The expression of the gene associated with β-glucan content, β-amylase and limit dextrinase synthesis as affected by post-heading heat stress in barley

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Research article

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Abstract

Background: Malt barley shows a dramatic deterioration of malt quality when exposed to heat or high temperature stress during grain-filling stage (post heading), and global change results in the more frequent occurrence of high temperature, posing a severe threat to high-quality malt barley production. In a previous study, we found heat stress during grain-filling stage caused the significant reduction of kernel weight, and the significant increase of protein and β-glucan content, and β-amylase and limit dextrinase (LD) activities, and the effect varied with barley genotypes and the time of heat stress exposure.

Results: In this study, we determined the relative expressions of HvCslF6 and HvCslF9 for β-glucan, HvBmy1 for β-amylase and LD gene for limit dextrinase of two barley cultivars ZU9 and Hua30 under the two heat stress (HS) treatments (32/26℃, day/night), initiated from the 7th day (early grain-filling stage) and the14th day (middle grain-filling stage) after heading. In comparison with normal temperature (24/18℃, day/night), HS treatments significantly up-regulated the relative expression of all four genes, and Hua30 being larger than ZU9. The change pattern of each examined gene for the two barley genotypes under heat stress treatments is completely consistent with that of corresponding malt quality trait as affected by heat stress.

Conclusion: The results indicate that the enhancement of β-glucan content, and β-amylase and limit dextrinase activities under high temperature during grain filling stage is at least in part attributed to increased expression of the relevant genes.

Background

Heat or high temperature stress is one of the most common natural disasters affecting crop production, and is becoming more severe because of global climate change, posing a great threat to agricultural sustainability [1]. Heat stress not only reduces crop yield due to its direct damage on plant growth and development, but also deteriorates quality of end products through disordering metabolisms and metabolite transportation [2–3].

Barley is one of the most important cereal crops in the world, ranking the fourth in terms of planting areas [1]. The end use of barley is universal, mainly used as animal feed and beer production. Beer quality is greatly dependent on malt quality, which is in turn related to malt barley. In general, malt quality is described by malt extract (ME), diastatic power (DP), viscosity (VC) and Kolbach index (KI) [4], and all these parameters are greatly affected by chemical components and enzyme activities. Among them, total protein and β-glucan contents, β-amylase and limit dextrinase (LD) activities are most important in determining malt quality [5–8].

It is well documented that these chemical components and enzymes are both controlled by genetic and environmental factors, including temperature. Barley is a temperate plant, being relatively sensitive to high temperature. Malt quality will be dramatically deteriorated when barley plants are exposed to post-heading heat stress. Heat stress resulted in significant enhancement of total protein concentration in

grains [9]. For β-glucan content, Narasimhalu et al (1995) planted the same batch of barley varieties in the eastern and western Canada respectively, and found the eastern regions with higher temperature during grain maturation had higher β-glucan content [10]. Similarly, Ellis et al (1997) reported that lower temperature and more precipitation during grain development tend to reduce β-glucan content in grains [11]. On the other hand, activities of the enzymes related to starch saccharification significantly increased under post-heading heat stress. For example, Wei et al (2009) found that β-amylase activity in the grains of two barley varieties was markedly increased when plants were exposed to heat stress (32/26℃, day/night) at grain filling stage, in comparison with the relatively lower temperature condition (26 – 20℃, day/night) [12]. Genetics of above-mentioned malt quality traits has been intensively investigated. For barley β-glucan biosynthesis, at least ten HvCslF genes are involved. However, only two of them, i.e. HvCslF6 and HvCslF9 were highly expressed in developing grains [13–14]. In addition, it was shown that β-glucan content would be substantially increased due to overexpression of HvCslF6 [15]. On the other hand, a specific mutation in the conserved region of HvCslF6 in a β-glucan-less (bgl) barley caused a dramatic reduction of β-glucan content [16]. Moreover, HvCslF6 showed consistently high expression during the whole endosperm development, while HvCslF9 reached a peak of expression at about 8 days after pollination (DAP) and then decreased to very low levels by 15 DAP. Endosperm-specific β-amylase is encoded by a Bmy1 gene located in the telomeric region on the long arm of chromosome 4H [17–18]. It has been confirmed that both thermostability types (A, B and C) and iso-electric focusing patterns (Sd1 and Sd2) of β-amylase were controlled by the Bmy1 gene [19–20]. It was found that the mRNA encoding limit dextrinase (LD) was present at lower level in the developing endosperm of immature grain, and abundant in germinating grains [21].

The genetic adaptation of plants to environmental stress could be reflected by the changed physiological and biochemical reactions, which are accompanied by up- and down-expression of the relevant genes. However, there is little research about the influence of post-heading heat stress on the expression of the genes controlling main malt quality traits. In this study, we determined the expressions of the genes encoding β-glucan, β-amylase and limit dextrinase in the two barley cultivars (ZU9 and Hua30) under the different temperature treatments after heading, so as to clarify the molecular mechanisms of malt quality deterioration under heat stress.

Results

Effect of post-heading heat stress on the expression of HvCslF6 and HvCslF9

The expression of CslF6 and CslF9 in both ZU9 and Hua30 grains was significantly up-regulated under heat stress (HS) relative to the control, and their relative expression levels were highest at the one day after heat stress treatment (Fig. 1). In C2 treatment (at early grain-filling stage), the relative expression level of CslF6 in ZU9 and Hua30 was 4.6 and 2.4 times higher than the control, respectively in the first day after HS treatment, while in C3 treatment (at middle grain-filling stage), the relative expression level of

CslF6 in the two genotypes showed a much less up-regulation in comparison with C2 treatment, reaching 1.8 and 1.7 times higher than the control at 15 day after heading (DAH), respectively. The results indicated that HS treatment caused the up-regulation in the expression of CslF6, with the effect being much larger in ZU9 than in Hua30, and early treatment (C2) than late treatment (C3). In addition, the relative expression level of CslF6 tended to reduce gradually upon reaching the maximum value at the first day after treatment and again showed a slight increase in the last 2 days of the HS treatment for both barley cultivars.

For CslF9, its relative expression level in ZU9 and Hua30 had the maximum value at the first day after HS treatment, being 3.7 and 2.7 times higher than the control for C2 treatment, and 2.1 and 1.8 times higher than the control for C3, respectively. Then the relative expression level of CslF9 remained a relatively higher level from 8 to 10 DAH for C2 treatment and from 15 to 17 DAH for C3 treatment. Like CslF6, the expression level of CslF9 was more up-regulated in ZU9 than in Hua30, and in C2 treatment (early grain-filling stage) than C3 (middle grain-filling stage).

Effect of post-heading heat stress on the expression of HvBmy1

The relative expression level of Bmy1 in the developing grains of the two barley cultivars also increased under heat stress relative to the control, and reached the highest at the first day after HS treatment (Fig. 2), when ZU9 and Hua30 was 5.6 and 9.3 times higher than the control for C2 treatment, and 2.3 and 2.8 times higher than control for C3 treatment. Obviously the effect of heat stress on the expression of Bmy1 was larger in C2 treatment than in C3 treatment, and in Hua30 than in ZU9 (Fig. 2b). Upon reaching the highest at the first day after HS treatment, the two cultivars showed a gradual decrease in relative expression level, and finally reached 2.7 and 3.5 times higher than the control for ZU9 and Hua30, respectively (Fig. 2a).

Effect of post-heading heat stress on the expression of LD gene

LD synthesis related genes appeared to have similar heat responses as CslF genes and Bmy1 did in their expression under heat stress. Post-heading high temperature enhanced LD gene expression, promoting the synthesis of limit dextrinase (Fig. 3). During HS treatment, the relative expression level of LD gene showed a gradual increase and reached the highest at the 5th day after treatment for the two heat treatments (C2 and C3). Again C2 treatment had the much larger effect on the expression of LD gene than C3 treatment. Thus, at the first day after treatment, the relative expression level of ZU9 and Hua30 was 6.4 and 7.9 times higher than the control for C2 treatment, respectively, and for C3 treatment the corresponding values were 2.9 and 3.1 times.

Meanwhile, the effect of heat treatment on the expression level of the LD gene differed dramatically between the two cultivars. For C2 treatment, all samples taken in the different time, except for 10 DAH showed significant difference in the relative expression level of the LD gene between ZU9 and Hua30. For C3, the two samples, taken in 15 DAH and 19 DAH showed significant difference between the two cultivars. On the whole, the relative expression of LD gene was more up-regulated in Hua30 than in ZU9 (Fig. 3).

Discussion

In this study, we examined the influence of post-heading heat stress on the expression of the genes associated with β-glucan content, and β-amylase and limit dextrinase activities in barley grains, as the three chemicals are closely related to malt quality [22–23]. High β-glucan content will deteriorate malt quality as it has the negative impact on filtering of wort and chill haze stability of beer [5, 24]. β-amylase and limit dextrinase are key important enzymes involved in complete decomposition of starch into sugars, and low activities of both enzymes cause low malt extract, which is directly related to beer quality and economic profits of malt or beer producers [8]. β-glucan content, β-amylase and limit dextrinase activities in barley grains are controlled by both genetic and environmental factors [10, 25–26]. The influence of environmental conditions on these malt quality traits have been intensively investigated [10, 26–28]. In a previous study, we investigated the impact of one-week heat stress (32/26℃, day/night) initiating from the 7th (HT7) and 14th (HT14) days after heading on some grain and malt quality traits of two barley varieties. In comparison with normal temperature (24/18℃, day/night), heat stress significantly reduced kernel weight, and increased total protein and β-glucan contents, and β-amylase and limit dextrinase activities, with HT7 having a larger effect than HT14 [29]. On the surface, heat stress has both positive and negative effect on malt quality traits, such as enhancing β-amylase and limit dextrinase activities, beneficial for starch decomposition, and increasing protein content and reducing grain weight, unfavorable for malt extract. Actually smaller grain weight, higher β-glucan content as well as protein content have more negative impact on malt extract and quality than the positive impact by higher β-amylase and limit dextrinase activities.

Among the HvCslF genes associated with β-glucan synthesis, HvCslF6 and HvCslF9 were chosen in this study, as the two genes have the high expression in developing barley endosperms [30], with CslF6 playing a unique role in controlling β-glucan biosynthesis [16]. Actually, HvCslF6 kept high relative expression level throughout endosperm development, although it reached the maximum value at the first day after HS treatment. Comparatively, HvCslF9 had smaller increase in its relative expression level under heat stress. In terms of β-amylase, it has been genetically confirmed that the endosperm-specific β-amylase in barley grains is encoded by HvBmy1, which is located on the long arm of chromosome 4H [18, 31–32]. LD is only enzyme for starch debranching in barley grains and encoded by a single gene i.e. LD gene [21]. In the current experiment, these genes associated with β-glucan, β-amylase and limit dextrinase synthesis were dramatically up-regulated under heat stress, which is completely consistent with the previous findings that heat stress increased β-glucan content, and β-amylase and limit dextrinase activities.

In addition, the influence of high temperature on the relative expression level of these genes associated with β-glucan, β-amylase and limit dextrinase synthesis also differed distinctly between the two barley cultivars and exposure time. In this study, Hua30 and C2 treatment (initiating from the 7th day after heading) showed greater change than ZD9 and C3 treatment (initiating from the 14th day after heading), respectively, which is on the whole consistent with the previous results that Hua30 and HT7 had more increase in β-glucan content, and β-amylase and limit dextrinase activities than ZD9 and HT14, respectively, under heat stress in comparison with control [29]. Therefore it may be concluded that the enhancement of β-glucan content, and β-amylase and limit dextrinase activities under high temperature during grain filling stage is at least in part attributed to increased expression of the relevant genes.

Conclusions

In conclusion, post-heading heat stress caused the dramatic increase in the expressions of the genes associated with β-glucan, β-amylase and limit dextrinase synthesis in barley grains in comparison with the control (normal temperature), and the effect varied greatly with barley genotypes and the time of stress exposure, with Hua30 showing larger effect than ZD9, and the treatment at early grain-filling stage being larger than that at middle grain-filling stage.

Methods

Experimental materials and design

Two malting barley cultivars, ZU9 and Hua30, which are widely planted locally and differ in the responses of malt quality traits to heat stress according to the previous studies [12], were used in this experiment. The seeds of both ZD9 and Hua30 were obtained from authors' laboratory, Zhejiang University, China. The seeds were imbibed in water for 3 h and sown into 10 L pots filled with 2.5 kg mixed soil (peat soil : vermiculite = 1:1) in early Nov, 2018 and the pots were placed in a greenhouse of Zijingang campus, Zhejiang University (Hangzhou, China, 120.2°E, 30.5°N). Compound fertilizer of 10 g (N : P_2O_5 : K_2O = 13:7:5) per pot was mixed uniformly with soil before sowing and 5 seedlings were remained in each pot through deleting extra seedlings at 2-leaf stage.

At heading stage, all pots with 5 plants per pot were moved into three growth chambers, referred to C1, C2 and C3, respectively thereafter. C1 was used as control, which was 18/24℃ (night/day, 12/12hours) of temperature during the whole maturity stage. C2 and C3 were the heat stress treatments, which remained the same temperature as the control during no heat treatment, and they were 26/32℃ (night/day, 12/12hours) from 7th and 14th day after heading and lasted for 9 days, respectively. All plants were well irrigated and the relative humidity was maintained at 75% and the light was supplied from 6:00 to 18:00 at a light intensity of 50000 Lux.

Grain samples were taken randomly from the two barley cultivars of both control and treatments on the day just before treatment, and 1st, 3rd, 5th, 7th and 9th day after treatments. The sampled grains were

immediately placed into liquid nitrogen and transferred to a refrigerator at -80℃ for use of RNA extraction.

Total RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted from the sampled grains with a TaKaRa MiniBEST Plant RNA Extraction Kit (TAKARA BIO INC, Beijing, China) according to manufacturer's instructions, and then RNA was treated by RNase-free DNase I (TAKARA BIO INC, Beijing, China) for digesting template DNA. The extracted RNA quality was identified by gel electrophoresis. The concentration of RNA solution was measured by Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific™, US) and then accurately diluted to 200 ng/μl. The 20 μl RT reaction solution system, consisting of 4 μl PrimeScipt RT Master Mix (TAKARA BIO INC, Beijing, China), 5 μl total RNA solution and RNase free H_2O, were used to prepare first-strand cDNA synthesis by reverse transcription (37℃, 10 min). The single-strand cDNAs were amplified using TB Green Premix Ex Taq (TAKARA BIO INC, Beijing, China) with gene-specific forward and reverse primers of HvCslF6, HvCslF9, HvBmy1 and limit dextrinase mRNA (Table 1), while actin gene (HvACT) was amplified with specific primers (forward:5'-GACTCTGGTGATGGTGTCAGC-3';reverse:5'-GGCTGGAAGAGGACCTCAGG-3') as an internal control for the reactive amount of RNA. The whole real-time PCR reaction was carried on the background of LightCycler480 System (Roche Diagnostics, Mannheim, Germany) with a de-naturation step of 30 seconds, followed by 40 cycles of 95℃ for 5 s, 60℃ for 30 s and melting curve analysis at 65℃ for 15 seconds. Three technical replicates were performed for both control and treatments. Meanwhile, the expression of malt quality genes was normalized by HvActin expression values. LightCycler480 Software (Roche, version 1.5.0) was used to analyze the raw data.

Statistical analysis

SPSS software version 22 (Chicago, IL, USA) was used in one way analysis of variance to analyze the difference of relative gene expression between two cultivars and among three treatments.

Abbreviations

Act
Actin gene;
CslFs
Cellulose synthase-like genes;
DAH
Days after heading;
DAP
Days after pollination;
DP

Diastatic power;
HS
Heat stress;
KI
Kolbach index;
LD
Limit dextrinase;
ME
Malt extract;
VC
Viscosity;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study, and the plant materials used in the presenting study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

GZ and SN conceived and designed the experiments; SN and HZ performed the experiments. SN analyzed the data. SN and GZ wrote the paper. All authors have read, edited and approved the current version of the manuscript.

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Tables

Table 1 Primers used in quantitative real-time RT-PCR expression analysis

Target gene	Correlation trait	GeneBank Accession No.	Sequence of primers (5'-3')	Amplicon size (bp)
HvCslF6	β-glucan	AB621331.1	Forward primer TTCGAGCTCAAGTCACACCC Reverse primer CGCTGCTTGATGTCATGCTC	131
HvCslF9	β-glucan	EU267184.1	Forward primer ACGAGAAAGAAGGGTGTGGG Reverse primer TGCAGGATGGTGTCCTTTCC	102
HvBmy1	β-amylase	KF302667.1	Forward primer TACGTCATGCTCCCTCTGGA Reverse primer GTAGGCGGACCAGTCATACG	174
LD gene	Limit dextrinase	AF252635.1	Forward primer GTACTGGGTGACGAGCGACC Reverse primer CTTCTGGGTCACGGTTTCCG	187
HvActin	Reference	AY145451.1	Forward primer GACTCTGGTGATGGTGTCAGC Reverse primer GGCTGGAAGAGGACCTCAGG	332

Figures

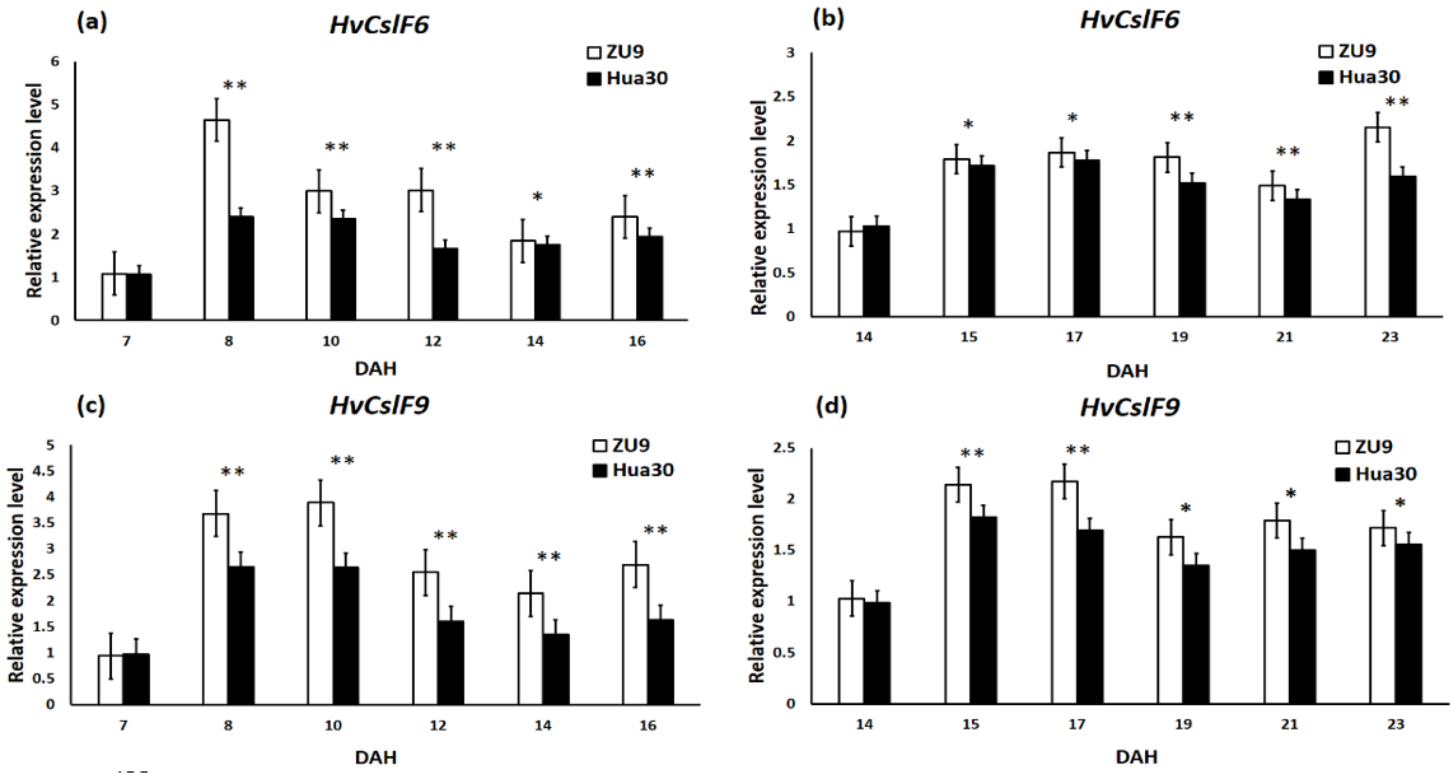

Figure 2

Relative expression level of HvCslF6 and HvCslF9 under the two heat stress treatments. (a) Relative expression levels of HvCslF6 for C2; (b) Relative expression levels of HvCslF6 for C3; (c) Relative expression levels of HvCslF9 for C2; (d) Relative expression levels of HvCslF9 for C3. **, highly significant; *, significant.

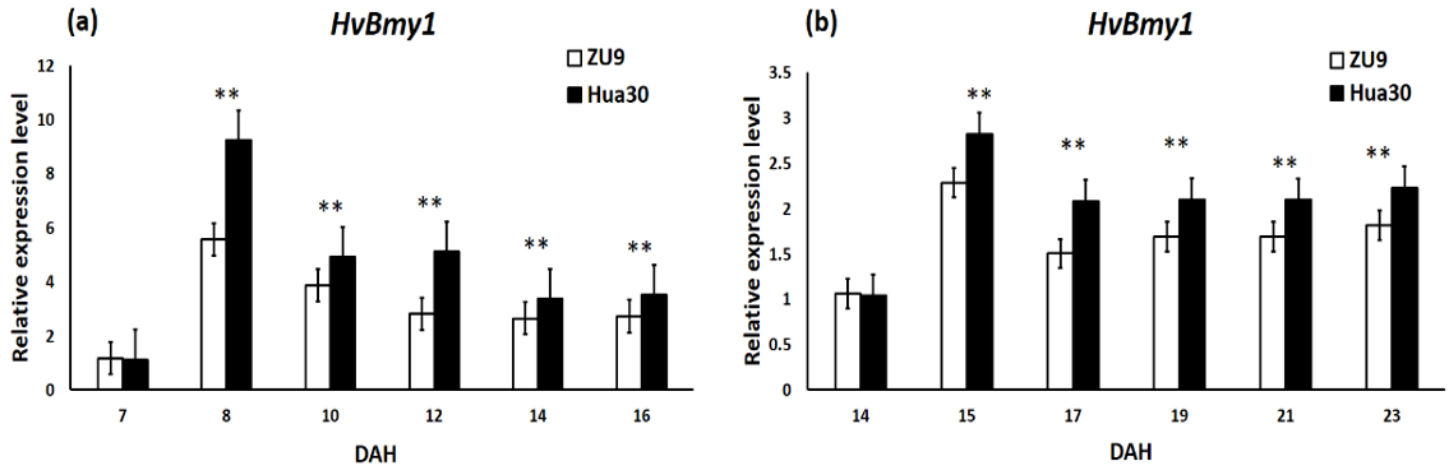

Figure 3

Relative expression level of HvBmy1 under the two heat stress treatments. (a) Relative expression levels of HvBmy1 for C2; (b) Relative expression levels of HvBmy1 for C2. **, highly significant; *, significant.

460
461

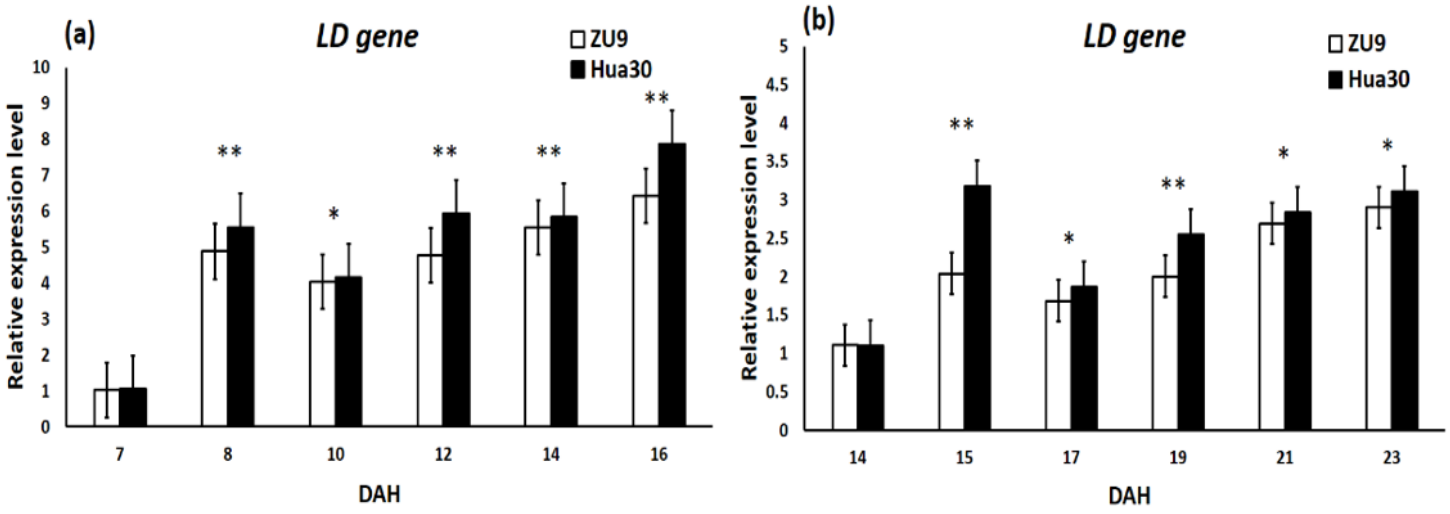

Figure 6

Relative expression level of LD gene under the two heat stress treatments. (a) Relative expression levels of LD gene For C2; (b) Relative expression levels of LD gene For C3. **, highly significant; *, significant.