the most often observed consequence of CO_2 enrichment is a decrease of the foliar nitrogen (N) concentration and also of the N content of seeds and grains (*4, 7, 8*), which has been summarized in a recent review (*9*). Such changes may have serious ecological, economic and nutritional consequences.

With respect to cereals and particularly wheat, bread-making quality might be changed as a consequence of a CO_2 induced reduction of grain N and protein. Grain protein concentration and composition are major determinants of grain nutritional value (*10*) as well as of flour functional properties (*11, 12*). Wheat flour protein consists of albumins and globulins (around 20%) and gluten proteins (around 80%). While albumins and globulins are mainly metabolic proteins, gluten proteins are storage proteins and responsible for the unique baking properties forming a viscoelastic network (gluten) during dough mixing. Their amount is highly correlated with baking performance (crumb structure, bread volume). Flour protein content correlates with bread volume (*11*) and determines the price of bread wheat at least in Germany. Gluten proteins can be divided into two main fractions according to their solubility in aqueous alcohols:

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Table 1. Crude Protein Content (Percent) and Relative Amounts (AU per Milligram) of Protein Fractions and Types As Affected by Nitrogen (N50/N100) and CO_2 Supply (BLOW/FACE)[a]

conditions	CP	ALG	GLI	ω	ω
N50/BLOW	6.70 ± 0.38 (12) a	230 ± 6 (4) a	509 ± 33 (8) a	18 ± 3 (8) a	21 ± 2 (8) a
N50/FACE	6.08 ± 0.25 (12) b	223 ± 6 (4) a	443 ± 27 (8) b	14 ± 1 (8) b	18 ±2 (8) b
N100/BLOW	9.12 ± 0.23 (11)* c	241 ± 12 (4) a	784 ± 48 (8) c	43 ± 6 (8) c	41 ± 5 (8) c
N100/FACE	7.82 ± 0.28 (12) d	231 ± 4 (4) a	628 ± 45 (8) d	28 ± 4 (8) d	30 ±3 (8) d

conditions	α	γ	GLU	$\omega\beta$	HMW
N50/BLOW	205 ± 17 (8) a	265 ± 16 (8) a	302 ± 16 (7)* a	8 ± 1 (8) a, c	66 ± 7 (8) a
N50/FACE	178 ± 15 (8) b	233 ± 13 (8) b	256 ± 17 (8) b	7 ± 1 (8) a	54 ± 5 (8) b
N100/BLOW	331 ± 31 (8) c	369 ± 4 (6)* c	425 ± 22 (8) c	14 ± 2 (8) b	115 ± 11 (8) c
N100/FACE	261 ± 27 (8) d	309 ± 12 (8) d	360 ± 28 (8) d	11 ± 2 (8) c	89 ± 10 (8) d

conditions	LMW	GLUT	SSP	GMP
N50/BLOW	228 ± 10 (7)* a	811 ± 48 (8) a	891 ± 53 (8) a	132 ± 4 (8) a
N50/FACE	195 ± 12 (8) b	699 ± 19 (8) b	781 ± 13 (6)* b	111 ± 5 (7)* b
N100/BLOW	296 ± 6 (6)* c	1209 ± 66 (8) c	1422 ± 55 (8) c	204 ± 4 (5)* c
N100/FACE	260 ± 8 (6)* d	988 ± 70 (8) d	1181 ± 93 (8) d	166 ± 5 (6)* d

[a] Mean values (on an "as is" basis) ± standard deviation of (*n*) measurements (one (ALG), two (all other fractions) and three (CP) determinations of four corresponding plots each); * outliers ($p = 0.05$) were eliminated (*29*). Values followed by the same letter within each column are not significantly different ($p < 0.01$, one-way ANOVA). Abbreviations: AU, absorbance units of HPLC; CP, crude protein; ALG, albumins/globulins; GLI, total gliadin; ω5, ω1,2, α, γ, and ωb, gliadin types; GLU, total glutenin; HMW, LMW, high and low molecular weight subunits; GLUT, total gluten proteins (gliadin + glutenin); SSP, SDS-soluble proteins; GMP, glutenin macropolymers.

the soluble gliadins and the insoluble glutenins (*13*). Both fractions consist of numerous, partially closely related protein components characterized by high glutamine and proline contents. Gliadins are mainly monomeric proteins with molecular weights in a range from 28,000 to 55,000 and can be classified according to their primary structures into ω5-, ω1,2-, α-, and γ-gliadins. The glutenin fraction comprises aggregated proteins linked by disulfide bonds. The largest glutenin aggregates termed "glutenin macropolymers" contribute significantly to dough properties; their amounts are strongly correlated with dough strength and bread volume (*11*). Glutenin subunits have been divided into high and low molecular weight types with molecular weights around 80000 and 33000, respectively. Additionally, the glutenin fraction contains small amounts of glutenin-bound ω (ωb)-gliadins. The α-, γ-, and low molecular weight types are major components of gluten with proportions of around 20−30% each, whereas ω5-, ω1,2-, ωb-, and high molecular weight types occur in much lower proportions (2−10% each). Amounts and proportions of gluten protein types in grain and flour are strongly influenced by genotype (variety) and fertilization. For example, the effect of N fertilization on gliadins is more pronounced than on glutenins; the proportions of ω5-, ω1,2-, and high molecular weight types are increased and those of γ- and low molecular weight types are decreased by high N levels (*14*).

Existing reports on the effects of elevated CO_2 concentrations on wheat grain quality are contradictory. Little or no changes of wheat quality were reported by two studies (*15, 16*). In contrast to this, significant decrease in the protein concentration along with reduced nutritional and breadmaking quality of wheat grown under increased CO_2 levels was found by several authors (*17–22*). In a growth chamber experiment with three winter wheat varieties, variable responses of quality parameters (crude protein, gluten content) to CO_2 enrichment were found (*23*). While nearly all the above-mentioned studies were conducted in some type of chamber environment, hardly any studies have been done under relevant agronomic conditions. A result of a free-air CO_2 enrichment (FACE) experiment from Arizona, USA revealed only a slight decrease of spring wheat grain protein at ample supply of soil N (*24*). However, in this study, high CO_2 treatment exacerbated deleterious effects of low soil N on grain protein. Based on this limited information it is a matter of debate, to what extend future CO_2 concentration will affect grain quality of cereals. Until today, there are no field studies addressing this question for European cereal growth conditions.

The present paper describes results of a field study with a German winter wheat cultivar exposed to elevated CO_2 concentrations under field conditions (FACE) in the course of a crop rotation. Effects of CO_2 enrichment on crude protein, Osborne fractions, single gluten protein types and glutenin macropolymer were examined. To our knowledge, this is the first report addressing CO_2 enrichment effects on wheat grain protein composition under realistic European growing conditions.

MATERIALS AND METHODS

Plant Cultivation and Free-Air CO_2 Enrichment. Winter wheat was grown on an experimental field site located at the Johann Heinrich von Thunen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, in Braunschweig, Germany, within a crop rotation consisting of winter barley, sugar beet and winter wheat. Agricultural management of the field was conducted according to local farm practices. Details of the soil conditions have been described elsewhere (*25*). Elevated CO_2 concentration was provided by a free-air CO_2 enrichment (FACE) system consisting of fumigation rings, each of 20 m in diameter, engineered by Brookhaven National Laboratory (*25, 26*). Treatments included two rings equipped with blowers and CO_2 enrichment (FACE), two controls operated with blowers at ambient air CO_2 level (BLOW) and two additional rings operated without blowers at ambient air CO_2 level (AMBI). The target CO_2 concentration in the FACE rings was 550 μL L^{-1} during daylight hours. No-enrichment criteria for CO_2 fumigation were wind speeds >6 m s^{-1} or air temperatures <5 °C. In order to investigate possible interactions between CO_2 enrichment and N supply on the plants, N fertilization was restricted to 50% (N50) of adequate N (N100) in each half of the rings. Total mineral N added to the experimental areas amounted to 84 kg N ha^{-1} (N50) and 168 kg N ha^{-1} (N100), respectively. For the sampling, each

half of a ring was additionally divided into two quarter sections resulting in 4 sampling plots per CO_2 and N treatment. The winter wheat cultivar 'Batis', which is one of the most frequently cultivated bread wheat varieties in Germany, was sown on October 26, 2004, in east−west rows spaced 0.12 m with a seeding density of about 360 plants m^{-2}. Mineral nutrients were added according to local fertilizing practices based on soil nutrient analysis in early springtime. Nitrogen fertilizer was added as urea/ammonium nitrate in March and two times in May with the last application ten days before anthesis (May 26). CO_2 enrichment was started on January 12, 2005, when plants were at the one leaf stage, and stopped on July 20, 2005 (one week before grain maturity). In order to avoid interaction effects of elevated CO_2 concentration and drought stress, the field was irrigated using a linear irrigation system to keep the soil water content above 50% of field capacity.

Grain Chemical Analysis. At grain maturity, plant samples were taken from a ground area of 1.0 m^2 in each quarter circle of the six rings resulting in four samples per CO_2 and N treatment. The harvested plant material was separated in straw, chaff and grains. Subsequently, kernels which did not strongly differ in individual weight between treatments were milled into white flour using a laboratory mill (Brabender, Duisburg, Germany) and sieved through a 0.2 mm sieve (Retsch, Haan, Germany). The crude protein content (N × 5.7) was determined according to Dumas (ICC standard method 167) using an FP-328 combustion instrument (Leco, Kirchheim, Germany). The average coefficient of variation derived from three determinations was ± 0.5%. For the quantitation of flour protein fractions and types, the following modified Osborne fractionation (*27*) was performed: Nondefatted flour (100 mg) was extracted stepwise with 0.4 mol L^{-1} NaCl + 0.067 mol L^{-1} $HKNaPO_4$, pH 7.6 (2 × 1.0 mL) for 10 min at ~20 °C (albumins/globulins), with 60% (v/v) ethanol (3 × 0.5 mL) for 10 min at ~20 °C (gliadins) and with 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol L^{-1}, pH 7.5), urea (2 mol L^{-1}), and 1% (w/v) dithioerythritol (2 × 1.0 mL) for 20 min at 60 °C under nitrogen (glutenins). For the determination of SDS-soluble and SDS-insoluble proteins, nondefatted flour (100 mg) was extracted stepwise with 1% (w/v) SDS + 0.05 mol L^{-1} sodium phosphate, pH 6.9 (2 × 1.0 mL) for 30 min at ~20 °C (*28*). Each extraction step was initiated with vortexing for 2 min at ~20 °C and continued with magnetic stirring. The suspensions were then centrifuged for 20 min at 6.000*g* and ~20 °C using a C412 centrifuge (Jouan, Dreieich, Germany). The corresponding supernatants of Osborne fractionation were combined and diluted to 2.0 mL with the respective extraction solvents. Supernatants of SDS extraction were combined, dried under a stream of N_2 and dissolved in 50% (v/v) 1-propanol (2 mL) containing Tris-HCl (0.05 mol L^{-1}, pH 7.5) and 1% (w/v) dithioerythritol under magnetic stirring for 20 min at 60 °C under nitrogen. The residue was extracted according to the glutenin extraction of Osborne fractionation (see above).

Aliquots (~0.5 mL) of the extracts were filtered through a 0.45 μm membrane and used for HPLC analysis under the following conditions (*27*): Instrument, solvent module 126 with a System Gold software (Beckman, Munich, Germany); column, Nucleosil 300-5 C_8, 4.6 × 240 mm (Macherey-Nagel, Düren, Germany); temperature, 50 °C; injection, 150 μL (albumins/globulins, glutenins), 100 μL (gliadins), 50 μL (SDS-solubles) or 200 μL (SDS-insolubles). 500 μL of 0.1% (v/v) trifluoroacetic acid (TFA) were injected before and after sample injection; elution system, A) TFA (0.1%, v/v), B) acetonitrile (99.9%, v/v) + TFA (0.1%, v/v); linear gradients, 0 min 20% B, 20 min 60% B (albumins/globulins), 0 min 28% B, 50 min 56% B (other protein fractions); flow rate, 1.0 mL min^{-1}; detection, UV absorbance at 210 nm; integration, System Gold. Albumins/globulins were determined by a single measurement. All other fractions were determined by two measurements (extraction + HPLC). The average coefficients of variation were ± 2.3% (gliadins), ± 2.6% (glutenins), ± 2.6% (SDS-solubles) and ± 1.9% (SDS-insolubles), respectively. Statistic evaluations of the data of the protein analyses were made with Slide Write Plus (Advanced Graphics Software Inc., Carlsbad, CA). Outlier tests ($p = 0.05$) were performed according to Gottschalk and Kaiser (*29*).

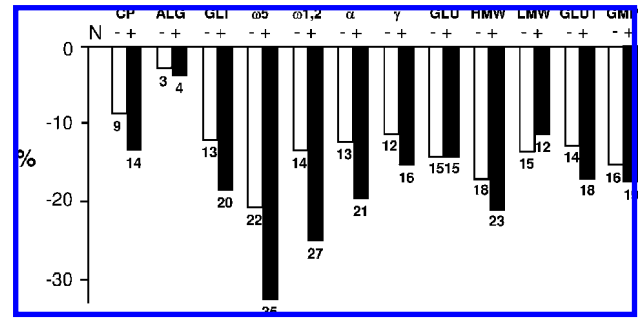

Figure 1. Percentage reduction of the amounts of protein fractions and types by elevated CO_2 (FACE) compared with ambient CO_2 (BLOW = 0%) (N− = N50, N+ = N100; for abbreviations see **Table 1**).

RESULTS

The relative amounts of protein fractions and types were derived from HPLC absorbance units (AU) determined at a wavelength of 210 nm, which had been shown to be strongly correlated with the amount of proteins (*27*). Statistical evaluation of quantitative data revealed a high correlation between the crude protein content and the sum of albumins/globulins, total gliadin and total glutenin ($r = 0.989$). Only a few outliers ($p = 0.05$) were identified, when all values from four corresponding plots were combined. Thus, only the combined mean values are discussed in the following. Significant differences between AMBI and BLOW samples could not be detected showing that blowers activated during day light had no additional effect. Therefore, only BLOW and FACE samples are compared in the Tables. **Table 1** summarizes the effects of nitrogen and CO_2 supply on the contents of crude protein and protein fractions. Different N fertilization had a strong influence on the content of crude protein and on the amounts and proportions of total gliadin and total glutenin and their protein types. Comparing N50 with N100 samples, crude protein increased by 36% in BLOW and by 29% in the FACE treatment. The effect on total gliadin (BLOW: +54%, FACE: +42%) was more pronounced than on total glutenin (+41/+41%), whereas albumins/globulins (+5/+4%) were scarcely influenced. Glutamine-rich proteins such as ω5-gliadins (+139/+100%) and ω1,2-gliadins (+95/+67%) were more sensitive to N fertilization than proteins with lower glutamine contents such as γ-gliadins (+39/+33%).

With the exception of albumins/globulins, elevated CO_2 concentration had strong significant effects ($p < 0.001$) on

Table 2. Proportions (Pecent) of Gluten Protein Types and Ratios of Gliadins to Glutenins and Low to High Molecular Weight Glutenin Subunits As Affected by Nitrogen (N50/N100) and CO_2 Supply (BLOW/FACE)[a]

conditions	GLI	ω5	ω1,2	α	γ	GLU	ωb	HMW	LMW	GLI/GLU	LMW/HMW
N50/BLOW	62.8 a	2.2a	2.6 a	25.3 a	32.7 a	37.2 a	1.0 a	8.1 a	28.1 a	1.69 a	3.45 a
N50/FACE	63.4 a	2.0a	2.6 a	25.5 a	33.3 a	36.6 a	1.0 a	7.7 a	27.9 a	1.73 a	3.61 a
N100/BLOW	64.9 a	3.6b	3.4 b	27.4 b	30.5 b	35.1 a	1.1 a	9.5 b	24.5 b	1.84 b	2.57 b
N100/FACE	63.6 a	2.8c	3.1 c	26.4 a, b	31.3 b	36.4 a	1.1 a	9.0 c	26.3 c	1.74 a	2.92 c

[a] Values followed by the same letter within each column are not significantly different ($p < 0.01$, one-way ANOVA). For abbreviations see **Table 1**.

crude protein and protein fractions and types resulting in a remarkable reduction of protein amounts (**Table 1**). In most cases, this reduction was more pronounced in samples with higher N supply (N100). Crude protein was lowered from 9.12 to 7.82% (N100) and from 6.70 to 6.08% (N50). The amount of total gliadin was reduced from 784 to 628 AU (N100) and from 509 to 443 AU (N50) and that of total glutenin from 425 to 360 AU (N100) and from 302 to 256 AU (N50). **Figure 1** summarizes percentage losses of crude protein and the different protein fractions and types. Differences between total gliadin (−13/−20%) and total glutenin (−15/−15%) were only small on the N50 level and more pronounced on the N100 level. However, no distinct differences were found between the AMBI and BLOW treatment, so the BLOW treatment data was chosen for comparison with the FACE data. Summarizing total gluten proteins (gliadin + glutenin), the reduction (−14/−18%) was much more pronounced than that of crude protein (−9/−14%), because the latter contained albumins/globulins (−3/−4%) and nonprotein N compounds that might be not affected by elevated CO_2. The effect on glutenin macropolymers (−16/−19%) was somewhat stronger than on total gluten proteins. Single gluten protein types were differently affected by elevated CO_2. Within the gliadin types, the reduction of ω5-gliadins (−22/−35%) and ω1,2-gliadins (−14/−27%) was higher than that of α-gliadins (−13/−21%) and γ-gliadins (−12/−16%). Within the glutenin types, high molecular weight subunits (−18/−23%) were more affected than low molecular weight subunits (−15/−12%). Glutenin-bound ω (ωb)-gliadins (−12/−21%) were less reduced than ω5- and ω1,2-gliadins. The proportions of gluten protein types were scarcely influenced by elevated CO_2 at the N50 level (**Table 2**), whereas the N100 level revealed significant effects. The proportions of ω5-, ω1,2-gliadins and high molecular weight subunits decreased and those of low molecular weight subunits increased. The ratio of total gliadin to total glutenin was lowered and that of low to high molecular weight subunits was enhanced.

DISCUSSION

In the present study, the effects of different CO_2 and nitrogen supply on the content and composition of proteins in winter wheat were investigated. Crude protein content was quite low even under nitrogen supply conventional for Central Europe (N100, 168 kg ha^{-1}). This may have resulted from the moderate nitrogen supply, the low mineralization rate of soil nitrogen (*25*) and the high grain yield obtained, which was more than 8 tons per hectare. In other European wheat growing areas, N fertilization may vary between less than 10 kg ha^{-1} in Bulgaria and 190 kg ha^{-1} in The Netherlands (*30*). Our findings for the effect of N fertilization on protein composition under the present CO_2 concentration are in agreement with previous studies (*14, 31*) showing that albumins/globulins were scarcely influenced, while proteins with a high glutamine content showed the greatest response (*14*). The elevated CO_2 concentration resulted in a decrease of crude protein content of grain which corresponds to several previous studies conducted on different crops and cultivars (*8, 9, 17, 19–22, 24*). However, our results also demonstrate that CO_2 enrichment changes the proportion of the different protein fractions and types in the flour. While the amount of albumins/globulins was unaffected by the CO_2 treatment, the gluten proteins were lowered more than overall protein level (**Figure 1**). Moreover, it turned out that ω5-gliadins, ω1,2-gliadins, α-gliadins and high molecular weight subunits were decreased by more than 20% in the N100 treatment. This result demonstrates that the CO_2 effect was closely linked with the different glutamine contents of the proteins (*13*) and thus, with the N requirement of proteins.

Numerous studies described in literature have shown that differences in amounts and proportions of gluten protein fractions and types have significant effects on dough properties (*32*). For example, the strength of dough was shown to be determined positively by the amount of total glutenin and negatively by the ratio of total gliadin to total glutenin (*33*). Because both total glutenin and the ratio of total gliadin to total glutenin were reduced by the elevated CO_2 concentration, it is difficult to estimate the effects on rheological dough properties. One of the most important characteristics for baking quality is bread volume, which has been shown to be strongly correlated with crude protein, total gluten proteins and glutenin macropolymers (*11*). All of these parameters were significantly reduced by CO_2 enrichment. A strong linear relationship exists between bread volume and a wide range of crude protein (10−18%). The results of the present study suggest that under future CO_2 concentrations crude protein content of flour will be clearly lowered. Consequently, a reduction of bread volume can be expected and it will be even more pronounced, when total gluten proteins and glutenin macropolymers are considered instead of crude protein due to their higher sensitivity to elevated CO_2 levels. It has been shown that this CO_2 effect might be counterbalanced by an excessive N fertilization (*24*). According to this, the extrapolation of data obtained for N50 and N100 levels indicates that around twice as much N fertilizer has to be supplied in midcentury in order to get wheat flours with high baking quality. This will be associated with higher costs, reduced nitrogen-use efficiency, an increase in environmental impact and will be almost impossible for organic farming. The nitrogen-use efficiency (NUE) is the product of N uptake efficiency and N utilization efficiency (*34*). The latter can be defined as the ratio between grains or above ground dry weight and grain or total plant N. Under elevated CO_2 concentrations, N utilization efficiency is generally increased as found in the present study and in many previous ones (see, e.g., refs *5, 7*, and *22*). N uptake efficiency (i.e., the ratio of total plant N and soil available N) was not affected by CO_2 enrichment in the present study, since at grain maturity soil mineral N content was similar among the CO_2 treatments (A. Pacholski, personal communication). However, N uptake efficiency is known to decrease with increasing N supply (*34*) and this effect outweighs the positive CO_2 effect on N utilization efficiency by far resulting in a decrease in NUE. There is evidence that the reduction in N content by elevated CO_2 concentrations can not completely be counterbalanced by an excessive N fertilization (*4, 19, 22*). Thus, breeding of high protein varieties (crude protein content ∼18%) could be an alternative strategy to prevent the impairment of baking quality. The present results clearly demonstrate the negative impact of CO_2 enrichment on wheat grain quality. However, only a single wheat cultivar was examined in one place and year. To get a more comprehensive picture of anticipated reductions in relevant grain proteins, it is necessary to conduct studies at different places and with further wheat cultivars.

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