Effect of *Bacillus subtilis* QM3 on β-amylase Isoenzyme in Early Germination of Wheat Seed

Ya-Jing Li¹ and Qing-Ping Hu¹*

¹School of Life Science, Shanxi Normal University, 1 Gongyuan Street, Linfen City 041004, China.

**Authors’ contributions**

This work was carried out in collaboration between both authors. Author YJL performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author QPH designed the study, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/SAJRM/2020/v6i230146

(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.

(1) Delian Elena, University of Agronomical Sciences and Veterinary Medicine from Bucharest, Romania.

(2) Janine Farias Menegaes, Federal University of Santa Maria (UFSM), Brazil.

Complete Peer review History: [http://www.sdiarticle4.com/review-history/57323](http://www.sdiarticle4.com/review-history/57323)

**Received 07 March 2020**

**Accepted 13 May 2020**

**Published 22 May 2020**

**ABSTRACT**

Seed germination is the basis and initial stage in the process of plant growth and development. *Bacillus subtilis* QM3 is a plant growth promoting rhizobacterium (PGPR) with function of growth promoting, promoting, prevention of pathogens and pests attack and can improve plant resistance to different stress factors. Through the measurement of the early germination rate of wheat (*Triticum aestivum* L.) seeds under *B. subtilis* QM3 treatment, the results showed that *B. subtilis* QM3 can significantly promote the germination of wheat seeds, which has reached a significant level at 6h after seeds sowing or at 6 h of treatment, during imbibition. The β-amylase isoenzyme in early period of wheat seed germination was measured by denaturing polyacrylamide gel electrophoresis. The results showed that the band width and brightness of β-amylase isoenzyme of wheat seeds treated with *B. subtilis* QM3 increased during three to 6 h of imbibition and especially the effect was significant at 6 h. In the early period, the band of α-amylase isoenzyme could not be detected. It is suggested that the increase of β-amylase isoenzyme early band may be one of the main reasons for *B. subtilis* QM3 to promote wheat seed germination. Through the combination of *B. subtilis* QM3 with free and binding states of β-amylase, it was found that the former can increase the activity of β-amylase by either increasing free β-amylase or releasing binding β-amylaseisoenzyme. β-amylaseisoenzyme inhibitors can significantly inhibit β-amylase activity.

*Corresponding author: Email: hqp72@163.com;*
nevertheless α-amylase activators and inhibitors have no significant effect on β-amylase isoenzyme, which further proved that the β-amylase exerted the effects in the early period of wheat seed more precisely during the imbibition of the seeds.

Keywords: B. subtilis QM3; early seed germination; β-amylase isoenzyme.

1. INTRODUCTION

Wheat (Triticum aestivum L.) is an important food crop and the second largest food crop after rice (Oryza sativa L.). It is widely distributed and has high yield and nutritional value. Wheat adds up to half of the calories in human food to meet people’s biggest nutritional needs. The output of wheat accounts for 22% of China’s crop output, and the planted area accounts for 25% of the crops. Wheat is the main food crop for human survival. China is a country with a large population, and wheat consumption accounts for most of the crop consumption [1]. The consumption of wheat in the world accounts for half of the consumption of food crops. In addition to being a major food crop, wheat is also an important industrial raw material and has important economic value. Each country in the world uses about 600 million tons of wheat for commercial purposes every year [2]. According to the estimates of the International Food Policy Research Institute (IFPRI), with the growth of the population and the progress of society, the living standards of human beings are getting higher and higher, and the requirements for food production and quality are growing, so, consumption, consumption of wheat will climb up, from 552 to 775 million tons by year 2020, ultimately results consumption up to 60% by the year 2050 [3].

Seed timely germination begins the life cycle of the crop. Timely germination and even germination are the key determinants of many crops in modern agricultural production systems. They are closely related to seed germination and subsequent plant growth and development, and ultimately affect the yield and quality of cereals. Germination is considered to be the most critical stage in the plant life [4]. Poor germination and decreased seedling growth result in poor establishment and occasionally crop failure [5]. As an important part of the life cycle, germination is the basis of crop growth. Starch is one of the main storage reserves of wheat seeds. Although the germination of cereal seeds is affected by many factors, the corresponding amylase is required to breakdown when the seeds germinate [6]. Therefore, the research on amylase in the early stage of wheat germination (imbibition period) is extremely important.

The extent of amylase activity probably determines germination ability of cereal seeds. Amylases have many isozymes in plants. Studies have shown that varieties with fast germination rate have more isoenzymes bands than those with slower germination rate, indicating that isoenzymes have a certain relationship with seed germination rate [7]. Starch enzymatic hydrolysis power is closely related to its activity and is the most critical enzyme in starch degradation. In wheat grains, β-amylase generally exists in two forms, free and bound [8]. The active center of β-amylase consists of specific groups, which are involved in the conversion of enzyme and substrate complexes and the process of enzyme-substrate binding [9]. Amylopectin produces less maltose than amylose. When β-amylase acts on glycogen, the amount of maltose converted is only 40% - 50% of the syrup. β-amylase has strong acid resistance but is not heat-resistant, and is completely inactivated by holding it in a constant temperature water bath at 70°C for 15 min.

Bacillus subtilis is a kind of biological control bacterium, which can act on the rhizosphere, body surface or plant body of the plant. It secretes antibacterial substances to inhibit its growth, and induce the plant to start the defense system, which can not only promote the growth of the plant, but also improve the plant stress resistance. B. subtilis QM3 is a strain with biocontrol effect isolated from Qinghai cow dung. It has strong reproductive capacity and is a good biocontrol bacterium [10,11]. Previous studies have shown that B. subtilis QM3 can promote the germination and growth of wheat seeds, and it can alleviate the growth of wheat under salt stress. B. subtilis QM3 promotes early amylase isoenzyme changes during seed germination.

The purpose of this study was to investigate the effects of B. subtilis QM3 treatment on early amylase isozymes in wheat seed germination and to promote physiological and biochemical processes during seed germination, by B. subtilis
QM3. Molecular aspects provide some theoretical guidance, which lays a foundation for B. subtilis QM3 bacterium to promote seed germination and further apply to agricultural production practices.

2. MATERIALS AND METHODS

2.1 Media

The commonly used media in this investigation were the beef extract peptone containing peptone 1.0%, beef extract 0.3%, sodium chloride 0.5%, agar 1.5% - 2.0%, pH 7.4 - 7.6 and sterilized 20 min at 121°C.

2.2 Bacterial Suspension Preparation

B. subtilis QM3 used in the present study come from the microbiological lab, the School of Life Science, Shanxi Normal University [11]. A culture of B. subtilis QM3 was obtained by transferring colony from the activated culture plate into a 250 mL flask containing 100 mL beef extract-peptone medium and shaking in an orbital shaker at 200 rpm at 37°C for 3 days. Then, it was diluted in sterile water solution in order to reach an optical density (OD)_{600} nm of 0.8 [10^6 colony-forming units (CFU)]. Dilute its bacterial suspension liquid 10 times and 100 times to reserve [12].

2.3 Seed Materials

Wheat seeds (Linhan N. 9) were used came from Shanxi Academy of Agricultural Sciences. Healthy seeds of similar size and mass were selected. Prior to germination, the seeds were surface-sterilized in 5% sodium hypochlorite for 10 min and rinsed in distilled water. Seeds were divided into two large groups that soaked in sterile water (CK), 10^7 CFU mL\(^{-1}\) B. subtilis QM3 (X0) and 10^6 CFU mL\(^{-1}\) B. subtilis QM3 (X) for 3 h, respectively, and then transferred to Petri dishes for germination. Seeds were taken every three hours until the end of 12 h. The followings conditions have been assured temperature incubator (25°C day:12 h; and 55% relative humidity). The experiment was repeated three times.

2.4 Germination Rate

Standard germination was conducted according to Ma [13]. Using 50 seeds for each repetition, repeated 3 times. The seeds were incubated in the growth box in the dark at 25°C for 12 h, and under light at 25°C for 12 h. Germination is based on the radicle penetrating the seed coat. From the beginning of seed germination, the germinated seeds have been counted every 3 h and the germination rate was recorded.

\[
\text{Germination percentage (GP)} = \frac{(G_t/T) \times 100\%}{G_t/T} \times 100\%
\]

Where Gt is the number of germinations in t days, and T is the total number of seeds used in the test.

2.5 Determination of Isoenzyme

2.5.1 Preparation of amylase extract

There were used 0.5 g and these were grinded in ice bath with 5 mL phosphate buffer (pH = 7.5). The homogenate was centrifuged at 10000 g for 30 min. The supernatant was collected in an Eppendorf tube and it represented the total amylase solution extract. Utilize the characteristics of α-amylase-resistant acid-resistant and heat-resistant β-amylase. Heated total amylase at 70°C for 10 min, and then centrifuged at 10000 g for 30 min. The supernatant obtained was designated as α-amylase. Added 4 mol\(\text{L}^{-1}\) HCl to total amylase to make its pH less than 4, then centrifuged at 10000 g for 30 min. The supernatant obtained was designated as β-amylase [14].

Cancellation excluded half a grain of wheat germ, the method of reference Guerin, ice bath using Tris - HCl grinding into homogenate, with gauze filtration, the filtrate in 10000 g of centrifuge 30 min, remove the supernatant, precipitated by the Tris-HCl suspension centrifugal, repeated three times, merge the supernatant, known as beta amylase free state of crude enzyme liquid precipitation and Tris-HCl suspension, the suspension is a combination of β-amylase thick enzyme fluid state [15].

2.5.2 Isozyme extract by native PAGE

Amylase electrophoresis was assayed by the photochemical method described by Ming J. Wu [16]. Slight change, ten micrograms protein (10 μL) in each extract from each variety was mixed with an 5:1 volume of sample buffer (150 mmolL\(^{-1}\) Tris-HCl pH 6.8, 20% glycerol, 0.001% bromophenol blue) without boiling, and then loaded onto a native-PAGE gel which had a 10% separation gel with acrylamide: Bisacrylamide at a ratio of 29:1 in 150 mmolL\(^{-1}\) Tris-HCl pH 6.8 and a 5% stacking gel using 150 mmol/mL Tris-HCl, pH 8.8. Both gel components contained no...
sodium dodecyl sulfate (SDS). Electrophoresis was carried out at 80 V per gel for 1h for the stacking step and 110V per gel for 2.5 h for the resolving step in running buffer (25 mmolL⁻¹ Tris, 400 mmolL⁻¹ glycine) until the dye had run to the edge of the gel.

2.5.3 Dyeing

After electrophoresis, transferred the gel to phosphate-buffered saline (PBS) buffer containing 1% soluble starch, incubated at 37°C for 3 h, rinse the gel surface with water, and immersed it in the configured I₂-KI solution for staining at 20 min, the results were photographed and recorded. The non-chromatographic band on the glue surface was the amylase isoenzyme band.

2.6 Data Analysis

All data are the average of three repetitions. The obtained results were analyzed statistically by SPSS (version 11.0) statistical software. Data were analyzed by the analysis of variance and treatment mean comparison by using least significance difference (p < 0.05) [17].

3 RESULTS

3.1 Effect of \textit{B. subtilis} on the Germination of Wheat Seeds

The germination of seeds is closely related to the growth of plants. In this experiment, the germination rate was measured early in the germination of wheat, as shown in Fig.1.

As can be seen from Fig. 1, in general, in the early stage of germination (0-12 h), with the progress of germination time, the germination rate of CK group wheat seeds in the control group did not increase significantly, while the treatment group X₀ and X treated wheat seeds the germination rate showed an upward trend. Compared with the control group, both X₀ and X had a significant effect on promoting wheat germination (P < 0.05). There was already a significant difference at the 6th h of germination, and the growth was most significant at 12 h and the highest germination rate of wheat seeds compared with other treatment groups, the largest increase in the X treatment group. It shows that \textit{B. subtilis} QM3 can obviously promote the early germination rate of wheat seeds, especially at a suitable concentration, that is, the concentration of \textit{B. subtilis} QM3 is 10⁶ CFU mL⁻¹.

3.2 Effect of \textit{B. Subtilis} QM3 on α/β-Amylase Isoenzymes of Whole Wheat Seeds at Early Germination

Based on the above test results, we selected \textit{B. subtilis} QM3 strain (10⁶ CFU mL⁻¹) (X) treated with sterile water as the CK group for electrophoresis to analyze α/β-amylase isozymes.

Changes in the number and brightness of amylase isozyme electrophoresis bands during wheat germination can largely reflect the activity of amylase. As can be seen from Fig. 2A and B, β-amylase has been present since the beginning of germination, but the presence of α-amylase
could not be detected. Three isozymes Aβ, Bβ, and Cβ are observed in the gel of Fig. 2A, and most β-amylase activity can be attributed to Cβ. The strip Aβ is a narrow band, and the widths of the Bβ and Cβ bands are relatively large. At different germination times, the number and brightness of bands in group X were significantly different from those in group CK. The number and brightness of bands in group X at 6 h after germination were significantly different from those in other germination stages. This shows that during the germination of wheat seeds, group X can improve the activity of β-amylase isozyme, and it has the most obvious promotion effect at 6 h after germination, especially the specific Bβ and Cβ bands. B. subtilis QM3 promotes the activity of β-amylase isoenzymes, thereby promoting wheat germination. In Fig. 2B, the activity of α-amylase isozyme did not change significantly in seeds treated with CK or B. subtilis QM3. The above conclusions indicate that B. subtilis QM3 can rapidly increase the content of β-amylase isozyme, but not of α-amylase.

In order to regenerate wheat seeds induced by B. subtilis QM3, β-amylase, rather than α-amylase, worked in the early stage of germination. Fig. 2C is the total amylase for the sample treated with B. subtilis QM3 for 6 h. Cut the slices into pieces and put them into Petri dishes contained α-amylase inhibitor ethylenediaminetetraacetic acid (EDTA), α-amylase activator (calcium chloride - CaCl₂) and β-amylase inhibitor (α-cyclodextrin, copper sulphate - CuSO₄, mercuric chloride - HgCl₂). Incubated at 25°C for 30 min, rinsed three times with water, and stained with iodine-potassium iodide. The results showed that β-amylase inhibitors had a significant inhibitory effect on the total amylase of wheat seeds, while α-amylase inhibitors had no significant change in the activity of total amylase in wheat seeds. So, B. subtilis QM3 induced β-amylase, rather than α-amylase activity in wheat seeds during imbibition.

![Fig. 2. A: Shows the effect of B. subtilis QM3 on β-amylase isozymes in the early germination of whole wheat seeds. CK and X represent sterile water treatment and B. subtilis QM3 (10⁶ CFU mL⁻¹) treatment, respectively. Aβ, Bβ and Cβ are three different β-amylase isoenzyme bands. 0, 3, 6, 9 and 12 h are the germination time of wheat seeds. B: Is the α-amylase activity during early germination. C: Shows the changes in the total amylase electrophoresis placed in inhibitors and accelerators.](image-url)
3.3 Effect of *B. subtilis* QM3 on A/B-Amylase Isoenzymes of Half-Grain Wheat Seeds at Early Germination

Using embryo-free half-grain wheat as the experimental material, and repeating the above experiment, we obtained similar results as shown in Fig. 3.

Fig. 3 A and B are the β-amylase isozymes in the early stage of wheat seed germination. The results of β-amylase isozyme and α-amylase isozyme of half-grain seeds are the same as those of whole wheat seeds. *B. subtilis* QM3 quickly induced an increase in the brightness and width of the β-amylase band in the early stage of seed germination, and basically detected less than the α-amylase band. It showed that *B. subtilis* QM3 had an effect on β-amylase, but not α-amylase, in the early stage of wheat seed germination. Fig. 3 C is *B. subtilis* QM3 treatment after 6 h of total amylase electrophoresis, placed in different activators or inhibitors, using α-amylase inhibitor (EDTA) and activator (CaCl₂) and β-amylase inhibitors (α-cyclodextrin, CuSO₄, HgCl). The results show that α-amylase inhibitors and activators have no significant effect on the total amylase isoenzyme band. β-amylase inhibitors can significantly inhibit the total amylase isozyme.

3.4 Effect of *B. subtilis* QM3 on Free and Bound B-Amylase

*B. subtilis* QM3 reacts with free β-amylase for 3, 6, 9, and 12 h.

![Fig. 3](image_url)

**Fig. 3.** A: Shows the effect of *Bacillus subtilis* QM3 on β-amylase isozymes in the early germination of half-grain seeds. CK and X represent sterile water treatment and *B. subtilis* QM3 (10⁶ CFU mL⁻¹) treatment, respectively. Aₐ, Bₐ and Cₐ are three different β-amylase isoenzyme bands. 0, 3, 6, 9 and 12 h are the germination time of wheat seeds. B: Is the alpha-amylase activity during early germination. C: Shows the changes in electrophoresis of total amylase placed in the inhibitor and accelerator.
The results are shown in Fig. 4 A. Compared with the CK group, the amylase bands in group X are significantly brighter than those in the control group (CK). In particular at 6 h, in the seeds treated with B. subtilis QM3, low-activity large-molecular-weight β-amylase monomers were depolymerized into high-active small-molecular-weight β-amylase monomers. It is suggested that the depolymerization of free β-amylase into monomer is one of the ways to improve the activity of amylase.

β-mercaptoethanol can dissociate the free β-amylase into monomers [18]. Treated the free β-amylase with low-concentration of β-mercaptoethanol for 3 h, then add B. subtilis QM3 to react for 3 h, the results are shown in the figure as shown in Fig. 4 B, the band brightness of the free β-amylase was not significantly different from that of the control, indicating that B. subtilis QM3 did not increase the free β-amylase monomer. In addition, we used B. subtilis QM3 to treat the crude enzyme solution of β-amylase binding state, as shown in Fig.4 C, and found that the band brightness of X was significantly higher than that of CK, indicating that β-amylase is elevated.

4. DISCUSSION

Starch is the main storage material in wheat and other cereal seeds. Amylase plays an important role in the process of seed germination, being a key enzyme of this physiological process [18,19]. The amylase activity affects the wheat seeds germination rate and the seedlings survival [20]. In the process of germination, the activity of amylase may be affected by various environments. Microorganisms can increase plant seed germination by increasing amylase activity during seeds germination [21]. Studies by Zhang and others found that β-amylase can increase the activity of β-amylase [8]. Sun and other studies found that β-amylase is one of the important factors to promote wheat seed germination [18]. According to different products, amylase can be divided into α-amylase and β-amylase, and β-amylase plays a major role in the early stage of wheat germination.

The experimental results showed that in the early stage of seed germination, with the increase of wheat germination time, the band of β-amylase isozyme in the seed increased, the band brightness increased and widened. Enzymatic enzyme bands have enhanced expression activity. After soaking with 10⁶ CFU/mL B. subtilis QM3, the β-amylase isoenzyme band in wheat seeds increased and widened, thereby enhancing the expression activity of the enzyme band. Among them, the sixth h of wheat seed imbibition, the brightness and number of bands increased the most. However, as the imbibitions progressed, the α-amylase isoenzyme band of wheat did not change significantly, even the

![Fig. 4. A: Is treated with B. subtilis QM3 for free crude β-amylase enzyme solution for 3, 6, 9, and 12 h, where CK is the control group, that is, seeds treated with sterile water, and X is treated with B. subtilis QM3. Of wheat seeds, 3, 6, 9, 12 h represent the reaction time with crude enzyme solution for 3, 6, 9, 12 h, respectively. B: Shows the treatment of free β-amylase with β-mercaptoethanol for 3 h, and then adding B. subtilis QM3 for 3 h, 2-ME for β-mercaptoethanol for 3 h, and 2-ME + X for β-mercaptoethanol. After 3 h, add B. subtilis QM3 for 3 h. C: Shows that the bound β-amylase was treated with B. subtilis QM3 for 6 h, indicated by X](image-url)
5. CONCLUSION

Soaking seeds with B. subtilis QM3 can increase the β-amylase activity during wheat seed germination, and promote the germination process by increasing in special of the free fraction of β-amylase enzyme. Choosing the appropriate concentration of bacterial solution is more conducive to promoting the germination of wheat seeds, thereby increasing the yield of crops. This phenomenon may be related to the combination of bacteria and wheat seeds, but the deeper reason needs further study.

ACKNOWLEDGEMENT

This work was financially supported by the Natural Science Foundation Project of Shanxi Province (201801D121206) and the Natural Science Foundation of Modern College of Humanities and Sciences of Shanxi Normal University (2018JCYJC01).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yu Y, Zhu D, Ma C, et al. Transcriptome analysis reveals key differentially expressed genes involved in wheat grain development. The Crop Journal. 2016;4(2):92-106. DOI: 10.1016/j.cj.2016.01.006
2. Albajes R, Cantero-Martinez C, Capell T, et al. Building bridges: An integrated strategy for sustainable food production throughout the value chain Molecular Breeding: new Strategies in Plant Improvement. 2013;32(4):743-770. DOI: 10.1007/s11032-013-9915-z
3. Ittersum V, Martin KJERoAE. Crop yields and global food security will yield increase continue to feed the world. European Review of Agriculture Economics. 2016;43(1):191-192. DOI: 10.1093/erae/jbv034
4. Van Ittersum MK. Integrated physiology and proteome analysis of embryo and endosperm highlights complex metabolic networks involved in seed germination in wheat (Triticum aestivum L.). Journal Plant Physiology. 2018;229:63-76. [Epub 2018/07/25]
5. Edmondson JL, Davies ZG, Gaston KJ, Leake JR, Kardol P. Urban cultivation in allotments maintains soil qualities adversely affected by conventional agriculture Journal of Applied Ecology. 2014;51(4):880-889. DOI: 10.1111/1365-2664.12254
6. Zakharova O, et al. The effects of CuO nanoparticles on wheat seeds and seedlings and Alternaria solani fungi: In vitro study [J]. Iop Conference. 2019;226(1):012036. DOI: 10.1088/1755-1315/226/1/012036
7. Wang J, Yu Y, Tian X. Effect of γ-ray irradiation on the germinating characteristics of wheat seed [J]. Radiation Physics and Chemistry. 2012;81(4):463-465. DOI: 10.1016/j.radphyschem.2011.12.024
8. Zhang H, Dou W, Jiang CX, Wei ZJ, Liu J. Hydrogen sulfide stimulates β-amylase activity during early stages of wheat grain germination. Plant Signaling and Behavior. 2010;5(8):1031-1033. DOI: 10.4161/psb.5.8.12297
9. Dicko M. Purification and characterization of β-amylase from Curculigo pilosa. Appl Microbiol Biotechnol. 1999;52:802–805. DOI: 10.1007/s002530051596
10. Hu QP, Xu JG, Zhang F, Tian CR. Changes of morphological parameter of wheat seed pretreatment by the biocontrol agents Bacillus subtilis QM3 during germination.
Communications in Soil Science and Plant Analysis. 2013;44(14):2168-72. DOI: 10.1080/00103624.2013.799684

11. Hu Q, Liu R, Liu J. Effects of *Bacillus subtilis* QM3 on germination and antioxidant enzymes activities of wheat seeds under salt stress. Open Access Library Journal. 2019;06(03):1-9. DOI: 10.4236/oalib.1105218

12. Hao Y, Wu H. Mitigative effect of *Bacillus subtilis* QM3 on root morphology and resistance enzyme activity of wheat root under lead stress. Advances in Microbiology. 2015;05(06):469-78. DOI: 10.4236/aim.2015.56048

13. Ma WG, Zhang ZH. Determination of tobacco (*Nicotiana tabacum*) seed vigour using controlled 324 deterioration followed by a conductivity test. Seed Science and Technology. 2020;48(1):1-10. DOI: 10.15258/sst.2020.48.1.01

14. Zhou L, Xia M. Toxic effect of perfluorooctanoic acid on germination and seedling growth of wheat (*Triticum aestivum* L.). Chemosphere. 2016;159:420-425. DOI: 10.1016/j.chemosphere.2016.06.045

15. Kristensen M. Large-scale purification and characterization of barley limit dextrinase, a member of the α-amylase structural family. Cereal Chemistry Journal. 1998; 75(4):473-479. DOI: 10.1094/cchem.1998.75.4.473

16. Wu MJ, McKay S, Howes N. Polymorphism and pedigree analysis of β-amylase isozymes in Australian wheat. Journal of Cereal Science. 2011;53(3):362-70. DOI: 10.1016/j.jcs.2011.02.007

17. Yu L. Exogenous hydrogen sulfide enhanced antioxidant capacity, amylase activities and salt tolerance of cucumber hypocotyls and radicles. Journal of Integrative Agriculture. 2013;12(3):445-56. DOI: 10.1016/s2095-3119(13)60245-2

18. Zhang H. A rapid response of β-amylase to nitric oxide but not gibberellin in wheat seeds during the early stage of germination. Planta. 2005;220(5):708-716. DOI: 10.1007/s00425-004-1390-7

19. Uchida K. Immunological characterization of amylase isozymes in developing and germinating wheat seeds. 1987;14(1):89-94. Available:http://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115

20. Monroe JD, Storm AR. β-Amylase1 and β-Amylase3 Are Plastidic Starch Hydrolases in Arabidopsis That Seem to Be Adapted for Different Thermal, pH, and Stress Conditions. Plant Physiology. 2014.166(4): 1748-1763. DOI: 10.1104/pp.114.246421

21. Yang H. Enhancing the NaCl tolerance potential of wheat on root morphology and osmoregulation substance by exogenous application of QM3. RGUHS Journal of Pharmaceutical Sciences. 2016;03(11):1-14. DOI: 10.4236/oalib.1103072

© 2020 Li and Hu; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57323