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Effects of osmopriming on tomato (*Lycopersicon esculentum* M.) hybrid seed vigor under aging and salinity stress

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This study investigates the effects of osmopriming on tomato (*Lycopersicon esculentum* M.) hybrid seed vigor under aging and salinity stress. Tomato seeds of the ZZ1 hybrid variety, stored for four years under natural (aged) or -20°C (unaged) conditions were primed in 10% (w/v) polyethylene glycol (PEG) solution for 2 d at 20±1°C in the dark. The seed vigor was evaluated at 25±1°C for 7 d under normal (water) and 100 mM NaCl conditions, respectively. Results show that the germination percentage (GP), germination index (GI) and mean germination rate (MGR) of primed, aged seeds were significantly enhanced with a substantial increase in the radicle length (RL), shoot length (SL) and total fresh weight (FW) compared with unprimed aged seeds. Similarly, the GI, MGR and FW significantly increased in primed seeds compared with unprimed seeds under salinity stress, while GP, RL and SL did not show significant differences. Furthermore, a decline in the relative electrolyte leakage (REL) and in malondialdehyde (MDA) was detected in primed seeds during the imbibition stage compared with unprimed seeds under aging and salinity stress. The negative correlations between seed vigor and REL and MDA were observed which suggests that seed priming improves seed vigor under stress conditions associated with a decrease in seed lipid peroxidation.

Key words: Tomato, seed priming, osmopriming, seed vigor, seed aging, salt tolerance.

INTRODUCTION

Tomato (*Lycopersicon esculentum* M.) is one of the most important vegetable crops throughout the world. Rapid and uniform seed germination is essential for increasing tomato crop yield and quality. However, seed germination might be problematic due to the decrease in seed vigor caused by deterioration during storage (Coolbear et al., 1984) and differences in seed vigor (non-uniformity; Ismail et al., 2005) and effects of abiotic stress (Wang et al., 2011). In tomato, salinity has become a great threat to

tomato growth since tomato cultivation was switched to greenhouses (Chen et al., 2009). Therefore, low and non-uniform seed vigor as well as salinity stress, if not properly managed might become limiting factors for synchronized stand seedling establishment in tomato. Developing an elite variety with high level seed vigor is the best way to solve the foregoing mentioned problems; while this strategy is difficult and time-consuming. However, seed priming treatment is an effective and low-cost method to improve seed vigor.

Seed priming is a technique that controls seeds hydration and drying to their original moisture content. After seed priming, the first step of seed germination is completed but radicle emergence does not occur. Mostly, seeds are partially hydrated to have a water content that can be controlled by priming osmotic solutions (osmopriming) or by limiting the imbibition time (hydropriming; Schwember and Bradford, 2010). Previous

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Abbreviations: PEG, Polyethylene glycol; GP, germination percentage; GI, germination index; MGR, mean germination rate; RL, radicle length; SL, shoot length; FW, total fresh weight; REL, relative electrolyte leakage; MDA, malondialdehyde.

Table 1. Seeds pre-germination treatments and germination conditions.

Treatment		Aging treatment	Priming treatment	Germination condition
Normal condition	T1	Unaged	Unprimed	Water
	T2	Unaged	Primed	Water
Aging condition	T3	Aged	Unprimed	Water
	T4	Aged	Primed	Water
Salinity condition	T5	Unaged	Unprimed	Salinity
	T6	Unaged	Primed	Salinity

studies have indicated that seed priming constitutes a successful strategy for improving seed longevity (Butler et al., 2009) and seed germination under stress conditions (Guan et al., 2009).

In tomato, previous studies have reported that priming benefits seed storability (Liu et al., 1996), seed germination under drought (Mauromicale and Cavallaro, 1995) and low temperature stress (Mauromicale and Cavallaro, 1997; Ozbingol et al., 1998). Hybrid tomato varieties are popular for commercial cultivation because they have many advantages compared with open pollinated varieties. However, little is known about the priming effects on hybrid tomato seed vigor under aging and salinity conditions.

During the seed hydration period, a variety of physiological and biochemical changes take place in seeds. In summary, the benefits of seed priming include the advancement of germination metabolism (Farooq et al., 2010), enhanced antioxidant activities (Baillly, 2004; Chen and Arora, 2011) and improved repair processes (Sivritepe and Dourado, 1995), and so on. Under stress conditions, the reactive oxygen species (ROS) can cause lipid peroxidation, which results in cell membrane damage and malondialdehyde (MDA) production (Fu and Huang, 2001; Mei and Song, 2010). Thus, the relative electrolyte leakage (REL) and concentration of MDA are good indicators for investigating the effects of priming under stress conditions (Guan et al., 2009).

However, few studies have considered the changes of lipid peroxidation in primed tomato seeds at imbibition stage, little are known about the effects of lipid peroxidation at seed imbibition stage on tomato successful seed germination and seedling growth.

The objectives of this study were to explore the beneficial effects of osmopriming using PEG as priming reagent on the vigor of tomato hybrid seeds. Therefore, several seed vigor traits such as germination percentage (GP), germination index (GI), mean germination rate (MGR), shoot length (SL), radicle length (RL) and total fresh weight (FW) were analyzed under normal, natural aging and salinity conditions, respectively.

Furthermore, using the REL and MDA indicators, the

changes of lipid peroxidation in primed hybrid tomato seeds during the seed germination process were further analyzed. These results may provide valuable information for improving seed vigor of tomato under stress conditions by PEG osmopriming treatment.

MATERIALS AND METHODS

Plant materials

The ZZ1 hybrid variety of tomato (*L. esculentum* M.) was used in this study. Seeds were produced and harvested at a mature stage by the China National Seed Group Co. LTD (Beijing, China) in 2005. After ripening at 30°C, 85% RH for 7 d, the original moisture content and germination of seeds were approximately 9.0 and 100% by seed testing, respectively. Then, seeds were stored in kraft paper bags under natural (aged) and -20°C (unaged) conditions in Nanjing (Jiangsu Province, China; E118°50', N32°02') for four years. Six treatments were conducted in this study (Table 1).

Seed priming

Seeds were surface sterilized with 0.1% HgCl₂ for 5 min, then placed in a Petri dish (9-cm diameter) on two sheets of filter paper with 10 mL of 10% (w/v) polyethylene glycol (PEG) 6,000 (molecular weight) solution, sealed with parafilm and stored at 25°C in the dark for 48 h. The primed seeds were then washed twice with distilled water and dried to their original moisture content (9.0%) at 25°C to perform germination tests.

Seed germination

Seeds were germinated under normal water or 100 mM NaCl conditions at 25±1°C for 7 d with a 12-h light/dark photoperiod. Briefly, 100 seeds per replication were surface sterilized with 0.1% HgCl₂ for 5 min. Then, they were placed in a Petri dish (9-cm diameter) on two sheets of filter paper with 10 mL of distilled water or 100 mM NaCl. Seeds were considered to have germinated when their radicle length reached more than 2 mm. The germinated seeds were observed each day. The percentage of germinated seeds at 7 d was referred to as the GP that represents seed germinability. The GI and MGR were calculated as $GI = \sum (Gt/t)$ and $MGR = 1/MGT$ (mean germination time), $MGT = \sum (Gt \times t) / \sum Gt$, respectively, where Gt is the number of germinated seeds on day t (Guan et al., 2010 a). Here, the GI represents germination speed and uniform and the

Table 2. The effects of priming on seed germination under normal, aging and salinity conditions.

Treatment ^a		GP (%) ^b	GI ^b	MGR ^b
Normal condition	T1	100±0.0 ^A	24.9±0.2 ^A	0.500±0.000 ^A
	T2	100±0.0 ^A	13.0±0.5 ^B	0.414±0.020 ^B
Aging condition	T3	61.1±4.0 ^C	4.3±1.1 ^C	0.233±0.006 ^C
	T4	87.8±6.9 ^B	11.7±0.9 ^B	0.417±0.002 ^B
Salinity condition	T5	99.3±1.2 ^A	12.5±0.4 ^B	0.244±0.004 ^C
	T6	98.9±1.9 ^A	21.0±0.5 ^A	0.500±0.000 ^A

^aT1: Unaged seeds germinated under water condition; T2: Primed unaged seeds germinated under water condition; T3: Aged seeds germinated under water condition; T4: Primed aged seeds germinated under water condition; T5: Unaged seeds germinated under salinity stress; T6: Primed unaged seeds germinated under salinity stress. ^bGP: Germination percentage; GI: Germination index; MGR: Mean germination rate; Means ± SD (standard deviation); Sample size n = 100, replications r = 4; Means within the same column followed by the same letters are not significantly different between treatments at the 1% level of probability according to Fisher's least significant difference (LSD) test (P<0.01, LSD).

MGR represents germination speed. The RL, SL and SW were measured after 3 and 7 d. The seed germination process was replicated four times.

Measurement of membrane permeability and MDA

After seeds germinated at 25°C for 1 d and 7 d in 10 mL of water or 100 mM NaCl, respectively they were washed with deionized water for the measurement of membrane permeability and MDA. Membrane permeability was estimated by measuring cell electrolyte leakage. Seeds or seedlings were placed in a test tube with 80 mL of deionized water and incubated at 25°C for 24 h. Subsequently, the electrolyte leakage was determined as E1 according to the method of Guan et al. (2009) using a conductometer (DDS-11A, Shanghai, China). Then, test tubes were kept in water at 100°C for 10 min and were cooled to 25°C when the second electrolyte leakage was measured as E2. The electrolyte leakage of deionized water was named E0. Finally, the REL of seeds was calculated according to the following formula: REL (%) = (E1-E0)/(E2-E0) × 100 (Guan et al., 2009).

The MDA content of seeds was measured by the thiobarbituric acid (TBA) reaction method (Guan et al., 2009). The seeds or seedlings were mixed with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 × g for 10 min; the supernatant (1 mL) was then mixed with 4 mL of 20% (w/v) TCA. The resulting mixture was heated at 95°C for 30 min and then quickly cooled on ice for 5 min. After centrifugation at 10,000 × g for 10 min at 4°C, the absorbance of the reaction mixture was measured at 450, 532 and 600 nm.

The MDA concentration (nmol/g DW) was calculated using the following equation: [MDA] = 6.45 × (A₅₃₂ - A₆₀₀) - 0.56 × A₄₅₀, where A₅₃₂, A₆₀₀, and A₄₅₀ represent the absorbance of the mixture at 450, 532, and 600 nm, respectively. The experiment was replicated four times and 100 seeds per replicate were used.

Statistical analysis

Experimental data were analyzed using the Statistical Analysis System (SAS) software, and treatment means were compared using a Fisher's least significant difference (LSD) test at 1% level of probability. The correlation of different traits was computed using

PROC CORR in the SAS software.

RESULTS

Effects of priming on seed germination

Compared to unaged seeds under normal condition (T1), there were significant effects of long-term natural storage (aged) on tomato seed germination (Table 2); the GP, GI and MGR of unprimed aged seeds (T3) decreased to 61.1, 4.3 and 0.233 after four years of storage, respectively. In contrast, under salinity condition, the GI and MGR of unprimed seeds (T5) significantly decreased to 12.5 and 0.244 compared to T1, respectively but non-significant effects on GP. Based on the curve of seed germination, the peak germination of T3 and T5 occurred on the 6th day; however, that of T1 occurred on the 2nd day (Figure 1). The seed germination could be improved by PEG priming under aging and salinity conditions, respectively. Under aging condition, significant differences were observed in GP, GI and MGR between unprimed (T3) and primed seeds (T4) (Table 2); the GP, GI and MGR of T4 increased by 26.7, 7.4 and 0.184, respectively compared with T3. Under salinity condition, significant differences were also observed in GI and MGR between unprimed (T5) and primed seeds (T6) but non-significant difference in GP; the GI and MGR of T6 increased by 8.5 and 0.256, respectively compared with T5. Based on the seed germination curve, the priming treatment reduced the number of days to the peak germination of seeds under aging and salinity conditions; the peak germination of T4 and T6 occurred on the 5th day and 2nd day (Figure 1). However, non-significant benefits of priming were observed under normal condition; the GI and MGR of T2 significantly decreased by 11.9 and 0.086, respectively compared with T1.

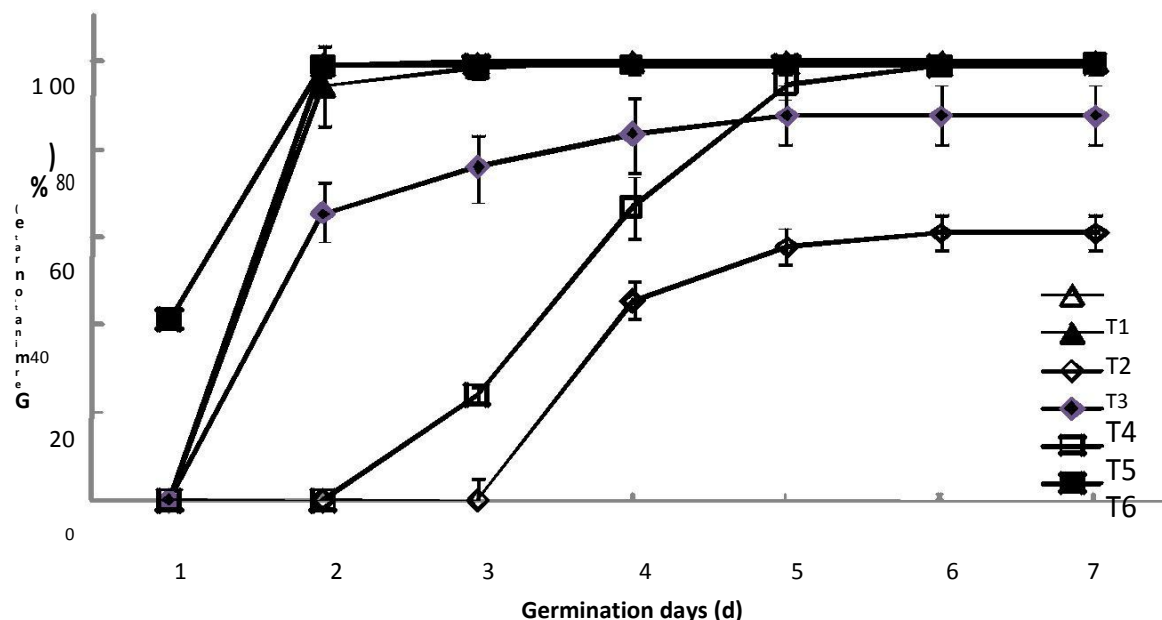


Figure 1. The effects of priming on seeds germination process under normal, aging and salinity conditions.

Effects of priming on seedling growth

Likewise, four years of natural storage (T3) significantly reduced seedling growth compared with the unaged seeds (T1); the SL, RL and FW of T3 on the 3rd and 7th d were reduced, respectively (Table 3). However, under salinity condition, only the FW of T5 significantly decreased on the 3rd and 7th d compared with T1; non-significant differences in SL and RL.

Similarly, the seedling traits also could be improved by PEG priming under aging and salinity conditions, respectively. Under aging condition, the priming treatment significantly increased the SL, RL and FW of T4 on the 3rd and 7th d compared to T3 (Table 3). Under salinity condition, priming treatment significantly increased the FW of T6 compared with T5, but non-significant difference in SL and RL. However, non-significant effects of priming were observed under normal condition; the SL, RL and FW of T2 were non-significant difference compared with T1. In general, after priming, the seedling performances under aging and salinity conditions were improved, which also reflected seed germination speed was enhanced by priming.

Effects of priming on the level of REL and MDA during seed germination process

To investigate the effects of priming on lipid peroxidation subject to aging and salinity stress, changes of REL and MDA at seed imbibition and seedling stages were evaluated. Upon exposure to either aging or salinity stress,

the REL of unprimed seeds (T3 and T5) significantly increased compared with that of the control (T1) at imbibition stage (Table 4). However, at seedling stage, the REL of T3 and T5 was significantly decreased and increased, respectively compared to T1. Furthermore, the MDA content significantly increased only in the aging treatment (T3) at both imbibition and seedling stages, the MDA content was approximately double that of T1; whereas in the salinity treatment (T5), the MDA content was comparable to that of T1.

Under normal, aging and salinity conditions, primed seeds (T2, T4 and T6), in general, exhibited a lower REL and MDA than unprimed seeds (T1, T3 and T5) (Table 4). Under normal condition, only the REL of T2 at imbibition stage was significantly decreased as compared to T1. Under aging condition, the REL and MDA of T4 significantly decreased at imbibition stage compared with T3, however, only the REL of T4 was significantly decreased at seedling stage. Upon exposure to salinity stress, only the REL of T6 was significantly decreased and increased as compared to T5 at imbibition and seedling stages, respectively whereas the MDA content of T6 was comparable to that of T5.

Correlations between the REL and MDA in imbibition seeds with seed vigor

In order to clear the effects of lipid peroxidation at seed imbibition stage on tomato successful seed germination and seedling growth by seed priming treatment, the correlations between REL and MDA in imbibition seeds

Table 3. The effects of priming on seedling growth under normal, aging and salinity conditions.

Treatment ^a		3 d ^b			7 d ^b		
		SL (cm)	RL (cm)	FW (mg/per line)	SL (cm)	RL (cm)	FW (mg/per line)
Normal condition	T1	3.7±0.3 ^A	4.3±0.5 ^A	19.5±1.7 ^A	10.2±1.4 ^A	8.8±0.7 ^A	40.4±1.2 ^A
	T2	3.4±0.2 ^A	5.1±0.3 ^A	20.1±1.0 ^A	10.8±2.8 ^A	6.4±0.5 ^A	38.3±4.6 ^A
Aging condition	T3	0.0±0.0 ^C	0.0±0.0 ^C	0.0±0.0 ^C	3.5±0.9 ^B	2.6±0.4 ^B	13.3±2.8 ^B
	T4	4.8±0.4 ^A	6.1±0.2 ^A	21.3±1.7 ^A	9.4±0.7 ^A	7.2±0.7 ^A	41.6±2.9 ^A
Salinity condition	T5	3.5±0.9 ^A	3.9±0.9 ^B	17.2±1.2 ^B	8.9±1.6 ^A	6.8±1.4 ^A	33.3±1.3 ^B
	T6	3.9±0.9 ^A	5.2±1.0 ^A	23.8±2.9 ^A	9.0±1.4 ^A	7.1±1.0 ^A	37.0±1.4 ^A

^aT1: Unaged seeds germinated under water condition; T2: Primed unaged seeds germinated under water condition; T3: Aged seeds germinated under water condition; T4: Primed aged seeds germinated under water condition; T5: Unaged seeds germinated under salinity stress; T6: Primed unaged seeds germinated under salinity stress. ^bSL: Shoot length; RL: Radicle length; FW: Total fresh weight; Means ± SD (standard deviation); Sample size n = 100, replications r = 4; Means within the same column followed by the same letters are not significantly different between treatments at the 1% level of probability according to Fisher's least significant difference (LSD) test (P≤0.01, LSD).

Table 4. The effects of priming on the level of REL and MDA at different germination stages under normal, aging and salinity conditions.

Treatment ^a		REL (%) ^b		MDA (nmol/g DW) ^b	
		1 d	7 d	1 d	7 d
Normal condition	T1	53.8±2.9 ^C	69.8±4.0 ^C	15.5±0.7 ^B	12.5±0.6 ^B
	T2	44.0±0.7 ^U	68.4±3.4 ^C	13.9±1.2 ^B	9.8±1.5 ^B
Aging condition	T3	95.8±7.2 ^A	48.4±2.0 ^D	31.0±1.6 ^A	34.2±1.7 ^A
	T4	62.5±6.5 ^B	35.7±1.7 ^E	15.5±0.8 ^B	33.5±1.5 ^A
Salinity condition	T5	69.3±2.7 ^B	79.9±2.0 ^B	14.0±0.7 ^B	7.8±0.3 ^B
	T6	13.8±1.5 ^F	92.9±0.5 ^A	13.0±0.7 ^B	7.9±0.4 ^B

^aT1: Unaged seeds germinated under water condition; T2: Primed unaged seeds germinated under water condition; T3: Aged seeds germinated under water condition; T4: Primed aged seeds germinated under water condition; T5: Unaged seeds germinated under salinity stress; T6: Primed unaged seeds germinated under salinity stress. ^bREL: Relative electrolyte leakage; MDA: Malondialdehyde; Mean ± SD (standard deviation); Sample size n = 100, replications r = 4; Means within the same column followed by the same letters are not significantly different between treatments at the 1% level of probability according to Fisher's least significant difference (LSD) test (P≤0.01, LSD).

with seed vigor traits under aging and salinity conditions were analyzed, respectively. Under aging condition, correlation analyses showed that the REL and MDA were significantly and negatively correlated with GP, GI, MGR, SL, RL and FW, respectively (Table 5). Under salinity condition, significant and negative correlations were observed in the relationships between REL with MGR and between MDA with GP, GI and MGR. The significant and negative correlations between REL and MDA with seedling traits were also observed, except between REL and SL and between MDA and FW at 7 d.

DISCUSSION

Seed deterioration is a major problem in agricultural

production. It has been estimated that 25% of the annual value of seeds in inventories might be lost because of poor seed quality (Schwember and Bradford, 2010). The rate of aging is strongly influenced by several environmental and genetic factors such as storage temperature, storage time, storage fungi, seed moisture content and seed quality (Harman and Mattick, 1976; Walters, 1998; Rajjou and Debeaujon, 2008).

In Nanjing (Jiangsu Province, China; E118°50', N32°02'), the average annual temperature is approximately 15°C, with the extreme lowest and highest values reaching -10 and 40°C, respectively and the precipitation period ranges from mid-June to July. In this study, after four years of storage under natural conditions in Nanjing, hybrid tomato seeds showed a decreased seed germinability, germination speed and germination uniformity.

Table 5. Correlation between the level of REL and MDA at imbibition stage with seed vigor under aging and salinity conditions.

Treatment	Trait	GP	GI	MGR	3 d			7 d		
					SL	RL	FW	SL	RL	FW
Aging condition	REL	-0.9231**	-0.6831**	-0.9136**	-0.7933**	-0.7703**	-0.9368**	-0.9817**	-0.9449**	-0.9990**
	MDA	-0.9516**	-0.7408**	-0.9439**	-0.7406**	-0.7153**	-0.9049**	-0.9940**	-0.9686**	-0.9993**
Salinity condition	REL	0.3786	-0.1058	-0.7157**	-0.9690**	-0.9995**	-0.9971**	0.2288	-0.9899**	-0.3660**
	MDA	-0.4703**	-0.5897**	-0.9449**	-0.6547**	-0.4752**	-0.5146**	-0.6857**	-0.7857**	0.1494

REL: Relative electrolyte leakage; MDA: Malondialdehyde; GP: Germination percentage; GI: Germination index; MGR: Mean germination rate; SL: Shoot length; RL: Radicle length; FW: Total fresh weight ** Significant at 0.01 level.

These results reveal that either protection of seed longevity or usage of aged seeds have significant impacts on crop production and conservation of plant biodiversity in hot and moist climate regions such as Nanjing.

However, if deterioration damages do not reach a critical level, the seed vigor can be improved by priming treatment (Goel et al., 2003). For example, Dell'Aquila et al. (1984) indicated that the germination performance of aged wheat seeds could be improved by a priming treatment. Similar results were found in this study that priming can effectively improve the vigor of aged tomato seeds.

Salinity adversely affects seeds germination and seed-ling growth, as reported in most crops (Sivritepe et al., 2003; Wang et al., 2011). In this study, similar results were observed that salinity significantly decreased germination speed, germination uniformity and total fresh weight of seedlings in tomato. However, priming could improve seed vigor by enabling rapid and uniform germination and improving seedling fresh weight. Similar results of the improvement of seed germination and seedling growth due to priming were observed in many crops under other stress conditions, such as chilling (Guan et al., 2009), heat (Schwember and Bradford, 2010) and drought stress (Kaur et al., 2002). Seed germination includes three phases, namely, imbibition, lag phase and protrusion of radicle through testa. Seed priming is the controlled hydration of seeds to allow the completion of imbibition and lag phase of germination. The benefits of seed priming are related to morphological, physiological and biochemical changes that occur in seeds during priming process, such as reducing the physical resistance of the endosperm during imbibition (Toorop et al., 1998), promoting cell membrane stability and integrity (Guan et al., 2009), facilitating repair of chromosomal damage (Sivritepe and Dourado, 1995) and increasing the activity of enzymes related to seed vigor (Baillly et al., 2000). Therefore, the significantly improved vigor of tomato seeds under aging and salinity stress by priming might be due to the reduction of germination lag phase, the early reserve breakdown and the increase in metabolic and recovery activities. For example, the beneficial effects of priming on improving germination speed and uniform

under aging and salinity stress could partially be explained by the increase in imbibition speed, reduction in lag phase and acceleration of metabolic activities in primed seeds. However, a detailed investigation is required to reveal the priming mechanisms in seed germination processes such as the seed imbibition step.

Indeed, early imbibition is a simple and rapid physical process, and a large amount of ROS is produced during this process (Liu et al. 2007). In this study, we investigated the effects of priming on the reduction of lipid peroxidation during seed imbibition stage under aging and salinity stress in tomato.

In general, the extent of lipid peroxidation was lower in primed seeds than that of unprimed seeds. Further correlation analyses showed that an increase in seed vigor under aging and salinity stress by priming was significantly correlated with a decrease in the levels of REL and MDA.

These results suggest that priming effects on tomato seed vigor under stress conditions might be closely related to the reduction of lipid peroxidation during seed imbibition stage. However, the ability to understand, manipulate and control the seed priming process at the molecular level is one of the greatest challenges of modern seed science (Gallardo et al., 2001; Schwember and Bradford, 2010). Further studies are required to clarify the molecular mechanisms of seed priming effects on seed vigor under stress conditions in tomato.

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