

# Changes in Antioxidant Enzyme Activity, Lipid Peroxidation and Seedling Growth of Cucumber Seed Induced by Hydropriming and Electric Field Treatments

Rukui Huang<sup>1\*</sup>, Sutevee Sukprakarn<sup>2</sup>, Lop Phavaphutanon<sup>2</sup>,  
Sunanta Juntakool<sup>3</sup> and Chaiwat Chaikul<sup>4</sup>

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## ABSTRACT

Two cucumber seed lots 'Bingo I' (high germination) and 'Bingo II' (low germination) were subjected to hydropriming and electric field treatments. Hydropriming, which was conducted by incubating seeds (moisture content adjusted to 25-30%) for 3 days under 25°C and saturated humidity, accelerated the speed of germination in both seed lots, also increased the germination percentage of 'Bingo II'. Electric field treatment, which was carried out by exposing 'Bingo I' for 3 min to 5 kV/cm electric field, or exposing 'Bingo II' for 5 min to 3 kV/cm electric field, enhanced the germination percentage in 'Bingo II', but had no effect on 'Bingo I'. Hydropriming accelerated seedling growth in 'Bingo I'; electric field treatment, however, did not improve the seedling growth. Furthermore, the activities of superoxide dismutase, catalase and ascorbate peroxidase were increased remarkably in association with the reduction of malondialdehyde accumulation in these two seed lots after the two treatments.

**Key words:** hydropriming, electric field, germination, enzyme activity, malondialdehyde accumulation

## INTRODUCTION

Seed priming is known as a hydration-dehydration process, which has beneficial effects on germination performance in various plant species. Priming initiates metabolic activities, such as protein, RNA, and DNA synthesis, DNA replication, and  $\beta$ -tubulin accumulation (McDonald, 2000). Recently, it has been suggested that priming could enhance the activity of antioxidative systems, resulting in lower rate of lipid peroxidation, contributing to seed

invigoration (Bailly *et al.*, 2000; Wang *et al.*, 2003).

Besides the intensive studies on seed priming, the exploring on electric field (EF) treatment, emerging as a seed enhancement technique since the 1960s (Sidaway, 1966), is drawing attention. Exposing seed to EF was reported to improve germination performance, e.g. in soybean (Zhao *et al.*, 1995), tomato (Moon and Chung, 2000), and cucumber (Zhu *et al.*, 2000). Regardless of the early starting, knowledge of the physiological and biochemical mechanism of EF

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<sup>1</sup> Vegetable Research Center, Guangxi Academy of Agricultural Sciences, Nanning 530007, China.

<sup>2</sup> Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

<sup>3</sup> Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

<sup>4</sup> Department of Electrical Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand.

\* Corresponding author, e-mail: hrui@excite.com

seed treatment is still scarce. Chiabrera and Bianco (1987) hypothesized that EF could influence the biochemical processes involving free radicals and antioxidant enzyme activity, resulting in seed invigoration. However, more evidence is needed to support this hypothesis.

Seed deterioration concerning free radicals has been discussed widely (Priestley, 1986). The highly aggressive free radicals produced by autooxidation in dry seed can react with the majority of biomolecules, causing cellular damage, e.g. membrane dysfunction, enzymes inactivation, etc. When seed is allowed to imbibe, the rapidly increasing respiratory activities elevate free radical production, resulting in oxidative stress to cellular components (McDonald, 2000; Bailly, 2004). Accordingly, the success of germination could largely depend upon the activity of antioxidative systems to prevent cellular components from being damaged by the free radicals. The activities of antioxidative enzyme such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were reported to increase after priming in bitter melon seed (Wang *et al.*, 2003), and after electric field treatment in cucumber seed (Zhu *et al.*, 2000). To understand the differences between these two seed enhancement technologies, we conducted a study to compare the effects on seed germination enhancement and seed antioxidative systems between hydropriming and electric field treatments.

## MATERIALS AND METHODS

### Seed materials

Two cucumber seed lots of cucumber namely 'Bingo I' (initial germination 94.0%, seed moisture content 5.5%) and 'Bingo II' (initial germination 61.5%, seed moisture content 5.8%) were obtained from Thai Seed & Agriculture Co. Ltd., Thailand.

### Hydropriming (HP)

Cucumber seeds were soaked for 30 min in 0.5% carbendazim (methyl benzimidazol-2-ylcarbamate 50% w.p.) for disinfection (Wright *et al.*, 2003). The disinfected seeds were then rinsed under tap water for 10 min, surface dried, and placed on metal meshes over water in airtight plastic boxes (relative humidity  $\approx$  100 %) for a three-day incubation at 25°C (based on preliminary study). After the incubation, the seeds were redried at ambient temperature to lower the moisture content to approximately 6-7%.

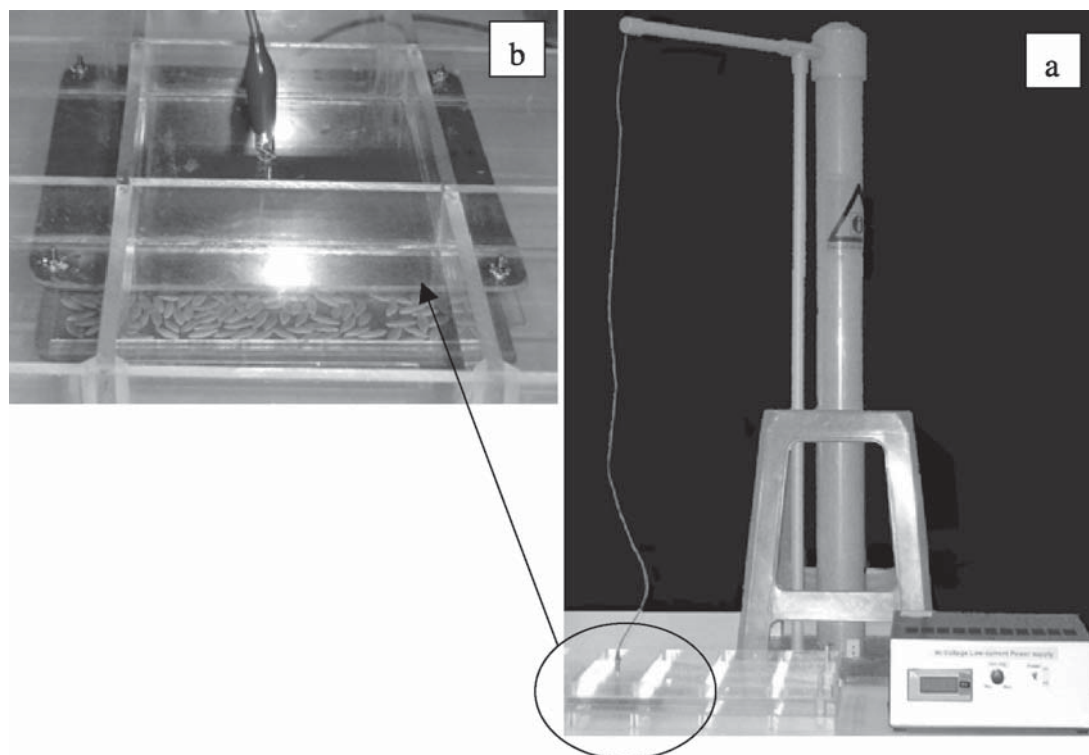
### Electric field treatment (EF)

The experimental set-up of electric field in the present study comprised two parts: the power supply and the test cell (Figure 1). The test cell consisted of two horizontal electrodes (copper square plates, side length 20 cm; interelectrode gap: 2 cm), which was connected to a fully adjustable AC high-voltage supply (20 kV, 50 Hz). The seed was loaded one layer in a shallow polyethylene (transparent high density polyethylene) tray with the cover of same material to avoid contact with the electrodes. No heating effect was noticed during the experiments, even when the maximum voltage was applied to the electrode system.

Seeds of 'Bingo I' were exposed for 5 min to EF under field strength of 3 kV/cm; and seeds of 'Bingo II' were exposed for 3 min to EF under field strength of 5 kV/cm. The conditions of EF were chosen based on the previous finding in which both seed lots were exposed to EF of the strength ranged from 1kV/cm to 7 kV/cm for 1 min to 5 min, and the best result in the subsequent germination test were induced by the conditions mentioned above.

### Germination test

Germination tests were carried out immediately after HP and EF treatments. Seeds were sown on top of the moistened blotter paper



**Figure 1** Experimental set-up for electric field treatments: **a.** electric field comprised power supply and the test cell; **b.** the test cell consisted of two pieces of copper plate, between which the electric field was generated.

in covered transparent polyethylene boxes (17×25 cm). A completely randomized design with four replications of 25 seeds per treatment was employed. The germination tests were conducted at 25°C (ISTA, 2003). Radicle protrusion to 4 mm was scored as germination; counts of the number of germinated seeds were made at 24 h intervals until no further germination was observed. Germination percentage was presented as the percentage of normal seedling (ISTA, 2003). Mean germination time (MGT) was calculated from the formula proposed by Ellis and Roberts (1980):

$$\text{MGT} = \sum T_i \cdot N_i / \sum N_i$$

Where  $N_i$  was the number of newly germinated seeds at time  $T_i$ .

### Seedling growth test

Immediately after HP and EF treatments, seeds were sown in seedling trays (cell size = 5 cm diameter × 10 cm depth) containing growing media (carbonized rice husk: coconut coir: manure = 1:1:1 by volume). Four replications of 50 seeds per treatment were tested, and untreated seeds of each seed lot served as the control. The experiment was carried out in a net-house in the summer season, the ambient temperature ranged from 32°C to 38°C. A seed was considered emergence when the cotyledons totally rose upon the surface of the media. The mean emergence time (MET) was computed based on daily counting of newly emerged seedling from the fourth day after sowing (DAS) until no further seedling emerged, using the formula of MGT calculation described above.

The percentage of seedling emergence was presented as the percentage of normal seedling. Seedling growth parameters were measured at 10 DAS, 20 DAS and 30 DAS, and the data of plant height, stem diameter, shoot dry weight and root dry weight were collected from five plants per replication in every treatment, the means of the five plants were used for statistical analysis.

### Enzyme activity analysis

The assay of enzyme activity and lipid peroxidation was carried out within seven days after seeds received HP and EF treatments. Three replications of 20 seeds per treatment were allowed to imbibe for 10 h, then decoated and hand-homogenized in an iced mortar with pestle in 4 ml of 0.1 M potassium phosphate buffer (pH 7.0), followed by centrifuging at 10,000 *g* for 20 min, the supernatant obtained was used for determining enzyme activity and total soluble protein (Wang *et al.*, 2003).

Superoxide dismutase (SOD, EC 1.15.11) activity was assayed using photochemical method as described by Steward and Bewley (1980). Every 1 ml of the reaction mixture contained 0-200  $\mu$ l enzyme extract, 1.3  $\mu$ M riboflavin, 13 mM methionine, 63  $\mu$ M nitroblue tetrazolium (NBT), 50 mM Na-phosphate buffer (pH 7.8), and 0.1 mM EDTA. The test tubes containing the reaction mixture were shaken and placed 30 cm below a light blank consisting of two 15-w fluorescent tubes. The reaction was allowed to run for 10 min, and then stopped by covering the tubes with a piece of black cloth. The reduction in NBT was followed by reading the absorbance at 560 nm. The non-irradiated reaction mixtures were used as the blank. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition to the initial reaction rate (the rate of reaction in absence of the enzyme).

Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decomposed rate of  $\text{H}_2\text{O}_2$ . Every 1 ml reaction mixture contained 100

ml enzyme extract, 0.25 mM  $\text{H}_2\text{O}_2$  and 50 mM Na-potassium phosphate buffer (pH 7.0). The reaction was started by adding  $\text{H}_2\text{O}_2$ , and the rate of decomposition of  $\text{H}_2\text{O}_2$  was measured by following the decrease in absorbance at 240 nm ( $\epsilon = 39.4 \text{ mM}^{-1}\text{cm}^{-1}$ ) for 1 min. The CAT activity was expressed as mmol  $\text{H}_2\text{O}_2$  decomposed  $\text{g}^{-1}$  fresh weight  $\text{min}^{-1}$  (Cakmak *et al.*, 1993).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by monitoring the oxidized rate of ascorbic acid. Every 1 ml of reaction mixture contained 50 mM Na-potassium phosphate (pH 7.0), 0.5 mM  $\text{H}_2\text{O}_2$ , 0.5 mM ascorbate, 0.1 mM EDTA and 25 ml of enzyme extract. The reaction was started by addition of  $\text{H}_2\text{O}_2$ , and the oxidation rate of ascorbate was estimated by recording the decrease of absorbance at 290 nm ( $\epsilon = 2.8 \text{ mM}^{-1}\text{cm}^{-1}$ ) for 1 min. The APX activity was expressed as mmol ascorbate oxidized  $\text{g}^{-1}$  fresh weight  $\text{min}^{-1}$  (Costa *et al.*, 2002).

The concentration of soluble protein was determined spectrophotometrically using the Bradford dye-binding assay, with bovine serum albumin as a standard (Bradford, 1976).

### Peroxidative products estimation

Lipid peroxidation was measured by the thiobarbituric acid (TBA) test that determines malondialdehyde (MDA) as an end product of lipid peroxidation (Costa *et al.*, 2002). Three replications of five seeds per treatment were allowed to imbibe for 10 h, then decoated, and hand-homogenized in a mortar with pestle in 4 ml of 5% (v/v) trichloroacetic acid (TCA), followed by centrifuging at 10,000 *g* for 20 min, the supernatants obtained were used for MDA determination. To 1 ml of the supernatant, 1 ml of 20% TCA containing 0.5% (w/v) TBA was added. The mixtures were heated at 95°C for 30 min, and then quickly cooled in an ice-water-bath to room temperature. The mixtures were then centrifuged at 10,000 *g* for 10 min, and the absorbance of the supernatants was read at 532 nm. The value of

non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was calculated from the extinction coefficient  $155 \text{ mM}^{-1}\text{cm}^{-1}$ .

### Statistical analysis

A completely randomized design was employed in all laboratory experiments, and a randomized completely block design was used in the seedling growth test. Data analyses were performed using analysis of variance (SAS statistical software Version 6.12). Multiple comparison tests were performed by least significant difference test (LSD) at the level of  $p < 0.05$ .

## RESULTS

### Seed germination

At  $25^{\circ}\text{C}$ , hydropriming increased germination percentages of 'Bingo II' (up to 26%), and significantly reduced both of their MGTs (Table 1). While EF treatments showed less effectiveness in germination enhancement, in

which up to 22% increase in germination was observed in 'Bingo II'. Nevertheless, EF treatments were found to have no impact on the MGT (Table 1).

Under ambient condition in the net-house, no significant enhancements in germination percentage of both 'Bingo I' and 'Bingo II' after hydropriming were found, however, the METs of primed 'Bingo I' and 'Bingo II' were reduced by 37.5% (1.96 day) and 44.1% (2.79 day), respectively. On the other hand, EF treatment showed no improvement on neither the percentage of seedling emergence nor MET, compared to the control (Table 2).

### Seedling growth

Seedlings of hydroprimed seeds grew faster and achieved larger plants in the high germination seed lot 'Bingo I', but no differences were observed in 'Bingo II'. EF treatments did not show any difference in seedling growth in both seed lots when compared to the control (Figure 2).

**Table 1** Cucumber seed germination at  $25^{\circ}\text{C}$  following hydropriming and electric field treatments.

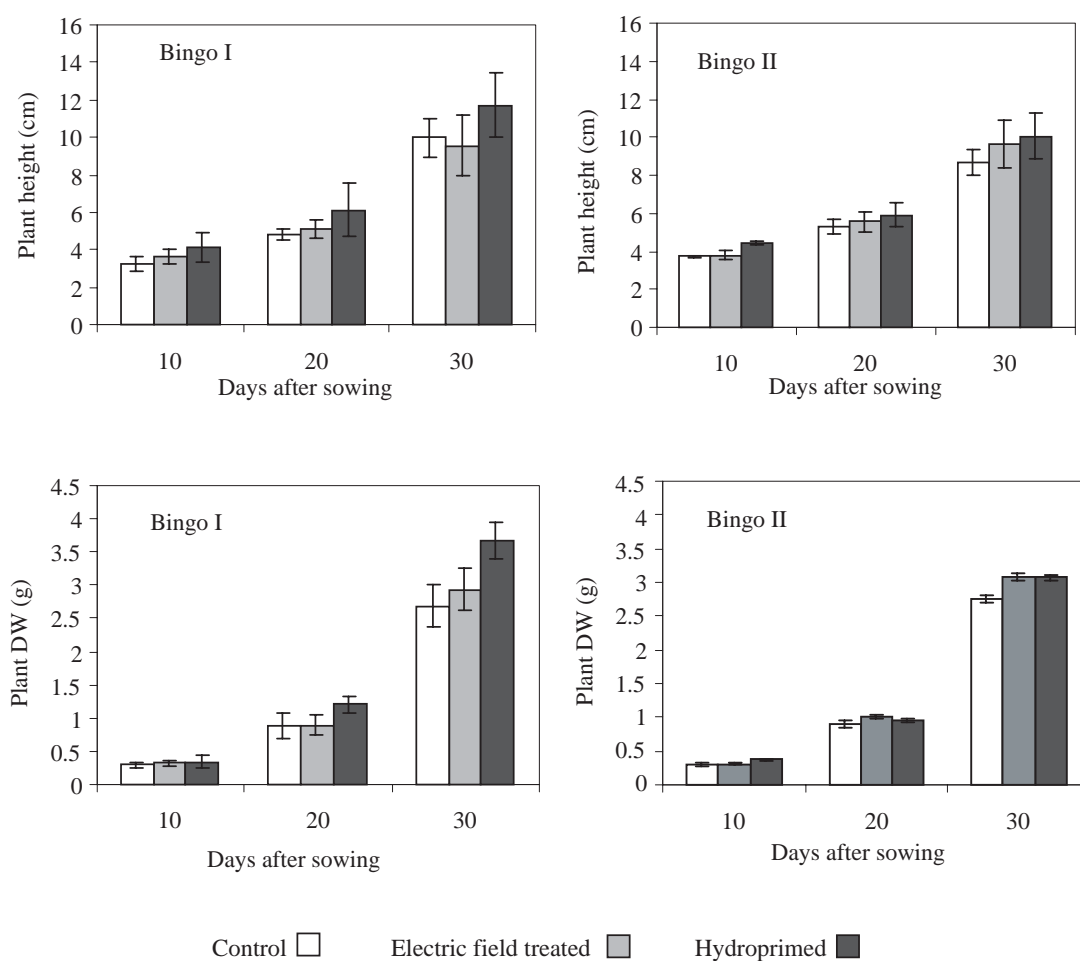
Treatments	Germination (%)		Mean germination time (day)	
	Bingo I	Bingo II	Bingo I	Bingo II
Control	$91.0 \pm 4.2 \text{ a}$	$45.0 \pm 11.0 \text{ b}$	$2.2 \pm 0.1 \text{ a}$	$3.3 \pm 0.1 \text{ a}$
Electric field	$93.0 \pm 6.0 \text{ a}$	$67.0 \pm 2.0 \text{ a}$	$2.1 \pm 0.1 \text{ a}$	$3.3 \pm 0.2 \text{ a}$
Hydropriming	$94.0 \pm 2.3 \text{ a}$	$71.0 \pm 6.6 \text{ a}$	$1.0 \pm 0.0 \text{ b}$	$1.3 \pm 0.1 \text{ b}$

Data were presented as mean  $\pm$  standard error; different letters within one column indicating significant differences at the level of  $p < 0.05$ .

**Table 2** Cucumber seedling emergence under ambient temperature in net-house following electric field and hydropriming treatments.

Treatments	Emergence (%)		Mean emergence time (day)	
	Bingo I	Bingo II	Bingo I	Bingo II
Control	$88.5 \pm 2.7 \text{ a}$	$45.4 \pm 3.5 \text{ a}$	$5.2 \pm 0.6 \text{ a}$	$6.3 \pm 0.7 \text{ a}$
Electric field	$89.6 \pm 6.9 \text{ a}$	$50.4 \pm 6.3 \text{ a}$	$5.1 \pm 0.5 \text{ a}$	$6.3 \pm 0.5 \text{ a}$
Hydropriming	$94.3 \pm 2.4 \text{ a}$	$55.3 \pm 1.8 \text{ a}$	$3.3 \pm 0.2 \text{ b}$	$3.5 \pm 0.2 \text{ b}$

Data were presented as mean  $\pm$  standard error; different letters within one column indicating significant differences at the level of  $p < 0.05$ .



**Figure 2** Changes in seedling growth after hydropriming and electric field treatments. Vertical bars indicating standard error.

### Antioxidant enzymes activities and lipid peroxidation

The enzyme activities of SOD, CAT and APX were increased by the treatments of HP and EF in both high and low vigour seed lots; and the improvement by hydropriming was greater than that by the electric field treatment. The soluble protein contents of both seed lots increased coincidingly with the enzyme activity (Table 3 and 4). Conversely, the MDA contents were reduced significantly by both HP and EF treatments, suggesting lower level of lipid peroxidation occurred in seeds after both HP and EF treatments.

### DISCUSSION

The germination and seedling emergence of 'Bingo I' and 'Bingo II' were improved by both HP and EF treatments, especially HP, by which the speed of seed germination and seedling emergence was remarkably accelerated (Table 1 and 2). The explanation of priming effect on accelerating germination might be attributed to the onset of the early metabolic events during hydration, leading the seed physiological status to the brink of radicle protrusion. Being retained largely after redrying of the seed, such

**Table 3** Changes in antioxidant enzymes activity and MDA accumulation in response to hydropriming and electric field treatments on cucumber 'Bingo I'.

Treatments	SOD activity (unit g <sup>-1</sup> FW)	CAT activity (μmol H <sub>2</sub> O <sub>2</sub> g <sup>-1</sup> FWmin <sup>-1</sup> )	APX activity (μmol ascorbate. g <sup>-1</sup> FWmin <sup>-1</sup> )	MDA content (μmol g FW <sup>-1</sup> )	Soluble content protein (μg g <sup>-1</sup> FW)
Control	247.9 ± 35.9 c	400.8 ± 52.9 b	308.2 ± 16.3 b	5.6 ± 0.1 a	468.7 ± 27.2 b
Electric field	396.2 ± 51.4 b	458.4 ± 13.7 b	331.5 ± 57.8 b	2.9 ± 0.4 b	494.0 ± 23.6 b
Hydropriming	745.0 ± 99.4 a	528.6 ± 32.1 a	437.5 ± 46.6 a	1.8 ± 0.1 c	618.1 ± 51.4 a

Data were presented as mean ± standard error; letters within one column indicating significant differences at the level of  $p < 0.05$ .

**Table 4** Changes in antioxidant enzymes activity and MDA accumulation in response to hydropriming and electric field treatments on cucumber 'Bingo II'.

Treatments	SOD activity (unit g <sup>-1</sup> FW)	CAT activity (μmol H <sub>2</sub> O <sub>2</sub> g <sup>-1</sup> FWmin <sup>-1</sup> )	APX activity (μmol ascorbate. g <sup>-1</sup> FWmin <sup>-1</sup> )	MDA content (μmol g FW <sup>-1</sup> )	Soluble content protein (μg g <sup>-1</sup> FW)
Control	215.6 ± 42.8 b	320.3 ± 9.5 b	190.3 ± 23.9 c	3.5 ± 0.1 a	293.2 ± 24.7 b
Electric field	303.6 ± 21.7 b	355.5 ± 18.1 b	298.7 ± 14.9 b	2.1 ± 0.3 b	357.7 ± 15.5
Hydropriming	463.0 ± 84.7 a	470.7 ± 22.3 a	463.2 ± 73.3 a	1.8 ± 0.0 c	491.4 ± 69.2 a

Data were presented as mean ± standard error; letters within one column indicating significant differences at the level of  $p < 0.05$ .

physiological advancement of primed seed results in faster germination upon rehydration (McDonald, 2000). Alternately, the germination speeds of 'Bingo I' and 'Bingo II' were not influenced by EF treatments (Table 1 and 2). This is in the contrary to a study of Zhu *et al.* (2000), in which the germination speed of cucumber cv. 'JY 7' was increased after EF treatment. It may indicate that the response of cucumber seed to the EF treatment is cultivar dependent.

Seedling growth of 'Bingo I' was improved by hydropriming. The faster germination of hydroprimed seed could result in further seedling emergence and seedling growth (Figure 2). Similar observations were reported in other cucumber cultivar, okra, and corn (Passam *et al.*, 1989; Conway *et al.*, 2001; Subedi and Ma, 2005). In addition, the seedling emergence of 'Bingo II' was not increased under net house condition, suggesting that even they were invigorated to some extent after HP and EF treatments, and were able

to germinate under favorable condition (25°C), the low vigour seeds could not survive under high temperature stress (32-38°C) in the net house.

Priming enhanced germination associated with the stimulation of enzyme activity was reported in bitter melon seeds by Wang *et al.* (2003) and Hsu *et al.* (2003), the activities of enzyme such as SOD, CAT, APX, peroxidase (POD) and glutathione reductase (GR) were elevated, coincidingly with the reduction of lipid peroxidation. Moreover, Bailly *et al.* (2000) found that the activities of SOD, CAT and GR were increased and the content of MDA was reduced during rehydration of primed sunflower seeds. In the present study, the enzyme activities of SOD, CAT, and APX were also increased remarkably, accompanied by the decrease of MDA content in both 'Bingo I' and 'Bingo II' seed lots after 10 h rehydration (Table 3 and 4), which is in agreement with the previous studies.

The mechanism of priming-induced



reduction in lipid peroxidation could be attributed to several reactions: first, as seed moisture content increases, the cells become hydrated, the presence of water slows down the access of oxygen to the sensitive sites; second, metal ions in cells are hydrated, thus their catalytic effectiveness become lower; third, the hydration makes antioxidants more efficiency by increasing their rate of diffusion; fourth, water also provides hydrogen bonding, which interferes with the decomposition of hydroperoxides (Priestley, 1986).

EF treatments elevated the activity of antioxidant enzyme and reduced lipid peroxidation in 'Bingo I' and 'Bingo II' (Table 3 and 4). This observation is in agreement with Zhu *et al.* (2000), who detected that the activity of SOD, CAT and APX was significantly increased in association with lower level of MDA accumulation after electric field treatment in cucumber cv. 'JY7' seeds. Similar results were reported in other crops such as soybean (Zhao *et al.*, 1995), and pumpkin (Wu *et al.*, 2004).

The polarization effect of high voltage electric field upon dielectric substance can cause the hydrogen bonding in water to bend or break (Chaplin, 2005); hence it is reasonable to assume that during the electric field exposure of seed, such polarization effect could also bend or break the hydrogen bonding in ultrastructural elements of the cell, e.g. enzymes, resulting in structural alteration of the macromolecules. This structural alteration may increase enzyme activity; or cause enzyme denaturizing, depending on the strength of the electric field and time of exposure. Furthermore, free radicals and metal ions bearing some kind of electrical charge might move under the EF force toward the electrodes, result in radical-radical recombination and the replacement of metal ions. As a result, the level of free radicals is decreased; the availabilities of metal ions to trigger the reaction of lipid peroxidation are reduced (Frankel, 1980); and the cell balance is improved.

## CONCLUSION

Hydropriming could increase the seed germination speed of both 'Bingo I' and 'Bingo II' under both 25°C and the ambient condition (32-38°C); the primed seeds of 'Bingo II' also had higher percentages of germination than the control at 25°C. On the other hand, the enhancement of electric field treatment was significantly higher only on germination percentages of 'Bingo II', but not 'Bingo I'. In addition, hydropriming accelerated seedling growth of 'Bingo I', but not 'Bingo II'. Electric field treatments, however, showed little improvement on seedling growth on both seed lots.

Both hydropriming and electric field treatments elevated enzyme activities of SOD, CAT and APX, and reduced the accumulation of MDA in 'Bingo I' and 'Bingo II'. However, the effects of hydropriming were more pronounced than that of the electric field treatments.

In summary, as seed treatment, both hydropriming and electric field treatment can improve cucumber seed germination but the effects of hydropriming on enhancing the germination speed and seedling growth are more pronounced than that of the electric field treatment. Alternatively, the application of hydropriming is time consuming and labor costing than that of the electric field. Therefore, electric field treatment should be considered for a commercial application in improving seed quality.

## ACKNOWLEDGEMENTS

The authors are pleasant to acknowledge the Tropical Vegetable Research Center and the Asian Region Center of Asian Vegetable Research and Development Center for providing facilities used in this research. Grateful thanks are due to Dr. Wachiraya Imsabai and Miss Korakot Chanjirakul of the Department of Horticulture, Faculty of Agriculture, Kasetsart University for



providing valuable advices and discussion in the biochemical assay. We specially thank Prof. Dr. Saichol Ketsa of the Department of Horticulture, Faculty of Agriculture, Kasetsart University for providing the chemicals used in the biochemical assay in this study.

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