Temperature stress during flowering time affects yield and quality parameters of waxy barley

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Abstract

Temperature affects growth and quality parameters of crops. This study investigates the effects of low and high temperatures during flowering time on yield and quality of waxy barley. Three waxy genotypes and the cultivar Lomerit with a non-waxy starch composition were exposed to three different temperature variants (10, 20 [control] and 30 °C) in growth chambers. Stress was applied at the beginning of heading and was stopped at the beginning of watery ripe. Different temperatures during flowering resulted in different yields, whereas these effects were also genotype specific. High temperatures (30 °C) at flowering resulted in a significantly reduced number of kernels and kernel yield per plant. Low temperatures at flowering (10 °C) mainly resulted in higher yields. In addition, a significant influence of the temperature at flowering and the genotype on protein content, starch content and starch composition was detected. High temperature at flowering resulted in a decreased starch and increased protein content for all genotypes. Low temperatures at flowering time were mainly associated with increased starch and decreased protein contents. Protein content was negatively correlated with starch content and positively correlated with the ß-amylase-activity. The waxy genotypes showed a higher temperature sensitivity regarding the investigated yield parameters than the non-waxy cv. Lomerit.

Keywords: *barley, temperature stress, flowering period, yield and quality parameters*

Zusammenfassung

Temperaturstress während der Blüte beeinflusst Ertrags- und Qualitäts-parameter bei waxyGersten

Temperatur hat einen Einfluss auf das Wachstum und die Qualität von Nutzpflanzen. In dieser Studie wurden die Auswirkungen von Temperaturstress während der Blüte auf Ertrags- und Qualitätskomponenten von waxyGersten untersucht. Hierfür wurden drei waxyGersten-Linien sowie eine Gerstensorte (Lomerit) mit einer normalen Stärkezusammensetzung verschiedenen Temperaturen (10, 20 [Kontrolle] und 30 °C) ausgesetzt. Der Temperaturstress wurde zu Beginn des Ährenschiebens eingeleitet und endete mit Beginn des Kornansatzes. Es konnte ein hoch signifikanter Einfluss der Blühtemperatur auf den Ertrag nachgewiesen werden. Hohe Temperaturen (30 °C) führten bei allen Gersten zu einer Reduktion von Kornanzahl und -gewicht pro Pflanze. Ertragssteigerungen wurden vor allem bei niedriger Blühtemperatur von 10 °C ermittelt. Der Protein- und Stärkegehalt sowie die Stärkezusammensetzung im Korn wurden ebenfalls von der Blühtemperatur und vom Genotyp beeinflusst. Alle Linien zeigten infolge einer hohen Temperatur einen erhöhten Proteingehalt bei gleichzeitiger Abnahme des Stärkegehalts im Vergleich zur Kontrolle (20 °C). Sinkende Protein- und steigende Stärkegehalte wurden hingegen bei niedrigen Blühtemperaturen ermittelt. Der Proteingehalt korrelierte negativ mit dem Stärkegehalt und positiv mit der ß-Amylaseaktivität. Verglichen mit Lomerit zeigten die waxyGersten eine stärkere Variation der Ertragsparameter in Abhängigkeit der Temperaturstufen und sind somit als temperatursensitiver einzuschätzen.

Stichwörter: *Gerste, Temperaturstress, Blüte, Ertrag, Qualitäts-parameter*

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Introduction

Barley *(Hordeum vulgare)* is the oldest cereal crop and one of the most important cereals in the world. It serves as animal feed and is a source of fermentable material for beer and distilled beverages. Furthermore, barley gains an increasing importance as a renewable resource. The starch of waxy barley contains a high amount of amylopectin (95 to 100 %) and can be a useful raw material for industrial purposes. Furthermore, waxy barley is characterized by a high content of ß-glucan, which makes barley flour suitable for baking and interesting for the production of functional food (Dieckmann et al., 2009). The waxy endosperm in barley is conditioned by a single recessive gene *(wx)* which results in a reduced expression of granule-bound starch synthase (Nakamura et al., 1995) leading to an eponymous waxy appearance of kernels.

Abiotic stress like water shortage and unfavorable temperatures highly affect yield and quality parameters of crops (Gusmao et al., 2012; Brandt et al., 2011; Clausen et al., 2011). Considering possible impacts of climate change it is very important to investigate environmental effects on crop yield and quality and to develop cultivars exhibiting high yield and quality in different environments (Haussmann et al., 2012). It is well documented that temperature – especially during grain filling – affects yield and quality of cereals. In earlier studies high temperatures during grain filling period of barley and wheat resulted in reduced yields and decreased starch accumulation (Shi et al., 1994; Savin et al., 1997; Zahedi et al., 2003). Grain filling periods are shorter under high temperature, probably as a result of the impairment of the catalytic activity of enzymes involved in starch biosynthesis (Jenner, 1994). Temperature stress studies with different barley mutants showed a lower temperature stability of starch mutants than of common barley cultivars (Tester et al., 1991; Jansen et al., 2001). However, temperature effects during the flowering period of barley are largely unknown although this vegetation period seems to be a sensitive phase as it was shown for wheat (Wheeler et al., 1996).

The aim of this study was therefore to analyze the effects of temperature stress during the flowering period on yield and quality parameters of waxy barley. For this purpose, three waxy barley genotypes and a cultivar with a normal starch composition were exposed to three different temperatures (10, 20 [control] and 30 °C). The stress application was initiated with the beginning of heading and finished with the beginning of watery ripe. To evaluate the effects of temperature, different yield and quality parameters were assessed.

Material and methods

Experimental design

Two hundred barley (*Hordeum vulgare*) plants were cultivated, three waxy genotypes, breeding line 1, breeding line 2 and cultivar Waxyma as well as cv. Lomerit with a "normal" starch composition (50 plants per genotype). The whole experiment was conducted in growth chambers. Before and after the stress period all plants were placed in the control chamber (20 °C). With the beginning of stress approximately 16 plants per treatment were further cultivated while ambient temperatures of 10 and 30 °C where used as stress treatments and 20 °C as control treatment. The stress was initiated with the beginning of heading (BBCH code 51; Meier, 1997) and finished with the beginning of watery ripe (BBCH 71). Time of stress treatment varied individually; the duration was between 10 (30 °C) and 33 (10 °C) days. The stress period in the chamber with 30 °C was considerably shorter than in the chamber with 10 °C due to the shorter flowering period.

Plant cultivation

Before sowing, the starch composition of single seeds was examined by a NIR (Near-infrared spectroscopy) method (Jansen et al., 2003). Kernels with an R-value (ratio of the absorbances of the iodine-starch-complex at wave length 620 nm for amylose content and at wave length 550 nm for amylopectin content) less than 0.7 were considered as waxy barley. Kernels were germinated in planting trays. After germination at 20 °C and a light exposure time of 8 h per day for 3 to 4 days plants were vernalized about six weeks by reducing the ambient temperature to 4 °C. After vernalization plants were planted into 13 x 13 cm pots (1 plant per pot) and randomly placed in a chamber with 20 °C. Relative humidity in the chamber was kept constant around 65 %. Standard soil (Einheitserdewerk Uetersen Werner Tantau) was used as substrate. The substrate was fertilized with 1 kg/m^3 of a NPK-fertilizer (14 % N, 16 % P_2O_5, 18 % K_2O) and 50 g/m^3 of an trace element fertilizer (B, Cu, Fe, Mn, Mo, Zn) to ensure optimal nutrient supply. The pH value of the soil corresponded to the plant requirements (about pH = 5.8). At first the light exposure time was according to short-day conditions (8 hours). After two months, conditions were changed to long-day exposure (16 hours) with 20 °C day temperature and 16 °C night temperature. At this time all plants were fertilized with a leaf micronutrient fertilizer (2 x Fetrilon, 2.5 g/l), calcium ammonium nitrate (1 x 4 granules/pot)) and a liquid iron chelate fertilizer (WUXAL SUPER, 0.2 %).

Analysis of plant material

Ears of all plants were harvested at BBCH 89. Yield parameters were determined per plant. To determine the percentage of unfertile spikelets the total and sterile spikelets were counted per ear and calculated per plant. The awns were then removed and the ears were threshed by a Multi-Chef Rot (Tupperware). Kernel yield was determined per plant by use of precision scales (Kern 440). The thousand-seed weight was calculated from the kernel yield and kernel number. Before estimating the quality parameters random composite samples were made. To achieve this, kernels of each genotype developed at the same temperature were combined to three composite samples. The grains per sample were dehulled using a compressed air oat-peeler (Friedrich Falke Maschinen- und Mühlenbau). After that the samples were ground using the Rotor Speed Mill - Pulverisette 14 (Fritsch

GmbH) to pass a 0.5 mm sieve. The whole meal was stored at room temperature (20 °C) with an average relative humidity of 11 to 12 %.

Protein content was analyzed by a quantitative determination of nitrogen in ground material developed by Kjeldahl (1883). Starch content was measured polarimetrically using the procedure of Ewers (1908); a method based on the optical activity of starch after hydrolyzing with acid. The starch composition was analyzed by determining the so-called R-value based on a method of Hovenkamp-Hermelink et al. (1988). This method utilizes the colorimetric differentiated reaction of the starch components amylose and amylopectin with iodine. The ratio of these components in starch was subsequently determined by a photometer. The content of ß-glucan (ICC standard method No. 166) was determined enzymatically according to McCleary (1985) using a corresponding kit (Megazyme International Ireland, Bray). The ß-amylase acti-vity was also measured enzymatically using the betamyl-3 method (Megazyme International Ireland, Bray) according to Mathewson & Seabourn (1983). Both methods are based on a multi-step enzymatic cleavage procedure in which the parameters are quantitatively measured in a colorimetric reaction.

Statistical analysis

Analysis of variance (GLM) was performed with IBM SPSS Statistics (version 20). Differences among means were evaluated using Tukey's post-hoc-test. P-values < 0.05 were considered to be statistically significant. Correlation coefficients were calculated with Pearson's correlation analysis.

Results

Yield parameters

The results showed a significant ($p \leq 0.01$) influence of the temperature at flowering and the genotype on the number and yield of kernels per plant, thousand-kernel weight and the ratio of unfertile spikelets to total number of spikelets per plant (Table 1). Interactive effects of genotype and temperature on yield parameters were also found. All genotypes showed a reduction of kernel number, thousand-seed weight and kernel yield per plant when temperature at flowering rose to 30 °C (Table 2). On the contrary at 10 °C the breeding line 2 and Waxyma showed higher kernel numbers and higher kernel yield per plant. A negative correlation between unfertile spikelets and kernel number ($r = -0.616$, $p < 0.01$) and kernel yield ($r = -0.627$, $p < 0.01$) per plant was found considering all genotypes and temperatures. In comparison to the control cultivar the waxy genotypes showed a higher variation of the yield parameters in dependence of the temperature (data not shown). The variation was found to be especially high for Waxyma.

Table 1
Influences of barley genotype, flowering temperature and genotype * temperature on yield parameters (GLM, p-values)

parameter (per plant)	genotype	temperature	genotype * temperature
spikekelet sterility	0.000	0.000	0.050
number of kernels	0.000	0.000	0.000
yield of kernels	0.000	0.000	0.013
Thousand-seed weight	0.000	0.000	0.000

Table 2
Means of yield characteristics of different barley genotypes in dependence of the temperature treatment (10, 20 and 30 °C) during flowering period

parameter (per plant)	T [°C]	breeding line 1		breeding line 2		Waxyma		Lomerit	
spikelet sterility [%]	10	21.5		16.5		28.8		17.8	
	20	20.2		25.6		35.0		14.8	
	30	32.8		44.7	*	61.0	*	28.9	
number of kernels	10	115.9		127.4	*	112.4	*	115.6	
	20	133.5		72.1		50.5		114.4	
	30	68.6	*	47.1	*	19.8	*	60.3	*
yield of kernels [g]	10	5.7		6.0	*	5.4	*	6.4	
	20	5.6		4.0		2.7		5.7	
	30	2.2	*	2.0	*	0.9	*	2.6	*
Thousand-seed weight [g]	10	49.9	*	47.3	*	48.2	*	56.4	
	20	41.8		55.5		55.6		50.5	
	30	35.6		44.5	*	43.7	*	44.4	*

* significant different ($p < 0.05$) in comparison to the control (20 °C)

Quality parameters

Significant effects of the genotype and the temperature were found for protein and starch content and the R-value (Table 3). The ß-glucan content, however, was neither affected by the genotype nor by temperature. The ß-amylase-activity varied depending on the temperature but was not significantly different between the genotypes. Significant interactive effects of genotype x temperature were found for all quality parameters except for the starch content.

A negative correlation ($r = -0.914$, $p < 0.01$) between protein and starch content was found. Protein content was positively correlated ($r = 0.962$, $p < 0.01$) with the activity of the ß-amylase. Rising protein contents combined with decreasing starch contents after flowering at 30 °C were found for all barley genotypes (Table 4). In tendency, the protein content decreased in all genotypes at 10 °C compared to the control (20 °C). The analysis of variance showed an effect of the temperature on the R-values. A lower R-value was found when Waxyma flowered at 30 °C. In general, all waxy genotypes showed relatively stable waxy properties (average R-va-

lue 0.70) under all temperatures analysed. A significantly higher ß-glucan content in the 30 °C variant was only detected for breeding line 2, whereas high temperature at flowering resulted in raised activities of ß-amylase of all genotypes. Activity of α-amylase was also analysed and no hints of pre-harvest sprouting were found (data not shown). In general, waxy genotypes did not show a higher variation of quality parameters than Lomerit in relation to the temperature at flowering time.

Table 3
Influences of barley genotype, flowering temperature and genotype * temperature on quality parameters (GLM, p-values)

parameter (per plant)	genotype	temperature	genotype * temperature
protein content	0.000	0.000	0.045
starch content	0.000	0.000	0.244
R-value	0.000	0.002	0.019
ß-glucan content	0.132	0.564	0.026
ß-amylase-activity	0.206	0.000	0.001

Table 4
Means of quality characteristics of different barley genotypes in dependence of the temperature treatment (10, 20 and 30 °C) during flowering period

parameter (per plant)	T [°C]	breeding line 1		breeding line 2		Waxyma		Lomerit	
protein content [%]	10	11.5		10.3		10.5		10.4	
	20	12.1		11.1		10.9		10.7	
	30	19.0	*	18.0	*	18.3	*	15.9	*
starch content [%]	10	53.6		56.9		55.5		58.5	
	20	53.7		55.4		56.3		58.0	
	30	46.4	*	49.7	*	48.9	*	53.2	*
R-value	10	0.69		0.70		0.72		0.98	
	20	0.70		0.68		0.73		0.95	
	30	0.69		0.68		0.70	*	0.96	
ß-glucan content [%]	10	5.8		4.4		4.9		4.3	
	20	5.5		4.8		4.9		4.2	
	30	4.0		5.9	*	5.7		5.1	
ß-amylase-activity [U/g]	10	1634		1488		1416		1853	
	20	1647		1539		1496		1731	
	30	3164	*	2887	*	3458		2527	*

* significant different ($p < 0.05$) in comparison to the control (20 °C)

Discussion

In the present study the temperature influence during the flowering period (beginning of heading up to beginning of watery ripe) of barley was investigated, whereas former studies focused on temperature stress during the grain filling period of barley. Although the stress ended before grain filling, differences of yield and quality characteristics were measured in dependence of temperature. Pronounced effects were found when the plants were treated with 10 °C higher temperatures than the control of 20 °C, whereas 10 °C lower temperatures than the control showed smaller effects. These results confirm those of a study of Ferris et al. (1998), in which a negative linear relationship between the maximal temperature at mid-anthesis and the kernel number per ears of wheat was detected.

Impacts of temperatures during grain filling on yield characteristics of various barley cultivars were found previously by Savin et al. (1996a, b), Savin et al. (1999) and Jansen et al. (2001). Savin et al. (1999) additionally described, that the reduction in grain yield was higher under heat stress than under drought stress. Different ambient temperatures during grain filling also affected quality parameters of barley, i.e.he protein content, starch content and starch composition as well enzyme activities and the content of ß-glucan (Johnson et al., 1985; Correll et al., 1994; Wei et al., 2009).

In the present study a higher protein content and a lower starch content was found in the 30 °C-variant. The results confirm data of Correll et al. (1994) and Jansen et al. (2001) who investigated these parameters when temperature stress was applied during grain filling.

Several authors assume an inhibiting effect of high temperatures on enzyme activity involved in starch biosynthesis as the reason for a reduced starch deposition in cereal kernel (e.g. Jenner et al., 1994). During ripening stage of rice the activity of granule bound starch synthase was lower at 29/35 °C than at 22/28 °C (Jiang et al., 2003).

The present study showed only small changes in starch composition (amylose/amylopectin ratio, R-value) under temperature stress. Long temperature stress duration under field conditions, however, reduced amylose contents considerably (Myllärinen et al., 1998).

The ß-glucan content of our investigated waxy barley lines was higher than in the cultivar Lomerit. This result was expected and is in accordance with data of a study of Ullrich et al. (1986) who compared ß-glucan contents of four waxy barley lines with those from isogenic lines with a normal starch composition.

The influence of temperature at flowering time on the ß-glucan content was not consistent in our experiment, which might be explained by the early end of the stress period before grain filling. Savin et al. (1997) documented decreasing ß-glucan contents after ambient temperatures of

27 and 30 °C in comparison to 21 °C during grain filling of malting barley cultivars. A possible explanation was the shorter time span for grain filling at higher temperatures. Swantson et al. (1997) affirm this assumption by describing that ß-glucan is mainly deposited during the late grain filling period.

Our results showed an increase of ß-amylase-activity after high temperatures which confirmed the results of Wei et al. (2009) who investigated temperature stress during the grain filling period of barley. The authors also revealed a positive correlation between the total protein content and the ß-amylase-activity and described a negative correlation between the ß-amylase-activity and kernel yield, which was also found in the present study.

Conclusions

The present study showed that yield and quality parameters of barley were affected by temperature stress before the grain filling period. High temperatures at flowering time have a negative impact on yield and quality of waxy barley; low temperatures seem to have an positive effect. In waxy barley lines temperature had a higher impact on yield parameters than on cv. Lomerit, whereas the variation of quality characteristics was similar for all genotypes. It should be noted, that here first results are reported and further studies using a higher number of genotypes are needed to investigate the role of temperature during the short period of barley anthesis. Furthermore, it would be interesting to include more temperature treatments in order to investigate the temperature effect quantitatively.

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