

Comparative transcriptome analysis of Arabidopsis and rapeseed under extreme temperature stress

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Abstract

Rapeseed (*Brassica napus* L.) is an important oil crop and it is cultivated in temperate regions. When the temperature rise above or fall under moderate range of temperature, rapeseeds would be under temperature stress. Temperature stress, which limits development and reproduction of plants, is very complex event controlled by enormous genes and it has limitation to understand exactly if several particular genes were studied only. To understand the responses of rapeseed under temperature stress, we have chosen 5 Korean rapeseed cultivars, Naehan, Youngsan, Yudal, Sandongchae and Tammi and examined the temperature stress responses in these cultivars. Firstly, cold and heat tolerance in these cultivars was compared. Naehan, Sandongchae and Yudal were relatively tolerant and Youngsan and Tammi were relatively sensitive to heat and cold stresses. Secondly, Naehan, a relatively tolerant cultivar, and Youngsan and Tammi, relatively sensitive cultivars were selected and the transcriptome analysis was performed using 1.6k Arabidopsis specialized cDNA microarray (for cold stress) or Arabidopsis 29k oligonucleotide microarray (for heat stress). Under heat and cold stress, change of gene expression level occurs widely. Therefore, we tried to divide the complex event into smaller events to interpret and compare the overall aspect of temperature stress. We constructed a database of gene set catalog using publically available microarray data and applied to our own transcriptome data.

Introduction

Plants, as sessile organisms, have evolved an enormous ability to realize their genetically predetermined developmental programmed despite ever-changing environmental conditions and are able to cope with environmental conditions such as extreme temperature stress (Shinozaki et al., 2003). Plants, including rapeseed, respond and adapt to these stresses through various biochemical and physiological processes, thereby acquiring stress tolerance (Harter et al., 2006). Here, we identified and analyzed the stress responsive genes to understand molecular backgrounds extreme temperature stress responses in 5 Korean rapeseeds.

Materials and Methods

Korean rapeseed cultivars

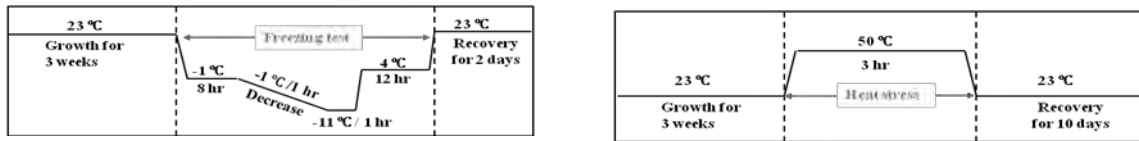
Table 1. Inbred line of rapeseeds.

Cultivar	Female	Male
Naehan	Erra	Tower
Sandongchae	B.napus	B. campestris
Yudal*	-	-
Youngsan	Erra	Tower
Tammi	Mangunjaerae/ Bronow-wski//Era	Erra

* Korean native rapeseed.

Freezing & Heat stress tolerance assay

The stress profile tests were performed according to the following schemes.



Cold stress treatment - Heat stress treatment -

Analysis of gene expression profile of rapeseed under extreme temperature stress

To monitor the changes of gene expression profile in response to extreme temperature stress treatment, RNA was prepared from rapeseed rosette leaves at various time point of stress treatment (0°C for cold stress, 45°C for heat stress). Microarray analysis was performed using 1.6k Arabidopsis specialized cDNA microarray for cold stress or Arabidopsis 29k oligonucleotide microarray for heat stress, and using GSEA method (<http://broad.mit.edu/gsea>) with co-expression gene set (Reiner et al., 2003, Subramanian et al., 2005).

Results & Discussion

Cold & Heat stress tolerance of 5 Korean rapeseed cultivars

Result

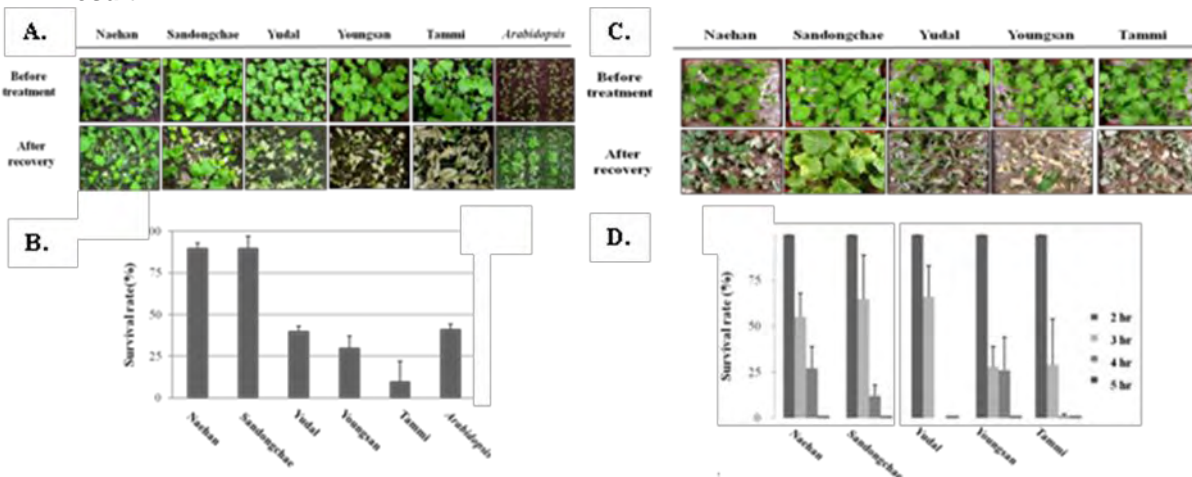


Figure 1. Physiology test under extreme temperature stresses. (A) 3-week-old rapeseeds were treated cold stress. (B) Survival rate after cold stress. (C) 3-weeks-old rapeseeds were treated heat stress. (D) Survival rate after heat stress.

Conclusion

Cold tolerance : Naehan ≥ Sandongchae >Yudal ≥ Arabidopsis > Youngsan > Tammi.
 Heat tolerance : Naehan ≥ Sandongchae > Yudal ≥ Youngsan > Tammi.

Analysis of gene expression of rapeseed under extreme temperature stresses.

Result

Microarray analysis was performed using 1.6k Arabidopsis. cDNA chip for cold stress or using. 29k Arabidopsis oligonucleotide microarray chip for heat stress (Table2).

Table 2. List of microarray data obtained in this study
(A) Cold stress response

Test ID	Cultivar	Treat condition	Test ID	Cultivar	Treat condition	Test ID	Cultivar	Treat condition
#1	Naeha n	Cold 1 hr	#11	Youngsan	Cold 1 hr	#21	Sandongch ae	Cold 1 hr
#2	Naeha n	Cold 3 hr	#12	Youngsan	Cold 3 hr	#22	Sandongch ae	Cold 3 hr
#3	Naeha n	Cold 8 hr	#13	Youngsan	Cold 8 hr	#23	Sandongch ae	Cold 8 hr
#4	Naeha n	Cold 24 hr	#14	Youngsan	Cold 24 hr	#24	Sandongch ae	Cold 24 hr
#5	Naeha n	Cold 72 hr	#15	Youngsan	Cold 72 hr	#25	Sandongch ae	Cold 72 hr
#6	Tammi	Cold 1 hr	#16	Yudal	Cold 1 hr	#26	Arabidopsis	Cold 1 hr
#7	Tammi	Cold 3 hr	#17	Yudal	Cold 3 hr	#27	Arabidopsis	Cold 3 hr
#8	Tammi	Cold 8 hr	#18	Yudal	Cold 8 hr	#28	Arabidopsis	Cold 8 hr
#9	Tammi	Cold 24 hr	#19	Yudal	Cold 24 hr	#29	Arabidopsis	Cold 24 hr
#10	Tammi	Cold 72 hr	#20	Yudal	Cold 72 hr	#30	Arabidopsis	Cold 72 hr

(B) Heat stress response

Test ID	Cultivar	Treat condition	Test ID	Cultivar	Treat condition	Test ID	Cultivar	Treat condition
#31	Naeha n	Heat 0 hr	#36	Tammi	Heat 24hr	#41	Yudal	Heat 3 hr
#32	Naeha n	Heat 3 hr	#37	Youngsan	Heat 0 hr	#42	Yudal	Heat 24hr
#33	Naeha n	Heat 24hr	#38	Youngsan	Heat 3 hr	#43	Sandongch ae	Heat 0 hr
#34	Tammi	Heat 0 hr	#39	Youngsan	Heat 24hr	#44	Sandongch ae	Heat 3 hr
#35	Tammi	Heat 3 hr	#40	Yudal	Heat 0 hr	#45	Sandongch ae	Heat 24hr

- *All microarray experiments performed in duplicate.

We counted the number of up or down-regulated genes, and then only genes induced or repressed more than 2-fold were considered to minimize the number of false positives related to data varieties due to extreme temperature stresses.

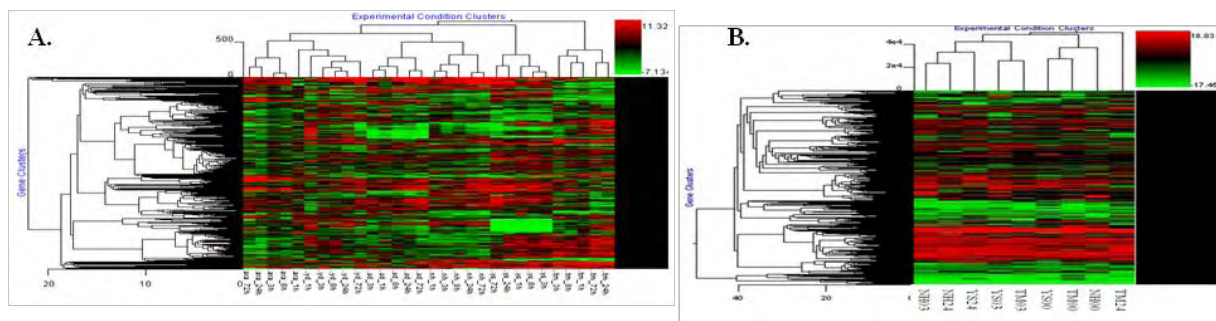


Figure 2. Hierarchical Clustering in rapeseed. (A) Hierarchical clustering analysis of gene expression patterns under cold stress condition rapeseed and Arabidopsis. (B) Hierarchical clustering analysis of gene expression patterns under heat stress condition rapeseed.

- **Conclusion**

Hundreds of genes were affected by extreme temperature stresses. Gene expression pattern of rapeseed under extreme temperature stresses are distinguished with the pattern of non-treated rapeseed.

Comparative analysis of early response of extreme temperature stresses.

- **Result**

In order to understand the process of extreme temperature stresses on a global scale, their stress responses were diagnosed using the co-expression gene sets. Using co-expression gene sets, Gene Set Enrichment Analysis (GSEA) was performed using GSEA software. We used the two different kind of microarray between these experiment, this is the reason for the using the different parameters.

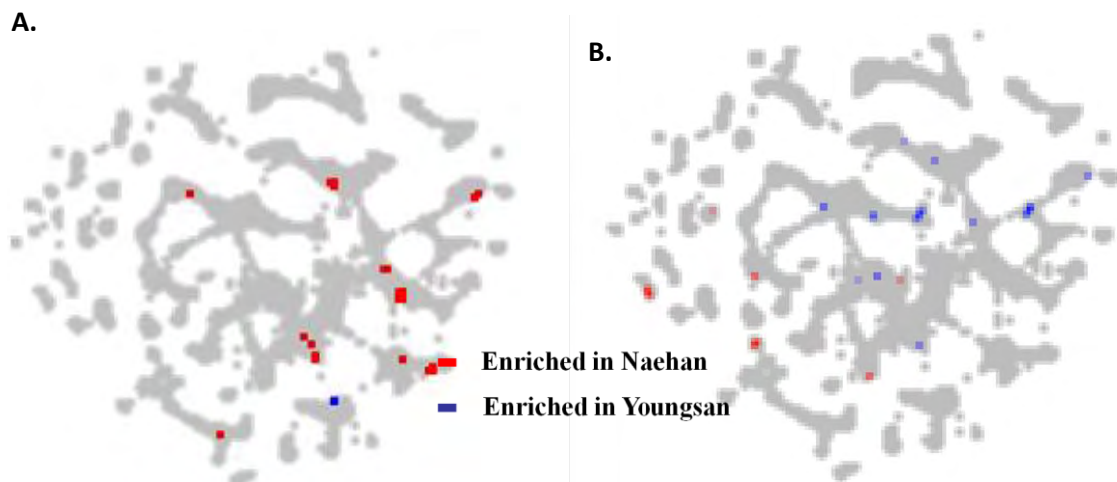


Figure 3. The enriched gene set from response of extreme temperature stresses between Naehan and Youngsan. (A) Comparative analysis of early cold response in the Naehan vs Youngsan (Early time : 1,3,8 h). (B) Comparative analysis of early heat response in the Naehan vs Youngsan (Early time : 0,3 h)

*Parameter : p-value < 0.05, False discovery rate (FDR) < 0.25 for cold stress.
p-value < 0.05, FDR < 0.05 for cold stress.

- **Conclusion**

According to previous experimental results, Naehan is the most tolerant to extreme temperature stresses. Youngsan is relatively sensitive to these stresses. The meaningful gene sets which would contribute to the enhancement of each extreme temperature stresses were selected and detail of these results from these approaches will be presented and discussed.

References

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