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Effects of accelerated aging and subsequent priming on seed quality and biochemical change of hybrid cucumber (*Cucumis sativa* Linn.) seeds

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The research was aimed to study beneficial effects of seed priming treatment on various deteriorated seeds under artificial accelerated aging. It was focused on seed quality and biochemical parameters which were germination percentage, speed of germination, and mean germination time, lipid peroxidation (malondialdehyde and total peroxide), and antioxidant activity (ascorbate, dehydroascorbate, catalase and %DPPH inhibition). Experiments were tested for seed priming and accelerated aging which conducted in the seed processing plant with the hybrid cucumber seeds of cultivar SPP012. Seeds were brought to incubate aging chamber to obtain 45°C and relative humidity of 100 % for 3, 6, 9, 12, 15, 18 and 21 days. Subsequently, all of the seeds were dried at 35 °C for 48 hours by the modified air-dryer to reduce seed moisture to 7-8%. Aged seeds were soaked in KH_2PO_4 and using PEG 6000 as the agent to adjust a potential to be -1 MPa at 15 °C for 72 hours. Primed seeds were rinsed with deionized water and adjusted to the initial seed moisture by air-drying at 35 °C for 48 hours. The samples were randomly selected to examine the effects of accelerated aging to monitor seed quality in the laboratory and greenhouse. Germination tests were performed to examine seeds quality. Biochemical analysis covered lipid peroxidation and antioxidant activity. The results found that seed priming was improved the quality of hybrid cucumber seeds and biochemical parameters at significant level. Level of germination defines effectiveness of seed priming in commercial purpose. Seed priming significantly reduced amount of malondialdehyde and total peroxide. The antioxidant activity, seed priming affects various results of biochemical changes were expressed by increasing the level of antioxidant activity.

Keywords : seed priming, seed enhancements, seed germination, antioxidatants, cucumber

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Introduction

Seed priming has been used as an enhancement for seed quality in commercial seeds such as tomato, pepper, onion, maize, sunflower, rice, cotton. This method improves germination for its rate and uniformity which yields quality seedling and accelerated germination rate. (Harris *et al*.,1999 ; Basra *et al*., 2005). The main principle of seed priming is the control of water solution potential at low level. (Stuart and Kevin ,1986). Chemical use included polyethylene glycol (PEG), $CaCl_2$, KNO^3 , KH_2PO_4, KCl , Na_2SO_4, mannitol, and sorbitol (Frett *et al*.,1991; Basra *et al*., 2005). This can delay water absorption of the seed which slow activity and allow membrane to be restored. Seed deterioration is related to loss of membrane integrity, changes in enzymatic activities, reduction in protein and nucleic acid synthesis, and lesions in DNA (McDonald, 1999). Such deteriorative changes are also related to activated oxygen species (AOS) induced oxidative injury (Hendry, 1993; McDonald, 1999). Because priming has affected which are associated with the restoration and production of nucleic acids. The effects are foster the increasing synthesis of protein and repairing of mitochondria and seed membranes. Moreover, priming helps to activate antioxidant mechanism in treated seeds (McDonald, 1999; Siri, 2013). Deteriorative change is a reverse correlation between antioxidant and lipid peroxidation. Whereas decreasing level in malondialdeyde (MDA) is related to increasing antioxidant and decreasing lipid peoxidation. As a result, seed priming yields the effectively development of embryo and accelerates emergence time. Seedling performance can be improved for observing from the seedling stands which are synchronized. This technique affects the quality of emergence in several horticultures (Bradford *et al*., 1990; Jett *et al*., 1996).

Cucumber is one of the major agricultural exports. Annual export was 78,000 kilograms and seed price ranging from 2,000-14,000 THB/kilogram or approximately $60-400/kilogram (Seed association of Thailand, 2010). Seed storing is problem in exporting quality control as 90% of emergence causes the defect from deteriorative seed. As Wilson and McDonald (1986) suggest, two of the main primary causes of deterioration of stored seed are lipid peroxidation and free-radical mediated. Therefore, seed priming is the solution to improve the quality of cucumber seed. The research was aimed to study effects of seed priming treatment on various deteriorated seeds on artificial accelerated aging. The study focused on seed quality and biochemical property. The findings could benefit seed industry in the future.

Materials and methods

Experiments were tested for seed priming and accelerated aging in the seed processing plant at the Faculty of Agriculture and the chemical analysis laboratory at the Department of Biotechnology, the Faculty of Technology, Khon Kaen University during March 2010 - October 2013.

Seed materials

The lot of commercially produced hybrid cucumber seed of cultivar SPP012 was purchased from AG Universal Company, Khon Kean, Thailand. It was stored at 15 °C (initial seeds moisture content 7-8%).

Aging and priming processes

Accelerated aging was carried out by following the method of Delouche and Baskin (1973). Thirty grams of seeds were placed on a wire mesh tray, which was then placed in distilled water in a sealed aging chamber to obtain 45°C and relative humidity of 100 % for 3, 6, 9, 12, 15, 18 and 21 days. Subsequently, all of the seeds were dried at 35 °C for 24 hours by the modified air-dryer (Model SKK-09) until seed moisture content was reduced to original moisture content of 7-8%. Seed germination and biochemical determination were evaluated. The eight treatments of aged seeds (included the 0-day-seeds as a control group) were soaked in KH2PO4 and using PEG 6000 as the agent to adjust a potential to be the final potential at -1 MPa. Treatment was conducted in 15 °C water for 72 hours in the incubator (Sanyo, Model Fr-6011). Primed seeds were rinsed with deionized water for 2 min and adjusted to the initial seeds moisture content at 7-8% by allowing the seeds to air-dry at 35 °C for 48 hours by the modified air-dryer (Model SKK-09). The seed qualities were determined by seed germination and biochemical evaluation.

Seed germination

Germination tests were performed on paper towels saturated with distilled water, at 25 °C under dark condition for 8 days. Four 50-seeds replicates for each treatment were used. Germination counts were performed daily for 8 days and germination was considered to have occurred if the radical protruded 5 mm or longer from the seed coat according to the ISTA rules (ISTA, 2004). The total germination percentage was expressed as the average of four replicates. The parameters of germination speed and mean germination time (MGT) were also calculated for supplementary explanations. MGT was calculated as follows: MGT = $\sum$nidi/n, where n is the total number of germinated seeds

during the germination test, ni is the number of germinated seeds on day di, and i is the number of days during the germination period (between 0 and 8 days). For germination under greenhouse, four replicates of 50 seeds from each aged or primed samples were tested on peat moss medium. The complete seedling was counted at 8 days after imbibitions. The evaluation criteria in this experiment were developed following the criteria suggested by the International Seed Testing Association (2004).

Malondialdehyde (MDA) and Total peroxide assay

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 10,000 x g for 30 min. The supernatants were used for MDA and total peroxide determinations. MDA was determined by adding 0.8 ml of 20%(w/v) trichloroacetic acid in 0.5% (w/v) thiobarbituric acid to 200 µl of extracted sample (supernatant) and 3.0 ml of distilled water and they were incubated at 95°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath for 10 min. and then centrifuged at 10,000 x g for 5 min. The absorbance of the supernatants was monitored at 532 nm.(Heath and Parker,1968).

Antioxidant activity by free-radical scavenging antioxidant activity

One hundred milligrams of seeds were hand-homogenized using a mortar and pestle with 5 ml of 80% ethanol at room temperature overnight, and then filtered through No. 1 filter paper, followed by centrifugation at 10,000 x g for 20 minutes. The supernatants were used for antioxidant activity. The total antioxidant activity was determined by adding 100 µl of sample solution, 1.9 ml of deionized water and 2 ml of 0.1 mM of DPPH. The reaction was carried out at room temperature for 30 min. and monitored by the spectrophotometer at 517 nm.

Ascorbate and Dehydroascorbate determinations

A modified method of Law *et al*.(1983) was employed to determine the total ascorbate. One hundred milligrams of seeds were hand-homogenized using a mortar and pestle with 4 ml ice-cold 5% (w/v) trichloracetic acid solution and centrifuged at 10,000 x g for 20 min. The supernatant was used for the determination of total ascorbate and ascobate. Total ascorbate was determined in a reaction mixture consisting of 200 µl of supernatant, 400 µl of 100 mM KH_2PO_4 buffer (pH7.4) containing 5 mM EDTA and 200 µl of 10mM dithiothreitol (DTT) to reduce dehydroascorbate to ascorbate. After 10 min at room temperature, 200 µl of 0.5%(w/v) N-ethylmaleimide was added to remove

excess DTT. Ascorbate was determined using the same procedure as for total ascorbate but without the dithiothreitol and N-ethylmaleimide additions. Colour was developed in both series of reaction mixtures with the addition of 100 µl of 10%(w/v) trichloracetic acid, 800 µl of 44% (v/v) phosphoric acid, 800 µl of α,α′- dipyridyl in 70% (v/v) ethanol and 400 µl of 3%(w/v) FeCl3.The reaction mixtures were incubated at 40 °C for 1 hr. and measured by spectrophotometrically at 525 nm. Dehydroascorbate was estimated by the difference of total ascorbate and ascorbate.

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 MgCl2 and 1 mM EDTA. The homogenate was centrifuged at 10,000 x g for 30 min at 4 °C and the supernatant was used for the enzyme assay. The CAT activity was estimated by the method of Aebi (1984). The reaction mixture contained 2.8 ml of 0.05 M potassium phosphate buffer pH 7.0, 0.98 ml of distilled water, 200 µl of sample solution and 13 µl of 486 µM H2O2. The solution mixture was then incubated at room temperature for 4-5 min and was detected by a spectrophotometer at 240 nm. The disappearance of peroxide was spectrophotometer followed by recording the decrease in absorbance at 240 nm for 3 min. One unit of catalase activity was defined as the amount of enzyme that used 1 µmol H_2O_2 per min.

Statistical analysis

The experiment was conducted using a Completely Random Design (CRD) with four 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 results concerning seed germination percentage were statistically analyzed by means of variance analysis and transformed according to $y = \arcsin(x)$.

Results

Effects of priming treatments on seed germination

The germination analysis of data (Table 1-3) indicated that the seed germination and seedling vigor were significantly different in every aging primed seed. To determine the radical emergence in the laboratory, it found that primed seed accelerated aging for 3, 6, and 9 days improve radical emergence

at 1.0-2.1%. While primed seed accelerated aging for 15 days improved at 6.0%. Whereas the primed seed accelerated aging for 21 days decreased the radical emergence at -6.3 % but still yield improvement from reducing mean of germination time (Table 3) from 4.36 to 3.73 days (14.4%). Overall, normal seedling emergence was likely to develop its change correlated to radical emergence. In the greenhouse , seed priming accelerated aging for 3, 6, 9, and 12 days was improved the germination, speed of germination , and mean of germination time for hypocotyls and normal seedling as same as the experiment in the laboratory. While seed priming accelerated aging for 18-21 days decreased speed of germination (Table 3).

Table 1. Effects of accelerated aging and subsequent priming on radical emergence, hypocotyls and normal seedling under laboratory and greenhouse condition of hybrid cucumber seeds

	Laboratory						Greenhouse					
Aging (days)	Radicle emergence[1/] (%)			Normal seedling (%)			Hypocotyl emergence[2/] (%)			Normal seedling (%)		
	A	P	+-[4/]	A	P	+-	A	P	+-	A	P	+-
0	100 a[3/]	100 a	(0.0)[4/]	99 a	99 a	(0.0)	94 a	91 bc	(-2.9)	92 a	91 a	(-0.8)
3	96 b	97 b	(1.0)	93 b	95 b	(2.2)	91 a	94 ab	(3.3)	91 a	92 a	(1.1)
6	95 b	97 b	(2.1)	90 b	92 bc	(2.2)	88 ab	97 a	(10.6)	84 b	94 a	(11.9)
9	94 b	94 c	(0.0)	88 b	88 c	(0.0)	83 b	85 c	(2.0)	82 b	85 b	(3.3)
12	88 c	89 d	(1.1)	75 c	75 d	(0.0)	41 c	44 d	(7.3)	39 c	41 c	(4.4)
15	67 d	71 e	(6.0)	57 d	63 e	(10.5)	18 d	15 e	(-15.0)	17 d	14 d	(-17.6)
18	43 e	44 f	(2.3)	15 e	16 f	(6.7)	17 d	9 ef	(-45.3)	13 de	8 e	(-38.5)
21	16 f	15 g	(-6.3)	9 e	9 g	(0.0)	11 d	5 f	(-51.8)	9 e	5 e	(-41.1)
F-test	**	**		**	**		**	**		**	**	
CV. (%)	6.9	3.2		7.1	5.3		7.1	8.0		6.7	4.7	

A= aging P= priming, [1/] Radicle emergence : radicle protrusion of 3 mm;[2/] Hypocotyl emergence : hypocotyl protrusion of 3 mm, ** significant at p □ 0.01 level. [3/]Means within

a column followed by the same letter are not significantly different by DMRT.[4/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming.

Table 2. Effects of accelerated aging and subsequent priming on speed of radicle emergence, hypocotyl emergence and normal seedling under laboratory and greenhouse condition of hybrid cucumber seeds

	Laboratory				Greenhouse			
Aging (days)	Radicle emergence[1/] (%)		Normal seedling (%)		Hypocotyl emergence[2/] (%)		Normal seedling (%)	
	A	P +-	A	P +-	A	P +-	A	P +-
0	50 a[3/]	50 a (0.0)[4/]	25 a	25 a (0.0)	23.6 a	23.0 d (-2.3)	23.1 a	22.7 a (-1.8)
3	48 ab	48 b (0.0)	24 ab	23 b (-4.2)	22.0 ab	35.3 b (60.5)	22.0 ab	23.1 a (5.0)
6	48 ab	48 b (0.0)	23 ab	23 b (0.0)	19.7 b	40.5 a (105.6)	19.5 b	23.8 a (21.9)
9	46 b	46 c (0.0)	23 ab	23 b (0.0)	19.6 b	30.0 c (53.2)	19.4 b	20.1 b (3.7)
12	28 c	29 d (3.6)	19 c	19 c (0.0)	7.7 c	11.1 e (44.0)	7.7 c	9.8 c (27.1)
15	21 d	23 e (9.5)	13 d	14 d (7.7)	3.3 d	3.4 f (2.4)	3.3 d	3.2 d (-3.6)
18	12 e	13 f (8.3)	7 e	8 e (14.3)	2.6 de	2.0 g (-24.0)	2.6 de	2.1 e (-20.2)
21	4 f	4 g (0.0)	3 f	2 f (-33.3)	1.7 e	1.2 h (-29.0)	1.7 e	1.0 f (-40.8)
F-test	**	**	**	**	**	**	**	**
CV. (%)	3.1	1.7	3.2	1.7	6.3	3.7	6.3	2.9

A= aging P= priming, [1/] Radicle emergence : radicle protrusion of 3 mm ; [2/] Hypocotyl emergence : hypocotyl protrusion of 3 mm, ** significant at p = 0.01 levl.. [3/] Means within a column followed by the same letter are not significantly different by DMRT, [4/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming.

Table 3. Effects of accelerated aging and subsequent priming on mean emergence time (MGT) of radicle emergence, hypocotyl emergence and normal seedling under laboratory and greenhouse condition of hybrid cucumber seeds

Aging (days)	Laboratory						Greenhouse					
	Radicle emergence[1/] (%)			Normal seedling (%)			Hypocotyl emergence[2/] (%)			Normal seedling (%)		
	A	P	+-	A	P	+-	A	P	+-	A	P	+-
0	2.00 e[3/]	2.00 d	(0.0)[4/]	4.05 e	4.05 e	(0.0)	4.2 d	4.2 b	(-0.2)	4.1 d	4.0 c	(-2.7)
3	2.00 e	2.00 d	(0.0)	4.14 e	4.13 de	(-0.2)	4.2 d	2.8 c	(-32.5)	4.2 d	4.1 c	(-1.2)
6	2.00 e	2.00 d	(0.0)	4.23 e	4.22 d	(-0.2)	4.3 d	2.5 d	(-41.5)	4.3 d	4.2 c	(-3.0)
9	2.07 e	2.05 d	(-1.0)	4.30 e	4.28 d	(-0.5)	4.6 c	3.0 c	(-34.8)	4.7 c	4.2 c	(-9.7)
12	3.14 d	3.12 c	(-0.6)	4.91 d	4.89 c	(-0.4)	5.5 b	4.2 b	(-23.6)	5.5 b	4.7 b	(-14.5)
15	3.30 c	3.14 c	(-4.8)	5.35 c	5.26 b	(-1.7)	5.5 b	4.6 ab	(-16.8)	5.5 b	5.0 b	(-9.6)
18	3.60 b	3.41 b	(-5.3)	6.78 a	6.54 a	(-3.5)	6.5 a	4.7 a	(-27.9)	6.5 a	4.8 b	(-26.4)
21	4.36 a	3.73 a	(-14.4)	6.44 b	6.36 a	(-1.2)	6.4 a	4.6 ab	(-27.9)	6.4 a	5.4 a	(-15.4)
F-test	**	**		**	**		**	**		**	**	
CV. (%)	0.9	0.8		1.5	1.2		1.3	3.0		1.3	2.7	

A= aging P= priming, [1/] Radicle emergence : radicle protrusion of 3 mm ; [2/] Hypocotyl emergence : hypocotyl protrusion of 3 mm, ** significant at p = 0.01 level,[3/] Means within a column followed by the same letter are not significantly different by DMRT, [4/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming.

Discussion

The results of this study are also related to many studies in horticultural because seed priming techniques have been accepted as they reduce the emergence time and improve emergence rate and seedling stand. (Bradford

et al., 1990; Jett *et al*, 1996) In addition, some field crops such as wheat and maize, seed priming is widely used (Dell'Aquilla and Tritto, 1990;Basra *et al.,* 2002). Similarly, the results for germination percentage confirmed the findings of Warley and Fernando (2004) who reported that priming improved the germination percentage and germination rate of un-aged and aged seeds of muskmelon. Besides, benefits of seed priming are used on pre-enlargement of embryo (Bradford *et al*., 1990) and extension speed of metabolites (Basra *et al.,* 2005). In another study, using seed priming on pot marigolds (Calendula officinalis) and sweet fennel (Foeniculum vulgare) with GA3 and NaCl treatments, the mean germination time (MGT) has decreased significantly (Mohammad *et al*., 2010).This significant reduction in MGT could be the result of shortening of the lag phase during priming (Khan *et al*., 2009). Foti *et al.* (2008) found significant difference of priming the caryopses seed. The unprimed took 4 days for germination while the primed ones began germination as soon as day 1. According to McDonald (1999), primed seeds could shorten their steps of DNA and RNA synthesis. Consequently, seeds physiology tends to be closer to germination than the unprimed seeds. On the other hand, Higher rates of germination index (GI) might be due to the result of recovered development of genetic repair mechanisms during priming operations (Bradford,1986).

Effects of priming treatments on lipid peroxidation

Malondialdehyde (MDA) and total peroxide were determined and the results showed in Table 4. MDA is the product of lipid oxidation and peroxidation. So, the content of the product in seeds can inform its deterioration degree. Hence, determination of MDA is a convenient method of quantifying the extent of lipid peroxidation. All seed priming accelerated aging significantly reduced the amount of MDA and total peroxide compare to the accelerated aging seed before priming. The amount of MDA decreased the most at 8.1 % as in seed priming accelerated aging for 12 days and total peroxide decreased the most at 36.9% in seed priming accelerated aging for 6 days. However the seeds with longer accelerated aging from 15-21 days treated in seed priming, it made MDA slightly decreased at 0.2-5.2%. Total peroxide started to decrease at accelerated aging for 9 days at 29.4% to the lowest at 12.6% at accelerated aging for 21 days. The results of this experiment show that MDA and total peroxide decreased after priming. It indicated that emzyme systems are repaired in primed seeds. They are consistent with changes in germination and seed vigor as shown in Table 2 and 3. Thus, seed performance after priming showed attribution to decrease lipid peroxidation when the seeds are under imbibitions stage (Hsu *et al*., 2003). Similarly, Siri *et al*.(2013)

reported that seed priming has reduced the total peroxide and inhibit of MDA. This experiment suggested how to restore the quality of sweet pepper by adopting seed priming technique. Seed priming is found used in maize seed (Ya-jing *et al*., 2009) and bitter gourd seed (Lin *et al*., 2005).

Table 4. Effects of accelerated aging and subsequent priming on Malondialdehyde (MDA) and Total peroxide content of hybrid cucumber seeds

Aging (days)	MDA(µg/g seed)			Total peroxide (µg/g seed)		
	A	P	+-	A	P	+-
0	3.6 g[1/]	3.6 c	(0.0)[2/]	6.4d	6.4 b	(0.0)
3	4.2 f	4.0 c	(-2.9)	6.6cd	4.4 e	(-33.5)
6	4.9 e	4.8 d	(-3.2)	7.0c	4.4 e	(-36.9)
9	5.1 de	4.9 d	(-3.2)	7.4b	5.2 d	(-29.4)
12	5.5 d	5.0 d	(-8.1)	7.5b	5.9 c	(-20.5)
15	6.1 c	6.0 c	(-2.6)	8.1a	6.3 b	(-21.7)
18	6.9 b	6.5 b	(-5.2)	8.2a	7.0 a	(-15.1)
21	8.4 a	8.4 a	(-0.2)	8.3a	7.3 a	(-12.6)
F-test	**	**		**	**	
C.V(%)	4.68	4.85		3.22	3.16	

A= aging,P= priming,** significant at p □ 0.01 level,
[1/] Means within a column followed by the same letter are not significantly different by DMRT, [2/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming.

The effects of priming treatments on catalase

Seed priming accelerated aging for 6 days found the maximum increasing of catalase at 50.3% and maintain to increase until the 15 days of aging at 40% while it dramatically decrease at 14.9% and 13.7% for the primed seeds at 18 and 21 days respectively (Table 5). It infers that decreased catalase is correlated with MDA and total peroxide because at 18 days and more of aging the amount

of MDA and total peroxide is increased. In plant, antioxidants (ascorbate, glutathione, phenolic compounds, tocopherols), scavenging enzymes (such as 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)

Effects of priming treatments on antioxidants

The maximum percentage of DPPH inhibition increased at 10.1% on the seed priming accelerated aging for 3 days (Table 5). The maximum ascorbate increased at 8.4 % on the seed priming accelerated aging for 12 days. The maximum decrease of dehydroascorbate is 66.7% on seed priming accelerated aging for 12 days (Table 6). All the changes of antioxidants are correlated to germination percentage and seed vigor. However, in the seeds with low germination percentage and seed vigor, the effect of seed priming is slightly improved. Increasing catalase and antioxidant is not sufficient to withhold MDA and total peroxide.

Table 5. Effects of accelerated aging and subsequent priming on Catalase activity and % DPPH inhibition of hybrid cucumber seeds

Aging (days)	Catalase (unit/gseed)			% DPPH inhibition		
	A	P	+-	A	P	+-
0	2.47 g[1/]	2.47 g	(0.0)[2/]	60.4b	60.4 e	(0.0)
3	2.9 f	3.39 f	(16.9)	60.1b	66.2 a	(10.1)
6	3.08 f	4.63 e	(50.3)	60.4b	62.4 d	(3.3)
9	4.01 e	5.55 d	(38.4)	60.5b	62.6 d	(3.4)
12	4.63 d	6.79 c	(46.7)	60.5b	63.7 c	(5.2)
15	6.17 c	8.64 b	(40.0)	61.5a	63.8 c	(3.8)
18	8.33 b	9.57 a	(14.9)	61.8a	64.7 b	(4.6)
21	8.95 a	10.18 a	(13.7)	60.1b	64.2 bc	(6.7)
F-test	**	**		**	**	

C.V(%)	6.5	6.8	0.56	0.74	

A= aging P= priming,** significant at p = 0.01 level, [1/] Means within a column followed by the same letter are not significantly different by DMRT, [2/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming

Table 6. Effects of accelerated aging and subsequent priming on Ascorbate and Dehydroascorbate of hybrid cucumber seeds

Aging (days)	Ascorbate(μg/gseed)			Dehydroascorbate (μg/gseed)		
	A	P	+-	A	P	+-
0	117.8 d[1/]	117.8 d	(0.0)[2/]	27.7 a	27.7 a	(0.0)
3	117.9 d	118.8 cd	(0.7)	23.4 b	24.1 b	(2.9)
6	118.4 cd	122.0 bc	(3.0)	18.3 c	17.7 c	(-3.2)
9	118.5 cd	124.8 b	(5.3)	15.5 d	13.8 d	(-11.2)
12	119.0 cd	129.0 a	(8.4)	4.1 e	1.4 e	(-66.7)
15	120.5 bc	129.1 a	(7.2)	0.9 f	1.7 e	(100.0)
18	121.4 b	129.5 a	(6.6)	1.4 f	1.6 e	(14.0)
21	123.8 a	131.3 a	(6.1)	1.0 f	0.9 e	(-10.3)
F-test	**	**		**	**	

C.V(%)	6.5	6.8	0.56	0.74

A= aging, P= priming, ** significant at p = 0.01 level, [1/] Means within a column followed by the same letter are not significantly different by DMRT, [2/] The number in parentheses are increased percentage change (+) and decreased percentage change (-) after seed priming.

In previous studies, seed priming advance germination, for example, in dry bitter gourd seeds, they were examining the anti-oxidative abilities by having them imbibed for 48 hours to observe the ascorbate-glutathione cycle. Thus, abilities of primed seeds showed significant advancement of germination. (Lin *et al*., 2005). In another study, Yeh *et al*. (2005) examined the anti-oxidative responses of imbibing bitter gourd seeds after being imbibed for 48 hours. It showed that TAA yielded positive relationship with anti-oxidative abilities of primed seeds. TAA is also related to germination percentage and germination speed. Primed bitter gourd seeds showed decreasing MDA and total peroxide accumulations which are the effects from priming-enhanced TAA. Siri et al.(2013) stated that the ascorbate system plays an important role in plant cells as it is a co-substrate for detoxification of ROS in cell. Priming stimulates the activity of CAT. Results suggested that priming enhances CAT in cells to eliminate H_2O_2. These results were similar with many experiments showing that CAT catalyzed the decomposition of H2O2 to H2O and O2 (Bailly *et al*.,2002; Lin *et al*., 2005).

Conclusion

It is concluded that seed priming has effects on radical emergence, germination speed, mean of germination time, lipid oxidation, and antioxidants. Germination analysis showed that the seed with 88-93% germination percentage has the improvement from 1-11.9%, after priming treatment. The speed of germination increases from 3.7-105.6% and mean of germination decreases from 0.2-41.5%. In the seed with lower germination (9-75%) after seed priming treatment, the germination percentage increases 1-10.5%, speed of germination increases 2.4-44% and mean of germination decreases from 0.4-27.9%. MDA was changed significantly after priming. It found that the seed with 12 days of accelerated aging decrease MDA at 8.1%. While total peroxide decreases the most at 36.9% for the accelerated aging 6 days. Percentage of DPPH inhibition is also increased. The most increased percentage of DPPH was at 10.1% on the accelerated seed for 3 days and treated in priming. Similarly, catalase percentage increased 50.3% on the accelerated seeds for 6 days and treated in priming. Ascorbate and dehydroascorbate are changed interchangeably in the reverse direction. Ascobate increases the most at 8.4% and dehydroascorbate decreases 66.7% for the seed with accelerated aging for

12 days and treated in priming. In the future study, it is suggested to examine the changes of seed storage such as carbohydrate and the related enzyme activity in germination such as amylase.

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