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# Improvement of quality, membrane integrity and antioxidant systems in sweet pepper (*Capsicum annuum* Linn.) seeds affected by osmopriming

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**Abstract**

Seed priming is a complex physiological and biochemical process and offers an effective means to improve seed quality. This study focuses on the effect of priming process on quality and biochemical changes in sweet pepper seed (*Capsicumn annuum* Linn.). The sweet pepper seeds were artificially aged by exposing to high temperature (42°C) and high humidity (100% relative humidity) for 0, 5, 10, 15, 20, 25 and 30 days. After that the seven treatments from different aging times were primed in a polyethylene glycol (PEG 6000) solution with the osmotic potential of -1.5 MPa for 6 days. Seed germination and biochemical changes of primed seeds were compared with aged seeds. Seed germination was improved in primed seeds. Malondialdehyde (MDA) and total peroxide concentration in primed seeds were decreased. The accumulation of total antioxidant activity (TAA), total ascorbate, dehydroascobate and catalase (CAT) activity in primed seeds enhanced the defense mechanism in protecting the cell membrane damage from reactive oxygen species. The enhanced seed germination possibly was resulted due to accumulation of antioxidants and the improvement of cell membrane integrity. These results pave the way to gain the insight into fact that how seed quality can be improved by the priming process.

**Keywords:** accelerated aging, ascorbate, catalase, lipid peroxidation, malondialdehyde, seed deterioration, sweet pepper seed.
**Abbreviations:** CAT_catalase; ER_emergence radical; EDTA_ethylenediaminetetraacetic acid; GE_gallic acid equivalent; GH_germination under green house; GL_germination under laboratory; GR_glutathione reductase; ISTA_international seed testing association; MDA_malondialdehyde; MPa_megapascal; ROS_reactive oxygen species; TAA_total antioxidant activity.

## Introduction

Seed priming is a technique for enhancing the seed quality and improving the overall germination and seed storage in a wide range of crop species (McDonald, 2000). Osmopriming is the commonly used method to prime the seeds. Seeds are soaked in priming solutions at a concentration diluted enough to permit seeds to imbibe and initiate pre-germination metabolism, but concentrated enough to prevent emergence of the radicles. The beneficial effects of priming are associated with the repair and building up of nucleic acid, increased synthesis of protein as well as the repair of both mitochondria and membranes (McDonald, 1999; McDonald, 2000). Moreover, priming also restored antioxidant mechanism in treated seeds. Nevertheless, the physiological reasons and biochemical changes behind the improved seed germination with priming technique are still uncertain. Many researchers have reviewed the possibility of membrane alteration during storage and suggested that lipid peroxidation of cell membrane may underline the loss of seed viability (Hsu et al., 2003; Chiu et al., 2006; Kaewnaree et al., 2011). Free radical reaction from reactive oxygen species (ROS) is a major cause of lipid peroxidation on unsaturated fatty acids in the cell membranes. This change is associated with the decrease in unsaturated fatty acids and the increase in peroxidation products, particularly, MDA and induces more membrane damage and electrolyte leakage from the cells (McDonald, 1999; Kaewnaree et al., 2011). Some protective mechanisms involving antioxidant in seed and peroxide scavenging enzymes such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD) play many important roles within the seed during membrane alteration (Goel et al., 2003). Priming can improve the germination of aged seeds (Van Pijlin et al., 1995; Bailly et al., 1998). Seed priming is associated with a lower rate of lipid peroxidation and improved germinability of aged sunflower seeds (Bailly et al., 1998). This apparently is caused by the restoration of antioxidant mechanism, in particular, CAT activity and GR activity (Wang et al., 2002; Hsu et al., 2003). In antioxidant systems, CAT and GR are key enzymes in scavenging $H_2O_2$ and producing the antioxidant glutathione, respectively, and are responsible for controlling the rate of lipid peroxidation initiated by ROS (Bailly et al., 1998; Bailly, 2004). These data suggested that the germinating ability of primed seeds

was related to the scavenging of toxic ROS by restoring the antioxidant system and preventing lipid peroxidation.

Sweet peppers are bell-shape vegetables featuring either three or four lobes with various colors (green, red orange, yellow, brown and purplish-black). They are used in spices, vegetables, medicine and cooking and are the source of nutritional benefits for health. Tropical environments such as Thailand have the optimum climate for fertilization and growing sweet peppers to produce sweet pepper seeds. Moreover, the quality of sweet pepper seed is very important in commercial seed value. Low ability of seed germination during seed production and rapid deterioration during storage of sweet pepper seed are the major problems that affect on seed quality.

To the best of our knowledge, the biochemical changes after different durations of accelerated aging and priming of sweet pepper seeds have not been reported yet. Therefore, the aim of this study was to determine the beneficial effect of priming on improving sweet pepper seeds germination. Moreover, the increase in primed seed germination can be explained by the accumulation of antioxidant system and repairing of cell membrane integrity. These results would gain insight into the fact that how biochemical changes could improve sweet pepper seeds quality after priming.

## Results

### *Effect of seed priming on emergence radical and seed germination*

Primed seeds with different durations of accelerated aging were compared with aged seeds for their effects on emergence radical (ER), germination under laboratory (GL) and germination under greenhouse (GH) (Fig. 1A, 1B and 1C, respectively). Aged seeds showed a statistically significant decrease in all germination tests. On the other hand, both ER and GL in primed seeds were higher than the aged seeds on accelerated aging for 10, 15 and 20 days. However, similar results were observed for the durations of 0, 5, 25 and 30 days of aging time. For GH determination, primed seeds exhibited higher germination percentage than the aged seeds on accelerated aging for 10 days, whereas the primed seeds at other aging durations did not show significant differences from the aged seeds.

### *Effects of seed priming on total peroxide and malondialdehyde content*

Total peroxide and MDA content increased with the accelerated aging time (0 to 30 days) (Fig. 2A and 2B). Sharp increases of the total peroxide and MDA were observed from 0 day to 10 days. After 10 days, these increases were still continued; however, with much slower speed. Primed seeds had much lower total peroxide and MDA than aged seeds, especially, for MDA. Its value was very close to zero during the all periods of accelerated aging.

### *Effect of seed priming on total antioxidant activity*

TAA increased from 100-300 µg g of seed-1 in primed seed as the seeds were aged from day 0 to day 10 (Fig. 3). This increasing trend was continued with much slower rate from 10 to 25 days, while after 25 days, a sharp decline in the TAA was observed. In contrast, the aged seeds had the TAA values of approximate-

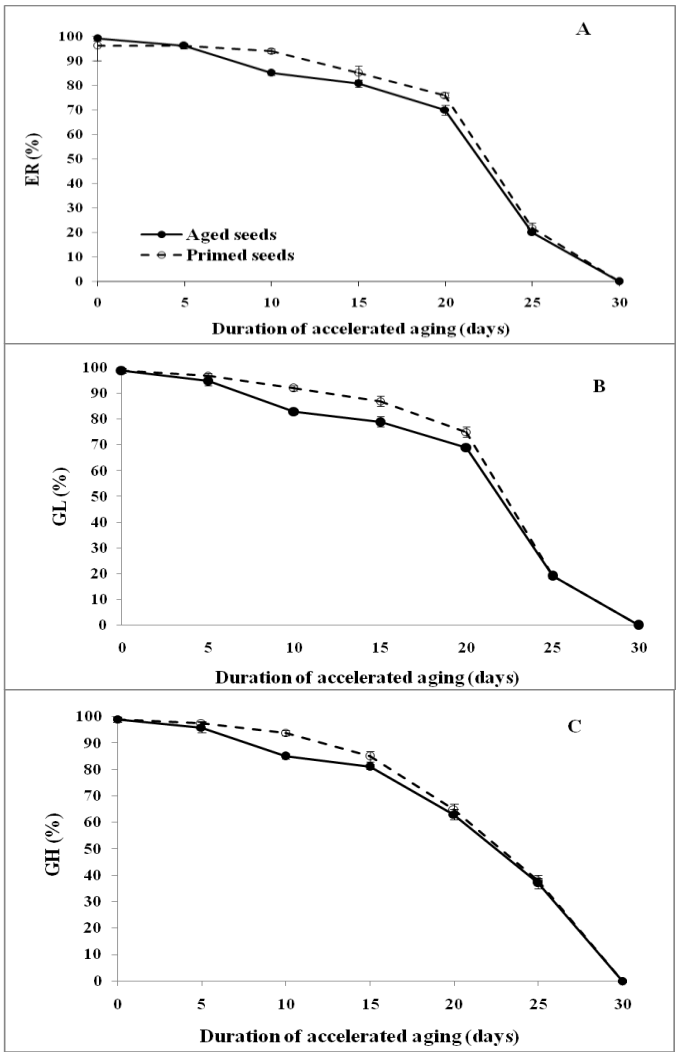


**Fig 1.** Changes in seed germination: emergence radicle (ER) (A), seed germination under laboratory (GL) (B) and seed germination under greenhouse (GH) (C) of aged and primed sweet pepper seeds. Vertical bars represent the mean and S.E. of three replications.

ly 100 µg g of seed$^{-1}$ across the durations of accelerated aging.

### *Effect of seed priming on ascorbate system*

Two ascorbate species (ascorbate and dehydroascorbate), including total ascorbate, were investigated in this study. Primed seeds had significantly higher total ascorbate than the aged seeds when the seeds were subjected to accelerated aging from 0-20 days (Fig. 4A).

However, the differences of total ascorbate were not significant when accelerated aging time was extended to 25 and 30 days. Both primed seeds and aged seeds showed a reduction in the total ascorbate, but primed seeds exhibited sharper reduction than the aged seeds largely due to the higher total ascorbate at the day 0 of accelerated aging. In contrast to the total ascorbate, primed seeds and aged seeds showed slight differences in ascorbate, and the significant difference was observed when the seeds were subjected to

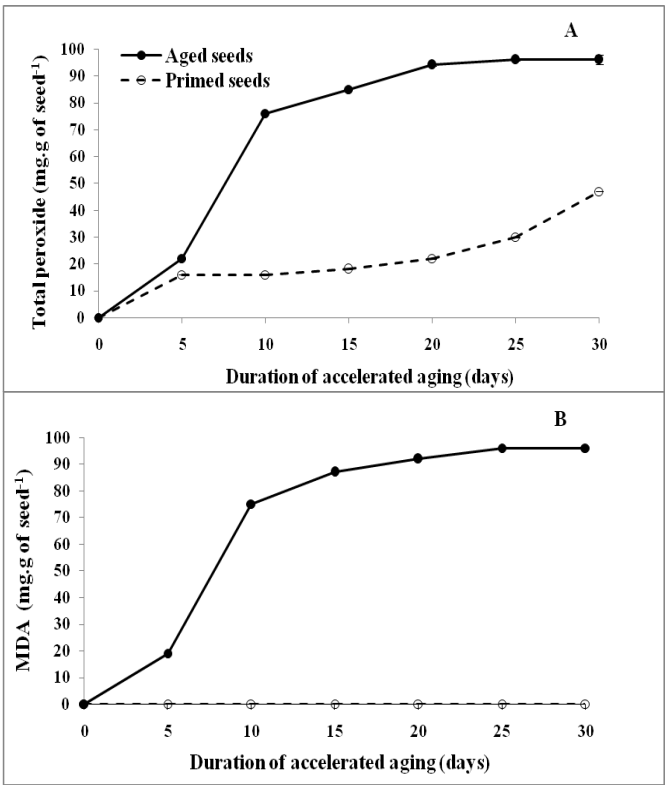


**Fig 2.** Changes in the total peroxide (A) and MDA content (B) of aged and primed sweet pepper seeds. Vertical bars represent the mean and S.E. of three replications.

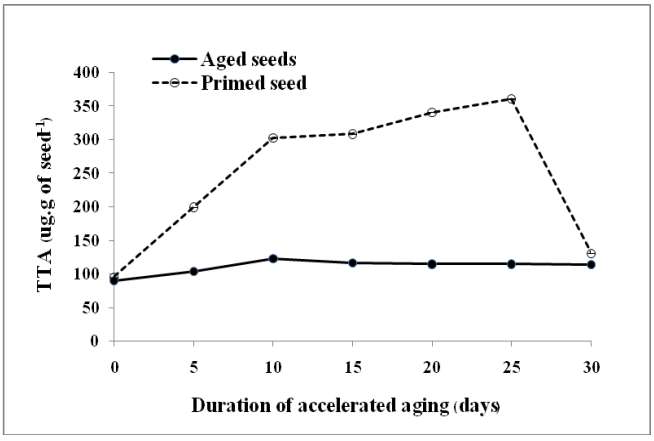


**Fig 3.** Changes in the total antioxidant activity (TAA) in aged and primed sweet pepper seeds. Vertical bars represent the mean and S.E. of three replications.

accelerated aging for 25 days (Fig. 4B). However, significant differences of dehydroascorbate between primed seeds and aged seeds were observed for most durations of accelerated aging except for 30 days (Fig. 4C), the results were similar to those of the total ascorbate.

***Effect of seed priming on catalase activity***

CAT activities for primed seeds and aged seeds increased with the durations of accelerated aging (Fig. 5). CAT activities for primed seeds and aged seeds were similar when seeds were subjected to accelerated aging for 0 and 5 days. However, significantly higher rates of increase in CAT activity were observed in primed seeds than in aged seeds, on day 10 to day 30 of accelerated aging.

**Discussion**

Seed deterioration is an important problem of crop production, which causes the seeds to be discarded. Therefore, the restoration of the seed quality is required in order to increase the optimum use of the seeds. The purpose of this research project was to explore whether seed priming could increase seed germination and whether the degree of seed deterioration could affect the restoration of seed quality by seed priming. The results of this study support the hypothesis that seed priming can restore the sweet pepper seed quality as indicated by the increases in ER and seed germination. Seed priming could increase ER and the rate of radical emergence in sweet corn (Chiu et al., 2003; Chiu et al., 2006). In addition, seed priming also improved seed germination and seedling emergence and reduced seed deterioration in tomato (Torres and Andrews, 2006). Restoration of seed quality; however, depends on the degrees of seed deterioration and the restoration could be achieved within very limited extent. Seeds with germination exceeding 90% did not require restoration, while the seeds with germination lower than 80% could not be restored.

How seed priming can restore seed quality is another question which needs to be answered. In this study, the total peroxide and MDA were investigated. The results showed that the total peroxide and MDA were involved in seed deterioration in sweet pepper, as these parameters were increased with the durations of accelerated aging. However, seed priming could reduce the total peroxide and inhibit the evolution of MDA and may partially explain why seed quality of sweet pepper could be restored by seed priming. Boonsiri et al. (2007) reported that the total peroxide and MDA content can explain the membrane degradation. Bailly et al. (1998) found that accelerated aging resulted in a rapid reduction in seed germination and an increase in MDA content and total peroxide in sunflower. The reduction in the total peroxide and MDA, as affected by seed priming in this study, also suggested that seed priming could reduce lipid oxidation in sweet pepper. Similar decrease in the total peroxide and MDA content as affected by seed priming was also reported in maize (Ya-jing et al., 2009) and bitter gourd (Lin et al., 2005), although different priming agents were used. Antioxidants can protect the cells against the damaging effects of ROS, such as singlet oxygen, superoxide, peroxyl and hydroxyl (Orhan et al., 2009). The increase in TAA of primed seeds (Fig. 3) was in agreement with other studies (McDonald, 1999; Lin et al., 2005; Chiu et al., 2006). They concluded that primed seeds exhibited higher total antioxidant than the aged seeds.

Also, priming has been shown to improve the germination of bitter gourd seeds and antioxidant activity (Wang et al., 2002; Hsu et al., 2003; Yeh et al., 2005), which were in close agreement with our results. Until now, ascorbate system has been considered almost uniquely for its antioxidant properties (Noctor and Foyer, 1998) since ascorbate system can react with ROS in non-enzymatic reactions. It is now clear that the ascorbate system also has a paramount role in plant cells as a co-substrate for detoxification of ROS in cells.

In plant, antioxidants (ascorbate, glutathione, phenolic compounds, tocopherols), scavenging enzymes (such as

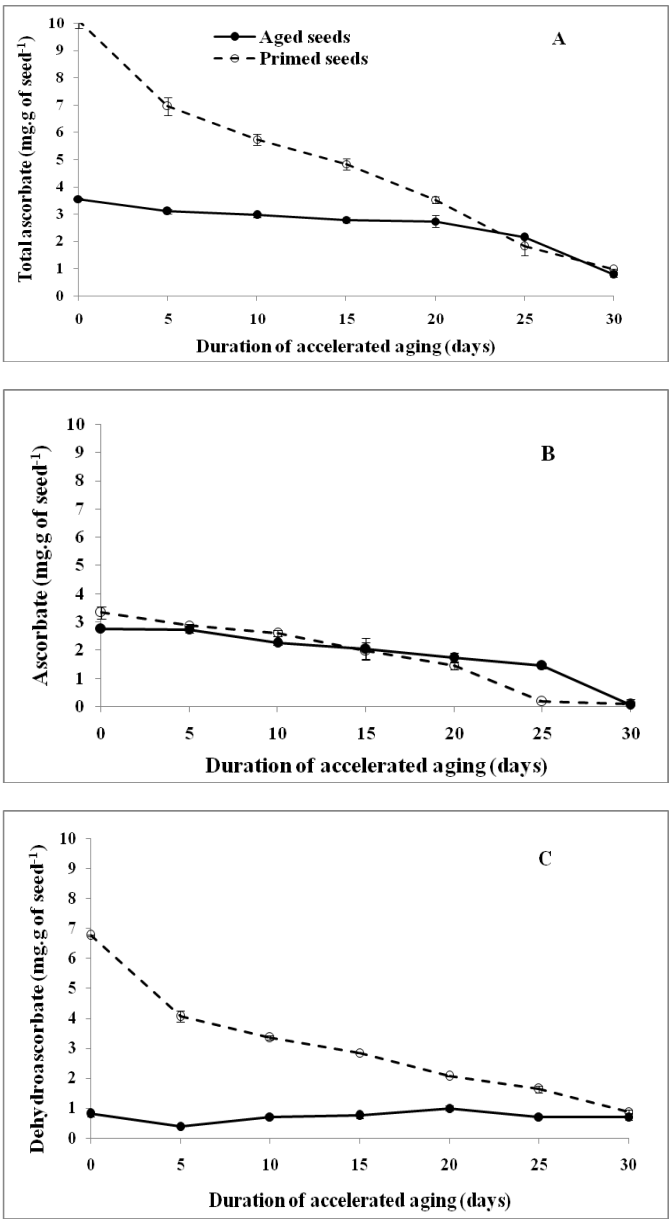


**Fig 4.** Changes in the total ascorbate (A), ascorbate (B) and dehydroascorbate (C) of aged and primed sweet pepper seeds. Vertical bars represent the mean and S.E. of three replications.

SOD, CAT, APX and AR) and regenerating the active forms of antioxidants have been used to control the level of ROS and to protect the cells (Yasar et al., 2008). Our results illustrated that the increase in the total ascorbate and dehydroascorbate in ascorbate system (Fig. 4) could be responsible for controlling the level of ROS after priming. The activities of CAT were substantially increased after priming (Fig. 5). The results suggested that CAT was enhanced in cells to eliminate $H_2O_2$. These results were in agreement with many findings that CAT catalyzed the decomposition of $H_2O_2$ to $H_2O$ and $O_2$ (Bailly et al., 2002; Lin et al., 2005; Chiu et al., 2006; Yasar et al., 2008). The CAT activity which was increased after maize seed priming also supported our results (Ya-jing et al., 2009). The overall conclusion could state that the possible renewal of antioxidant system was initiated with the repair of cell membrane and organelle development, which are required

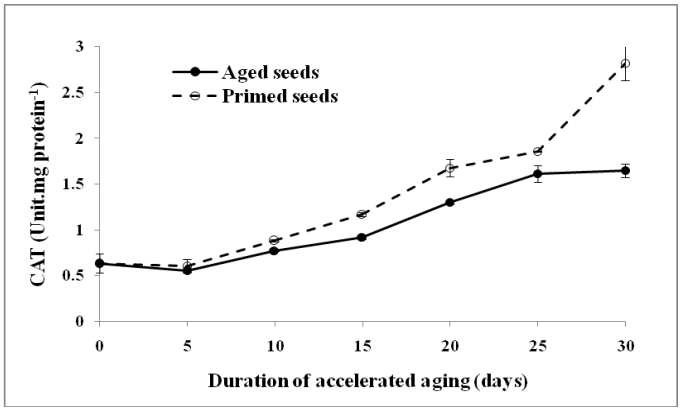


**Fig 5.** Changes in catalase (CAT) activity in aged and primed sweet pepper seeds. Vertical bars represent the mean and S.E. of three replications.

for germination and growth demands by the seedlings.

## Materials and Methods

### *Plant materials*

Hybrid sweet pepper seeds TPP004 used for aging and priming were purchased from AG Universal Company, Khon Kaen, Thailand and were stored at 15 °C.

### *Aging and priming processes*

Accelerated aging was conducted at the Seed Testing Laboratory, Seed Processing Plant, Faculty of Agriculture, Khon Kaen University. Accelerated aging technique was performed as described by Walters et al. (2005). Seeds were artificially aged for 0, 5, 10, 15, 20, 25 and 30 days in an accelerated aging chamber and incubated in plastic boxes with 100% relative humidity (R.H.) at 42 °C. Subsequently, all of the seeds were dried at 32 °C for 6 hours by the modified air-dryer (Model SKK-02) until seed moisture content was reduced to original moisture content of 6%. Seed germination and biochemical determinations were evaluated. The seven treatments of aged seeds (included 0 day as a control) were soaked in non-aerated polyethylene glycol (PEG 6000) solution at 15 °C in the incubator (Sanyo, Model Fr-6011) for 6 days to achieve a final osmotic pressure at the equivalent potential of -1.5 MPa (Chiu et al., 2006). Primed seeds were rinsed with deionized water for 2 min and adjusted to the normal moisture content by allowing the seeds to air-dry at 32 °C for 6 hours by the modified air-dryer (Model SKK-02). The seed qualities were determined for seed germination and biochemical evaluation.

### *Seed germination*

The samples were evaluated for seed germination consisting of ER, GL and GH described as following: Three replicates of one hundred seeds from each treatment of aged seeds and primed seeds were kept on paper towels and incubated at 24±1 °C under dark conditions. The 0.5 cm radical protrusion, in terms of ER, was counted and the complete seedling of GL was counted at 14 days after imbibitions (Goel et al., 2003). For GH, three replicates of one hundred seeds from each aged or primed samples were tested on peat moss medium. The complete seedling was counted at 14 days after imbibitions.

The evaluation criteria in this experiment were developed following the criteria suggested by the International Seed Testing Association (ISTA) (2004).

***Determination of lipid peroxidation, total antioxidant activity and ascorbate system***

One hundred milligrams of seeds were hand-homogenized using a mortar and pestle with 4 ml of ice-cold 5% (w/v) trichloroacetic acid (TCA) at 4 ◦C to precipitate proteins and then centrifuged at 14,000 x g for 20 min. The supernatant was used for lipid peroxidation, TAA and ascorbate system determination. Lipid peroxidation was evaluated by spectrophotometer measurements of MDA and total peroxide concentrations according to the methods of Heath and Packer (1968) and Sagisaka (1976), respectively. TAA was measured as the percentage scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Blois, 1958) which was used gallic acid in range 10-50 mg $l^{-1}$ as standard. TAA units were expressed as microgram of gallic acid equivalent per gram of seed (µg GE g of $seed^{-1}$). A modified method of Law et al. (1983) was employed to determine the ascorbate (reduced form) and dehydroascorbate (oxidized form). The supernatant was used for the determination of total ascorbate and ascorbate. Total ascorbate was determined by adding 5 µl sodium phosphate buffer (100 mM, pH 7.4), 2.5 µl dithiothreitol (10 mM), 2.5 µl 0.5% (w/v) N-ethylmaleimide, 10 µl 10% (w/v) TCA, 10 µl 44% (w/v) $H_3PO_4$, 10 µl 4% (w/v) bipyridyl in 70% (v/v) ethanol and 5 µl 13% (w/v) $FeCl_3$ to 5 µl of the supernatant. The reaction was monitored by the rate of change in the absorbance at 525 nm, and the total ascorbate was calculated from a standard curve, in which ascorbate equivalent present was plotted against the rate of change in the absorbance at 525 nm. Ascorbate was determined using the same procedure as the total ascorbate without adding dithiothreitol and N-ethylmaleimide solutions. Dehydroascorbate content was calculated from the difference between total ascorbate and ascorbate contents.

***Measurement of catalase activity***

Two hundred milligrams of seeds were ground in a chilled mortar and homogenized with 10 ml of 0.1 M phosphate buffer (pH 7.8) containing 0.2 g polyvinylpyrrolidone (PVP), 10 mM β-mercaptoethanol, 10 mM KCl, 1 mM $MgCl_2$ and 1 mM EDTA. The homogenate was centrifuged at 20,000 x g for 15 min at 4 ◦C and the supernatant was used for the enzyme assay and protein determination. The CAT activity was estimated by the method of Aebi (1984). Soluble proteins extracted from seeds were determined using Lowry's method (Lowry et al., 1951).

***Statistical analysis***

The experiment was conducted using a Completely Random Design (CRD) with three replicates of each biological sample. Data were statistically analyzed by ANOVA and the significance of the differences between means at $p<0.05$ was estimated by Duncan's new multiple range test (DMRT). The variation of the data of the seeds from each level of aged seeds and primed seeds was analyzed by standard deviation.

**Conclusion**

The correlation of the improvement of seed germination and biochemical changes in sweet pepper seed by priming was proposed in this study. The increase in germinability of primed seed was explained by the decrease in the total peroxide and MDA content, but increase in total ascorbate, dehydroascorbate and catalase activity. The results suggest that the priming process by PEG can improve seed deterioration corresponding to the repair of the membrane, which results in reduction of lipid peroxidation and the increased rate of antioxidant system synthesis responsible for eliminating ROS from the cells. Our data indicates that improved germinability of primed sweet pepper seed is related to membrane integrity restoration and enhanced antioxidant defense system within a cell.

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