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Effect of N-fertilization, fungicide treatment, seed density and abiotic stress factors on the total ß-glucan content of six-rowed winter barley (*Hordeum vulgare L.*)

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Summary

In Germany, the first six-rowed waxy winter barley cultivar (cv.) 'Waxyma' was registered in 2008. Besides changes in starch composition, waxy barley is rich in ß-glucans offering new applications in the food industry as ß-glucans reduce the blood cholesterol level. To investigate the stability of the ß-glucan content, cv. 'Waxyma', three waxy breeding lines and the non-waxy cv. 'Lomerit' were grown in field trials, shelter-, green house- and growth chamber experiments. Besides this, 'Waxyma' was grown in field trials at varying nitrogen levels and different seed rates, both with and without fungicide treatment. Waxyma showed a significant increase of the ß-glucan content under optimal nitrogen fertilization and fungicide treatment, but the influence of the seed density was not significant. Under shelter and greenhouse conditions, the influence of drought stress during grain filling was analyzed. The increase of the ß-glucan content under drought stress in the shelter and under rising temperatures in growth chambers was only significant for the non waxy cv. 'Lomerit' while no influence of drought stress was observed under green house conditions. In summary, the high ß-glucan content of cv. 'Waxyma' seems to be relatively stable with respect to growing conditions making it a suitable raw material for human nutrition.

Introduction

Barley (*Hordeum vulgare L.*) is an old crop for the brewing and feed industries. But high amounts of ß-glucans, especially soluble ß-glucans, which are the major soluble fiber component in cereals in which barley and oat are especially rich (AMAN and GRAHAM, 1987), cause problems in brewing and in animal nutrition (MACGREGOR et al., 1993). Comparisons of oat and barley revealed a similar (LEE et al., 1997) or even higher ß-glucan content in barley than in oat (JEROCH et al., 1999; STROBEL et al., 2001). Due to the high ß-glucan content, which is supposed to have a positive effect on human health, barley is becoming more important in human nutrition (ALMINGER et al., 2008), also in developed countries (GÜLER, 2003). ß-Glucans are valuable fibers which can lower the blood cholesterol level and reduce the risk of coronary heart disease. Therefore, EFSA released a health claim for barley (EFSA, 2011). WOOD et al. (2003) found a higher ß-glucan content in waxy barley (6.1 %, having a high amylopectin content of 95 - 100 % in starch) than in conventional barley (4.5 % with approximately 75 % amylopectin content in starch). The same fact, that waxy cultivars of barley generally have a higher ß-glucan content is reported e.g. in spring waxy barley cv. 'Sindy' and cv. 'Magdalena' compared with normal cv. 'Annabell', 'Kinnan', 'Iver' and 'Tyra' at different temperatures during grain filling (ANKER-NILSSEN et al., 2008) and in winter waxy barley cv. 'Waxyma' compared with normal winter barley (DIECKMANN et al., 2009).

The ß-glucan content in barley is mainly determined by the genotype, but to some extent is also influenced by environmental factors (STUART et al., 1988; ZHANG et al., 2001). The ß-glucan content in barley cultivars varies between 3.5 % in Spain (PEREZ-VENDRELL, 1996) to around 4.5 % in Canada (NARASIMHALU, 1995) and China (ZANG et al., 2002). Besides this, the ß-glucan content in barley is influenced by nitrogen fertilization (GÜLER et al., 2003; JACKSON et al., 1994), temperature during the growing period (ANKER-NILSSEN et al., 2008) and drought stress (COLES et al., 1991). In contrast to this, no impact of nitrogen fertilization on the ß-glucan content was detected in oat (SAASTAMOINEN et al., 2004).

The objective of this study was therefore (i) to determine the effect of different agricultural measures (N-fertilizer, seed rate and fungicide treatment) on the ß-glucan content of the German waxy barley cultivar 'Waxyma' in field trials and (ii) to analyze the effect of abiotic stress factors (drought stress and different temperatures during the grain filling period) on the ß-glucan content on different cultivars in shelter-, greenhouse- and growth chamber experiments in order to get information on the stability of the high ß-glucan content in waxy barley which is important with respect to human nutrition.

Materials and methods

Plant material

Seeds of cv. 'Lomerit' (non-waxy) and cv. 'Waxyma' (waxy) as well as three waxy breeding lines were supplied by Dieckmann GmbH & Co. KG (Nienstädt, Germany).

In order to ascertain that in all experiments with a limited number of plants analysed, i.e. in green house and growth chamber experiments, only waxy kernels are included, they were examined by using a two-wavelength colorimetric method with sodium hydroxide as solvent according to JANSEN et al. (2011).

Field trials

In 2009 and 2010 field experiments with respect to the impact of nitrogen fertilization on the ß-glucan content of cv. 'Waxyma' were carried out in Bernburg, Germany (Northern latitude: 51.82, Eastern longitude: 11.78) at the Professor Hellriegel Institute e.V. Grain samples of four replications were collected from a randomized block design. Nitrogen levels of 0 kg N, 60 kg N, 120 kg N (60 kg N + 60 kg N) and 180 kg N (60 kg N + 60 kg N + 60 kg N) were applied in plots of 12.0 m^2 with a seed rate of 300 grains/m^2. The N-fertilizer was applied at BBCH-Code 22/23 (tillers detectable), BBCH-Code 31/32 (node 2 and 3 at least 2 cm above node 1 and 2, respectively) and BBCH-Code 45-49 (booting).

In 2010 and 2011 additional field experiments concerning the influence of different intensities, i.e. seed rate, nitrogen fertilization and fungicide treatment, were conducted with cv. 'Waxyma' in plots of 10.2 m^2 and 13.3 m^2, respectively, in Sülbeck (Northern latitude: 52.30, Eastern longitude: 9.15) at Dieckmann GmbH & Co. KG. The complete experimental design is described in Tab. 1. Nitrogen levels of 80 kg N, 130 kg N (70/60), 180 kg N (70/70/40/0), 180 kg N (70/70/0/40) and 230 kg N (70/90/0/60), seed rates of 300 grains/m^2 and 230 grains/m^2 with and without fungicide treatment were used. The N-fertilizer was applied at BBCH-Code 21(beginning of tillering), BBCH-Code 30 (beginning of stem elongation) and BBCH-Code 39 (flag leaf stage, end of stem elongation) or BBCH-Code 49 (first awns visible).

Shelter experiments

In 2009, the influence of drought stress on the ß-glucan content was analyzed under field conditions in a rain-out shelter. Four waxy barleys (cv. “Waxyma” and three waxy breeding lines) and the non waxy barley cv. “Lomerit” were grown in the rain out shelter and under irrigated field conditions in single rows containing about 20 plants/ 3m in 4 replications. Terminal drought stress was applied by turning off the irrigation in the shelter at BBCH-Code 49 (first awns visible).

Green house experiments

The drought stress experiment in the greenhouse was carried out with three waxy breeding lines and non waxy cv. ‘Lomerit’. Half (waxy breeding lines, see above) and whole grains (non waxy cv. ‘Lomerit’) were germinated in planting trays and after germination at 20 °C plants were vernalized for six weeks at 4 °C. Experiments were conducted with one plant per pot (13 x 13 cm in standard soil with a pH of 5.8). For fertilization, 1 kg NPK-fertilizer (14 % N, 16 % P_2O_5, 18 % K_2O) /m³ soil and 50 g micro nutrient fertilizer (B, Cu, Fe, Mn, Mo, Zn) /m³ soil were applied. Additionally for optimal nutrient supply all plants were fertilized with a leaf micronutrient fertilizer (2 x Fetrilon, 2.5 g/l), calcium ammonium nitrate (1x4 granules/pot) and a liquid iron chelate fertilizer (WUXAL SUPER, 0.2 %, 1 x 30 ml/per pot). All pots (50 pots per variant) were randomly placed in the greenhouse at 20 °C and optimally supplied with water until flowering. From flowering to harvest, plants were cultivated in a control variant (60 % water capacity of the soil) and a stress variant (30 % water capacity of the soil).

Growth chamber experiments

The temperature stress experiment was carried out using the same genotypes as in the greenhouse experiment in 2009 in growth chambers by applying 15 °C, 20 °C and 25 °C from the beginning of heading until harvest. After germination at 20 °C plants were vernalized for six weeks at 4 °C. One plant per pot each was transferred to a 13 x 13 cm pot filled with standard soil (50 pots per line or cv. and variant) and then cultured at 20°C until stress application. For fertilization Plantosan ® COMPACT was used.

Sample collection

Grain samples were taken from field-, shelter-, greenhouse- and growth chamber experiments. Ears of field plots and shelter experiments were threshed by a plot combine harvester. Single ears of greenhouse and growth chamber experiments were threshed by a Multi-Chef Rot (company Baumann Saatzuchtbedarf). The grains were dehulled using a compressed air oat peeler (company Friedrich Falke Maschinen- und Mühlenbau) and ground using a Rotor Speed Mill Pulverisette 14 (company Fritsch) to pass a 0.5 mm sieve. Before estimating the quality parameters of the growth chamber and greenhouse experiments random grain samples of 50 plants per variant were combined to three composite samples.

The generated whole meal with a humidity of 11 to 12 % was stored at 20 °C until analysis.

Analysis of total ß-glucans

The content of total ß-glucan was determined according to McCleary and Glennie-Holmes (1985). For this, a mixed linkage test kit for the measurement of 1,3 and 1,4-ß-D-glucan in cereal grains (ICC Standard No. 166) from Megazyme International Ireland was used. The ß-glucan content is given in % in dry matter of whole meal (% in DM).

Statistical Methods

To assess the effects of different treatments and abiotic stress factors on the total ß-glucan content of six-rowed winter barley a generalized linear model for the analysis of variance was applied, using the GLM procedure of the software package SAS (version 9.3) followed by a Tukey-test ($\alpha = 0.05$) for comparing means. The data sets were analyzed separately. The influence of fungicide treatment was calculated with a covariance test.

Results

The effects of different nitrogen levels on the ß-glucan content of the waxy barley cv. ‘Waxyma’ were significant. Increased nitrogen levels positively affected the ß-glucan content of ‘Waxyma’ both in trials conducted in Bernburg in 2009 and 2010 (Fig. 1) and in field experiments in Sülbeck in 2010 and 2011 (Fig. 2). However, applying a high amount of nitrogen (220 kg N / ha) did not increase the ß-glucan content significantly compared with nitrogen levels of 180 kg N / ha and 130 kg N / ha (Fig. 2). In the trials conducted in Bernburg, the ß-glucan content varied between 5.1 and 5.6 % in dry matter (DM) in 2010 and between 5.2 and 5.9 % in DM in 2009 (Fig. 1). In Sülbeck the mean ß-glucan content varied between 5.4 and 5.8 % in DM in the years 2010 and 2011 (Fig. 2).

Tab. 1: Experimental design of field trials in 2010 and 2011 in Sülbeck, variety ‘Waxyma’, four replications.

Seed rate (grains/m²)	Fungizide treatment (kg N/ha)	Total N-fertilization BBCH 21	First N-application BBCH	Second N-application 30/31	Third N-application BBCH 37/39	Fourth N-application BBCH 47/49
300	without	80	80			
300	optimal	130	70	60		
300	optimal	180	70	70		40
300	optimal	180	70	70	40	
300	optimal	220	70	90		60
230	without	80	80			
230	optimal	130	70	60		
230	optimal	180	70	70		40
230	optimal	180	70	70	40	
230	optimal	220	70	90		60

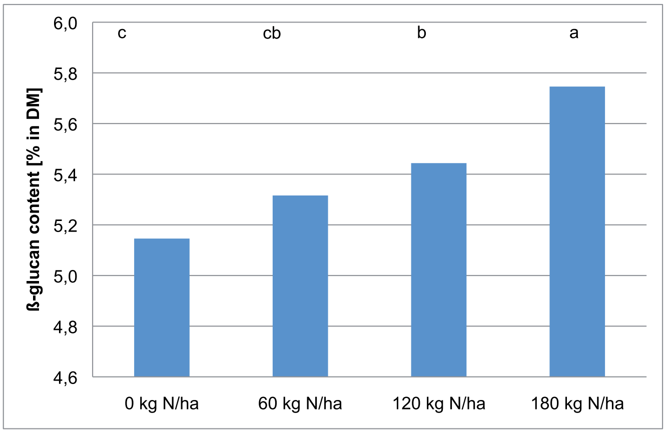
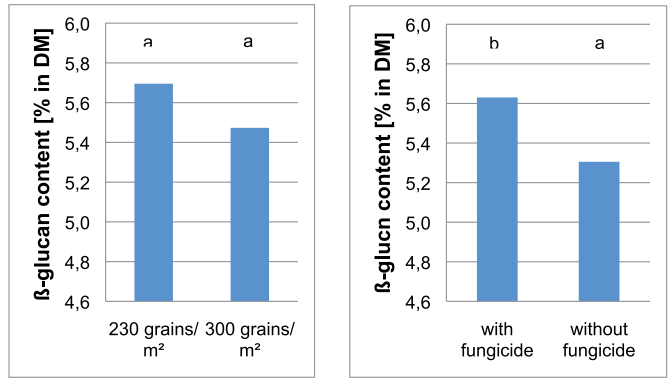


Fig. 1: Response of the ß-glucan content of the waxy barley cultivar 'Waxyma' to N fertilization in Bernburg (means of 2009/2010), different lower case letters indicate significant differences in ß-glucan contents).



A **B**

Fig. 3: Effect of seed rate and fungicide treatment on the ß-glucan content of waxy barley variety 'Waxyma' in Sülbeck (means of 2010/2011, different lower case letters indicate significant differences in ß-glucan contents).
A: Response of seed rate
B: Response of fungicide treatment

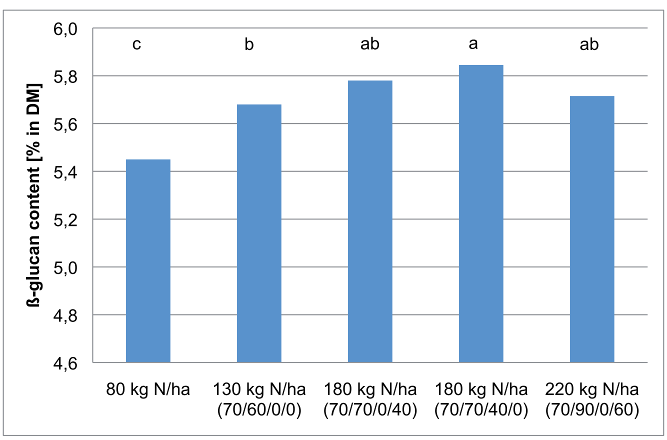


Fig. 2: Response of the ß-glucan content of waxy barley cultivar 'Waxyma' in Sülbeck (means of 2010 and 2011, different lower case letters indicate significant differences in ß-glucan contents).

Different seed rates did not influence the ß-glucan content significantly (Fig. 3A). But without fungicide treatment the ß-glucan content was significantly decreased compared with fungicide treatment (Fig. 3B). Mean values of ß-glucan content concerning seed rate varied between 5.5 and 5.7 % in DM. The lower ß-glucan content at higher seed rate was not significant. The lower ß-glucan content (from 5.6 % to 5.3 % in DM) without fungicide treatment was significant.

Both experiments, under drought stress conditions in the 'Shelter' in 2009 (Tab. 2) and under drought stress conditions in the green house in 2010 (Tab. 3) showed no significant influence of drought on the ß-glucan content of waxy six-rowed barley. Only the ß-glucan content of the non waxy cv. 'Lomerit' increased significantly under drought stress in rain out shelter conditions.

In addition the long-term temperature experiments with different temperatures of 15 °C, 20 °C and 25 °C between heading and harvest in growth chambers also had no significant effect on the ß-glucan content of waxy barley. Again, only the non waxy cv. 'Lomerit' showed a significant increase of the ß-glucan content with increasing temperatur (Tab. 4).

Tab. 2: Mean ß-glucan content in the shelter (drought stress) and in the control field in 2009.

Cultivar or breeding line	Control	Stress	P
Breeding line 1	5.4	5.5	0.6078
Breeding line 2	5.4	5.9	0.1651
Breeding line 3	5.7	5.8	0.8275
Lomerit	4.1	4.6	0.0255
Waxyma	4.9	5.5	0.1073

$P \leq 0.05$ significant

Tab. 3: Variance table of the drought stress experiment in the green house (2010).

Source	Degrees of freedom	Mean square	F-statistic	Pr>F
Variety	3	2.8883	147.88	<.0001
Stress	1	0.0069	0.35	0.5588
Variety*Stress	3	0.0050	0.26	0.8565

Pr>F = 0.05 significant

Tab. 4: Mean ß-glucan content in growth chamber experiments at different growth temperatures.

Cultivar or breeding line	15 °C	20 °C	25 °C
Breeding line 1	5.7 ± 0.1 a	5.7 ± 0.2 a	5.6 ± 0.7 a
Breeding line 2	6.0 ± 0.1 a	6.2 ± 0.2 a	6.0 ± 0.4 a
Breeding line 3	6.0 ± 0.1 a	6.0 ± 0.1 a	6.0 ± 0.1 a
Lomerit	5.6 ± 0.1 a	5.6 ± 0.2 a	6.5 ± 0.4 b

Statistical comparison line wise (different lower case letters indicate significant differences in ß-glucan contents)

Discussion

Concerning various nitrogen levels, the differences in the ß-glucan content of the German waxy barley 'Waxyma' were significant. The highest grain ß-glucan content was obtained using 180 kg N/ha. However, the ß-glucan content differed only between 5.2 % and 5.9 %, so that an increase due to N-fertilization amount is only 0.7 %. This is in agreement with results obtained for the effect of nitrogen on the ß-glucan content by Gühler et al. (2003) and Jackson et al. (1994). Gühler et al. (2003) found in a two-rowed barley cultivar a variation of the ß-glucan content between 4.8 and 5.8 % using nitrogen levels between 0 and 80 kg N/ha without irrigation. Jackson et al. (1994) detected a variation of the the ß-glucan content of waxy hulless barley between 6.2 and 7.6 % in response to nitrogen fertilization between 0 kg N/ha and 101 kg N/ha. In contrast to this, Saastamoinen et al. (2004) reported about a trial with Finnish oat cultivars in which the N-fertilization did not increase the ß-glucan content. With respect to barley, it may be concluded from our results and those from literature that nitrogen fertilization (up to 180 kg N/ha) increases the ß-glucan content in barley grains.

Nothing is known in literature about the influence of the seed rate and fungicide treatment on the ß-glucan content of barley. Our experiments show that the seed rate and a fungicide treatment influence the ß-glucan content of the six-rowed waxy barley 'Waxyma' only very little.

Furthermore, no significant influence of drought stress on the ß-glucan content was observed, although the ß-glucan content was slightly higher under drought stress. Similar results were obtained by Perez-Vendrell et al. (1996), who detected also a slightly higher ß-glucan content under low rainfall conditions. In contrast to this Coles et al. (1991) revealed that in the barley variety 'Triumpf' the grain ß-glucan contents decreases with increasing drought stress.

Anker-Nilssen et al. (2008) reported about the influence of different temperature during grain filling on the total ß-glucan content. The grain ß-glucan varied from 4.0 % to 7.4 % and was significantly affected by temperature. Furthermore, significant interaction between cultivar and temperature during grain filling was observed. The waxy cultivar 'Cindy' showed the highest ß-glucan content of 7.4 % at 18 °C and the lowest content of 5.8 % at 9 °C. 'Tyra', a normal two-rowed barley cultivar varied between 4.6 % at 9 °C and 4.9 % at 21 °C. These results agree with our results for the non waxy cv. 'Lomerit' in the long term stress experiment in which we detected a significant increase of the ß-glucan content with increasing temperature. In contrast to this applying different temperature only at the beginning of heading and beginning of watery ripe (Reinhardt et al., 2013), led to a significant increase of the ß-glucan content only in one breeding line in the 30 °C variant in comparison to the 10 °C variant. Savin et al. (1997) even detected a lower ß-glucan content at temperatures of 27 °C and 30 °C in comparison to 21 °C during grain filling of malting barley cultivars.

The ß-glucan content of German six-rowed winter waxy barley can be increased by optimal agronomic measures, but overall nitrogen fertilization, optimal seed rate, fungicide treatment and stress conditions (temperature and drought) had only a little effect on the ß-glucan. The high ß-glucan content of German six-row waxy barley cultivars and breeding lines seems to be relatively stable under different agronomic practices and stress situations and thus this cultivar maybe suited to produce barley which may be beneficially used in human nutrition.

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