



Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress^{*}

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Abstract: Low temperature stress during germination and early seedling growth is an important constraint of global production of maize. The effects of seed priming with 0.25%, 0.50%, and 0.75% (w/v) chitosan solutions at 15 °C on the growth and physiological changes were investigated using two maize (*Zea mays* L.) inbred lines, HuangC (chilling-tolerant) and Mo17 (chilling-sensitive). While seed priming with chitosan had no significant effect on germination percentage under low temperature stress, it enhanced germination index, reduced the mean germination time (MGT), and increased shoot height, root length, and shoot and root dry weights in both maize lines. The decline of malondialdehyde (MDA) content and relative permeability of the plasma membrane and the increase of the concentrations of soluble sugars and proline, peroxidase (POD) activity, and catalase (CAT) activity were detected both in the chilling-sensitive and chilling-tolerant maize seedlings after priming with the three concentrations of chitosan. HuangC was less sensitive to responding to different concentrations of chitosan. Priming with 0.50% chitosan for about 60~64 h seemed to have the best effects. Thus, it suggests that seed priming with chitosan may improve the speed of germination of maize seed and benefit for seedling growth under low temperature stress.

Key words: Seed priming, Chitosan, Low temperature stress, Germination, Physiological changes, Maize

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INTRODUCTION

Low temperature is one of the most important limiting factors in the productivity of plants. Maize (*Zea mays* L.) is a thermophilic crop. Low temperature frequently causes injuries to maize seed germination and seedling growth, thus being detrimental to early spring planting (Parera and Cantliffe, 1994). Previous reports indicated that the most conspicuous changes occurred in cellular membranes and enzymes under low temperature stress conditions (Zhou and

Leul, 1998; Lukatkin, 2003).

Seed priming has been shown to improve seed performance under sub-optimal temperature conditions (Lin and Sung, 2001). Priming increases the environmental range suitable for germination, and provides faster and synchronous seedling emergence (McDonald, 1999). Common priming methods such as polyethylene glycol (PEG) treatment are not suitable for large scale cereal crop production because farmers in developing countries could not cover the costs. PEG priming is frequently applied to vegetable seeds in laboratory and field experiments. Other priming methods such as drum priming need special equipment, and some methods are not easy to operate or not cheap enough to be used in large scale (Hu *et al.*, 2005).

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Chitosan is an abundant and comparatively cheap organic compound in China. It is a large cationic polysaccharide mainly obtained from waste materials from seafood processing. Chitosan treatment of wheat seeds induced resistance to certain disease and improved seed quality (Reddy *et al.*, 1999). Seed soaked with chitosan increased the energy of germination, germination percentage, lipase activity, and gibberellic acid (GA_3) and indole acetic acid (IAA) levels in peanut (Zhou *et al.*, 2002). Seed coated with chitosan may accelerate seed germination and improve the tolerance to stress condition of hybrid rice seedlings (Ruan and Xue, 2002) and restrain the growth and reproduction of sclerotinia rot in carrot (Cheah *et al.*, 1997). Seed priming with two different acidic chitosan solutions improved the vigor of maize seedlings (Shao *et al.*, 2005). Thus, it seems that chitosan is a promising material for seed treatments. In the present study, the effects of seed priming with different concentrations of chitosan solutions were investigated. For better understanding the results, chilling-tolerant and chilling-sensitive maize inbred lines were used to determine germination and the seedling growth in relation to physiological changes under low temperature stress after the seed priming.

MATERIALS AND METHODS

Materials

Seeds of two maize (*Zea mays* L.) inbred lines, 'HuangC' (chilling-tolerant) and 'Mo17' (chilling-sensitive) (Zheng *et al.*, 2006), from Zhangye Seed Company (Gansu, China) were used. Their original moisture contents were 12.3% and 11.7%, respectively.

Seed priming

Chitosan solutions (Yuhuan Chemicals, Zhejiang, China) of 0.25%, 0.50%, and 0.75% (w/v) concentrations (pH 5.1) were used for seed priming. Four replicates of 50 seeds each were used for each treatment. Fifty seeds were placed on a 3-layer blotter wetted with 12 ml chitosan solution in a 12-cm diameter petri dish. The petri dishes were sealed with parafilm. The seeds were primed at 15 °C in darkness for 58 h (0.25%) and 64 h (0.50% and 0.75%) for Mo17, and 48 h (0.25%) and 60 h (0.50% and 0.75%)

for HuangC, respectively. Following the priming, seeds were dried back to their original moisture contents at room temperature. The un-priming dry seeds were used as control (CK).

Measurement of germination and physiological characteristics of seedlings

After priming, seed germination tests were carried out. Fifty seeds each for each treatment were placed in a plastic germination box (12 cm×18 cm) with sand bed, and each experiment was replicated four times. Then, seeds were incubated in a germination chamber at 25 °C under alternating cycle of 12 h illumination and 12 h darkness for 5 d (Zheng *et al.*, 2006), followed by low temperature stress at 5 °C for 3 d. After that, the seedlings were transferred to 25 °C for recovering growth for 3 d. Seeds were considered germinated when there was a visible coleoptile protrusion through the sand bed. The germinated seeds were counted daily for 11 d. Then, the germination percentage was calculated on Day 11. The germination index and mean germination time were calculated as $GI = \sum(G_i/T_i)$ and $MGT = \sum(G_i \times T_i) / \sum G_i$, respectively (Zhang *et al.*, 2007; Kaya *et al.*, 2008), where G_i is the number of germinated seeds on Day t , T_i is time corresponding to G_i in days.

Root length and shoot height were measured manually with a ruler and dry weights of shoot and root were determined after drying at 80 °C for 24 h. Relative electrolyte leakage as a measurement for membrane permeability was determined according to the method of Li (2001). Leaves of seedlings were cut into segments of about 5 mm. Each replication (0.2 g fresh weight (FW)) was collected and washed with deionized water, and then placed in the test tube with 10 ml deionized water and covered with a plug. After incubation at 25 °C for 6 h, the relative electrolyte leakage of the leaves was measured using a conductometer (DDS-11A, Shanghai, China), and the value was named E_1 . Subsequently, the test tube was kept in water at 100 °C for 30 min, and then cooled to 25 °C, and the second relative electrolyte leakage was measured as E_2 . The relative electrolyte leakage of deionized water was named E_0 . The relative electrolyte leakage (REL) was calculated as follows: $REL (\%) = (E_1 - E_0) / (E_2 - E_0) \times 100$. The concentrations of malondialdehyde (MDA), proline, and soluble sugar in leaves were measured using thiobarbituric acid

(TBA) reaction method, colorimetric method, and anthrone colorimetric method, respectively (Li, 2001). Leaf peroxidase (POD) activity was determined by guaiacol method (Zhu and Zhong, 1990), and leaf catalase (CAT) activity was determined by the method described by Fu and Huang (2001). All measurements mentioned above were made after seed germination for 8 d (at 25 °C under alternating cycle of 12 h illumination and 12 h darkness for 5 d, followed by low temperature stress at 5 °C for 3 d) on 20 randomly selected seedlings per measurement.

Statistical analysis

All data were analyzed by means of analysis of variance (ANOVA) using Statistical Analysis System (SAS) software; percentage data were arcsin-transformed before analysis according to $y = \arcsin \sqrt{x/100}$. Significant level $P=0.05$ was used.

RESULTS

Effects of seed priming with chitosan on germination

There were no significant differences in germination percentage among treatments irrespective of variety (Table 1). All the three concentrations of chitosan significantly increased the GI and reduced the MGT when compared with controls in both lines tested. The GI and the MGT of 0.50% chitosan

priming treatment were significantly higher and lower than those of 0.75% chitosan treatment, respectively, but were not significantly different from those of 0.25% chitosan treatment in Mo17; however, there were no significant differences among the three priming treatments with chitosan in HuangC.

Effects of seed priming with chitosan on seedling growth

All priming treatments with chitosan significantly increased the shoot height and shoot dry weight compared with the control in Mo17 (Table 2). Seed priming with 0.50% and 0.25% chitosan significantly increased the root dry weight and the root length as compared with the control, respectively. The most pronounced effect was recorded in 0.50% concentration.

For HuangC, seed priming with all the three concentrations of chitosan significantly increased the shoot height and shoot dry weight compared with the control, and there were no significant differences among the three treatments with chitosan (Table 2). The three priming treatments with chitosan increased root length, and the increase of root length by priming with 0.25% and 0.75% chitosan reached a significant level compared with the control. Seed priming with 0.50% chitosan significantly increased the root dry weight compared with the control, and there were no significant differences among 0.25% and 0.75% chitosan treatments and the control in both lines.

Table 1 Effects of seed priming with various concentrations of chitosan on germination percentage (GP), germination index (GI), and mean germination time (MGT) of maize after germination for 11 d including 3 d of low temperature stress and 3 d of recovering growth

Variety	Treatment	GP (%)	GI	MGT (d)
Mo17	CK	98a	68.22c	7.17a
	0.25% chitosan	97a	77.57ab	6.91c
	0.50% chitosan	98a	80.09a	6.89c
	0.75% chitosan	96a	73.23b	7.01b
HuangC	CK	97a	69.09b	7.13a
	0.25% chitosan	98a	81.18a	6.89b
	0.50% chitosan	99a	80.63a	6.88b
	0.75% chitosan	97a	80.67a	6.85b

Different letter(s) following the values indicated significant difference ($P=0.05$, LSD) among treatments within the same variety

Table 2 Effects of seed priming with various concentrations of chitosan on shoot height (SH) and root length (RL), shoot and root dry weights (SDW and RDW) of maize seedlings after germination for 8 d including 3 d of low temperature stress

Variety	Treatment	SH (cm)	RL (cm)	SDW (mg/shoot)	RDW (mg/root)
Mo17	CK	7.23c	12.47b	16.03c	18.15b
	0.25% chitosan	10.80a	13.48a	19.23b	17.75b
	0.50% chitosan	10.61a	12.49ab	21.43a	21.05a
	0.75% chitosan	9.32b	12.69ab	19.67b	17.90b
HuangC	CK	10.50b	15.26b	26.13b	15.60b
	0.25% chitosan	12.99a	18.61a	32.40a	18.33ab
	0.50% chitosan	12.65a	17.53ab	35.07a	20.43a
	0.75% chitosan	12.88a	19.17a	32.53a	17.57ab

Different letter(s) following the values indicated significant difference ($P=0.05$, LSD) among treatments within the same variety

Effects of seed priming with chitosan on relative permeability of plasma membrane and MDA concentration

The relative permeability of the plasma membrane after the 0.50% and 0.75% chitosan treatments was significantly lower than that of the control in Mo17 (Table 3). For HuangC, only the relative permeability of plasma membrane of 0.50% chitosan treatment was significantly lower than that of the control.

All the three priming treatments with chitosan reduced MDA concentration and the decrease of MDA by priming with 0.75% chitosan reached a significant level compared with the control in Mo17 (Table 3). For HuangC, the MDA concentrations of all the three treatments with chitosan were significantly lower than that of the control, and there were no significant differences among the three treatments.

Table 3 Effects of seed priming with various concentrations of chitosan on relative permeability of plasma membrane (RPPM) and MDA concentration (C_{MDA}) of maize seedlings after germination for 8 d including 3 d of low temperature stress

Variety	Treatment	RPPM	C_{MDA} (nmol/g FW)
Mo17	CK	0.364a	4.08a
	0.25% chitosan	0.258ab	3.74ab
	0.50% chitosan	0.207b	3.64ab
	0.75% chitosan	0.198b	3.36b
HuangC	CK	0.207a	5.55a
	0.25% chitosan	0.184ab	4.67b
	0.50% chitosan	0.172b	4.35b
	0.75% chitosan	0.188ab	4.25b

Different letter(s) following the values indicated significant difference ($P=0.05$, LSD) among treatments within the same variety

Effects of seed priming with chitosan on concentrations of soluble sugar and proline

Priming treatments with 0.25% and 0.50% chitosan significantly increased the soluble sugar concentration compared with the control in Mo17 (Table 4). Seed priming treatment with 0.50% chitosan significantly increased proline concentration compared with the control and 0.75% chitosan treatment, and there was no significant difference between 0.25% and 0.50% chitosan treatments in Mo17.

For HuangC, all treatments with chitosan significantly increased the concentrations of soluble

sugar and proline compared with the control, but there were no significant differences among all the three treatments with chitosan (Table 4).

Table 4 Effects of seed priming with various concentrations of chitosan on the concentrations of soluble sugar (C_{SS}) and proline (C_P) of maize seedlings after germination for 8 d including 3 d of low temperature stress

Variety	Treatment	C_{SS} (mg/g FW)	C_P (μ g/g FW)
Mo17	CK	18.37b	15.58b
	0.25% chitosan	22.74a	18.73ab
	0.50% chitosan	22.46a	21.61a
	0.75% chitosan	20.41ab	17.61b
HuangC	CK	32.47b	13.14b
	0.25% chitosan	36.20a	14.84a
	0.50% chitosan	37.09a	15.60a
	0.75% chitosan	39.18a	15.38a

Different letter(s) following the values indicated significant difference ($P=0.05$, LSD) among treatments within the same variety

Effects of seed priming with chitosan on POD and CAT activities

In Mo17, the POD activity of the 0.25% chitosan treatment was higher than that of the control but higher concentrations of chitosan did not significantly affect POD activity (Table 5). There was no significant effect of chitosan treatment on the CAT activity of this variety either.

In HuangC, seed priming with 0.50% chitosan had no significant effect on POD activity, but significantly increased CAT activity in comparison with the control (Table 5). Between the three chitosan treatments there was no significant difference in CAT activity.

Table 5 Effects of seed priming with various concentrations of chitosan on POD and CAT activities of maize leaves after germination for 8 d including 3 d of low temperature stress

Variety	Treatment	POD activity (U/(g FW·min))	CAT activity (U/(g FW·min))
Mo17	CK	1063b	16.0a
	0.25% chitosan	1259a	15.3a
	0.50% chitosan	1216ab	19.3a
	0.75% chitosan	1108ab	19.0a
HuangC	CK	732ab	16.5b
	0.25% chitosan	606b	19.5a
	0.50% chitosan	849a	21.0a
	0.75% chitosan	732ab	24.0a

Different letter(s) following the values indicated significant difference ($P=0.05$, LSD) among treatments within the same variety

DISCUSSION

Priming is used to control the hydration level within seeds so that the metabolic activity necessary for germination can occur, but radicle emergence is still prevented. There are many factors associated with the effects of seed priming, but the concentrations of priming solutions as well as the time and temperature during priming were crucial (Khan, 1992). Sometimes, priming effects and conditions varied with varieties (Adegbuyi *et al.*, 1981; Khan, 1992). In the present study, we found that the combinations of priming time and concentration were different between the two maize lines, Mo17 and HuangC.

Seeds of the two inbred maize lines showed different responses to chitosan priming under cold-stress. Among the three different concentrations, priming with 0.50% chitosan had the best enhancement effects for chilling-sensitive Mo17, while no significantly different effects among the three concentration treatments were observed for chilling-tolerant HuangC. This difference may be related to the differences in antioxidant enzyme activities, proline concentration, and plasma membrane permeability in these two maize inbred lines (Gao *et al.*, 2006).

Low temperature induces damage to cell membranes and also affects physiological functions of plants. Relative permeability of the plasma membranes has been used to illustrate the damage degree of low temperature stress (Lukatkin, 2003). The lower relative permeability of plasma membrane under low temperature stress means less damage from low temperature stress. Priming with chitosan reduced the relative permeability of the plasma membranes of the two lines under low temperature stress, indicating that chitosan priming alleviated the chilling injury of the seedlings. Similar observations have been made previously by seed film coated with a cold-tolerant agent on maize subjected to low temperature stress (Li *et al.*, 2004).

Proline and soluble sugar are small molecules, which are synthesized after suffering intimidation action (Hu *et al.*, 2006). These molecules are strongly hydrophilic, and alleviate stress damage in plant cells

by reducing the water potential and keeping the activities of some biological macromolecules (Rathinasabapathi, 2000). Accumulation of organic compounds such as proline in the cytoplasm also plays an important role in osmotic adjustment in plants (Watanabe *et al.*, 2000). After sand priming, seed germination and seedling growth of alfalfa were improved, and soluble sugar and proline concentrations under high salt concentration stress were also increased (Hu *et al.*, 2006). Similar effects were also achieved in this experiment, which suggests that priming with chitosan improved chilling tolerance in maize seeds.

Cell membrane stability was affected by lipid peroxidation caused by active oxygen species under various stress conditions (Fu and Huang, 2001), and the concentration of MDA was an indicator of lipid peroxidation in plant cells (Feng *et al.*, 2003). The decline of the MDA concentrations in the two primed maize lines under low temperature stress suggested that seed priming with chitosan reduced lipid peroxidation. MDA was produced when polyunsaturated fatty acids in the membrane underwent peroxidation (Meloni *et al.*, 2003). The MDA accumulation was reduced after seed film was coated with a cold-tolerant agent in maize under low temperature stress (Li *et al.*, 2004), and the decline of the MDA accumulation was achieved after sand priming in alfalfa seedlings under salt stress condition (Hu *et al.*, 2006). Our observations are consistent with these earlier reports.

The accumulation of MDA may be related to the POD and CAT enzyme activities, the lesser degree of membrane damage (as indicated by low MDA concentration), and the higher activities of antioxidative enzymes (Meloni *et al.*, 2003). Chilling conditions may cause an increase in reactive oxygen species (ROS), starting oxidative damage to the membrane system of plants (Wise, 1995). Cooperation of protective enzymes such as POD and CAT could eliminate ROS and keep a homeostasis between producing and cleaning of ROS and reduce the level of free radicals. Finally, injury to cells could be declined or avoided (Hu *et al.*, 2006). The three concentrations of chitosan used here increased the POD activity of Mo17 and significantly increased CAT activity of HuangC. It seems that for the chilling-sensitive maize

seedlings, POD plays a key role in protecting seedlings from low temperature stress, and for the chilling-tolerant maize seedlings, CAT plays a key role.

In conclusion, this study shows that the chitosan priming increased the chilling tolerance of maize seedlings demonstrated by improving germination speed and shoot and root growth and maintaining membrane integrity and higher activities of antioxidative enzymes. The 0.50% chitosan seems to be a suitable concentration for seed priming. However, seed priming effect for other crops warrants further study.

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