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Wheat seed germination based on α-amylase activity to study promoting mechanism of *Bacillus subtilis* QM3

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ABSTRACT: The mechanism of promoting wheat seed germination by *Bacillus subtilis* has been paid great attention by many scholars. The germination rate and α-amylase activity of wheat seeds were significantly increased after germinating with *Bacillus subtilis* QM3 in this paper. Inhibitor and promoter of α-amylase were used to study relationship between bacteria and α-amylase. Compared with inhibitor group (10 mmol.L^{-1} EDTA), α-amylase activity of seeds treated by *B. subtilis* QM3 (10^6 CFU.mL^{-1}) increased by 19.8%. It indicates that the inhibition has been alleviated. Similarly, α-amylase activity of co-treated group (2 mmol.L^{-1} $CaCl_2$ and 10^6 CFU.mL^{-1} *B. subtilis* QM3) was higher than that of the promoter alone, reaching 14.9%. Furthermore, the results of α-amylase isozyme electrophoresis showed that there were three isozyme types in the gels, and the expression of α-amylase isoenzyme was significantly increased after treatment with *B. subtilis* QM3 (10^6 CFUmL.$^{-1}$), which was reflected in the width and brightness of band mainly, especially band Cα. In addition, germination rate, α-amylase activity and isozyme electrophoresis of other three kinds of wheat seeds were also tested and similar results were obtained. Therefore, one of the possible mechanisms by which *B. subtilis* QM3 promotes seed germination is as a potential exogenous factor that can enhance activity and expression of α-amylase.

Index terms: amylase, *Bacillus subtilis*, germination rate, promoting mechanism, wheat.

RESUMO: O mecanismo de promoção da germinação de sementes de trigo por *Bacillus subtilis* tem sido bastante estudado. A taxa de germinação e a atividade de α-amilase de sementes de trigo foram significativamente aumentadas após a germinação com *Bacillus subtilis* QM3 neste estudo. Um inibidor e um promotor de α-amilase foram utilizados para estudar a relação entre bactérias e α-amilase. Em comparação com o grupo inibidor (10 mmol.L^{-1} EDTA), a atividade da α-amilase das sementes tratadas com *B. subtilis* QM3 (10^6 CFU.mL^{-1}) aumentou 19,8%, indicando que a inibição foi reduzida. Da mesma forma, a atividade da α-amilase do grupo co-tratado (2 mmol.L^{-1} $CaCl_2$ e 10^6 UFC.mL^{-1} *B. subtilis* QM3) foi superior à do promotor sozinho, chegando a 14,9%. Além disso, os resultados da eletroforese da isoenzima α-amilase mostraram que havia três tipos de isoenzimas nos géis, e a expressão da isoenzima α-amilase foi significativamente aumentada após o tratamento com *B. subtilis* QM3 (10^6 UFC.mL^{-1}), o que se refletiu principalmente na largura e brilho das bandas, especialmente Cα. Além disso, a taxa de germinação, atividade de α-amilase e eletroforese de isoenzimas de outros três tipos de sementes de trigo também foram testados e resultados semelhantes foram obtidos. Portanto, um dos possíveis mecanismos pelos quais *B. subtilis* QM3 promove a germinação de sementes é como um potencial fator exógeno que pode aumentar a atividade e a expressão da α-amilase.

Termos para indexação: amilase, *Bacillus subtilis*, taxa de germinação, mecanismo promotor, trigo.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important crop in China and even in the world. Because it is easy to artificially cultivate and maintain normal growth in the laboratory, it is widely used as a model organism to study the influence of environmental factors on seed germination. Seed germination is the beginning and crucial stage of plant growth and development, which is strongly related to seedling survival rate and grain yield (Han et al., 2017). Seed germination is the process in which seeds recover embryo growth and grow into seedlings (Bewley and Black, 1995), and it is a precise interaction process of catabolism and anabolism (Bewley, 1997).

The main storage substance in wheat seeds is starch, which belongs to starch seed. Amylase hydrolyzes the storage material in the endosperm to provide energy and substrate for seed germination (Zhang et al., 2010). Amylase is generally considered to be the key enzyme for crop seed germination. Amylases include α-amylase, β-amylase and limiting dextrin. B-amylase (α-1,4-glucan malt hydrolase, E.C. 3.2.1.2) is an exo-hydrolase, that cannot work inside starch molecules. However, β-amylase can hydrolyze the non-reducing ends of α-1,4 glycosidic bonds on starch, and sequentially cut off a maltose unit to release β-maltose and β-limit dextrin (Wu et al., 2011). A-amylase (EC number 3.2.1.1: 1,4-α-D-glucan glucanohydrolase), an endo-hydrolase which belongs to glycoside hydrolase family 13, acts on α-1,4-glycoside linkages of starch. A-amylase is synthesized and secreted by the scutellum and aleurone layer, and it plays a key role in starch degradation during seed germination (Cheng et al., 2014; Elarbi et al., 2009).

Studies on wheat germination mainly focused on: (1) The effects of signal molecules on seed germination such as: NO (Zhang et al., 2009), CO (Liu et al., 2010), reactive oxygen species (Hayat and Bailly, 2008) and so on; (2) The effects of hormones on seed germination (Lei et al., 2014). *B. subtilis* QM3 is a rhizosphere growth promoting bacteria. Previous studies have shown that *B. subtilis* QM3 can promote the germination and growth of wheat seeds and enhance the activity of amylase (Hu et al., 2013; Hu et al., 2019). In the early stage of wheat germination, *B. subtilis* QM3 can increase β-amylase activity (Li and Hu, 2020a). The purpose of this study was to based on α-amylase activity and α-amylase isoenzyme to study promoting mechanism of *B. subtilis* QM3 on wheat seed germination and lays a foundation for *B. subtilis* QM3 further use to agricultural production practices.

MATERIAL AND METHODS

Bacteria materials

B. subtilis QM3 used in the present study come from the microbiological lab, College of Life Science, Shanxi Normal University, China (Hao et al., 2015). 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. Centrifuged for 10 min in 3000 $r.min^{-1}$. Then, the supernatant was discarded, and the cells were washed by sterile water three and resuspended in sterile water adjusting to OD_{600} of 0.8 colony-forming unit (10^8 $CFU.mL^{-1}$ *B. subtilis* QM3) (Li and Hu, 2020a). Dilute its bacterial suspension liquid 10 times, 100 times, 1000 times and 10000 times to reserve.

Seed material

Seeds of the Jinmai N°.101, Jinmai N°.97, Linhan N°.9, Jinmai N°.47 (*Triticum aestivum* L.) came from Shanxi Academy of Agricultural Sciences.

Seed germination

Select the wheat seeds with full and uniform grain size, sterilize them with 5% NaClO for 5 min, then rinse them 3-5 times with sterile water (Li and Hu, 2020a). The seeds were soaked for 3 h. In a glass petri dish lined with filter paper

and gauze, add 5 mL of the corresponding solution or bacterial liquid, place the seeds on a petri dish gauze and add 5 mL of the corresponding solution or bacterial liquid every 12 hours. All the seeds were soaked and germinated in darkness at 25 °C.

Different treatments of seed germination

The seeds (Jinmai N°.47) were soaked in sterile water, 10 mmol.L^{-1} EDTA, 2 mmol.L^{-1} $CaCl_2$ for 3 h. The seeds soaked in sterile water were cultivated with sterile water and 10^6 CFU.mL^{-1} *B. Subtilis* QM3, respectively. The seeds soaked in EDTA were cultivated with sterile water and 10^6 CFU.mL^{-1} *B. Subtilis* QM3, respectively. The seeds soaked in 2 mmol.L^{-1} $CaCl_2$ were cultivated with 2 mmol.L^{-1} $CaCl_2$ and 10^6 CFU.mL^{-1} *B. Subtilis* QM3, respectively. The seeds (Jinmai N°.101, Jinmai N°.97, Linhan N°.9) were soaked in sterile water for 3 h, cultivated with sterile water and 10^6 CFU.mL^{-1} *B. Subtilis* QM3. The seeds were collected at different germination stages (0, 12, 24, 36, 48, 60 and 72 h) for determination of α-amylase activity.

Determination of 1000-seed weight and morphological observation during seed germination

The seeds (Jinmai N°.47) were germinated in sterile water and were collected at seven different germination stages (0, 12, 24, 36, 48, 60 and 72 h). The changes of 1000 seeds weight were tested. The morphological changes of seeds at seven time points of germination were observed.

Determination of germination rate

After sowing, the number of germinated seeds was recorded every 12 h. The germination rate was calculated by continuously recording the number of germinated seeds for 72 h.

Germination percentage (GP) = (Gt/T) ×100%. Where Gt is the number of germinations in t h, and T is the total number of seeds used in the test.

Determination of α-amylase activity

The activities of amylase were measured by the method of 3,5-dinitrosalicylic acid (Elarbi et al., 2009). The absorbance was measured at 540 nm. The corresponding maltose curve is obtained from the standard curve and the enzyme activity is calculated according.

Determination of α-amylase isoenzyme

The seed 0.5 g was put into mortar containing 5 mL phosphate buffer solution (pH = 7.5) and ground to homogenate in ice-bath. The homogenate was collected in a centrifuge tube and centrifuged for 30 min at 10000 r.min^{-1} (Li and Hu, 2020b). The supernatant was the total amylase solution. Taking advantage of the heat intolerance of β-amylase, the total amylase solution was placed in a constant temperature water bath at 70 °C for 15 minutes, which was α-amylase solution. A-amylase isoenzyme in wheat seeds were determined by polyacrylamide gel electrophoresis (Li and Hu, 2020b).

Determination Statistics analysis

All data are the average of three repetitions. The number of seeds per replications is 150. The obtained results were analyzed statistically by SPSS statistical software.

RESULTS AND DISCUSSION

Seed water absorption promotes the absorption of nutrients and seed germination. With the seed germination, the seed morphology also changed (Figure 1a). Wheat seed inflated gradually, but appearance was relatively unchanged in the first 12 h. At 24 h, the structures surrounding the embryo were penetrated by the radicle, and both radicle and bud

were visible at 48 h. At 72 h, the plumule becomes greenish and the radicle is extended to three radicles, indicating the completion of seed germination and the beginning of seedling growth. It can be seen that abundant early imbibition establishes a basis for the mobilization of water-soluble metabolites, which provide the continuous energy for the seed germination and subsequent seedling growth.

During the germination process, seed weight increased with imbibition (Figure 1b). Initially, water uptake by the dry seed is rapid during germination. Thus, the seed weight increased quickly during the first 12 h of imbibition (Phase 1), after which the seed weight increases slowly from 12 to 24 h. Seed weight increase steadily from 24 to 72 h. What is interesting is that this result is different from the triphasic pattern of seed imbibition proposed and defined by Bewley and Black (1995) and Bewley et al. (2013). The triphasic pattern of seed imbibition is not a general rule and is usually more suitable to assess individual seeds, rather than seed lots or seed samples (Pereira et al., 2022). Toorop (2015) proposed an alternative pattern to describe the seed imbibition process, by splitting the original second phase into three others related to testa and endosperm integrity. In a world, we can describe the curve of Figure 1b as two phase. The first Phase 1 (Figure 1b) is characterized by seed water absorption due to potential differences between the seed and the germination medium and by low intensity metabolic activity (Bewley et al., 2013). The second phase is divided into three parts: Phase 2a (identical to the classic phase 2, from 12 h to 24 h) is characterized by little to no seed weight variation and intense metabolic activities involved in preparing the seed for germination and seedling growth, with testa rupture (phase 2b, form 24 to 48) followed by endosperm rupture and, lastly, embryo growth at 2c (from 48 to 72).

The data shown in Table 1 are the changes of germination percentage of wheat seeds treated with different concentrations of *B. subtilis* QM3. It can be seen from the table that the germination rate of wheat seeds treated with different concentrations of *B. subtilis* QM3 is obviously different. *B. subtilis* QM3 can promote the germination of wheat seeds, especially in A3 and A4 treatment groups. Compared with control group, the germination rate of wheat seeds increased obviously in A3 and A4 treatment groups ($p < 0.05$), the germination percentage of wheat seeds treated with A3 and A4 respectively increased by 7%-19% and 5%-14%. Seed germination is the initial stage of plant growth and is easily affected by various factors (Rifna et al., 2019). It has also been reported in the literature: Exogenous biological and abiotic factors could improve the germination rate of seeds (Sheng et al., 2018).

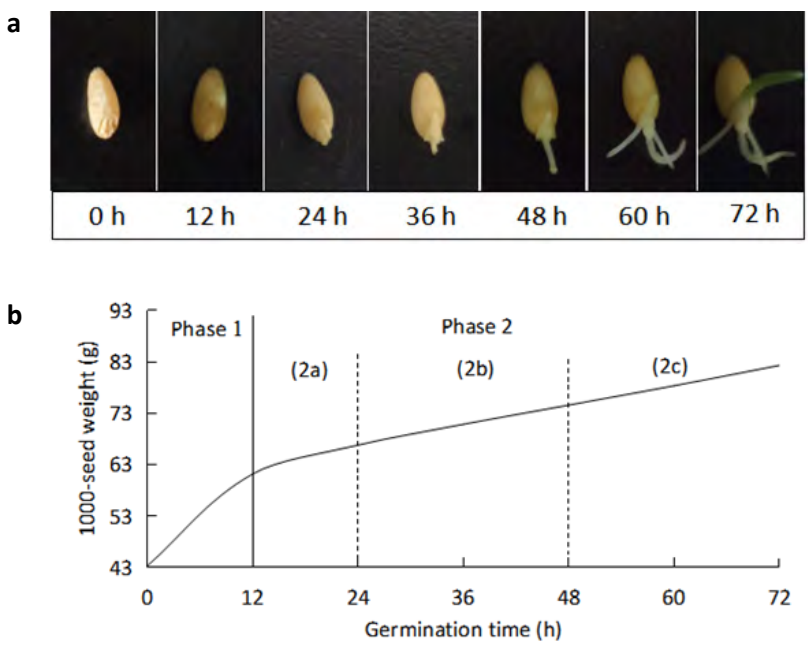


Figure 1. Grain morphology and water content changes during seed germination. a. Morphology changes. b.1000-seed water content changes.

Table 1. Effect of *B. subtilis* QM3 on the germination rate of wheat seeds.

Germination time (h)	Germination rate (%)					
	CK	A1	A2	A3	A4	A5
24	53.5 ± 1.5 d	58.0 ± 1.0 bc	58.7 ± 1.2 bc	63.7 ± 1.5 a	61.0 ± 1.7 b	56.0 ± 1.7 c
36	63.3 ± 1.5 d	68.0 ± 1.0 bc	66.3 ± 1.5 c	71.0 ± 1.7 a	69.3 ±1.5 ab	65.7 ± 0.6 cd
48	77.3 ± 1.5 d	82.0 ± 1.0 b	82.7 ± 1.5 b	86.3 ± 1.7 a	84.3 ± 1.5 a	79.7 ± 0.6 c
60	83.7 ± 0.6 d	86.3 ± 2.1 bc	87.3 ± 1.2 cd	91.3 ± 1.5 a	89.7 ± 0.6 ab	85.0 ± 2.0 cd
72	91.0 ± 1.0 d	93.7 ± 0.6 bc	94.7 ± 0.6 bc	97.0 ± 1.0 a	95.3 ± 0.6 ab	92.0 ± 1.7 cd

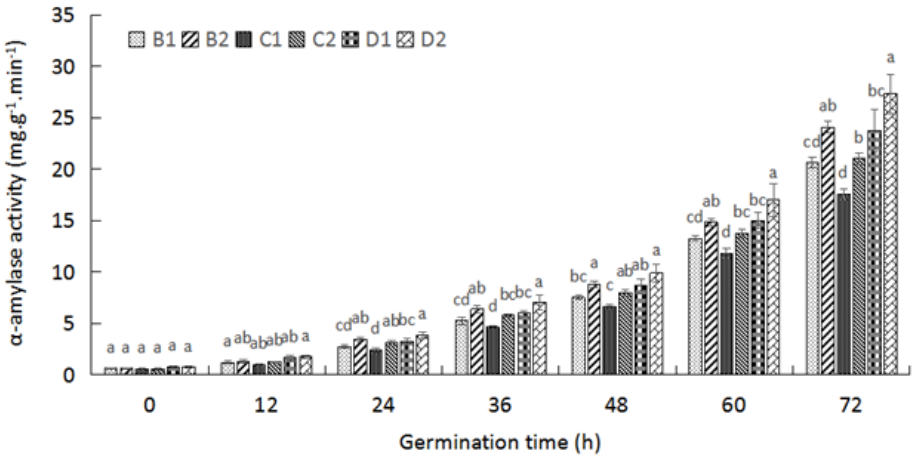
CK, A1, A2, A3, A4 and A5 represent soaked in sterile water, cultivated with sterile water (CK), 10^4 CFU.mL^{-1} *B. Subtilis* QM3, 10^5 CFU.mL^{-1} *B. Subtilis* QM3, 10^6 CFU.mL^{-1} *B. Subtilis* QM3, 10^7 CFU.mL^{-1} *B. Subtilis* QM3, 10^8 CFU.mL^{-1} *B. Subtilis* QM3. The different letters represented the significant difference between different bacterial solution treatments at the same time ($p < 0.05$).

Figure 2 shows the changes of α-amylase activity under different treatments during wheat seed germination. With the increase of germination time, α-amylase activity of wheat seeds continuous increased, and α-amylase activity was the highest at 72 h. Compared with the control group, 10^6 *B. subtilis* QM3 can significantly increase the activity of α-amylase ($p < 0.05$), The α-amylase activity of 10^6 *B. subtilis* QM3 group increased by 16.9%. Compared with the inhibitor group (10 mmol.L^{-1} EDTA), α-amylase activity of seeds treated by 10^6 CFU.mL^{-1} *B. subtilis* QM3 increased by 19.8%. It indicates that the inhibition has been alleviated. The amylase activity of the co-treated group (2 mmol.L^{-1} $CaCl_2$ and 10^6 CFU.mL^{-1} *B. subtilis* QM3) was higher than that of 10^6 *B. subtilis* QM3 group, reaching 14.9%. In general, α-amylase is a key enzyme in seed germination. The stimulation of exogenous regulators on seed germination is mainly due to the increase of amylase expression and activity (Ansari et al., 2019).

The changes including the number and brightness of electrophoretic bands of α-amylase isoenzymes during wheat seed germination can reflect the activity and content of the α-amylase largely. Based on the above experiment results, *B. subtilis* QM3 (10^6 CFU.mL^{-1}) (B) were selected and treated with sterile water as the control group (CK) for electrophoresis analysis of α-amylase isoenzymes (Figure 3).

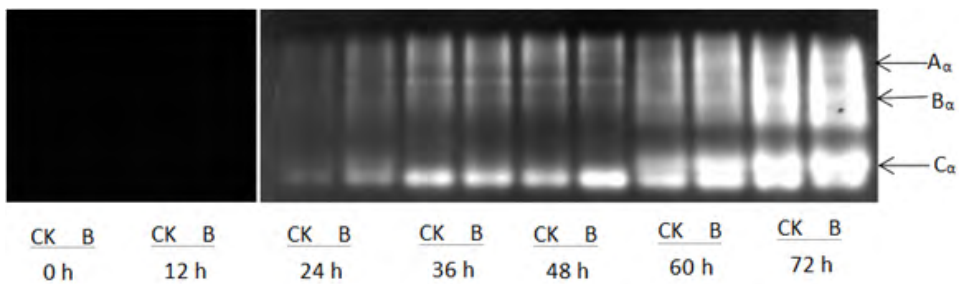
There were three isozyme types, Aα, Bα and Cα were observed in the gels, and most of the α-amylase activity could be attributed to Cα. The band of Aα and Bα are narrow band, and the width of Cα bands is relatively greater. No bands were detected at 0-12 h of germination, and the bands appeared from 24 h, and the width and brightness of bands increased with the extension of germination time. At different germination times, the number and brightness of bands in Group B were different from those in the control group. At 0-12 h, no isozyme bands were detected in Group B seeds. At 24-72 h, the number and brightness of bands in Group B seeds were significantly different from those in CK Group, in particular, at germination 48 h-72 h. The results showed that B treatment could improve the activity and content of α-amylase isozymes in wheat seed germination, especially the specific Cα band of α-amylase isozymes. *B. subtilis* QM3 may increase the expression of α-amylase isozymes by increasing the brightness and width of Cα band, thus promoting wheat seed germination.

In order to explore the increase of germination rate of wheat seeds was related to α-amylase under the action of *B. subtilis* QM3 (10^6 CFU.mL^{-1}). Three wheat varieties (Jinmai N°.101, Jinmai N°.97, Linhan N°.9) were selected and their seed germination rate, α-amylase activity and α-amylase isozymes were measured. The Table 2 showed that the germination rate of wheat seeds soaked by sterile water and *B. subtilis* QM3 (10^6 CFU.mL^{-1}) during germination. Compared with X1, Y1 and Z1, X2, Y2, and Z2 the germination rate of seeds increased by 7-18%, 7-19%, 6-16%. Figure 4 showed that the α-amylase activity of wheat seeds soaked by sterile water and 10^6 CFU.mL^{-1} *B. subtilis* QM3 at 72h. Compared with X1, Y1 and Z1, X2, Y2, and Z2 the α-amylase activity of seeds increased by 8.8%, 14.6%, 12.1%. Figure 5 showed the α-amylase isozymes of wheat seeds soaked by sterile water and *B. subtilis* QM3 (10^6 CFU.mL^{-1}) at 72 h. Compared with X1, Y1 and Z1, X2, Y2 and Z2, the brightness of the α-amylase bands of seeds increased, especially the Cα band of α-amylase isozymes bands.



B represent soaked in sterile water, B1 represent cultivated with sterile water, B2 represent cultivated with 10^6 CFU.mL^{-1} *B. Subtilis* QM3; C represent soaked in 10 mmol.L^{-1} EDTA, C1 represent cultivated with sterile water, C2 represent cultivated with 10^6 CFU.mL^{-1} *B. Subtilis* QM3; D represent soaked in 2 mmol.L^{-1} $CaCl_2$, D1 represent cultivated with 2 mmol.L^{-1} $CaCl_2$, D2 represent cultivated with 10^6 CFU.mL^{-1} *B. Subtilis* QM3. The different letters represented the significant difference between different bacterial solution treatments at the same time ($p < 0.05$).

Figure 2. Effect of *B. subtilis* QM3 on the α-amylase activity of wheat seeds.



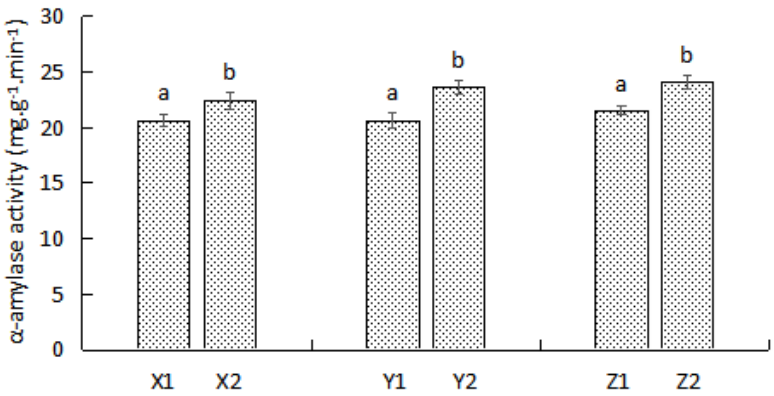
CK and B represent sterile water treatment and *B. subtilis* QM3 (10^6 CFU.mL^{-1}) treatment, respectively. Aα, Bα and Cα are three different α-amylase isozyme bands. 0 h, 12 h, 24 h, 36 h, 48 h, 60 h and 72 h are germination time of wheat seed.

Figure 3. Effect of *B. subtilis* QM3 on the α-amylase isozymes.

Table 2. Seed germination rate of three wheat species.

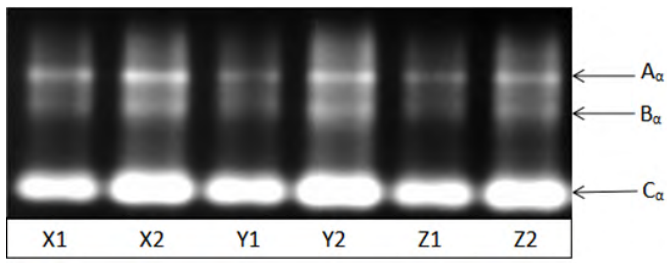
Germination time (h)	Germination rate (%)					
	X1	X2	Y1	Y2	Z1	Z2
24	59.3 ± 1.5 a	67.0 ± 1.7 b	60.0 ± 1.7 a	68.0 ± 1.0 b	60.7 ± 2.5 a	69.3 ± 1.5 b
48	68.0 ± 2.0 a	79.7 ± 1.2 b	68.7 ± 2.1 a	82.0 ± 3.6 b	70.3 ± 2.5 a	81.7 ± 3.2 b
72	87.0 ± 2.0 a	93.3 ± 1.5 b	87.7 ± 1.5 a	94.0 ± 1.7 b	89.3 ± 2.1 a	95.0 ± 1.0 b

X, Y, Z represent Jinmai N°. 97, Jinmai N°. 101, Linhan N°. 9. X1, Y1, Z1 represent germinated by sterile water. X2, Y2, Z2 represent germinated by 10^6 CFU.mL^{-1} *B. subtilis* QM3. The different letters represented the significant difference between different treatments at the same wheat variety and time ($p < 0.05$).



X, Y, Z represent Jinmai N°. 97, Jinmai N°. 101, Linhan N°. 9. X1, Y1, Z1 represent germinated by sterile water. X2, Y2, Z2 represent germinated by 10^6 CFU.mL^{-1} *B. subtilis* QM3. The different letters represented the significant difference between different treatments at the same wheat variety and time ($p < 0.05$).

Figure 4. Effect of *B. subtilis* QM3 on the α-amylase isozymes.



X, Y, Z represent Jinmai No. 97, Jinmai No. 101, Linhan No. 9. X1, Y1, Z1 represent germinated by sterile water. X2, Y2, Z2 represent germinated by 10^6 CFU.mL^{-1} *B. subtilis* QM3. The different letters represented the significant difference between different treatments at the same wheat variety and time ($p < 0.05$). Aα, Bα and Cα are three different α-amylase isozyme bands.

Figure 5. A-amylase isozymes of three wheat species at 72 h of germination.

CONCLUSIONS

Exogenous *B. subtilis* QM3 can effectively promoted wheat seed germination as a potential factor by increasing α-amylase activity and of α-amylase isozymes expression.

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REFERENCES

BEWLEY, J.D.; BRADFORD, K.; HILHORST, H.; NONOGAKI, H. *Seeds: physiology of Development, Germination and Dormancy*. New York: Springer Science & Business Media, 2013. 392p.

BEWLEY, J.D.; BLACK, M. Seeds: physiology of development and germination. *Journal of Ecology*, v.83, n.6, p.1053, 1995. https://doi.org/10.1016/0031-9422(95)90295-3

BEWLEY, J.D. Seed germination and dormancy. *The Plant Cell*, v.9, n.7, p.1055-1066, 1997. https://doi.org/10.1105/tpc.9.7.1055

CHENG, C.R.; OLDACH, K.; MRVA, D. Analysis of high pI a-Amy-1 gene family members expressed in late maturity a-amylase in wheat (*Triticum aestivum* L.). *Molecular Breeding*, v.33, n.3, p.519-529,2014.https://xueshu.baidu.com/usercenter/paper/show?paperid=1n470ay0y65t0ag0wx3m0aq046160922

ELARBI, M.B.; KHEMIRI, H.; JRIDI, T.; HAMIDA, J.B. Purification and characterization of α-amylase from safflower (*Carthamus tinctorius* L.) germinating seeds. *Comptes Rendus Biologies*, v.332, p.426-432, 2009. https://wenku.baidu.com/view/4ccd2d8f680203d8ce2f2435.html

HAN, C.; ZHEN, S.; ZHU, G.; BIAN, Y.; YAN, Y. Comparative metabolome analysis of wheat embryo and endosperm reveals the dynamic changes of metabolites during seed germination. *Plant Physiology and Biochemistry*, v.115, p.320-327, 2017. https://www.docin.com/p-1914821323.html

HAO, Y. WU, H.T.; LIU, Y.F.; HU, Q.P. Mitigative effect of *Bacillus subtilis* QM3 on root morphology and resistance enzyme activity of wheat root under lead stress. *Advances in Microbiology*, v.5, n.6, p.469-478, 2015. https://www.researchgate.net/publication/281336078

HAYAT, E. M.; BAILLY, C. Oxidative signaling in seed germination and dormancy. *Plant Signaling and Behavior*, v.3, p.175-182, 2008. https://www.docin.com/p-1378050374.html

HU, Q.; XU, J. G.; ZHANG, F.; TIAN, C.R. Changes of morphological parameter of wheat seed pretreatment by the biocontrol agents *Bacillus subtilis* QM3 during germination. *Communications in Soil Science and Plant Analysis*, v.44, p.2168-2172, 2013. https://doi.org/10.1080/00103624.2013.799684

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*, v.6, n.3, p.1-9, 2019. https://www.researchgate.net/publication/331550860

LEI, C.; DING, T.L.; WANG, B.S. Ameliorative effects of exogenous GA_3, ABA and $Ca(NO_3)_2$ on germination of wheat seeds under salt stress. *Acta Botanice Boreali-0ccidentalia Sinica*, v.4, n.4,p.583-587, 2004. http://europepmc.org/article/CBA/399050

LI, Y.; HU, Q. Studying of the promotion mechanism of *Bacillus subtilis* QM3 on wheat seed germination based on β-amylase. *Open Life Sciences*, v.15, n.1, p.553–560, 2020a. https://doi.org/10.1515/biol-2020-0062

LI,Y.; HU, Q. Effect of *Bacillus subtilis* QM3 on β-amylase isoenzyme in early germination of wheat seed. *South Asian Jounal of Research in Microbiology*, v.6, n.2, p.24-32, 2020b. https://doi.org/10.9734/sajrm/2020/v6i230146

LIU, Y.; XU, S.; LING, T.; XU, L.; SHEN, W. Heme oxygenase/carbon monoxide system participates in regulating wheat seed germination under osmotic stress involving the nitric oxide pathway. *Journal of Plant Physiology*, v.167, n.16, p.1371-1379, 2010. https://www.sciencedirect.com/science/article/abs/pii/S0176161710002981

NSARI, F.A.; AHMAD,I.; PICHTEL, J. Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming *Bacillus pumilus* strain FAB10. *Applied Soil Ecology*, v.143, p.45-54, 2019. https://doi.org/10.1016/j.apsoil.2019.05.023

PEREIRA, W.V.S; JOSÉ, A.C.; TONETTI, O.A.O.; MELO, L.A.; FARIA, J.M.R. Imbibition curve in forest tree seeds and the triphasic pattern: theory versus practice. *South African Journal of Botany,* v.144, p.105-114, 2022. https://doi.org/10.1016/j.sajb.2021.08.032

RIFNA, E.J.; RAMAMANAN, R.; RADHAKRISHNAN, M. Emerging technology applications for improving seed germination. *Trends in Food Science and Technology*, v.8, p.95-108, 2019. https://doi.org/10.1016/j.tifs.2019.02.029

SHENG, Y.; XIAO, H.; GUO, C.; WU, H.; WANG, X. Effects of exogenous gamma-aminobutyric acid on α-amylase activity in the aleurone of barley seeds. *Plant Physiology and Biochemistry*, v.127, p.39-46, 2018. https://www.sciencedirect.com/science/article/abs/pii/S0981942818301128

TOOROP, P.E. Nitrate controls testa rupture and water content during release of physiological dormancy in seeds of *Sisymbrium officinale* (L.) Scop. *Seed Science Research*, v.25, p.138–146., 2015. https://doi.org/10.1017/S0960258514000397

WU, M.J.; MCKAY, S.; HOWES, N.; HEGEDUS, E.; CHIN, J. Polymorphism and pedigree analysis of β-amylase isozymes in Australian wheat. *Journal of Cereal Science*, v.53, n.3, p.362-370, 2011. https://www.sciencedirect.com/science/article/abs/pii/S0733521011000427

ZHANG, C.F.; JIANG, D.; LIU, F.L.; DAI, T.B.; LIU, W.C.; JING, Q.; CAO, W.X. Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environmental and Experimental Botany*, v.67, p.222-227, 2009. https://doi.org/10.1016/j.envexpbot.2009.05.002

ZHANG, H.; DOU, W.; JIANG C.X.; WEI,Z.J.; LIU, J.; JONES, R.L. Hydrogen sulfide stimulates β-amylase activity during early stages of wheat grain germination. *Plant Signaling and Behavior*, v.5, p.1031-1033,2010. https://www.docin.com/p-1377080840.html