Association between drought-related genes expression and physiological indicators in different drought tolerrance varieties (*Brassica napusL.*)

Qingsheng Xiao, Guangyuan Lu*, Z. Li, Y. Chen, P. Y. Zheng, X. K. Zhang
Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Key Laboratory of Oil Crop Biology of the Ministry of Agriculture, Wuhan 430062, China
*the authors contributed equally to the article.

Correspondence
X. K. Zhang
Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan 430062, China
Tel: +86 27 86824573
Email: zhang.xk@139.com

Abstract: Eight drought-related genes in eight drought-tolerant rapeseed lines were chosen to study the transcription analysis of drought tolerance. The suggested that relative expression level of *Btg-26* was significantly higher in drought-tolerant lines after drought stress for 24 h. Drought-tolerant varieties had significantly higher expression levels of Δ^1-pyrroline-5-carboxylate synthetase (*P5cs*), betaine aldehyde dehydrogenase (*Badh*) and *Bnsos* gene than other varieties after 9 hours. Transcript accumulation was up-regulation of *Bnd22* in all cultivars during drought stress. In this study, The expression level of *Btg-26* after 24 hours was negatively correlated with relative electrolyte leakage (REL) and positive correlation with relative water content (RWC). The expression levels of *P5cs* gene and *Adc* gene after 3 hours were positive correlation with water content (WC) and drought index (DI) but negatively correlated with REL. The expression of *Badh* after 3 hours was negatively correlated with RWC and that of *Bnsos* after 24 hours was negatively correlated with H_2O_2 content. The results suggest that different physiological, expression characteristics should be applied in the selection towards drought resistance in the case of different drought resistance rapeseed varieties.

Keywords : *Brassica napus* L., Drought, Gene expression, physiological indicators

Introduction

Drought is a serious problem not only in arid and semi-arid areas but also in the Yangtze River region, the largest rapeseed-producing region in the world, and rapeseed yield may be reduced drastically both in autumn (September–December) and spring (February).

Plant growth and productivity are affected by abiotic forms of stress such as cold, heat, high salinity, and drought, or and plants must respond and adapt to such stress to survive. Stress due to abiotic factors induces several physiological and biochemical changes during the process in which plants acquire stress tolerance (Qin et al., 2007). Drought is considered to be one of the most important forms of abiotic stress that limit crop yield and affect crop productivity (Szira et al., 2008). Adaptation to drought is undoubtedly a complex process, involving changes in a number of genes and the induction of some genes. Exposure to drought leads to cellular dehydration, which causes osmotic stress: water moves cytoplasm into the extracellular space, thereby lowering the cytosolic and vacuolar volumes (Dorothea and Ramanjulu, 2005). The mechanisms of adaptation to drought are similar across a number of plant species and involve a series of physiological, cellular, and molecular processes culminating in stress tolerance (Kazuo and Kazuko, 2007). During drought, a large number of genes involved in adaptation to water stress are activated.

The study of drought tolerance in rapeseed has so far involved molecular features such as gene transcript accumulation or physiological features. However, we chose to focus on the association between physiological characters and transcript accumulation of drought-related genes in different cultivars during drought. We also analyzed the expression profiles of drought-related genes at different stages during drought stress.

Materials and methods

Plant materials and drought treatment

Eight rapeseed varieties with different levels of drought tolerance and different genetic backgrounds were used in the experiment. These varieties are Zhu3, Y8056, y27842, Zs6, S95-3, S9548, T44, and Q2. Drought stress was simulated and modified by adding 15% PEG-6000 (Szira et al., 2008).

Measurements of physiological characteristics

The relative leaf water content (RWC) was determined as 100 × (FW–DW) / (TW–DW), where FW is fresh weight, DW is dry weight, and TW is turgid weight. To measure TW, Water content (%) was calculated as (DW/FW) × 100 (Rapacz et al., 2010; Sergi and Josep, 2003). The content of MDA in the leaves was determined by the method described by Zhang et al. (2003). Relative electrolyte leakage was measured following and modifying the protocol of Blum and Ebercon (1981). The electrical conductivity (EC_1) of the solution was measured with a conductivity meter. After that, the cotyledon pieces were immersed in boiling water for 10 min, cooled to room temperature, and electrical conductivity (EC_2) measured again. Relative electrolyte leakage was expressed as a ratio of EC_1 to EC_2 in percentage terms. To determine the content of hydrogen peroxide (H_2O_2), plant material was ground in 2 ml of 1 M perchloric acid. Hydrogen peroxide in each extract was determined as described by Doulis et al. (1997). The plants were scored for drought tolerance on a scale of 0 to 4 as follows: 0, normal plants, no leaf wilting; 1, up to 20% of leaves wilted; 2, 20–50% leaves wilted; 3, almost all the leaves withered but the plant tops were still alive; and 4, the entire plant withered and nearly died. Drought index (DI) was calculated as follows: DI (%) = (1 × S1 + 2 × S2 + 3 × S3 + 4 × S4) / (TN × 4), where S1, S2, S3, and S4 represent the number of plants at each level of the scale and TN = total number of plants (Wang, 2009).

Analysis of drought-related genes transcripts accumulation during drought

Samples (the second leaf) were collected at the following times after adding PEG-6000 for simulating drought stress: 0 h (control), 3 h, 9 h, 24 h, and 48 h. PCR amplification was performed using a Bio-Rad IQ5 real-time PCR system (Bio-Rad, USA) in 96-well plates, using SYBR Green method (Ju et al. 2009). The cycling parameters of PCR were 94 °C for 5 min followed by 40 cycles of incubation at 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 30 s. All primers were designed using primer Express 3.0 (Applied Biosystems) on the basis of *Brassica napus L*. Relative (to *Actin*) expression of drought-related genes during drought was calculated for each replication with the $2^{-\Delta Ct}$ method as described by Livak and Thomas (2001) using instrumental replication for calculation of mean C_t value.

Results

Difference in levels of drought tolerance in eight rapeseed cultivars

Each of the eight rape cultivars had a different level of drought tolerance, cultivars Zs6 and S95–3 being the least tolerant and cultivars S9548 and Q2 being the most tolerant.

Table 1 Data (mean±S.D.) of studied physiological parameters measured in eight cultivars of *B. napus*) after 48 h of drought stress

Cultivar	DI (%)	REL (%)	MDA (μM)	WC (%)	RWC (%)	H_2O_2 (%)
Zs6	25.9g±1.0	71.7a±0.7	2.556a±0.10	6.8f±0.14	71.9cd±0.8	19.4c±0.4
S95–3	26.0g±0.9	59.8d±1.2	1.691c±0.05	7.3e±0.44	73.2c±0.9	15.2de±1.0
Y27842	48.1f±1.8	64.9c±1.0	1.852b±0.01	6.7f±0.12	67.6e±1.0	5.1e±1.1
Zhu3	51.7e±2.1	69.3b±0.4	1.348d±0.03	7.2e±0.06	70.4d±2.2	18.5c±1.2
T44	60.0d±1.6	47.1f±0.6	0.947fg±0.04	9.2d±0.12	78.2b±0.5	4.5d±0.4
Y8056	67.7c±2.3	55.4e±0.6	1.04ef±0.17	10.3c±0.16	77.0b±1.4	23.8b±0.4
S9548	79.3b±0.6	45.7f±0.3	1.143e±0.02	12.1a±0.06	84.5a±0.7	31.3a±1.0
Q2	86.7a±1.9	30.0g±1.4	0.914g±0.01	11.6b±0.12	85.7a±2.6	14.8de±0.5

Means followed by the same superscript letter within a column are not significantly different as determined by Fisher's Least Significant Difference (LSD) test at ($P < 0.05$).

The profiles of drought-related genes in different drought-tolerant cultivars

The expression profile of *Btg*-26 shows that *Btg*-26 had the highest expression level in the eight cultivars after 24 h of drought. Almost eight cultivars' relative expression value increased during the first 24 h of drought stress and declined thereafter. Among the eight cultivars, Q2 and S95-3, the two drought-tolerant cultivates, showed the highest level of expression of the gene during drought stress. The profile of *P5cs* showed little change in the first 3 h; after that, it showed two distinct patterns: in cultivars except S95-3 and S9548, the expression was much higher than that in the other cultivars after 9 h of drought stress and the highest expression value were at 9h drought stress. Whereas the expression in the other cultivars kept rising up to the highest expression value at 24 h but changed little down thereafter. It was noteworthy that cultivars Q2 and Y8056 showed steeper expression

curves between 3 h and 9 h of drought than those of other cultivars. This may indicate that *p5cs* is expressed quickly in Y8056, and Q2 in response to drought stress. *CaM* was highly expressed in all eight cultivars initially (9–24 h of drought stress) but declined thereafter. Specially, Q2 has a greater slope than others cultivars in 0h to 9h. The expression profile of *Bnd22* varied considerably with time as well as with the cultivar. The highest expression level was reached 9 h after drought in Q2, 24 h after drought in S9548, and 48 h after drought in T44 and Zs6. Although the exact values were different, all eight cultivates showed a similar pattern, namely little change up to 9 h and consistent increase with time thereafter, an increase that was, however, more rapid in Q2.

The association between transcript accumulation and physiological features

We analyzed the relationship between these physiological features and the accumulation of drought-related genes. Significant correlation was observed between the transcript accumulation of *Btg-26* and REL and RWC and between that of *P5cs* and DI, REL, and WC. Significant correlations were also observed between transcript accumulation of *Badh* gene and RWC, between that of *Bnsos* and H_2O_2 content, and between that of *Adc* gene and DI, REL, and WC. However, no significant correlations were found between transcript accumulation of *Cam*, *Plc*, or *Bnd22* and any of the physiological features.

Discussion

The results of this study showed that different drought-related genes had different patterns of temporal and spatial expression in the eight cultivars of rapeseed during drought stress, and some physiological characteristics of drought tolerance were significantly correlated with levels of transcript accumulation of some drought-related genes, a link that furthers our understanding of the mechanism of drought tolerance and helps in selecting for drought tolerance in *Brassica napus*.

The results presented here indicate that expression of drought-related genes is indeed important for the development of drought tolerance in rapeseed. Transcript accumulation of Btg-26 gene was higher in drought tolerant cultivars Q2 and S9548 than in other cultivars. Transcript accumulation of *Bnd22* was higher during drought stress and the highest in leaves (Downing, 2008), and we found the transcript level of *Bnd22* to be high in almost all the cultivars during drought, and especially high in the drought tolerant cultivars Q2 and 9548. Some related drought-induced proteins were accumulated as a result of the higher transcript accumulation of *Btg-26* and *Bnd22* genes, which was good for cell osmotic pressure. It is therefore likely that drought tolerant rapeseed cultivars are better able to adjust to osmotic stress. In this study, levels of transcript accumulation of *P5cs* changed greatly during drought stress. We also found that the drought tolerant cultivars Q2 and T44 recorded higher levels of transcript accumulation of *Badh* gene after 9 h of drought stress. These results indicate that the drought-tolerant cultivars adjust to changes in osmotic pressure by improving the transcript accumulation of *P5cs* and *Badh*.

In terms of the relationship between physiological characteristics and drought-related genes, cell membranes are one of the first sites to be affected because of stress, and it is generally accepted that maintenance of the integrity and stability of cell membranes under water stress is a major component of drought tolerance in plants (Mohammed et al., 2001). The degree of injury to cell membranes induced by water stress is easily estimated by measuring REC from the cells. In this study, REL was negatively correlated with the transcript accumulation of *Btg-26*, *P5cs*, and *Adc* genes, probably because cell membranes had been injured only slightly, and accumulation of *Btg-26*, *P5cs* and *Adc* genes was not seriously affected.

Water content and relative water content both are important components of water relations in leaves. Accumulation of *Btg-26* was positive correlation with RWC. WC was positive correlated with the *P5cs* and Adc transcript level. The results appear contradictory and may be explained as follows: drought-tolerant cultivars are better than drought-sensitive cultivars at absorbing and retaining water. MDA is a decomposition product of polyunsaturated fatty acids and used as a physiological parameter to measure lipid peroxidation (Patel and Vora, 1985). Although we found no correlation between MDA and the transcript level of drought-related genes, accumulation of *Bnsos* was negatively correlated with H_2O_2 content.

The results of this study described that the expression profile of drought-related genes during drought stress, which was good for understanding the mechanism of plants during drought. The study also indicated that the well-described molecular and physiological characteristics associated with drought tolerance. All this will be performed in the future for a better understanding of the drought tolerance complexity and being useful in molecular approaches to breeding for drought tolerance.