

The Response Analysis of BR Related Genes at Low Temperature in Potato Sprout Regulation

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Abstract Brassinosteroids (BR) plays an important role in regulating plant growth. We found that the expression of BR synthesis and signal transduction genes were up-regulation when potato dormancy was released, and suckering agents can inhibit the expression of Methylsterol monooxygenase 1 (SMO1), Delta24-sterol reductase 1 (DWF1), Protein BR insensitive 1 (BR11), BR-signaling kinase (BSK) and Cyclin D3 (CYCD3) genes in potato tuber. Besides, we found that the storage period among different cultivars are significant different at low temperature. Low temperature can obviously extend cultivar ‘Favorita’ storage time than cultivar ‘Mira’. In order to explore the response of BR genes to potato sprout at low temperature. This study will analyze the expression of BR synthesis, signal transduction and regulation genes at from cultivar ‘Favorita’ and cultivar ‘Mira’ which both cultivars are storage at low temperature (4°C) and room temperature (23±2)°C environment by qRT-PCR technique. The results showed that the expression of DWF1 and BR11 are decreased in both cultivars by low temperature treatment. The expression of *SMO1*, *BSK* and *CYCD3* genes are decreased in cultivar ‘Favorita’ by low temperature treatment. While, the expression of *SMO1*, *BSK* and *CYCD3* genes in ‘Mira’ are increase by temperature treatment. It shows that low temperature influences the expression of *SMO1*, *BSK* and *CYCD3* genes in cultivar ‘Favorita’, which results in the long dormancy period of the cultivar ‘Favorita’. These results will provide the theoretical basis in elucidating the BR mechanism of potato sprout, breeding variety selection and new technology research and development of potato storage regulation.

Keywords Potato; Dormancy; Sprout; Low temperature; Brassinosteroids

Background

As the fourth largest grain crop in China, potato is a both grain and vegetable crop, which is rich in amino acids, vitamins, mineral elements, etc. (Tian et al., 2017). Potato storage plays an important role in potato industry. However, about 4.1 million tons of potatoes are lost each year in China because of improper storage (Zhang et al., 2012). The dormancy period of potato is affected by temperature, humidity, and growth regulator. 85% of the humidity was considered to be the optimum humidity for prolonging the storage time of the potato, and the 4 mg/L abscisic acid treatment could also significantly prolong the storage time of the potato (Zhong et al., 2017). At present, low temperature is often used as the main method to prolong the storage time of potato (Si et al., 2007, Chinese Potato Journal, (2): 104-107). The dormancy period of potato is not only affected by external conditions, but also closely related to its own genotype (Bamberg, 2010; Fan et al., 2009). We found that brassinosteroids (BR) plays a key role in dormancy release and germination of potato.

As polyhydroxyl steroid plant hormones, brassinosteroids is involved in the regulation of plant growth and development, including promoting seed germination, inducing flowering, plant photomorphogenesis and improving plant stress resistance (Clouse and Sasse, 1998; Steber and McCourt, 2001). Zou et al. (2017) analyzed the expression of BR synthesis and signal transduction in potatoes of different dormancy, which are storage at suckering agents and room temperature, and found that the genes expression of BR synthesis Delta24-sterol

reductase 1 (*DWF1*), Methylsterol monooxygenase 1 (*SMO1*), Protein BR insensitive 1 (*BRI1*), BR-signaling kinase (*BSK*) and Cyclin D3 (*CYCD3*) were up-regulation when potato dormancy was released. BRs play an important role in the release of dormancy and germination of tubers. Besides, we found that the suckering agents can inhibit the expression of above genes in potato tuber.

In order to explore the response of BR genes *SMO1*, *BRI1*, *DWF1*, *BSK* and *CYCD3* to potato sprout at low temperature, this study analyzed the expression of BR synthesis, signal transduction and regulation genes at from cultivar ‘Favorita’ and cultivar ‘Mira’ which both cultivars are storage at room temperature (23±2)°C and low temperature (4°C) environment. We observed the growth of apical sprout by stereoscopic microscope, and measured its length. qRT-PCR technique was used to analyze the expression of the gene under different dormancy and germination stages.

1 Results and Analysis

1.1 Sprout length measurement of ‘Favorita’ and ‘Mira’

Observed the microscopy of potato apical sprout in ‘Favorita’ at room temperature and low temperature (Figure 1), we found that at room temperature the apical sprout length was less than 1 mm at the time of storage of 0~19 d, on the 20th day, the apical sprout was gradually extended, and on the 37th day, it was more than 2 mm, the dormancy was released. At the low temperature, the apical sprout length was less than 1 mm at the time of storage of 0~19 d, it was more than 1 mm, but less than 2 mm at the time of storage of 20~48 d, and on the 49th day, it was more than 2 mm, the dormancy was released.

Observed the microscopy of potato apical sprout in ‘Mira’ at room temperature and low temperature (Figure 2), we found that at room temperature the apical sprout length was less than 1 mm at the time of storage of 0~21 d, on the 22th day, the apical sprout was extended slowly, and on the 48th day, it was more than 2 mm, and the dormancy was released. At the low temperature, the apical sprout length was less than 1 mm at the time of storage of 0~21 d, it was more than 1 mm, but less than 2 mm at the time of storage of 22~52 d, and on the 53th day, it was more than 2 mm, the dormancy was released. Through the changes of apical sprout of the two cultivars at room temperature and low temperature, we found that low temperature can obviously prolong the germination time of ‘Favorita’, but has little effect on the dormancy time of ‘Mira’ (Table 1; Table 2).

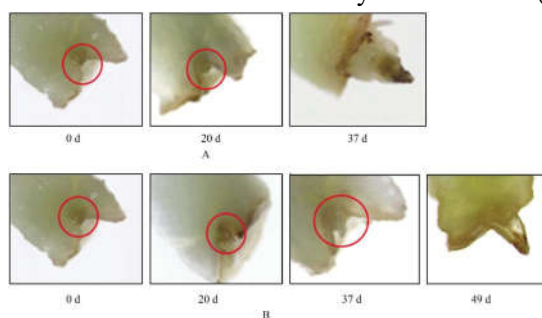


Figure 1 The microscopy of potato apical sprout in ‘Favorita’ at room temperature and low temperature

Note: A: The microscopy of potato apical sprout in ‘Favorita’ at room temperature; B: The microscopy of potato apical sprout in ‘Favorita’ at low temperature

1.2 The expression of BR synthesis genes in ‘Favorita’ and ‘Mira’

Analyzed the *DWF1* and *SMO1* genes expression which were treated with different temperature (Figure 3), we found that the expression of these two genes in ‘Favorita’ is similar at room temperature. At the time of 0~29 d, the expression of two genes was low, but it was increasing sharply at the time of 30~49 d. While, the expression of *SMO1* genes in ‘Mira’ were up-regulation when dormancy was released, and reached maximum on the 53th day. *DWF1* tended to be stable at 0~43 d and increased sharply on the 44th d until dormancy was released. We found that the expression of *SMO1* and *DWF1* was different in different cultivars at low temperature. The expression of *DWF1* gene in ‘Favorita’ is decreased at 0~50 d; the expression of *SMO1* gene is decreased at 20~49 d, and on the

5th day is the peak. The expression of *DWF1* gene in ‘Mira’ is decreased at low temperature, *SMO1* gene showed a low expression peak at 15~44 d. The two genes are involved in BR synthesis at room temperature, which affects the growth and development of apical sprout. The expression of *DWF1* gene in both cultivars was inhibited at low temperature. The expression of *SMO1* gene was inhibited by low temperature in ‘Favorita’, but not by low temperature in ‘Mira’. The result showed that *SMO1* has a specific response to low temperature in different potato cultivars.

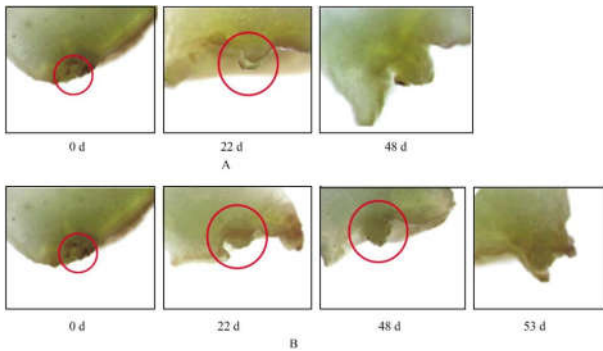


Figure 2 The microscopy of potato apical sprout in ‘Mira’ at room temperature and low temperature

Note: A: The microscopy of potato apical sprout in ‘Mira’ at room temperature; B: The microscopy of potato apical sprout in ‘Mira’ at low temperature

Table 1 Sprout length measurement of “Favorita”

Treatment	Growth length (mm)				
	0~19 d	20~36 d	37 d	38~48 d	49 d
Room temperature (23°C±2°C)	Sprout length<1	1<sprout length<2	Sprout length>2	Sprout length>2	Sprout length>2
Low temperature (4°C)	Sprout length<1	1<sprout length<2	1<sprout length<2	1<sprout length<2	Sprout length>2

Table 2 Sprout length measurement of “Mira”

Treatment	Growth length (mm)				
	0~21 d	22~47 d	48 d	49~52 d	53 d
Room temperature (23°C±2°C)	Sprout length<1	1<sprout length<2	Sprout length>2	Sprout length>2	Sprout length>2
Low temperature (4°C)	Sprout length<1	1<sprout length<2	1<sprout length<2	1<sprout length<2	Sprout length>2

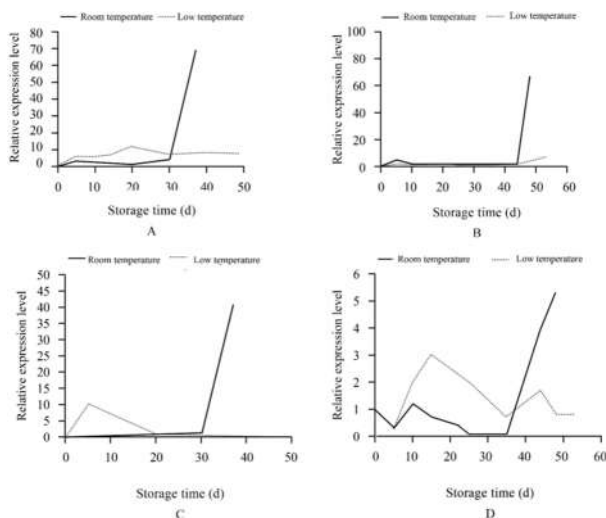


Figure 3 *DWF1* and *SMO1* genes expression of ‘Favorita’ and ‘Mira’ which were treated with room temperature and low temperature

Note: A: *DWF1* gene expression in ‘Favorita’; B: *DWF1* gene expression in ‘Mira’; C: *SMO1* gene expression in ‘Favorita’; D: *SMO1* gene expression in ‘Mira’

1.3 The expression of BR signal transduction genes in ‘Favorita’ and ‘Mira’

The expression of *BR11* and *BSK* in ‘Favorita’ and ‘Mira’ were up-regulation when dormancy was released at room temperature (Figure 4). On the 30th day, the expression of *BR11* and *BSK* in ‘Favorita’ was increasing sharply. While, the expression in ‘Mira’ was increasing sharply on the 44th day at dormancy. The expression of *BR11* tended to be stable in two cultivars at low temperature; it showed a low expression peak on the 12th day in ‘Favorita’. The expression of *BR11* was increased in ‘Mira’ at 0~15 d, decreased on the 15th day, and then tended to be stable until dormancy was released, it decreased again. The expression of *BSK* gene was significantly different between the two cultivars treated with low temperature. The expression of *BSK* gene in ‘Favorita’ was increased at 0~19 d, on the 20th day, *BSK* expression was decreased inhibited by low temperature. *BSK* gene in ‘Mira’ showed the first low expression peak on the 15th day treated with low temperature, and increased sharply on the 44th day. The results showed that *BRI* and *BSK* genes played an important role in potato germination. The expression of *BR11* in ‘Mira’ and ‘Favorita’ was inhibited by low temperature. The expression of *BSK* was inhibited by low temperature when stored in ‘Favorita’ at 22~49 d, while the expression of *BSK* was not inhibited by low temperature in ‘Mira’. *BSK* had a specific response to low temperature in different potato cultivars.

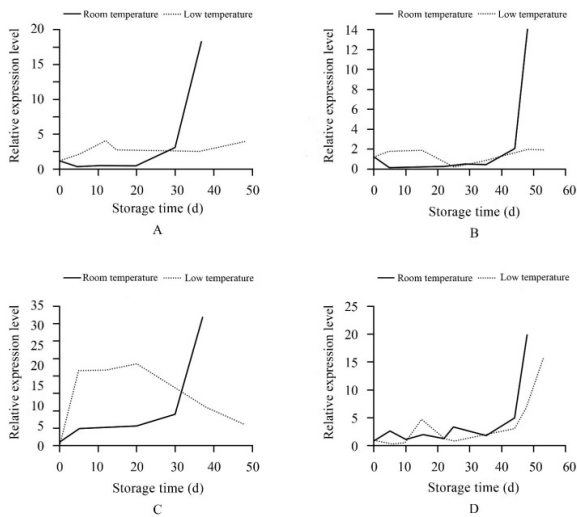


Figure 4 *BR11* and *BSK* genes expression of ‘Favorita’ and ‘Mira’ which were treated with room temperature and low temperature
 Note: A: *BR11* gene expression in ‘Favorita’; B: *BR11* gene expression in ‘Mira’; C: *BSK* gene expression in ‘Favorita’; D: *BSK* gene expression in ‘Mira’

1.4 The expression of BR signal regulation genes in ‘Favorita’ and ‘Mira’

The *CYCD3* gene expression in ‘Mira’ showed the first peak on the 26th day treated with room temperature, and was increasing sharply on the 35th day. While, *CYCD3* gene expression in ‘Mira’ showed the first peak on the 10th day treated with low temperature, and was increasing sharply on the 44th day (Figure 5B). The *CYCD3* gene expression in ‘Favorita’ was decreased treated with room temperature, and was increasing sharply on the 30th day. But it was decreased inhibited by low temperature (Figure 5A). The results showed that *CYCD3* gene has a specific response to low temperature in different potato cultivars.

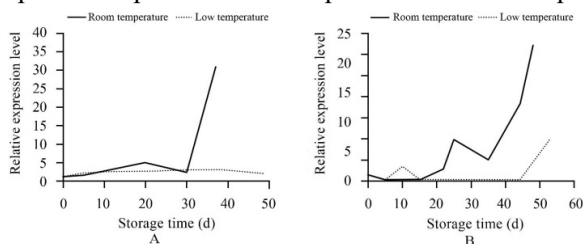


Figure 5 *CYCD3* gene expressions of ‘Favorita’ and ‘Mira’ which were treated with room temperature and low temperature
 Note: A: *CYCD3* gene expression in ‘Favorita’; B: *CYCD3* gene expression in ‘Mira’

2 Discussion

The results of the microscopy of potato apical sprout in 'Favorita' and 'Mira' at room temperature and low temperature showed that low temperature could inhibit the growth rate of potato apical sprout and prolong its storage period. The storage characteristics of potato were closely related to the cultivars (Pu et al., 2016). And according to the germination of 'Favorita' and 'Mira' at room temperature and low temperature, we could find that the sensitivity of different potatoes to low temperature was significantly different. Suttle (2008) applied auxin to dormant potato and found that the sensitivity of potato to exogenous auxin was different in different dormant period, and the later stage of dormancy was more sensitive to exogenous auxin. From this we could speculate that sensitivity of different cultivars of potato to low temperature in germination stage is different, which results in different effects of low temperature inhibition on germination of different potato.

BR is a necessary signal for tuber to release dormancy and germination. If its synthetic gene expression is inhibited, tuber germination will be blocked. The signal transduction and regulation between BR synthesis-related *SMO1*, *DWF1* members *BRI1*, *BSK*, and *CYCD3* is a necessary process for the transformation of plant physiological state (Zhang et al., 2009). Besides, BR plays an important role in improving plant stress resistance at low temperature. Ma (2015) treated corn seeds pretreated with EBR under low temperature stress, and found that the exogenous EBR obviously promoted the germination of the corn seeds and the growth rate of the seedlings at low temperature. This study analyzed the BR synthesis, signal transduction, and activated genes (*SMO1*, *DWF1*, *BRI1*, *BSK*, *CYCD3*) expression of 'Favorita' and 'Mira' at different dormancy period with different temperature, and found that these 5 genes expression of 'Mira' and 'Favorita' which were treated with room temperature were up-regulation when potato germination. This is consistent with the results of Zou et al. (2017). The BR synthesis signal transduction gene expression of 'Favorita' was inhibited by low temperature during germination. While the *SMO1*, *BSK*, and *CYCD3* genes expression of 'Mira' was not inhibited by low temperature during germination. Therefore, we speculated that the *SMO1*, *BSK*, and *CYCD3* genes expression of two cultivars could be affected by the low temperature, which leads to the difference of the effect of two cultivars. And we can effectively inhibit the growth of potato sprouts by inhibiting their expression in the germination of the potato. The results showed that the BR synthesis metabolism is closely related to the outside temperature in addition to its effect on potato tuber dormancy and germination.

3 Materials and Methods

3.1 Materials and reagents

Potato cultivars 'Favorita' and 'Mira' were provided by the potato research and development center of the Agricultural College of Sichuan Agricultural University.

Reverse transcription kit: RevertAid First Strand cDNA Synthesis, TRIzol, DEPC, and SGExcel FastSYBR Mixture.

3.2 Materials handling

The new potato cultivars 'Favorita' and 'Mira' of the same size (7~10 g/per) were selected, and washed with clean water, putting them into closed cartons after healing at room temperature. Each carton was filled with 40 potato seeds, and the cartons were stored at room temperature (23 ± 2)°C until the middle and late dormancy stages of two cultivars respectively, then stored at room temperature (23 ± 2)°C and constant temperature freezer (4°C) respectively. The above steps were repeated three times. The results of the microscopy of potato apical sprout was measured when the sprout was less than 1 mm, between 1 mm and 2 mm, and more than 2 mm. Taking the sprout eye as the center, collecting a potato cylindrical tissue with a height of 5 mm, and a diameter of 3 mm. This sample was quickly placed in liquid nitrogen and stored at -80°C for RNA extraction.

3.3 Gene expression determination by qRT-PCR

TRIzol was used to extract RNA from sample. High salt, ethanol, freeze-thaw, and other treatments were used to remove polysaccharides from potato tubers. The reverse transcription system: The mixture of 1 µL Oligo dT, 8 µL

DEPC H₂O, and 3 μL RNA was placed in water at 65°C, and cooled on ice for 2 min immediately after 5 min. Add 4 μL 5× reaction buffer, 2 μL 10 mmol/L dNTPs mixture, 1 μL RNase inhibitor (20 U/μ), 1 μL Reverse Transcriptase (200 U/μL) respectively. Reaction was stopped after 60 min at 42°C, and 10 min at 72°C. Oligo 6.0 software was used to design its specific primers according to each gene sequence (Table 3). *EF1aL* gene was selected as internal reference. 25 μL Fluorescence quantitative PCR: 12.5 μL 2×SGExcel FastSYBR mixture, 10.5 μL RNase-Free H₂O, 0.5 μL upstream and downstream primers, and 1.0 μL cDNA. The expression of each gene was detected by the Bio-Rad CFX Connect PCR machine. $2^{-\Delta\Delta C_t}$ was used to calculate the detection results of relative expression of each gene. The ΔC_t value of ‘Favorita’ and ‘Mira’ was used as a control at the time of storage for 0 d, respectively. PCR techniques were repeated 3 times (Zou et al., 2017).

Table 3 Primer sequences genes for qRT-PCR test

Gene	ID	Primer
Elongation factor 1 alpha-like (Reference gene)	PGSC0003DMT400014674	CTTGACACACGCTAAGGAG GTCAATGCAAACCATTCCTTG
Delta24-sterol reductase 1	PGSC0003DMT400030799	ACATTATGGACCTTCTTGTC AGATGCATTCTATCCTTAAG
Methylsterol monooxygenase 1	PGSC0003DMT400033236	GAGAGAACTGAGAATGGAGG GTGCTTGATAGAGAAACACAAC
Protein BR insensitive 1	PGSC0003DMT400066895	AGGTGCCTGTTCCACATAGT GGAGAAGACTTGCTTAGAATTA
BR-signaling kinase	PGSC0003DMT400052467	AGCCATTATTATTGACACC GAAAGCAGCATCACCAT
Cyclin D3	PGSC0003DMT400020648	TGATAGGCTTAAACCTTGC TAACAGATGTAGCCACTGAC

Authors' contributions

DXN carried out the experimental research, completed the data analysis, and drafted the manuscript. DMS and ZX participated in the experimental design, data analysis and drafted the manuscript. LYN participated in the experimental design and carried out the experimental research. NS participated in the result analysis and draft revision. WXY was in charge of the project, guided experiment design and draft revision. All authors read and approved the final manuscript.

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