Effect of high night temperature on storage lipids and transcriptome changes in developing seeds of oilseed rape

Longhua Zhou[1,2], Tao Yan[1], Xin Chen[1], Zhilan Li[1], Dezhi Wu[1], Shuijin Hua[3] and Lixi Jiang[1,*]

[1] Institute of Crop Science, Zhejiang University, Yu-Hang-Tang Road 866, Hangzhou 310058, China

[2] Biotech Research Institute, Shanghai Academy of Agricultural Sciences, Bei-Di Road 2901, Shanghai 201106, China

[3] Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Shiqiao Road 198, Hangzhou, 310021, China

[*] Correspondence: jianglx@zju.edu.cn

Introduction

Global warming causes a faster increase of night temperature than of day temperature in tropical and subtropical zones. Little is known about the effect of high night temperature on storage lipids and transcriptome changes in oilseed rape. This study aimed to determine the significant transcriptome changes resulting from high night temperature (NT) treatment and a mechanism interfering with lipid catabolism in seeds during the night.

Experiment

Two oilseed rape (*Brassica napus* L.) cultivars, namely Zheyou-50 (ZY) and Jiuer-13 (JR), were used in the study. ZY has a relatively high seed oil content (SOC) (50%) and nearly zero erucic acid (EA) (C22:1), whereas JR is an old local cultivar which had an SOC of ~35% and an EA proportion >30%.

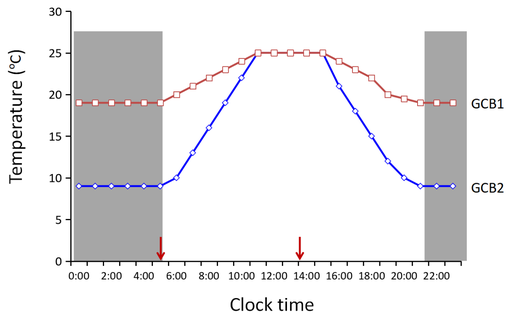

Fig.1. Design of the temperature treatment experiment in growth chambers. Plants were moved to growth chambers after the opening of the first flower. Different daily temperature cycles were set in two growth chambers, namely growth chamber 1 (GCB1) and GCB2. Curves indicate the daily temperature changes in the low (diamonds) and high (squares) NT chambers. The gray shaded area shows the time zone between 21.00 h and 05.00 h when the light was off in the chambers. Light in the chambers was on during 05.00–21.00 h. The arrows point to the time when developing seeds for RNA extraction were collected.

The **Fig.1** showed the design of the temperature treatment experiment in growth chambers. This study compared the total fatty acids and fatty acid compositions in seeds of two oilseed rape cultivars between high and low night temperatures by bioinformatics and biological analysis.

Results & Discussion

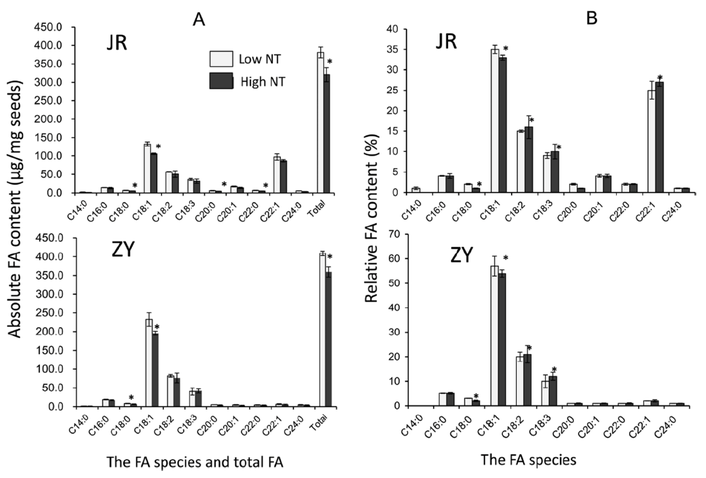

Fig.2. Comparisons of total FAs and FA compositions between low and high NT treatments. (A) Absolute values based on the means of biological repetitions showing total FA contents and various FA species. (B) Relative percentages of FAs in seed samples treated with low and high NTs. Asterisk indicates significant difference at P≤0.05 levels between the low and high NT treatments by *t-test*.

High NT caused low total FAs in both cultivars(**Fig.2A**). In particular, resulted in significantly lower C18:0, C18:1, C20:1 and C22:0 in JR seeds and significantly lower C18:0 and C18:1 in ZY seeds than those of low NT. The significant reduction of C18:0 and C18:1 and the non-significant decrease in the other FAs altered the various FA proportions in seeds (**Fig.2B**). High NT decreased the relative proportions of C18:0 and C18:1 but increased those of C18:2 and C18:3 in both cultivars.

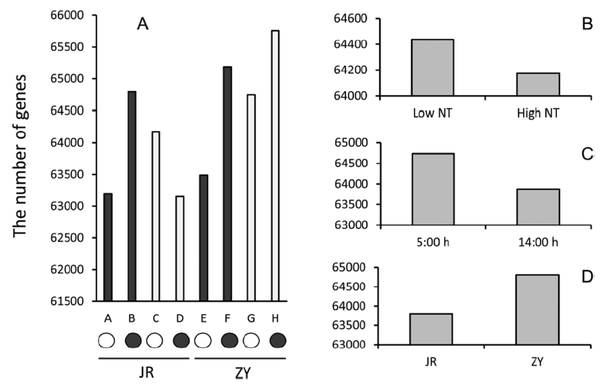

Fig.3. Overall number of expressed genes detected and gene expression level. (A) Comparison of the expressed gene numbers between eight treatments (A–H), (B) low and high NT treatments, (C) different time points when samples were harvested, and (D) two cultivars. The letters A, B, E, F represent low NT and C, D, G, H represent high NT on the X-axis in (A) represent high and low NT treatments, respectively. The open and filled circles in (A) indicate the harvest time at 05.00 h and 14.00 h, respectively. Values are denoted in means of two repetitions in (A) and four treatments with two repetitions each in (B–D).

The total number of genes expressed in each treatment ranged from 63,061 to 65,788 according to the threshold set(**Fig.3A**). On average, the samples treated with high NT displayed 266 less expressed genes than those treated with low NT (**Fig.3B**). Samples harvested at 14.00 h in the daytime showed 861 less expressed genes than those collected at 05.00 h in darkness (**Fig.3C**).

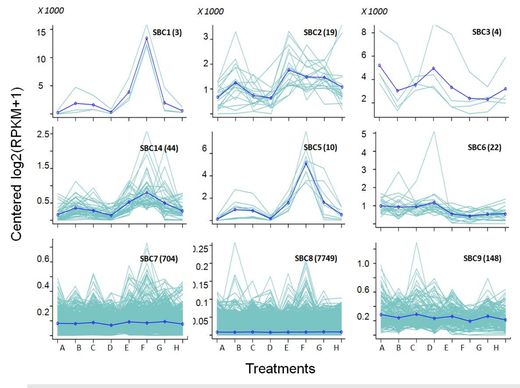

Fig.4. K-means cluster analysis for transcriptional changes in the oilseed rape genome against treatments A-H defined in Table 1. Nine groups of genes were classified as subcluster 1 (SBC1) to SBC9. The number of genes in each SBC is indicated in parentheses. The scales on the y-axis should be magnified 1000 times.

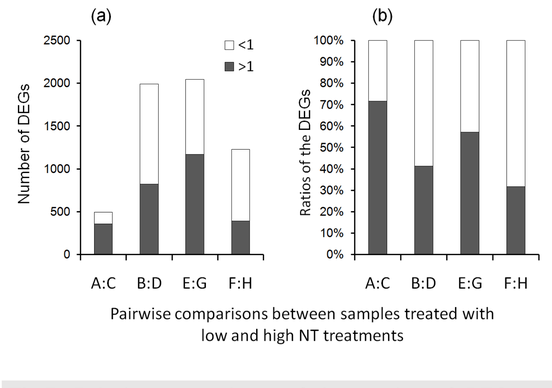

Fig.5. Pairwise comparisons of the number of DEGs and ratios of DEGs between the low and high NT treatments (A) Number of DEGs. (B) Ratios of up and down-regulated genes. White and dark gray bars indicate lower and higher gene expression in samples treated with high NT relative to those treated with low NT, respectively. A-H represent the eight treatments.

Nine clusters were classified on the basis of expressional changes against the eight treatments (A–H) (**Fig.4**). Genes exhibited significant differences in expression between NT treatments in clusters SBC1, SBC2, SBC3, SBC4, SBC5, and SBC6. The total number of DEGs caused by different NTs was compared between A and C, B and D, E and G, and F and H (**Fig.5A**). Results showed higher amounts of DEGs in ZY than in JR. High NT caused more up-regulations in samples harvested at 14.00 h (A, C, E, and G) than that at 05.00 h (B, D, F, and H) (**Fig.5B**).

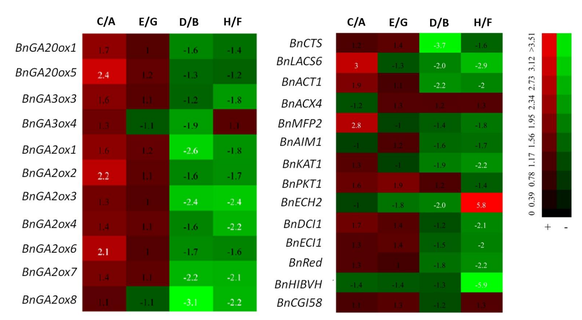

Fig.6. Comparison between RT-qPCR and RNA-seq results for DEGs caused by different NTs. (A and C) Results from RT-qPCR. (B and D) Results from RNA-seq. (A) and (B) are DEGs on the GA synthesis pathway, and (C) and (D) are DEGs on the β-oxidation pathway.

To confirm the RNA-seq results, several genes found in GA biosynthesis and glyoxylate metabolism were selected for RT-qPCR. RT-qPCR results generally matched well with the RNA-seq analysis results. High NT resulted in high expression of genes in the samples harvested at 05.00 h and low expression of genes in the samples harvested at 14.00 h in both cultivars. Only a few exceptions existed, as shown in **Fig.6**.

Conclusion

High night temperature significantly affected the total fatty acids and fatty acid compositions in seeds of both low and high oil content cultivars, namely Jiuer-13 and Zheyou-50, thereby resulting in 18.9% and 13.7% total fatty acid reductions, respectively. In particular, high night temperature decreased the relative proportions of C18:0 and C18:1 but increased the proportions of C18:2 and C18:3 in both cultivars. In-depth analysis of transcriptome profiles revealed that high night temperature up-regulated gibberellin signaling during the night-time. This up-regulation was associated with the active expression of genes involved in fatty acid catabolism, such as those in β-oxidation and glyoxylate metabolism pathways. Although the effect of temperature on plant lipids has been previously examined, the present study is the first to focus on night temperature and its effect on the fatty acid composition in seeds.

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