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Chapter

An Insight into the Responses of Early-Maturing *Brassica napus* to Different Low-Temperature Stresses

Xin He

Abstract

Rapeseed (*Brassica napus* L.) is an important oil crop worldwide, responds to vernalization, and shows an excellent tolerance to cold stresses during vegetative stage. The winter-type and semi-winter-type rapeseed were typical winter biennial plants in Europe and China. In recent years, more and more early-maturing semi-winter rapeseed varieties were planted across China. Unfortunately, the early-maturing rapeseed varieties with low cold tolerance have higher risk of freeze injury in cold winter and spring. The molecular mechanisms for coping with different low-temperature stress conditions in rapeseed recently had gained more attention and development. The present review gives an insight into the responses of early-maturing *B. napus* to different low-temperature stresses (chilling, freezing, cold-acclimation, and vernalization), and the strategies to improve tolerance against low-temperature stresses are also discussed.

Keywords: *Brassica napus*, low-temperature, early-maturing

1. Introduction

Low-temperature is a major environmental stress that adversely affects plant growth and development, limiting the productivity and regional distribution of crops [1, 2]. Rapeseed is an important oil crop worldwide, with planting area of 37.58 million hectares producing 75.00 million tons of oilseeds in 2018 (http://www.fao.org/faostat/). Based on vernalization requirement, rapeseed is divided into three main ecotypes, i.e., winter, semi-winter and spring types [3–5]. The winter type rapeseed is mainly grown in Europe and is sown in late summer, which requires strong vernalization and flowerings in spring, exhibiting a classical winter annual and with excellent cold tolerance during vegetative stage [3, 6]. The semi-winter type rapeseed is mainly grown in China only needs moderate or weak vernalization to promote flowering in spring, and with week cold tolerance [3, 7]. The semi-winter type rapeseed excessive exposure to low temperature stress in winter will lead to plant damage at vegetative stage and finally cause yield loss [8]. Yangtze River basin is the major region for planting semi-winter rapeseed in China, which accounts for at least 90% of the nation's total production [9]. The semi-winter rapeseed is usually sown in late September and early October shortly after the harvest of rice, and harvested in May before the cropping of rice in this area [10].

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However, in recent years, due to the delay of rice harvest which leads to the postpone of rapeseed sowing until late October or early November, therefore, more and more early-maturing semi-winter rapeseed varieties were planted across Yangtze River basin. Unfortunately, the early-maturing rapeseed varieties with low cold tolerance have higher risk of freeze injury in cold winter and spring [11]. Hence, it is vital to compare early-maturing rapeseed varieties tolerant to cold and evaluate molecular mechanisms that adapt to different low-temperature stress conditions.

2. Morphophysiological mechanism of rapeseed in responses to low-temperature stress

Cold (low-temperature) stress included chilling stress (>0°C) and freezing stress (<0°C) [12]. Chilling stress (0–15°C) causes the membrane to rigidify, destabilizes protein complexes and impairs photosynthesis, eventually made plant stop growing, whereas freezing stress (<0°C) causes intracellular and extracellular ice crystal formation, and results in mechanical injury, and plant death [13–15].

Despite the fact that winter and semi-winter rapeseed is an overwintering oil crop, cold stress can still affect rapeseed development and ultimately lead to a decrease in production [8, 11]. The suitable temperature scope is 10 ~ 20°C for the growth of winter and semi-winter rapeseed. The rapeseed flower number was reduced below 10°C and the rapeseed flowering was arrested when the temperature decreased to 5°C. The rapeseed growth was arrested below 3°C and rapeseed leaves was injured below 0°C [8]. The delay of rapeseed sowing results in poor germination [16], decreased seedling biomass [17, 18], delay of floral initiation and floral bud differentiation processes [17, 19], and decreased flower number, effective pod number, pod length, and seed yield [17, 20, 21] due to low-temperature stress. In January 2008, South China was exposed to an extremely ice-frozen weather, which caused serious injuries to winter rape, affected 77.8% of the overall winter rape area in China and resulted in 10.9% yield losses [22]. Due more and more early-maturing semi-winter rapeseed varieties were planted across Yangtze River basin, rapeseed faces increased risks from continuous low temperature overcast and rainy weather in March. Continuous low temperature overcast and rainy weather during the rapeseed flowering stage or after flowering decreased the ratio of effectual silique, seeds per silique and oil content [23]. In March and April 2010, the middle and lower reaches of the Yangtze River region were exposed to continuous low temperature overcast and rainy weather, which resulted in 10–20% yield losses [23].

To date, many studies have investigated the morphological and physiological changes of low-temperature stressed rapeseeds. Leaves are the main organ to perceive low temperature stress and transmit stress signal in plants [24]. The morphological changes (dehydrated and wilting) of leaves became increasingly evident with the decrease of temperature, due to the total water content in leaves of rapeseed decreased [25, 26].

In winter rapeseed, prolonged cold acclimation led to increased thickness of young leaf blades and leaf cell walls, modified dimensions of mesophyll cells, numerous invaginations of plasma membranes and large phenolic deposits in chloroplasts, large vesicles or cytoplasm/tonoplast interfaces [27, 28]. Unlike cold acclimation, transient freezing treatment reduced the thickness of leaf cell walls and phenolic aggregates, caused reversible disorganization of the cytoplasm and chloroplasts swelling [27, 28]. Obvious gaps existed in the chloroplast grana and starch grains increased in quantity and volume [25]. In general, cold-tolerant winter rapeseed usually grows slowly, having small thick creeping deep-green waxy leaves and large root system.

Low temperature-induced thermodynamic constraints on carbon metabolism was the primary reason for lower photosynthetic activity in plants [24]. Photosynthetic efficiency is a good indicator for Low temperature tolerance in plants [10]. Just like in other crop plants, a marked reduction of photosynthetic activity is observed in rapeseed leaves when treated with low temperature [24, 29]. Tough the photosynthetic activities were reduced both in the cold-stressed leaves of cold-tolerant and cold-sensitive rapeseed cultivars, the chlorophyll a, chlorophyll b and photosynthetic activities in the young leaves of cold-tolerant cultivar all were higher than that in cold-sensitive cultivar [24].

Simultaneously, low-temperature stress caused the overproduction of reactive oxygen species (ROS), elevated H_2O_2 level and increased malondialdehyde (MDA) content in plants, which leads to a necrosis of plants. Plants possess an effective antioxidant system includes superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) enzymes, whose combined activities play an important role in elimination of destructive effects of ROS [24, 30]. Furthermore, under natural cold stress in field, the proline, soluble sugar, soluble protein, MDA contents and SOD, POD, CAT activities changed obviously in functional leaves of rapeseed. CAT and SOD activity reached the highest when temperature dropped to 5 and 3°C, respectively. The proline and soluble sugar contents increased when mean daily temperature decreased to 5°C and reached the maximum when temperature was below 0°C. The contents of soluble protein and MDA showed a trend to decrease at first and then increase when mean daily temperature dropped to 10, 5 and 0°C [30]. The SOD and APX activities were both increased by low temperature in the young leaves of cold-tolerant rapeseed cultivar. However, the APX activity was decreased by low temperature in the young leaves of cold-sensitive rapeseed cultivar. While, in the cold-stressed mature leaves, both cold-tolerant and cold-sensitive rapeseed cultivars represented similar antioxidant capacities [24].

Under chilling and freezing stress, the increment of proline accumulation, soluble sugar and protein contents were enhanced in cold-tolerant cultivar compared with cold-sensitive cultivar [24, 26]. Leaf abscisic acid (ABA) was enhanced in cold-tolerant cultivar under chilling and freezing stress [26].

3. Molecular mechanisms influencing responses to different low-temperature stresses in rapeseed

Plants showed increased freezing tolerance during exposure to chilling and low nonfreezing temperatures in a phenomenon known as cold acclimation [31]. The molecular mechanism of cold acclimation and cold tolerance in *Arabidopsis* and winter cereals has been extensively studied. Cold acclimation is a very complex trait involving an array of physiological and biochemical modifications, and these altered processes involved changes in gene expression patterns via phytohormone and the ICE (Inducer of CBF Expressions)-CBF (C-repeat binding factors)-COR (cold-responsive) signaling pathway [13, 32, 33].

3.1 ICE-CBF-COR signaling

In most plant species, CBF transcription factors could bind directly to the promoters of *COR* genes and induce the expression of *COR* genes [34–36]. The *COR* genes protected plant cells against cold-induced damage, repaired cold-rigidified membranes and stabilized cellular osmotic potential by encoding cryoprotective proteins and key enzymes for osmolyte biosynthesis [37]. In Arabidopsis, the basic

helix-loop-helix transcription factor ICE1/2 were induced by cold stress, could bind directly to the promoters of CBF and induced CBF expression under low-temperature stress [38–40].

Similar as other plants, the expression of CBF and COR genes were induced by chilling and freezing stresses in different ecotypes rapeseed with different cold tolerance [11, 36, 41–45]. CBFs (BnaAnng34260D/BnaCnng49280D/BnaC03g71900D/BnaC07g39680D), *Kin1* and COR15 all were upregulated in both winter and semi-winter ecotype rapeseeds after cold stress (4°C for 7 days), while *BnaA08g30910D* (a CBF-like gene) and *BnCOR25* were not varied in expression in any cold stressed rapeseeds [41]. Furthermore, COR15A/B, COR413-PM1 and nine CBF1/2/4 genes all were strongly upregulated in winter and spring ecotype rapeseeds after cold stress (4°C for 12 h) [42]. While *ICE1* and COR413-PM2 was downregulated in both winter and spring ecotypes after cold stress, two *CBF3* genes were not induced by cold stress [42]. Most of *COR15A* and *COR15B* were significantly induced in both cold-tolerant and cold-sensitive semi-winter early-maturing rapeseeds after chilling (4°C for 12 h) and freezing (−4°C for 12 h) stress, regardless of cold acclimation (4°C for 14 days) [11]. Ten of 12 *CBF* genes were strongly induced by freezing stress more than chilling stress, regardless of cold acclimation [11]. Unlike other CBFs, BnaC03g71900D was induced by chilling stress more than freezing stress and BnaA03g13620D was induced by freezing stress but suppressed by cold acclimation [11]. However, two *ICE1* genes were not induced by chilling and freezing stress [11], as opposed to *AtICE1* [38]. Inexplicably, no CBF genes were identified in responsive to freezing stress in freezing-tolerant rapeseed as reported by Pu [25].

BnCOR25 were significantly induced by cold and osmotic stress treatment in rapeseed, overexpression of BnCOR25 in Arabidopsis enhances plant tolerance to cold stress [46]. Overexpression of two rapeseed CBF-like transcription factors BnCBF5 and BnCBF17 in spring rapeseed resulted in increased constitutive freezing tolerance, increased photochemical efficiency and photosynthetic capacity [29]. However, constitutively overexpressing *BNCBF5/17* in rapeseed resulted in various degrees of dwarf habit and longer time to flower [29]. The multi-gene (NCED3, ABAR, CBF3, LOS5, and ICE1) transgenic rapeseed plants exhibited pronounced growth advantage under both normal growth and stress conditions [47].

3.2 ABA signaling

Abscisic acid (ABA) is a vital plant hormone that plays a key role in stress resistance during plant growth and development [48–50]. It was reported that ABA levels are increased after cold stress in plants and exogenous application of ABA can induce plant cold tolerance [11, 51, 52]. OST1/SnRK2E, a serine-threonine protein kinase in ABA core signaling pathway, acted upstream of CBFs to positively regulate freezing tolerance via phosphorylating ICE1 to prevent its 26S proteasome-mediated degradation by HOS1 [53]. OST1 phosphorylated basic transcription factors 3 (BTF3) and BTF3-like factors, and facilitated their interactions with CBFs to promote CBF stability under cold stress [54].

27 ABA biosynthesis genes (nine-*cis*-epoxycarotenoid dioxygenase (NCED3/4/5/9), ABA DEFICIENT 1/2 (ABA1/2), abscisic aldehyde oxidase 1/2/3 (AAO1/2/3) and carotenoid cleavage dioxygenase 1 (CCD1)) were regulated by cold stress in winter and/or spring rapeseed. Additionally, many genes involved in ABA signal transduction, such as ABA INSENSITIVE 1/5 (ABI1/5), ABA-responsive element binding protein 3 (AREB3), ABA responsive element-binding factor 1/2/3 (ABF1/2/3), highly ABA-induced PP2C gene 1/2/3 (HAI1/2/3), OPEN STOMATA 1 (OST1), PYR1-like 4/6/7/10 (PYL4/6/7/10), regulatory component of ABA receptor 1/3 (RCAR1/3), SNF1-related protein kinase 2.2/2.5/2.7/2.10 (SnRK2.2/2.5/2.7/2.10),

all were differentially expressed in winter and/or spring rapeseed after cold stress [42]. Furthermore, in freezing-treated (−2°C) leaves of cold-tolerant winter rapeseed line 2016TS(G)10, one PYL gene and one ABI5-like gene were up-regulated, while 13 PP2C and 4 ABI5-like genes were down-regulated [25]. 72.8% ABA signaling genes (94/129) were regulated by chilling and/or freezing in both cold-tolerant and cold-sensitive rapeseed plants but they presented different expression profiles [11]. The ABA receptors *PYL5/7* genes were both induced by cold and freezing stress, while the *PYL1/9* genes were suppressed. The *PYR1/4* genes were only induced by chilling stress but not by freezing stress. The *PYL6* were induced by chilling stress and freezing stress following cold acclimation. ABA co-receptor *ABI1* and *HAB1* were suppressed by all low-temperature treatments, while *HAB2* was upregulated. The *SnRK2B* and *SnRK2D* were induced by all low-temperature treatments, while *SnRK2C* was suppressed. *SnRK2F* and one ABI5 were both induced only by freezing treatment. The *OST1* was induced only in cold-tolerant but not in cold-sensitive rapeseed [11]. While 13 ABI5-like genes have a complex expression pattern in response to different low-temperature conditions, including up-regulated, down-regulated and no changed. Exogenous application of ABA significantly improved the rapeseed seedlings freezing tolerance [11]. Overexpression of *BnaABI3* leads to improved embryo degreening following frost exposure and enhanced pod strength in rapeseed [55].

3.3 Ca^{2+} signaling

Calcium (Ca^{2+}) is an important second messenger of signal transduction in the plant stress responses, plant growth and development. Ca^{2+} signaling were detected and transmitted by calmodulin/calmodulin-like proteins (CaM/CML), calcium-dependent protein kinase (CDPK) and calcineurin B-like proteins (CBLs) [56, 57]. The level of cytosolic Ca^{2+} was transiently increased in plants under cold stress [57–59]. In rice, COLD1 interacts with the G-protein α subunit and activates the Ca^{2+} channel, results the increment of expression of CBF under low-temperature stress [60]. In *Arabidopsis*, overexpression of CaM3 repressed the expression of *COR* genes (*RD29A*, *KIN1* and *KIN2*) [61]. CaM-binding transcription activators (CAMTAs) bind to the promotor of CBF2, promoting CBF2 expression and plant freezing tolerance [62, 63].

88 of 129 *CaM/CML* genes were regulated by cold stress in semi-winter rapeseed cultivar ZS11 [64]. 91 of 129 *CaM/CML* were regulated by chilling and/or freezing stress in semi-winter early-maturing rapeseed, and most of them were strongly induced by freezing stress [11, 64]. Additionally, 22 rapeseed CDPK genes were up-regulated by freezing treatments [11]. Furthermore, there were 91 genes involved in Ca^{2+} signaling (35 *CDPK*, 38 *CaM*, 16 *CIPK* and 2 Ca^{2+}-ATPase) were differentially expressed in winter rapeseed after cold stress, while 79 genes (44 *CDPK*, 19 *CaM*, 15 *CIPK* and 1 Ca^{2+}-ATPase) were differentially expressed in spring rapeseed after cold stress [42]. In Zhang's report, there were 5 CBL, 7 CIPK and 7 CDPK genes were regulated by cold treatment [65, 66].

4. Future directions

Rapeseed is one of the most important oil crops in the world and China and is affected by chilling and freezing stress. In recently years, several studies have tried to identify the main signaling pathways and genes responsible for low-temperature stress (chilling and/or freezing; cold acclimation and/or cold shock) in different rapeseeds (winter, semi-winter and spring type; cold-sensitive and cold-tolerant;

late maturing and early maturing) based on transcriptomics, metabolomics, lipidomics, and QTL analyses [11, 25, 41, 42, 45, 67–69]. Tough there were so many candidate genes involved in the response to low-temperature stress have been identified, only few genes' functions in cold tolerant have been tested and verified in rapeseed [10, 29, 55, 70, 71]. It is a pity that constitutive overexpression of rapeseed BnCBF5 and BnCBF17 resulted in various degrees of dwarf habit and longer time to flower, tough which resulted in increased freezing tolerance remarkably in spring rapeseed "Westar" [29]. There is still much work to be performed to understand rapeseed plants' responses to low-temperature stress and breed cold-tolerant rapeseed.

Genome editing is an efficient approach for crop improvement either by loss or gain of gene function and several different strategies have been developed [72]. Tough there were a few studies using CRISPR/Cas9 system for editing genes associated with plant/pod development, fatty acid synthesis and biotic stress response [72], no application of CRISPR-Cas9 for editing genes involved in chilling and freezing tolerant in rapeseed. It is expected that the newly emerging genome editing system will make a contribution to future gene function research and molecular design breeding in cold-tolerant rapeseed.

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