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NAME: **Ms. Rukui Huang**

THIS THESIS HAS BEEN ACCEPTED BY

S. Sukprakarn — THESIS ADVISOR
(**Assistant Professor Sutevee Sukprakarn, Ph.D.**)

Lop Phavaphutanon — COMMITTEE MEMBER
(**Assistant Professor Lop Phavaphutanon, Ph.D.**)

S. Juntakool. — COMMITTEE MEMBER
(**Assistant Professor Sunanta Juntakool, Ph.D.**)

Chaiwat Chaikul — COMMITTEE MEMBER
(**Associate Professor Chaiwat Chaikul, M.S.**)

Somnuk Wongtong — PROGRAM CHAIRMAN
(**Associate Professor Somnuk Wongtong, Ph.D.**)

APPROVED BY THE GRADUATE SCHOOL ON April 5, 2006

Vinai Artkongharn — DEAN
(**Associate Professor Vinai Artkongharn, M.A.**)

THESIS

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON THE GERMINATION RESPONSE OF CUCUMBER SEEDS TO HYDROPRIMING AND ELECTRIC FIELD TREATMENTS

RUKUI HUANG

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Hydropriming (HP) and electric field (EF) treatments are techniques for seed germination enhancement. They both have the potential to improve seed quality, however, the practical handling of the two techniques are different. To investigate differences in the mechanism of these two techniques, a series of experiments were conducted.

In the first part of the study, three cucumber seed lots with different initial vigour, namely non-deep dormant, high vigour and low vigour were subjected to the treatments. The appropriate conditions of HP and EF treatments on cucumber seed germination enhancement were investigated. The effects of these two treatments were compared and their mechanisms were discussed. HP increased the speed of germination in all three seed lots, and the germination percentage of the non-deep dormant seed lot was also elevated by 17%. EF enhanced the germination percentage in both low vigour and non-deep dormant seeds, but had no effects on the high vigour seeds. Both treatments significantly reduced the electrical conductivity of the low and the high vigour seed lots, whereas slightly increased that of the non-deep dormant seed lot.

The second part of the study evaluated the effects of HP and EF treatments on seedling growth of the high and the low vigour cucumber seed lots. The changes in enzyme activities of superoxide dismutase, catalase and ascorbate peroxidase were also investigated. HP increased the percentage of seedling emergence of the low vigour seeds at 25°C, but had no enhancement at the ambient temperature (32-38°C). The activities of antioxidant enzymes were significantly increased, and the accumulation of malondialdehyde (one of the end products of lipid peroxidation) was reduced after the seeds were treated with HP and EF treatments.

Storage potential of HP and EF treated seeds were evaluated in the third part of this study. Primed seed appeared declining in storability when they stored up to six months. This negative effect was severer under unfavorable condition, which up to 51.1% of germination was reduced. Conversely, EF treatment showed no prejudicial effects on storability.

Rukui Huang
Student's signature

S. Sukprakarn 28 / 3 / 06
Thesis Advisor's signature

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Rukui Huang

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TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cond't)

LIST OF ABBREVIATIONS

AA	=	Accelerated aging
AC	=	Alternating current
APX	=	Ascorbate peroxidase
CAT	=	Catalase
DAS	=	Day after sowing
DC	=	Direct current
EC	=	Electrical conductivity
EF	=	Electric field
FW	=	Fresh weight
HP	=	Hydropriming
MC	=	Moisture content
MDA	=	Malondialdehyde
MET	=	Mean emergence time
MGT	=	Mean germination time
RH	=	Relative humidity
ROS	=	Reactive oxygen spices
SOD	=	Superoxide dismutase
v/v	=	volume /volume
w/w	=	weight/weight
w.p.	=	Wetable powder

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON THE GERMINATION RESPONSE OF CUCUMBER SEED TO HYDROPRIMING AND ELECTRIC FIELD TREATMENTS

INTRODUCTION

General Introduction

Today's plant breeding has produced abundance of advanced crop varieties that provide either high yield or superior quality, which attracting consumers' attention. These new varieties have become more costly, thus the growers' expectations of seed quality have also increased. Because of these factors, seed producers have become much more aware of the potential benefit and impact of any seed amendment on seed quality and storability. Seed technology, especially germination enhancement technology, is thus drawing great emphases.

Among the various technologies of germination enhancement, priming has been intensively studied. The term of priming is defined as a hydration-dehydration process, in which seeds are allowed to imbibe water to initiate the early events of germination, and then redried them before the germination completed to prevent radicle protrusion. Priming can be classified into different categories according to the imbibing medium as osmopriming (seeds are imbibed in osmotic solution), hydropriming (seeds are imbibed in water), and matricpriming (seeds are mixed with moistened solid matrix media). Regardless of the particularity of each priming method to control and manipulate seed hydration, the principle of priming is postulated as: "Enhancement of physiological and biochemical events in seeds during controlled hydration which the germination (radicle protrusion) is suspended below water potential of the imbibing medium" (Claudinei and Anwar, 2000). It has been well documented that seed priming could benefit the germination performance in

various plant species (McDonald, 2000; Warren and Bennett, 1997). Most researches suggested that priming providing time and moisture for seeds to 'repair' the damage from deteriorative events that associated with mitochondrial dysfunction, enzyme inactivation, membrane perturbations and genetic damage incurred during seed storage and aging (McDonald, 2000). However, a drawback to this germination enhancement technology is that the appropriate condition to which priming is applied differs largely among species, varieties and even seed lots of the same variety (McDonald, 2000), thus pretest to determine the treatment condition for every particular batch is essential. In addition, the hydration-dehydration process is a labor costing practice when large-scale seed treatment being involved.

Another germination enhancement technology, which recently has been gaining attention, is the electric field treatment. Exposing seed to electric field reportedly could stimulate seed germination and seedling growth, e.g. the germination speed of tomato seed was accelerated by applying AC electric field (Moon and Chung, 2000). Regardless of the early starting in the 1960s (Sidaway, 1966), the research on electric field in seed germination enhancement is still scarce, little has been done thus far to explore the mechanism of which the seed germination was altered, except that a hypothesis of Chiabrera and Bianco (1987) assumed the electric field invigorating seeds through influencing the biochemical processes involving free radicals.

The application of electric field on seed treatment involves exposing seed to a precisely modified electric field with specific field strength and exposure time. This treatment requires no altering of seed moisture content, and is apparently easier to handle than the hydration-dehydration process of priming, especially for large scale application. Therefore, strong interesting in electric field would be raised by the seed industries in pursuing seed enhancement technologies. In this prospect, a study to compare some physiological and biochemical changes of seeds in response to priming and electric field treatments was carried out, and it might provide a better understanding on the mechanism of electric field that effecting seed quality.

Purpose of Study

The present study was conducted to examine the influence of hydropriming and electric field treatments on seed germination performance, seedling growth, and some physiological and biochemical changes. The objectives of this study were specified as following:

1) To identify the appropriate conditions of hydropriming and electric field treatments for seed germination enhancement.

2) To investigate the physiological and biochemical changes in seeds after hydropriming and electric field treatments, e.g. the changes in cell membrane integrity and the activity of some antioxidant enzymes.

3) To study the effects of different storage conditions on the longevity of hydropriming and electric field treated seeds.

LITERATURE REVIEW

1. An Overview on Seed Priming and Electric Field Treatments

After harvest, the great majority of seeds is processed to enhance their physiological performance during or after germination, extend longevity, and modify the composition of the batch as a whole. Germination enhancement techniques, such as priming and electric field treatment, aiming to enhance germination percentage or speed, are usually applied after the routine process to enhance seed quality or value.

1. 1 Seed Priming

Seed priming is now a widely used commercial process for accelerating germination and improving seedling uniformity in many crops and ornamental plants. Seed priming can result in significant germination enhancement, especially when the seeds are grown under unfavourable environmental conditions, or in soils infested with pathogens such as *Pythium* or *Rhizoctonia* (Halmer, 2003; Rush, 1991; Taylor and Harman, 1990).

1.1.1 Concepts and techniques of seed priming

Priming is a hydration-dehydration process, in which seeds are exposed to restricted water availability under controlled conditions to initiate the early events of germination, and then redried to lower the moisture content, before the radicle protrusion, (Welbaum *et al.*, 1998; Warren and Bennett, 1997). There are at least three techniques to achieve priming (McDonald, 2000; Halmer, 2003).

Hydropriming: The technique covers the approaches of a gradual addition of limited amount of water to seeds or imbibiting seeds in water for a short period (also known as steeping), followed by incubation in humid air (Wright *et al*., 2003), and redrying before radicle protrusion. This technique minimizes the usage of chemical and avoids discarding materials that might be undesirable and incompatible with the

environment. Hydropriming can be accomplished by incubating seeds in a rotating drum with specified amount of water addition as fine mist to insure uniformly hydration. The duration of incubation can be several hours to several days depend on the physiological and biochemical status of the seeds. This process was termed 'drum priming' (Rowse, 1996). Other approaches to hydropriming can be as simple as allowing seeds to imbibe water from moistened blotter paper or gel for specific durations, followed by redrying (Myers and Mitchell, 1998). Hydropriming has been successfully applied to a number of crops, including many vegetables, field crops and flowers. Because the amount of water absorbed by the seed is precisely controlled during hydropriming to ensure that germination is not completed, the enhanced seed germination performance resulting from this treatment is thus considered as the same physiological mechanism as that of other priming techniques.

Osmopriming: Historically, the most widely used priming technique is osmopriming (or osmoconditioning), in which the controlled hydration is achieved by immersing seeds in aerated osmotic solutions of low water potentials. The seeds are then rinsed and redried after a predicted duration of soaking. The osmotica includes inorganic salt e.g. KNO_3 (Demir and Mavi, 2004), NaCl (Sivritepe *et al.*, 2003); high molecular compound such as polyethylene glycol (PEG) (Capron *et al.*, 2000), and organic materials such as mannitol (Passam *et al.*, 1989) etc. The osmotic solution is usually aerated to ensure oxygen supply, especially when PEG is used. Seeds can be soaked in the osmotica for several hours to several weeks before being redried, depends on the species.

Matripriming: Matripriming is carried out by mixing seeds with insoluble solid matrix particles and water in a predetermined ratio. The seeds are allowed to imbibe to an equilibrium hydration level before the solid matrix particle being sieved away, and the seeds being redried. The ideal characteristics of the solid matrix particles described by Khan *et al.* (1992) include: a. negligible water solubility; b. high water holding capacity; c. a high surface-to-volume ratio; d. nontoxicity to the seed; and e. the ability to adhere to the seed surface. Natural substances with such characteristics are vermiculite and peat moss. Celite and Micro-Cel, which are diatomaceous silica

products available commercially, were also used as the solid matrix particles (Dawidowicz-Grzegorzewska, 1997). Matripriming mimics the natural water imbibition process of seed from soil particles; the aeration condition of this technique is the best among three priming techniques. However, a considerable drawback to large-scale application of matripriming is the storage and disposal of the large quantity of the solid matrix particle.

1.1.2 Mechanism of seed priming to improve germination performance

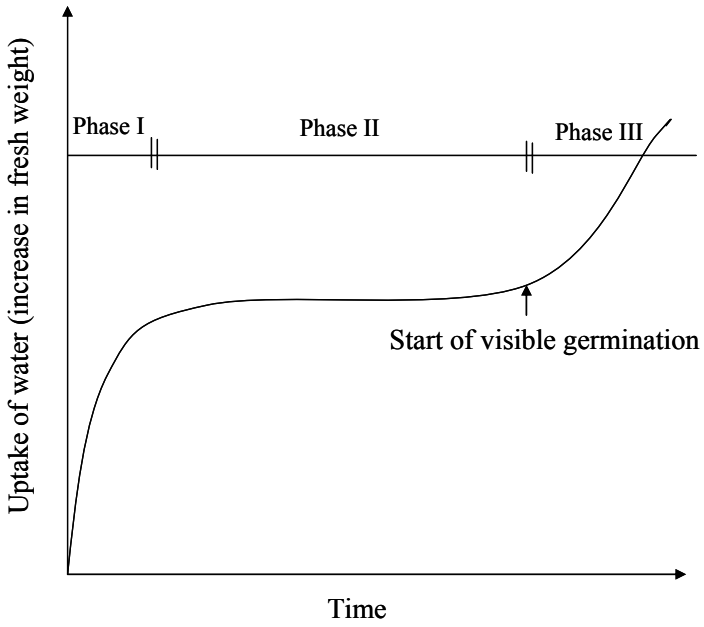

Figure 1 Triphasic pattern of water uptake by germinating seeds

Source: Bewley and Black (1978), physiology and biochemistry of seeds in relation to germination

When seeds are subjected to germination, they typically exhibit a triphasic pattern of water uptake (Figure1). This pattern starts with a rapid imbibition (phase I), followed by a lag phase in which there is little change in water uptake (phase II), and finally a sharp increase in water absorption coinciding with radicle growth (phase III) (Bewley and Black, 1994). During phase II, physiological and anatomical changes occur, preparing the seed for extensive growth. Thus, phase II is a critical phase of seeds germination (Bradford, 1990).

Seeds also process through phase I and phase II of water uptake during priming, as that of the germination; the process does not continues to phase III, because the uptake of additional water needed for the initiation of extensive embryo growth is blocked by redrying. By controlling water potential and temperature, the duration of phase II can be prolonged by priming, permitting further advance of the physiological and anatomical changes. This advancement can be mostly retained after the redring of the seeds, resulting in enhancing vigour such as faster germination when the primed seeds being subjected to rehydration (Welbaum *et al.*, 1998).

Synchronization and rapid seed germination are the commonly recognized benefits of seed priming. Particular advancements of seed priming treatment include improving germination potential and stand establishment, increasing germination speed and field emergence, and enhancing seed performance under stress conditions such as suboptimal temperature and salt stress (Sachs, 1977; Demir and Van de Venter, 1999; Sivritepe *et al.*, 2003; Claudinei and Anwar, 2000). Crop risk due to environmental conditions or insect and disease problems during field emergence, especially under adverse conditions could be minimized by using primed seeds. Short time hydration-dehydration treatment during long storage to improve seed longevity has also been reported elsewhere on various spices, e.g. soybean (Saha and Basu, 1984); carrot and lettuce (Pan and Basu, 1985); and tomato (Van Pijlen *et al.*, 1996).

The metabolic repairing during priming treatments was acknowledged in a battery of literatures. Metabolic events such as protein, RNA, and DNA synthesis are initiated within minutes after seeds starting imbibition (Osborne, 1983). In rye and most graminaceous seeds that lacking of dormancy, DNA repairing to maintain genetic integrity, which is considered critical for successful germination, occurred early after hydration, (Elder and Osborne, 1993). In seed of Brussels sprouts and cauliflower, aerated hydration advanced the onset of DNA replication and initiated repairing of DNA damage that accumulated in embryos (Thornton *et al.*, 1993). Post-storage priming treatment in lettuce and pea seeds were shown to reduce the frequency of chromosomal aberrations and morphologically abnormal seedlings, possibly due to the extension of the lag phase of imbibition permitting more complete repairing of age-

induced DNA damage (Rao, *et al.*, 1987; Sivritepe and Dourado, 1994). Osmotic priming also increased number of mitochondria in leek seeds (Bray, 1995).

Morphological changes in seeds were reported as another effect of priming treatments, e.g. free space around the embryo of tomato seeds was increased following priming due to the endosperm degradation, which may allow greater water uptake and greater turgor, leading to faster growing through the embryo and earlier penetration of the enclosing tissue by the radicle (Argerich and Braford, 1989). Increasing intercellular space and reducing seed coat puncture resistance were observed in bitter gourd (*Momordica charantia* L.) seed following matripriming, and this resulting in faster seedling emergence (Lin and Sung, 2001). The morphological changes of primed seeds may cause the breaking of dormancy, e.g. Cantliffe *et al.* (1984) observed an irreversible initiation of cell elongation in lettuce seeds after hydropriming and osmopriming, and consequently released thermodormancy. Aroonrungsikul (2001) reported the release of dormancy in cucumber seed after hydropriming and osmopriming corresponding to the accelerated degradation of the perisperm and endosperm layers.

In some species, priming treatments allow seeds to continue growing and developing after being harvested from the mother plants, enable the continuing maturation of immature embryo, result in embryonic development. For example, carrot embryos grew during priming as endosperm reserves being mobilized and absorbed (Wiebe and Tiessen, 1979). The embryo of tomato seed developed during priming as radicle protruding partially or completely through the seed endosperm (Liptay and Zariffa, 1993). Nerson *et al.* (1985) reported the increase of embryo size in tetraploid watermelon seeds following priming. Germination of immature seeds of muskmelon (Welbaum and Bradford, 1991) and watermelon (Demir and Oztokat, 2003) seeds were found enhancing by priming with salt solution. These afterripening effects may as well result in breaking dormancy.

Cellular membrane integrity has been considered one of the most critical factors of seed viability (Prestley, 1986). Seed priming has been shown to improve the

membrane integrity in the form of reducing electrolyte leakage, e.g. in sweet corn, osmopriming and matripriming resulted in decreased conductivity (Sung an Chang, 1993); lower electrical conductivity following hydropriming (indicating reduced membrane leakage) was reported in seeds of eggplant and radish (Rudrapal and Nakamura, 1988), and onion (Choudhuri and Basu, 1988). These beneficial effects may be due to the stimulation of the activity of peroxide-scavenging enzymes, which preventing the phospholipid bilayer from being attacked by free radicals, and in turn preserving the membrane integrity (Chiu *et al.* 1995). Improvement in mitochondrial membrane integrity of pea seeds after priming was also investigated by Benamar *et al.* (2003).

1. 2 Electric Field Treatments

1.2.1 Concepts and techniques of electric field seed treatments

All living things on the earth might expose to an electric field from time to time. Electric fields are abundant in our ambient environment, such as in between the cloud and the ground; in the living spaces of developed society; in industrial factories as well as in medical diagnostics and therapeutics. The impact of these electric fields may be positive or negative to biological systems, depending upon the prevailing conditions where the events taking place (Moon and Chung, 2000; Katancevic, 2001).

The extremely low frequency (ELF) electric fields are generally confined to the electric fields produced by the current frequencies of less than 100 Hz. The research of ELF field started approximately in 1967. Since then, numerous pertinent studies have appeared thereafter. Some of the studies were performed as part of an evaluation of the environmental impact of a proposed antenna. Because of the ELF range includes the power frequencies of most nations; ELF radiation is pervasively present as artificial components of the earth's electromagnetic background and this has prompted some studies of the human health consequences. But in most instances, the studies of ELF field were a result of increasing general interest in the effects of electricity on growth and physiology (Marino and Becker, 1977).The technology of

using electric field for seed treatments involves exposing seed to an electric field (AC or DC electric field) with predicted field strength for a specific interval. Both AC electric field (connected with alternating current), and DC electric field (powered by direct current), are in the category of the ELF electric fields.

1.2.2 Mechanism of electric field treatment to improve seed germination performance

The effects of electric field on plant were found stimulating or retarding plant growth (Murr, 1965; Wen *et al.*, 1995). As seed treatment, electric fields were investigated improving seed germination on various hard and dormant seeds (Wheaton *et al.*, 1971; Zhang and Hashinaga, 1997). Morar *et al.* (1999) discovered that the germination of bean seeds (*Phaseolus vulgare*) was enhanced by exposing them to electric field (field strength in the range of 2 to 16 kV/cm). Additionally, the germination of tomato seeds was increased by exposing the seeds to an AC electric field of 4 kV/cm to10 kV/cm field strength for 15 s to 45 s; while the germination was reduced when the field strength being higher than 12 kV/cm and the exposure longer than 60s (Moon and Chung, 2000).

After being graded by an electric separating unit, the germinations of rice, rape and sesame seeds were improved (Yu *et al.*, 1996). It was suggested that the separation unit conveyed energy from the electric field to the seeds, elevating their energy status, inducing phyciological and biological reaction, and enhancing seed germination. The amount of energy content conveying to seed depends on the strength of the electrical field and seed electrical properties (Lynikiene and Pozeliene, 2003). Better results obtained by immature or dormant seeds comparing to high vigour seeds in various physical treatments were also reported (Putincev and Platonova, 1997)

Some researchers claimed that electric field treatments enhance seed vigour by influencing the biochemical processes involving free radicals, and stimulating the activity of enzymes (Morar *et al.*, 1988; 1993; Kurinobu and Okazaki, 1995). Wu *et al.* (2004) found the improvement of germination and seedling growth and the reduction

of electrolyte leakage associated with the increase of antioxidant enzyme activities and seedling respiration rate in electric field treated pumpkin seeds in compared to the untreated control. Similar results were found in cucumber (Zhu *et al.,* 2000), soybean (Zhao *et al.*, 1995, 1998; Cao *et al.,* 2004), and *Oenothera biennes* (Wang *et al.*, 1997), etc.

In the view of physics, Chen *et al.,* (2003) hypothesized the mechanism of electric field that inducing biochemical activities in seeds is due to the polarization effect accruing to all dielectric substances when subject to electric field. On the cellular level, the electric field force may polarized the macromolecules such as protein bodies and enzymes inducing a series of movement and reorientations, allowing combination of some free radicals, stimulating the enzyme activities. Cellular membranes, which some perturbations may exist in the low moisture level, are also benefited from the polarization initiated lateral movement of the phospholipid molecules, hence induce reorientation in the phospholipid bilayers, and consequently reduce electrolyte leakage upon hydration.

However, the effect of electric field on seed is not always desirable, the high field strength and prolonged exposure may cause irreversible damage to the cell and results in failure of germination, e.g. the germination of morning glory seed was reduced by half after being exposed to an electric field (5 kV/cm) for 60 min. The seed water status during germination of the electric field treated morning glory seeds showed different to the untreated seed by NMR study, and the consequence was attributed to the reorientation of membrane components induced by the electric field treatment that preventing normal recovery of membrane systems and causing disorder of membrane structure (Isobe *et al.,* 1999).

Seed surface disinfection is also one of the positive effects of electric field. Ozone generation by partial discharges between seeds was the main sterilizing agent; and certain concentration of ozone can kill pathogens on the surface of seed coat without damaging the seed, ensuring better field establishment (Morar *et al.,* 1999).

2. Physiological and Biochemical Activities in Relation to Seed Quality

2.1 Cellular Membrane Integrity and the Detecting Methods

The integrity of cellular membrane is crucial importance to the maintenance of seed viability. Membranes perform functions that are essential for the survival of any hydrated organism. By regulating the flow of materials between subcellular compartments, membranes supply a means for the control of intermediary metabolism; they provide a dynamic framework for numerous enzymic activities and maintain separations between mutually incompatible cellular compartments. Some degree of membrane disruption may be evident in poor seed quality. The inability of the membrane to contain solutes within badly deteriorated cells entails two immediate consequences: the cell is unable to respond osmotically, failing to maintain proper turgor, and a substantial efflux of seed metabolites is likely to stimulate potentially pathogenic organisms in the microflora within and about the seed. In this concern, the study on membrane structure has gained great emphasis (Priestley, 1986).

The determination of cellular membrane integrity can be manifest through a breakdown in normal cellular permeability properties. To detect the membrane permeability, many indirect methods have been developed, e.g. the use of electron microscope to reveal membrane structure; the use of staining techniques in seed testing, or X-ray techniques to analyze the uptake of barium salts in deteriorated seeds (Priestley, 1986). Most recently, the technique of Electron Spin Resonance (ESR) has been employed in the detection of membrane permeability (Golovina and Tikhonov, 1994; Golovina *et al.*, 1997; 1998; 2001). Besides those methods mentioned above, a most convenient and widely recognized way is the electrical conductivity test. Seeds that have poor membrane structure release more cytoplasmic solutes into the imbibition medium than those with intact membrane structure; these solutes with electrolytic properties carry an electrical charge that can be detected by a conductivity meter (Copeland and McDonald, 1985).

2.2 Free Radical Metabolism and Lipid Peroxidation in Relation to Seed Quality

Many polyunsaturated fatty acids in seeds are highly susceptible to peroxidative degradation, as a result, not only the lipid itself is destroyed, but also a complex series of reactions generates a variety of potentially toxic products. It was suggested that metal ions often initiate the process of peroxidation by reacting with oxygen to form the superoxide anion:

$$M^{+} + O_2 \rightarrow M^{2+} + O_2^{-} \quad (1)$$

The addition of a proton produces a peroxy radical ($OH_2^{\bullet}$), which serves as an effective chain initiator (Frankel, 1980). Superoxides have also been implicated in lipid peroxidation occurring within hydrated, metabolically active systems, where they are formed as a consequence of electron transport or other enzymatic processes. Reaction of superoxide with H_2O_2 yields the hydroxyl radical ($^{\bullet}OH$), which readily exabstracts hydrogen from a lipid methylene group, beginning the chain of peroxidative degradation (Leibovitz and Siegel, 1980). Following hydrogen abstraction, and after diene conjugation and addition of oxygen, a peroxy radical ($ROO^{\bullet}$) is formed, which, by reaction with another unsaturated fatty acid, forms lipid peroxide as the primary oxidative product. The lipid peroxide decomposes readily in the present of metal ions, generating new free radicals and ultimately leading to highly complex mixture of olefins, alkfins, alkanes, alcohols and carbonyl compounds (Priestley, 1986).

Free radical scavengers in seeds play a key role in preventing or terminating the reaction sequence of lipid peroxidation. In dry seed stage, non-enzymatic free radical scavengers, such as tocopherols (vitamin E), are effective in quenching both superoxide and lipid peroxy radicals (Leibovitz and Siegel, 1980; Sattler *et al.,* 2004). Antioxidant enzymes, however, normally function in soluble fractions of fully hydration cells, their activities are critical to preserve membrane and other organelles form free radical attack when seeds starting imbibition (McDonald, 2000).

2.3 Enzyme Activities and Seed Quality

When dry seed starts imbibition, many previously latent enzymes become immediately detectable. The activities of enzymes are often considerably reduced in low vigour seeds. For example, Kang and Saltveit (2001; 2002a, b) reported the 2,3,5-triphenyltetrazolium chloride (TTC) viability index and diphenyl--picrylhydrazyl (DPPH)-radical scavenging activity were found higher in high vigour seeds than that in low vigour seed of cucumber. The radicels of high vigour cucumber seeds better in chilling tolerance in comparison to low vigour radicles, corresponding with the higher activity of ascorbate peroxidase (APX), catalase (CAT) enzymes and superoxide dismutase (SOD).

The accumulation of free radicals leads to the loss of seed quality, this process can be reduced by a hydration-dehydration cycle, which causes seed invigoration to some extents, especially in partly aged seeds (Priestley, 1986). Although unclear whether any direct physiological benefit necessarily accrues, it was suggested to be the consequence of greater dehydrogenase activity and lower peroxide formation (Rudrapal and Nakamura, 1988). In bitter gourd, sweet corn and peanut priming, free radical scavenging enzymes such as SOD, CAT, and peroxidase; the glyoxysome enzymes such as isocitrate lyase and malate synthase, were found to increase through hydration (Sung and Chang, 1993; Sung and Jeng, 1994; Hsu *et al.*, 2003). Gidrol *et al.* (1994) observed the accumulation of CAT activity during soybean seed germination, and attributed this to the generating of reactive oxygen species during hydropriming, which in turn stimulating the synthesis of CAT to minimize cell damage. Further more; Gallardo *et al.* (2001) identified a hydropriming-specific protein in arabidopsis seed, which the quantity increasing during hydropriming and the radicle emergence stage, to be an isoform of CAT.

3. Storability of Seeds After Germination Enhancement Treatments

Seed storability is an important element that has attracted great emphases in the practice of seed enhancement. Priming can increase the speed and uniformity of

germination when the treated seeds are subsequently planted (Taylor *et al.*, 1998). Nevertheless, primed seeds often exhibit reducing longevity in storage, particularly under adverse storage conditions, e.g. in lettuce (Tarquis and Bradford, 1992), and pepper (Sarocco *et al.*, 1995). Several studies have shown that long-term stored seeds are subjected to reactive oxygen spices (ROS) injury (Hendry, 1993; McDonald, 1999). In fresh primed sweet corn seeds, ROS was kept at low level by the priming-enhanced cooperative catalysis of the ROS-scavenging systems (Sung and Chiu, 2001). However, long-term storage results in impairment of the antioxidative systems in primed sweet corn seeds (Chang and Sung, 1998).

Some treatments can be applied after the priming and prior to the dehydration to restore longevity to the primed seeds without loss of the benefits gained from priming. In certain species of pepper, impatiens (*Impatiens walleriana*), and pansy seeds, the longevity restoring methods consisted of a. PEG incubation of seeds on the brink of germination; b.slow drying of primed seeds by means of exposure to air of 75% RH; c. incubation in high RH at 32^{o}C of primed seeds, with a certain seed moisture content (20-38%), and d. a heat shock treatment of 3 h at 40^{o}C (Bruggink *et al.*, 1999). A heat shock respond protein — the immunoglobulin binding protein (BiP) was detected increasing in quantity associating with the post-priming heat shock treatment by Gurusinghe *et al.* (2002), who attributed it to the consequence longevity restoring in primed tomato seeds.

Besides using post-priming treatments to restore longevity, storage condition plays an important role in the storage life of primed seeds. Alvarado and Bradford (1988) studied the impact of storage condition on tomato seeds primed with 3% KNO_3 and PEG, and found that p the benefits obtained from priming, such as reduction of mean germination time, was retained for at least 18 months when the primed seeds were stored at 20^{o}C or lower temperature; while lost vigour and viability more rapidly than the untreated seeds when they were stored at 30^{o}C. Chiu *et al.* (2003) discovered that vacuum storage could extent the longevity of primed sweet corn *sh-2* seeds, whereas the activities of the ROS-scavenging systems decreased in non-vacuum stored primed *sh-2* seeds. Similar result was found in bitter gourd (Yeh

et al., 2005). Low storage temperature was found favorable to retain the benefit of priming (Alvarado and Bradford, 1988; Huang *et al.,* 2002).

MATERIALS AND METHODS

Seed Materials

Three cucumber seed lots supplied by Thai Seed & Agriculture Co. Ltd., Thailand with the following initial seed quality were used in this study:

Seed lot	Initial germination (%)	Initial seed moisture content (%)
'HB128' (non-deep dormant)	84.0	5.6
'Bingo I'(high vigour)	94.0	5.5
'Bingo II'(lowe vigour)	61.5	5.8

The seeds were packed in zip-lock plastic bags and kept on laboratory bench at ambient condition prior to seed treatments. 'Bingo I' and 'Bingo II' were used throughout the whole study, however, 'HB128' was employed in the early the experiment of the study only (in item 1.1, 1.2 and 2.1 of methods) due to the released of dormancy after one month storage at ambient condition.

Methods

1. Investigation of Appropriate Treatment Conditions of Hydropriming and Electric Fields Treatments on Cucumber Seed Germination Enhancement

1.1 Hydropriming (HP)

Determining the seed moisture content and duration of incubation: Seeds of each seed lot were placed on blotter paper wet with 10 ml deionized water, in covered transparent polyethylene boxes (17×25 cm). Two replications of 25 seeds of each seed lot were removed from the blotter paper, surface dried, and their moisture content measured by a MA40 moisture analysis balance (Sartorius), and the average of the

two measurements was used to graph the imbibition pattern of each seed lot. The practice was carried out once every two hour until radicle protrusion was observed.

Prior to incubation, seeds of each seed lot were soaked for 30 min. in 0.5% carbendazim (methyl benzimidzol-2-ylcarbamate 50% w.p.) suspension for disinfection (Wright *et al.*, 2003; Zhao *et al.*, 2004), then rinsed with running tap water for 10 min., surface dried with blotter paper. The surface-dried seeds were placed on metal messes over water in airtight plastic boxes (RH ≈ 100%), and incubated at 25^{o}C for a varied duration of one day, two days, and three days. The moisture content (MC) of the seed batches was determined at the end of the incubation using oven method as described by the International Seed Testing Association (ISTA, 2003). Samples of incubated seeds were redried at ambient condition in the laboratory for two days to the MC in the range of 6% to 7%. The controls were the untreated dry seed of each seed lot.

1.2 Electric Field Treatment (EF)

The experimental set-up: The experimental set-up of electric field comprised of the power supply and the test cell (Figure 2), The test cell of the electric field as shown in Figure 3 consisted of two horizontal electrodes (20×20 cm square plates of copper; interelectrode gap = 2 cm), connected to a fully adjustable AC high-voltage supply (20 kV, 50 Hz). The seeds were loaded one layer on a shallow polyethylene (transparent high-density polyethylene) tray with cover of the 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.

Determining EF field strength and exposure time: Seeds of three seed lots were exposed to EF with the field strength of 1 kV/cm, 3kV/cm, 5 kV/cm and 7 kV/cm; the time of exposure was varied as 1 min., 3 min. and 5 min. (Zhu et al., 2000).

Variation of seed moisture content: Seed moisture content might influence the effect of EF treatment. To examine this, seeds of 'Bingo I' and 'Bingo II' were pre-humidified by incubating in a tightly sealed plastic box with controled RH of 75% modified by saturated salt (NaCl) solution (Creahan, 1991). The seeds were kept at 75% RH for 24 h prior to EF treatment, the seed MC at the end of humidifying reached approx. 8.5%. Control was the dry seeds of each seed lot (5.5% MC).

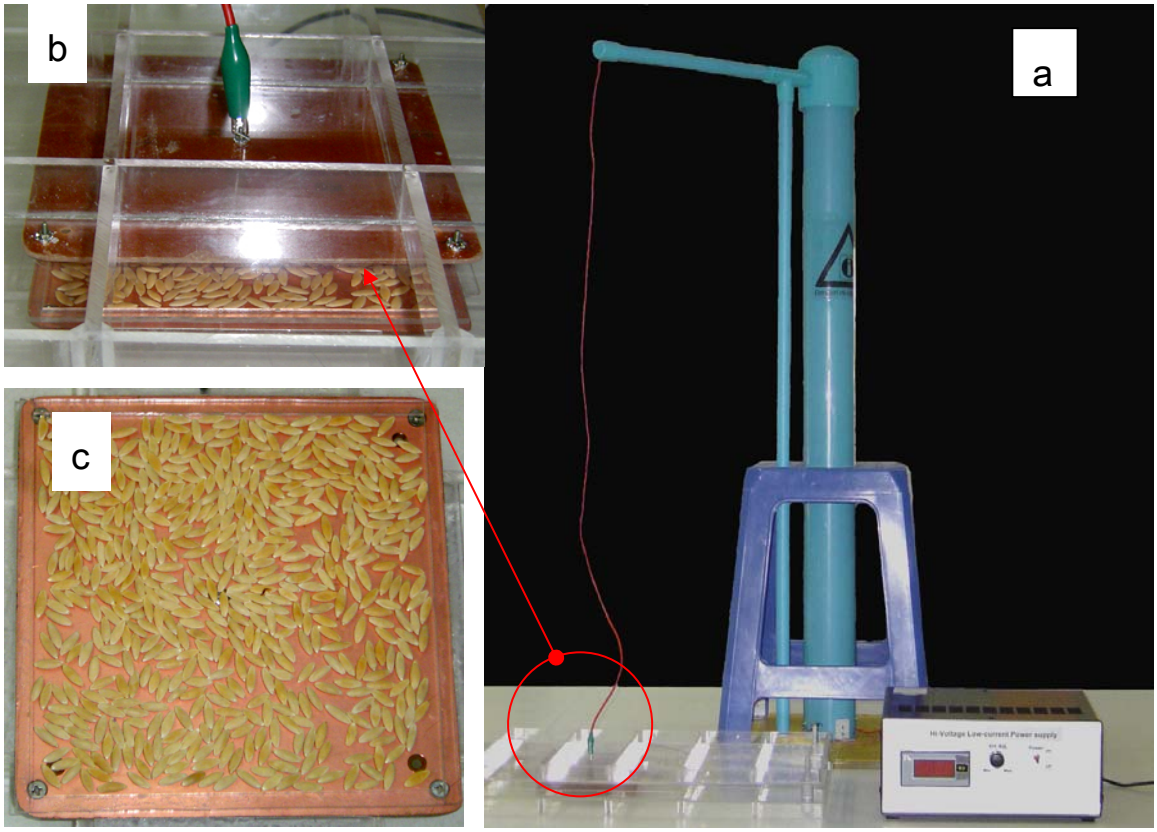

Figure 2 Experimental set-ups for electric field treatments in the present study. (a. electric field comprised by power supply, b. the test cell comprises of two pieces of copper plates where the electric field was generated; c. seeds were loaded one layer on a transparent polyethylene tray, and covered by a piece of same insulating material during the treatment.)

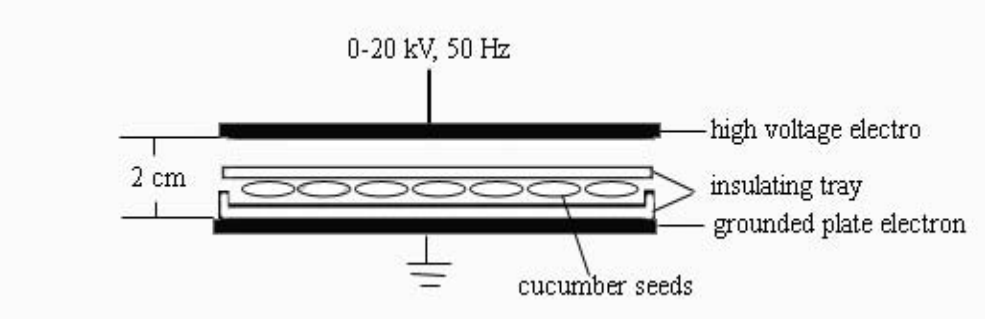

Figure 3 Schematic of the test cell (colour picture in Figure 2 b) used to impose electric field treatments on cucumber seeds

Determining effects of disinfection of primed seed by electric field vs. chemical treatment: To the chemical treatment, one batch of seeds of each lot was soaked for 30 min. in 0.5% carbendazim (methyl benzimidzol-2-ylcarbamate 50% w.p.) suspension as the disinfection treatment; another batch of seeds of each lot, which served as the non-disinfection control, was soaked in water for 30 min. The samples of both disinfected and control seeds were rinsed with running tap water for 10 min. and surface dried with blotter paper, then incubated at 25^{o}C for one to three days, redried at the end of incubation to the moisture content of approx. 6-7%, as described above in item 1.1.

To the electric field disinfection, seeds of 'Bingo I' and 'Bingo II' were primed and redried to 6-7% MC, as described above in item 1.1, except that the process of chemical treatment was left out. The primed seeds were then exposed to EF with field strength 1 kV/cm, 2 kV/cm, and 3 kV/cm; the time of exposure ranged from 15 s to 60 s, at intervals of 15 s. The samples of seeds were germinated immediately after the EF treatment. Primed seeds of each seed lot that had not been treated by EF served as the control.

1.3 Laboratory Germination Test

Germination tests were carried out immediately after treatments, according to the International Rules of Seed Testing (ISTA, 2003), except that only 100 seeds per treatment were used. Seeds were sown on top of moistened blotter paper in covered

transparenct polyethylene boxes (17×25 cm). Each box contained 25 seeds, and there were four replicate boxes per treatment. The boxes were placed in germination chamber at 25^{o}C, 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. The mean germination time (MGT) was calculated from the equation (Ellis and Roberts, 1980):

$$MGT = \sum Ti.Ni / \sum Ni \quad (2)$$

Where Ni = the number of newly germinated seeds at time Ti.

Seedling evaluation was carried out at the eighth day of the germination test, normal seedling, abnormal seedling, dead seed and fresh un-germinated seeds were scored (ISTA, 2003). Germination percentage was computed using the following equation:

$$\text{Germination (\%)} = (\sum \text{normal seedling} / \sum \text{tested seed}) \times 100 \quad (3)$$

2. Physiological and Biochemical Studies of Cucumber Seed Germination After Hydropriming and Electric Field Treatments

According to the previous experiments of HP and EF described in item 1.1 and 1.2, the best conditions of HP and EF treatments for each seed lot could be obtained after a germination test (Table 1), these treatment conditions were employed in the subsequent experiments.

2.1 Electrical Conductivity Test

Seeds of ‘Bingo I’, ‘Bingo II’ and ‘HB 128’ were treated by HP and EF with the conditions specified in Table 1. However, to the treatments of HP, in order to eliminate the confusion that these practices might lower the electrical conductivity by

rinsing away the electrolytes rather than the HP treatments, the processes of soaking in fungicide suspension and rinsing in tap water were omitted.

Table 1 Treatment conditions of hydropriming and electric field for three different cucumber seed lots

Seed lots	Hydropriming	Electric field treatment
'HB 128'	1. Soaking in 0.5% carbendazim (methyl benzimidzol-2-ylcarbamate 50 % w.p.) suspensions for 30 min. 2. Rinsing with running tap water for10 min. 3. Incubating in saturated RH for two days 4. Redrying at ambient condition for two days	Field strength of 1 kV/cm, Exposure time of 1 min.
'Bingo I'	Same as 'Bingo I' except that the incubation duration was three days	Field strength of 5 kV/cm, Exposure time of 3 min.
'Bingo II'	Same as 'Bingo I'	Field strength of 3 kV/cm, Exposure time of 5 min.

The electrical conductivity (EC) was tested immediately after the treatments. Dry seeds of each seed lot served as the control. The seeds were weighed to 0.01 g accuracy, and placed in covered glass jars containing 125 ml deionized water (20°C). The jars were then placed in a germination chamber at 20°C for 24 hours. Every jar contained 50 seeds, and there were four replicate jars for each treatment. The EC of

the soaking solutions were measured by a Cyberscan Con 500 electrical conductivity meter (EUTDCH). The EC of the deionized water was measured and used as the EC of the blank. The EC of seed sample per gram was then computed using the following formula (ISTA, 2003):

$$EC\ (\mu S\ cm^{-1}g^{-1}) = (\text{recorded EC of the sample} - \text{EC of blank})/\ \text{seed weight} \quad (5)$$

2.2 Enzyme Activity Analysis

Seeds of 'Bingo I' and 'Bingo II' were used in this experiment. The assay of enzyme activity and lipid peroxidation was carried out within seven days after the seed received HP and EF treatments. Control was the dry seeds of each seed lot. The HP and EF treated seeds and the control seeds were allowed to imbibe for 10 h, then decoated and hand-homogenized in iced mortars with pestles in 4 ml of 0.1 M potassium phosphate buffer (pH 7.0) followed by centrifugation at 10 000 *g* for 20 min., the supernatant obtained was used for the determination of enzyme activities and total soluble protein (Wang *et al.*, 2003). There were three replications of 20 seeds in each treatment.

Superoxide Dismutase (SOD, EC 1.15.11) assay: SOD activity was assayed using photochemical method as described by Steward and Bewley (1980), 1 ml of the reaction mixture containing 0-200 µl enzyme extract, 1.3 µM riboflavin, 13 mM methionine, 63 µM nitroblue tetrazolium (NBT), 50 mM Na-phosphate (pH 7.8), and 0.1 mM EDTA. Test tubes were shaken and placed 30 cm below a light bank consisting of two 15-w fluorescent tubes. The reaction was allowed to run for 10 min, then stopped by switching off the light and covered the tubes with a piece of black cloth. The reduction of NBT was followed by reading absorbance at 560 nm using a Lambda 20 UV/VIS spectrophotometer (PerkinElmer). The non-irradiated reaction mixtures were used as blank. One unit of SOD activity is defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50% of the initial rate of the reaction in the absence of enzyme.

Catalase (CAT, EC 1.11.1.6) assay: CAT activity was assayed as the rate at which H_2O_2 is decomposed, 1 ml reaction mixture containing100 µl enzyme extract, 0.25 mM H_2O_2 and 50 mM Na-potassium phosphate buffer (pH 7.0). The reaction was started by adding the H_2O_2, and the decomposition of H_2O_2 was measured by following the decrease in absorbance at 240 nm (ε = 39.4 $mM^{-1}cm^{-1}$) for 1 min. The CAT activity was expressed as µmol H_2O_2 decomposed g^{-1} fresh weight min^{-1} (Cakmak *et al*., 1993).

Ascorbate peroxidase (APX, EC 1.11.1.11) assay: APX activity is determined as the rate which ascorbate acid was oxidized, 1 ml of reaction mixture containing 50 mM Na-potassium phosphate (pH 7.0), 0.5 mM H_2O_2, 0.5 mM ascorbate, 0.1 mM EDTA and 25 µl of enzyme extract. The reaction was started by addition of H_2O_2. The oxidation rate of ascorbate was estimated by monitoring the decrease of absorbance at 290 nm (ε = 2.8 $mM^{-1}cm^{-1}$) for 1 min. The APX activity was expressed as µmol ascorbate oxidized g^{-1} fresh weight min^{-1} (Costa *et al.*, 2002).

Soluble protein concentration is determined spectrophotometrically by the Bradford dye-binding assay with bovine serum albumin as a standard (Bradford, 1976).

2.3 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). The primed, electric field treated and control seeds of 'Bingo I' and 'Bingo II' were allowed to imbibe in dionized water for 10 h. Three replications of five seeds from each treatment were decoated, then hand-homogenized using mortars and pestles with the presence of 4 ml 5% (v/v) trichloroacetic acid (TCA), the homogenates were centrifuged at 10 000 *g* for 20 min., the supernatants obtained were used for MDA determination. To 1 ml of the aliquot of the supernatant, 1 ml of 20% TCA containing 0.5% (w/v) TBA was added. The mixture is heated at 95^{o}C for 30 min and then quickly cooled down to room temperature in an ice water bath. The content was then

centrifuged at 10000 *g* for 10 min, and the absorbance of the supernatant was read at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.4 Seedling Growth Test

Cucumber seeds of 'Bingo I' and 'Bingo II' were used in the following experiment. Seeds were treated by HP and EF, under the conditions given in Table 1, prior to sowing. Dry seeds of each seed lot served as the control.

Seedling emergence under optimal (laboratory) and high temperature (net house):

Under optimal temperature: The seeds were sown one centimeter deep in moistened sand (sand: water = 10:1 w/w) in covered plastic boxes. One box contained 50 seeds, and there were four replicate boxes for each treatment. The boxes were incubated at 25^{o}C in a germination chamber.

Under high temperature: The experiment was conducted in a nursery net house operated under ambient condition in the summer season of Thailand, the temperature ranged from 32^{o}C to 38^{o}C, providing a high temperature stress. Immediately after HP and EF treatments (Table 1), the seeds were sown in seedling trays (cell size = 5 cm diameter × 10 cm depth), which were filled with growing media (carbonized rice husk: coconut coir: manure = 1:1:1 by volume). Four replications of 50 seeds per treatment were employed. Untreated seeds of each seed lot served as the control.

A seed was considered emerged when the cotyledons rose above the sand or the growing media. The numbers of seedling emergence were counted at one day intervals until no further emergence was observed. The term of mean emergence time (MET) was used to describe the speed and uniformity of the seedling emergence, and was computed using the equation as following:

$$MET = \sum Ti.Ni / \sum Ni \quad (4)$$

Where Ni = the number of newly emerged seeds at time Ti.

The seedlings of laboratory germination were evaluated after eight days of germination. Normal seedling, abnormal seedling, dead seed and fresh un-germinated seeds were scored. Germination was calculated based on the number of normal seedling using the formula (3) as described above in item 1.3 (ISTA, 2003).

Seedling growth test: Seedlings in the net house were watered twice daily in the morning and afternoon. Foliar fertilizer was applied when necessary. Seedling growth parameters were measured at 10 days after sowing (DAS), 20 DAS and 30 DAS, and data of plant height (from cotyledon to the topmost growing terminal bud), stem diameter (1 cm above cotyledon), shoot dry weight and root dry weight (dried in hot air oven at $80^{o}C \pm 2^{o}C$ for three days) were collected from five plants per replication in every treatment, the means of the five plants were used for statistical analysis.

3. Studies of the Storability of Hydropriming and Electric Fields Treated Cucucmber Seeds Under Different Storage Conditions

3.1 Accelerated Aging (AA) Test

The AA test is a rapid, inexpensive, simple and useful method to evaluate seed storability, it subjects the unimbibed seeds to conditions of high temperature and relative humidity for short periods, the seeds are then removed from the stress conditions and placed under optimum germination condition (Copeland and McDonald, 1985). The potential of seed storability can often be qualified by the survival of seed in AA test, in which seed storability is strongly reduced by exposing to the stress conditions (Powell and Matthews, 1984a, b).

The seeds of 'Bingo I' and 'Bingo II' were used in this experiment. The seeds were either primed or treated by electric field in the condition described in Table 1 prior to AA test. Untreated seeds of each seed lot served as the control. The treated and control seeds were incubated in an environment of saturated RH, which was achieved by placing them on wire mesh screens suspending over water inside tightly sealed plastic boxes (ISTA, 2003). The boxes were placed in an aging chamber at the temperature of 41 ± 1^{o}C for 72 h. There were four replicate boxes for each treatment. Germination tests were carried out immediately after the accelerated aging as described in item 1.3.

3.2 Study of Storability under Different Storage Conditions

In commercial propose, the question of whether or not the seeds can retain the benefits obtained from seed treatments in the subsequent storage for a certain period, and the impact of seed longevity after enhancements are heavily considered in determining a successful seed treatment. Therefore, in the following section of experimentation, the germination performance of HP and EF treated seeds after storage was evaluated. To examine the impact of storage temperature on the longevity of HP and EF treated seed, the experiment was carried out at differential temperature conditions: 15 ± 3^{o}C and the ambient condition (25-36^{o}C, with an average of 32^{o}C) of the laboratory.

HP and EF treated seeds were kept at ambient condition on laboratory bench for three days to reach the equilibrium moisture content (approx. 6%) prior to packing in polyethylene zipper bag. Untreated seeds of each seed lot were also packed as the control. The seeds bags were divided in to two batches, then kept at 15 ± 3^{o} C in a temperature controlled cool room and the ambient condition of the laboratory, respectively. Germination tests were carried out as described in item 1.3 at the intervals of 30 days.

4. Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) (SAS statistical software Version 6.12); Data of germination were arcsin transformed before ANOVA, untransformed values were shown in the tables. Multiple comparison tests were performed by least significant difference test (LSD) at the level of $p< 0.05$ or $p< 0.01$ as indicating in each table.

5. Place and Duration

The experiments were carried out in the Tropical Vegetable Research Center, Kamphaeng Saen Campus, Kasetsart University Kamphaeng Saen, Nakhon Phathom, Thailand, from August 2003 to June 2005.

RESULTS AND DISCUSSION

1. Investigation of Appropriate Condition for Treatments of Hydropriming and Electric Fields Treatments on Cucumber Seed Germination Enhancement

1.1 Hydropriming

Effects of incubation duration: The imbibition pattern of the three seed lots showed similar trend as demonstrated in Figure 4. The seeds imbibed water rapidly during the first two hours, resulted in a quick increase of seed MC, which was elevated from less than 6% up to higher than 24%. The MC remained relatively constant in the range of 25% to 30% during the second hour to the twentieth hour, indicating phase II of the triphasic imbibition pattern. A proportion of seeds of 'Bingo I' (5%) and 'HB128' (15%) showed radicle protrusion at the time of 22 hours after the on set of the imbibition, indicating the start of phase III of these two seed lots. Nevertheless, 'Bingo II' remained the status of phase II after 24 hours of imbibition.

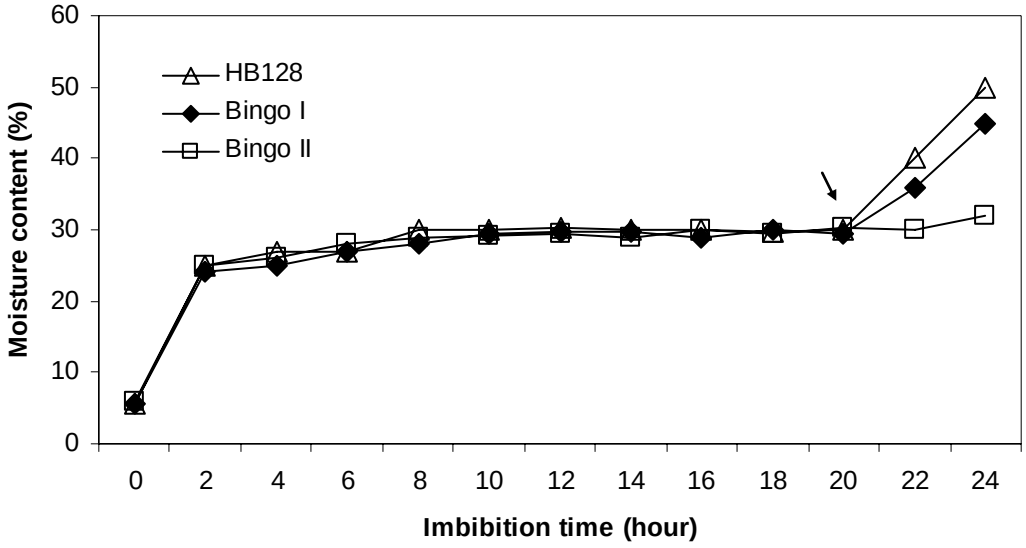

Figure 4 Triphasic pattern of 'HB128', 'Bingo I' and 'Bingo II' cucumber seed imbibition (arrow indicting radicle protrusion).

Based on the imbibition pattern, the MC of each seed lot at phase II of the triphasic imbibition pattern was retained at the range of 25% to 30%, this could be achieved by the handling of the disinfection process (30 min. soaking in carbendazim suspension, then 10 min. rinsing with running tap water), in which the MC of each seed sample was brought up to approx. 25%. During the incubation period, the MC increased steadily in a small rate, indicating the imbibition status of the seeds was kept in the phase II of the triphasic imbibition. The phase II was completed at the the fourth day, when a small portion of seeds of each lot (20% of 'HB 128', 5% of 'Bingo I') showed radicle protrusion, the seed moisture content increased to a higher level (Figure 5).

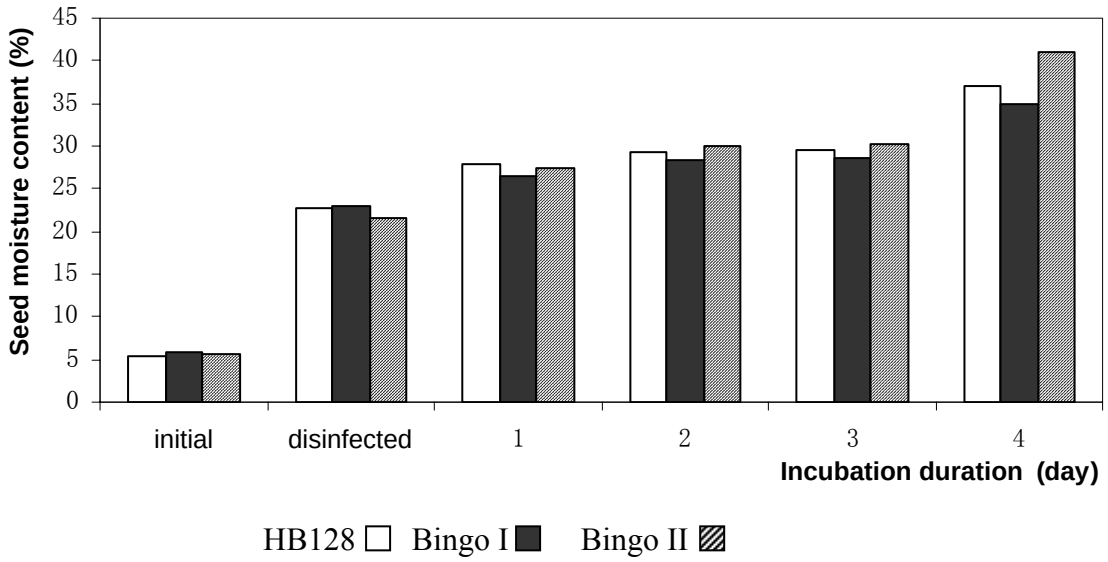

Figure 5. Changes on seed moisture contents of 'Bingo I', 'Bingo II' and 'HB128' cucumber seeds during incubation.

After being dried back to 6-7% MC, primed seeds of each seed lot were germinated, no significant differences in germination percentage were evident among the treated samples and control samples of 'Bingo II' and 'Bingo I'; whereas a remarkable increase in germination percentage of 'HB128' was found, and up to 17% higher in germination was obtained after 2-day and 3-day incubation treatments (Figure 6). Those fresh ungerminated seeds of 'HB128' in the control treatment remained fresh after one month of the germination test, and were classified as

dormant seeds by a tetrazolium staining test (ISTA, 2003). Thus, the increase of germination percentage in 'HB128' by priming could be attributed to the dormancy breaking mechanism. It was known that priming could break dormancy in some Thai cucumber seeds by accelerating the degradation of the perisperm envelope enclosing the embryo. The degradation of the perisperm might allow easier air and water movement into the embryo, resulted in faster embryo growth (Aroonrungsikul, 2001). In addition, as observed in several other crops, e.g. tomato and muskmelon (Argerich and Bradford, 1989; Oluoch and Welbaum, 1996), the degradation in the perisperm layer may cause the decrease of resistant tissue layer around the embryo, to which easier radicle protrusion was expected.

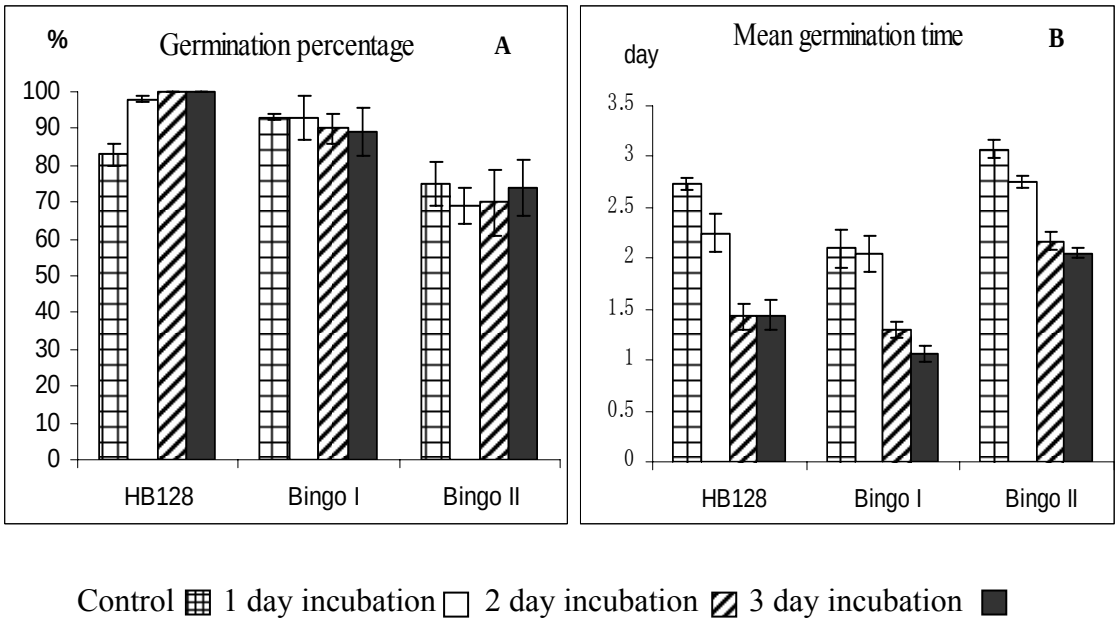

Figure 6. Changes in seed germination performance after hydropriming treatments (Vertical bars indicate standard error**).**

The germination speeds of the three seed lots were accelerated remarkably, as shown by the reduction of MGT. One day incubation noticeably reduced the MGT of 'Bingo I' and 'HB 128'; however, 2-day incubation was required for 'Bingo II' to result significantly. The MGT of each seed lot declined further with the increase of incubation time. The greatest reduction of MGT in each seed lot was 49.3% (1.05 d), 33.07% (1.02 d), and 47.66% (1.30 d) for 'Bingo I', 'Bingo II' and 'HB128',

respectively, as compared to their control. The optimum duration of incubation for each seed lot was different, 3-day incubation brought the most pronounce effect to ‘Bingo I’ and ‘Bingo II’; while 2-day incubation was sufficient to release dormancy in ‘HB128’ (Figure 6).

According to McDonald (2000), the effect of accelerating seed germination speed is a common benefit of priming, this has been observed in various crops. To this phenomenon, the most wildly accepted explanation attributes it to the accomplishment of the early germination metabolic events, e.g. the reorientation of cellular membrane, the reparation of cellular damage due to deterioration. Such accomplishment could be retained largely after the seed being redried, resulting in earlier radicle protrusion upon rehydration (McDonald, 2000). In addition, priming could advance the maturity of heterogeneously matured seed lot, thus improving the uniformity of germination (Demir and Oztokat, 2003; Demir and Mavi. 2004).

1.2 Electric Field Treatments

Electric field strength and time of exposing: Three seed lots responded differently when subjected to EF treatments as presented in Table 2. The germination of ‘Bingo I’ was not affected by EF treatments with the field strength ranging from 1 kV/cm to 7 kV/cm, and exposure time from 1 min. to 5 min. While the germination of ‘Bingo II’ was increased up to 17.3% after exposing to the EF of 3 kV/cm and 5 kV/cm; especially for a 3-minute exposure to 5 kV/cm EF. The seed lot of ‘HB128’ also responded markedly to EF treatments, and up to 10% increase in germination after exposing to the EF of 1 kV/cm and 3 kV/cm was observed. The effects on germination speed were varied among seed lots, no influences on the MGT of both ‘Bingo I’ and ‘Bingo II’ were observed, alternately, the MGT of ‘HB128’ after EF treatments tended to increase.

The EF treatments increased the germination of the low vigour and the dormant seed lot, but showed no effect on the high vigour seed lot. This is not unexpected, as reported by Hofmann and Steiner (1994), the low vigour seeds

exhibited larger cellular damage during seed deterioration compared to high vigour seeds, greater benefit gained from the repairing action by seed treatments could also be resulted. In the contrary, the high vigour seeds possessed less detrimental effect of deterioration, the improvement of seed treatment is thus less obvious, this phenomenon was in the agreement with the observation of Zhu *et al.* (2000), who treated cucumber seeds cv. 'JY 7' with an electric field. The dormant seeds, on the other hand, might possess some inhibitors, such as embryo immaturity, physical barrier surrounding the embryo, or impermeability of the cellular membrane to water or oxygen (Baskin and Baskin, 2001), which prevent the seeds from germinating even under favorable conditions. The consequences of electric field treatments that improved final germination of dormant seeds 'HB 128' could be attributed to the dormancy breaking mechanism, however, how this mechanism undergoing is still unknown. The decline in MGT of seed lot 'HB128' is unexpected, and it is in the contrary of the research of Zhu et al., (2000), which the MGT of cucumber cv. 'JY 7' was accelerated by EF treatment. This might suggest that the response of seeds to the EF treatment is cultivar dependent (Table 2).

Seed moisture content: According to the hypothesis of Lynikiene and Pozeliene (2003), being exposed to EF, seeds receive a quantity of energy transferred from the EF. Such energy stimulates the bioactivities within cells, leading to beneficial responses such as improvement of germination performance. The amount of energy transfers to a particular seed depends on the strength of the EF and seed electrical properties that affected by seed moisture content (Horyński, 2001). Thus, seeds with varied moisture contents could receive different amount of energy transfer, and might perform differently in germination. Nevertheless, the data demonstrated in Table 3 does not support the conjecture, the seed with differential moisture content showed no variation in germination performance in respond to the EF treatment. The only noticeable improvement observed in 'Bingo II' was apparently caused by the strength of EF (Appendix table 2). One should notice that, in order to prevent confusion with priming, the elevation of moisture content was limited to approx. 3% higher than the initial seed moisture content in this experimentation, and this may not be sufficient to stimulate differences obvious enough to be observed.

Table 2 Germination of cucumber seeds after exposure to electric field of varied field strength and exposure time.

Strength (kV/cm)	Time (min)	Germination (%)			Mean germination time (day)		
		HB128	Bingo I	Bingo II	HB128	Bingo I	Bingo II
1	1	94±6.9^{ab}	89 ±6.8	70 ±8.3^{ab}	2.6 ±0.2^{abc}	2.2 ±0.1	3.3 ±0.1
	3	94±2.3^{ab}	93 ±3.8	62 ±6.1^{bc}	2.8 ±0.4^{a}	2.1 ±0.1	3.5 ±0.5
	5	90±6.9^{abc}	94 ±2.3	71 ±6.1^{ab}	2.7 ±0.2^{ab}	2.1 ±0.1	3.5 ±0.0
3	1	94±5.2^{ab}	90 ±2.3	68 ±4.0^{ab}	2.7 ±0.2^{ab}	2.1 ±0.1	3.3 ±0.4
	3	94±5.2^{ab}	92 ±3.3	78 ±8.3^{a}	2.5 ±0.3^{abc}	2.1 ±0.1	3.8 ±0.3
	5	91±5.2^{abc}	94 ±2.3	64±8.4^{abc}	2.1 ±0.2^{d}	2.1 ±0.1	3.5 ±0.3
5	1	87±8.9^{bc}	89 ±2.0	71 ±4.6^{ab}	2.4 ±0.4^{abcd}	2.1 ±0.1	3.4 ±0.1
	3	87±3.8^{bc}	91 ±8.3	79 ±2.3^{a}	2.5 ±0.2^{abc}	2.1 ±0.1	3.4 ±0.1
	5	85±6.0^{bc}	93 ±3.8	78 ±8.3^{a}	2.2 ±0.2^{cd}	2.2 ±0.1	3.7 ±0.3
7	1	90±8.9^{abc}	94 ±2.3	50 ±7.9^{c}	2.3 ±0.2^{cd}	2.1 ±0.1	3.1 ±0.1
	3	89±3.3^{abc}	97 ±3.8	68 ±4.0^{ab}	2.4 ±0.3^{bcd}	2.1 ±0.1	3.4 ±0.1
	5	87±6.0^{bc}	95 ±3.8	64 ±9.0^{abc}	2.4 ± 0.3^{bcd}	2.1 ±0.1	3.3 ±0.2
Control		84±9.5^{bc}	94 ±5.2	62 ±7.2^{bc}	2.3 ±0.1^{cd}	2.1 ±0.1	3.1 ±0.3

Data were presented as mean ± standard error;

Different letters within column indicate statistical difference at level of $p< 0.05$. The interaction of field strength with time is not significant (a two-way ANOVA showing the interaction between electric field strength and the exposing time was presented in Appendix Table 1).

Table 3 Influence of different seed moisture contents on electric field treatments to enhance cucumber seed germination.

Moisture content (%)	Strength (kV/cm)	Time (min)	Germination (%)	
			'Bingo I'	'Bingo II'
5.5	3	1	96 ± 5.7	76 ± 9.8 [ab]
		3	93 ± 2.0	81 ± 5.0 [a]
		5	98 ± 4.0	74 ± 5.6 [ab]
	4	1	95 ± 2.0	71 ± 6.8 [ab]
		3	93 ± 3.8	80 ± 5.7 [a]
		5	93 ± 5.0	76 ± 4.6 [ab]
	5	1	97 ± 6.0	76 ± 3.3 [ab]
		3	97 ± 2.0	68 ± 5.7 [b]
		5	95 ± 3.8	76 ± 10.3 [ab]
8.5	3	1	90 ± 8.3	70 ± 13.7 [ab]
		3	94 ± 5.2	77 ± 3.8 [ab]
		5	99 ± 2.0	72 ± 3.3 [ab]
	4	1	94 ± 4.0	82 ± 10.1 [a]
		3	93 ± 3.8	78 ± 10.6 [ab]
		5	97 ± 3.8	76 ± 11.8 [ab]
	5	1	96 ± 3.3	73 ± 10.5 [ab]
		3	95 ± 2.0	82 ± 10.1 [a]
		5	93 ± 2.0	75 ± 10.5 [ab]
Cont. (5.5 % MC)			97 ± 3.8	62 ± 4.5 [b]
Cont. (8.5 % MC)			94 ± 4.0	69 ± 3.8 [b]

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p < 0.05$.
The interaction of MC, with field strength and time is not significant (a three-way ANOVA showed the interaction among MC, electric field strength and the exposing time was presented in Appendix Table 2).
SMC = seed moisture content; Strength = the strength of electric field; Time = duration of seed exposed to electric field.

Disinfection of primed seed by electric field vs. chemical treatment: HP incubates seeds in highly humid environment, which favors the development of pathogen, thus the seeds are exposed to a high risk of pathogen attack if they were contaminated prior to HP (Figure 7). Data in Table 4 showed that the percentage of fungi infected seeds increased with the incubation period. Similar observations were reported by Wright *et al.* (2003) and Zhao *et al.* (2004), who attempted to eliminate the activity of pathogens during priming by fungicide treatment. In the present study, soaking the seeds in 0.5% carbendazim (methyl benzimidzol-2-ylcarbamate 50% w.p.) suspension prior to incubation can significantly reduce the number of fungi infected seed and ensure the desirable consequences of priming (Table 4). There was no evident of toxic effect of the chemical on the seedling observed in this experiment.

Chemical seed disinfection usually causes environmental problems and it is unacceptable in organic agriculture. In response to the future trend of sustainable agriculture, many physical seed disinfecting methods have been taken into concern, hence, to explore the potential of EF treatment for seed disinfection as a substitution for the chemical treatment is worthwhile.

Figure 7 Pathogen grew on seeds during incubation of hydropriming

Table 4 Effects of chemical disinfection on hydroprimed cucumber seeds.

Treatment	'Bingo I'		'Bingo II'		'HB 128'	
	Germination (%)	Infection (%)	Germination (%)	Infection (%)	Germination (%)	Infection (%)
Control	93 ± 3.8	0 ± 0 ^{e}	70 ± 8.9 ^{abc}	20 ± 4.6 ^{b}	83 ± 6.0 ^{d}	3 ± 3.8 ^{de}
HP 1 d	93 ± 11.5	75 ± 10.0 ^{c}	53 ± 5.0 ^{d}	93 ± 3.8 ^{a}	92 ± 5.7 ^{bc}	94 ± 4.0 ^{ab}
HP 2 d	90 ± 6.9	86 ± 9.5 ^{b}	68 ± 8.3 ^{cd}	94 ± 4.0 ^{a}	100 ± 0 ^{a}	91 ± 3.8 ^{b}
HP 3 d	83 ± 11.0	97 ± 2.0 ^{a}	56 ± 11.8 ^{cd}	94 ± 6.9 ^{a}	100 ± 0 ^{a}	98 ± 2.3 ^{a}
Ch Cont.	91 ± 5.0	0 ± 0 ^{e}	74 ± 10.6 ^{ab}	0 ± 0 ^{d}	91 ± 3.8 ^{c}	0 ± 0 ^{e}
Ch.HP 1d	92 ± 5.6	3 ± 3.8 ^{de}	61 ± 15.5 ^{bcd}	10 ± 5.2 ^{c}	96 ± 3.3 ^{ab}	6 ± 2.3 ^{cd}
Ch HP 2d	94 ± 4.0	8 ± 3.3 ^{de}	69 ± 8.7 ^{abc}	9 ± 3.8 ^{c}	100 ± 0 ^{a}	9 ± 3.8 ^{c}
Ch HP 3d	95 ± 5.0	11 ± 6.8 ^{d}	79 ± 11.5 ^{a}	12 ± 5.7 ^{c}	100 ± 0 ^{a}	9 ± 6.0 ^{c}

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$. The interaction of chemical treatment with incubation time is significant (the ANOVA of Factorial in CRD was presented in Appendix Table 3).
Ch = chemical treated; HP = hydropriming; Ch. Cont = chemical treated, non-primed; Control= soaking in water for 30 min. prior to germination.

After a HP treatment without pre-chemical disinfection, both samples of primed 'Bingo I' and 'Bingo II' were significantly increased in the number of infected seed in a subsequent germination test (Table 5). Exposing primed seed to EF remarkably reduced number of infected seed, coincided with the increase of germination percentage. Although no significant difference on germination of 'Bingo I' was observed after exposure to EF with different treatment conditions (strength of 1, 2 and 3 kV/cm, and exposure time of 15 s, 30 s, 45 s, and 60 s), the maximum disinfection effect (47.8 % reduce in infection) was found in the treatment condition of 1 kV/cm field strength and exposure for 60 s, as compared with the control. On the other hand, the treatment condition of 1 kV/cm field strength and 15 s exposure yielded the best consequences on 'Bingo II', which the germination was increased up to 24.2% and the infection was lowered up to 41.8% (Table 5). The mechanism of

disinfection by EF might be attributed to ozone generation by partial discharges between seeds (Morar *et al.*, 1999).

Table 5 Disinfection effects of electric field treatments on hydroprimed cucumber seeds.

Strength (kV/cm)	Time (s)	'Bingo I'		'Bingo II'	
		Germination (%)	Infection (%)	Germination (%)	Infection (%)
1	15	93 ± 3.8 ^{ab}	65 ± 6.8 ^{ef}	77 ± 12.4 ^{a}	57 ± 8.9 ^{c}
	30	91 ± 6.8 ^{ab}	64 ± 7.3 ^{f}	71 ± 9.5 ^{ab}	74 ± 6.9 ^{b}
	45	96 ± 5.7 ^{a}	69 ± 8.7 ^{def}	64 ± 5.7 ^{bc}	75 ± 7.6 ^{b}
	60	96 ± 3.3 ^{a}	48 ± 8.6 ^{g}	65 ± 3.8 ^{bc}	75 ± 7.6 ^{b}
2	15	92 ± 8.6 ^{ab}	85 ± 15.1 ^{abc}	64 ± 4.6 ^{bc}	78 ± 2.3 ^{b}
	30	95 ± 2.0 ^{a}	75 ± 10.0 ^{cdef}	72 ± 8.6 ^{ab}	78 ± 13.3 ^{b}
	45	91 ± 3.8 ^{ab}	80 ± 6.5 ^{abcd}	65 ± 8.3 ^{bc}	79 ± 14.7 ^{b}
	60	93 ± 3.8 ^{ab}	92 ± 9.8 ^{a}	64 ± 8.6 ^{bc}	75 ± 6.0 ^{b}
3	15	95 ± 3.8 ^{a}	71 ± 12.4 ^{def}	55 ± 6.0 ^{c}	83 ± 2.0 ^{b}
	30	94 ± 4.0 ^{ab}	91 ± 8.3 ^{ab}	58 ± 5.2 ^{c}	76 ± 10.8 ^{b}
	45	96 ±3.3 ^{a}	78 ± 13.3 ^{bcde}	60 ± 4.6 ^{c}	80 ± 13.5 ^{b}
	60	96 ± 3.8 ^{a}	90 ± 5.2 ^{ab}	56 ± 8.0 ^{c}	86 ± 14.8 ^{ab}
Control (primed)		88 ± 3.3 ^{b}	92 ± 8.6 ^{a}	62 ± 8.3 ^{bc}	98 ± 4.0 ^{a}

Data were presented as the mean of 4 replicates ± standard error;
Values within one column followed by different letters are significantly different (p< 0.05). The interaction of field strength with time is not significant (the ANOVA of Factorial in CRD was presented in Appendix Table 4).

Although the electric field disinfection showed less effective than that of the chemical treatment, when the environmental impact, economic input (the cost of

using and disposing of the chemical), labor cost, etc. are taken into concern in long run, the potential of this environmentally friendly method is still worthy of further study.

2. Physiological and Biochemical Studies of Cucumber Seed Germination after Hydropriming and Electric Field Treatments

2.1 Electrical conductivity

Effects of hydropriming on membrane leakage: Low vigour seed lot 'Bingo II' showed higher EC than that of the high vigour seed lot 'Bingo I', suggesting severer membrane leakage occurring in low vigour seed lot. Whereas the leakage of non-deep dormant seed lot 'HB128' was noticeably lower than that of 'Bingo I', which indicated lower membrane permeability. HP reduced the leakage of seed lots 'Bingo I' (up to 23.15%) and 'Bingo II' (up to 12.15%), and the reduction went greater as the incubation duration was prolonged from 1 d to 3 d, except that 3 d incubation in 'Bingo II' showed some reverse effect. Nevertheless the influence of priming on 'HB128' was insignificant; it increased slightly (up to 10.8%) as the incubation was prolonged (Figure 8).

Cellular membrane is considered as one of the primary sites of lethal damage in cell when subjected to desiccation and deterioration (Priestley, 1986). Changes in seed moisture content could induce membrane phase transition in a double-way manner: when seed moisture content higher than 20%, the membrane stays in a fully hydrated state – the fluid phase, which could transit to a more compressed state – the gel phase in dry seed when the water content is low, and back to the fluid phase upon hydration of the seed. Reorientation of membrane components could take place during the fluid – gel phase transition (Bryant *et al.,* 2001). Such reorientation of membrane components may induce damage repair and preserve membrane integrity. The hydration – dehydration process of priming allows membrane transition to occur, thus, the reductions of electrical conductivity of the primed 'Bingo I' and 'Bingo II' might be the consequences of membrane reorientation (Table 4).

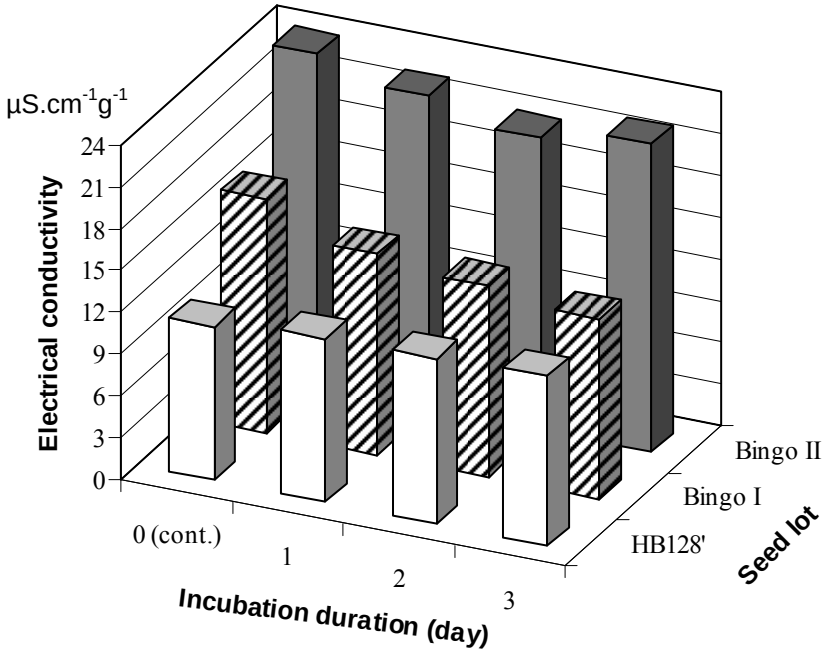

Figure 8 Effects of hydropriming with different incubation durations on the electrical conductivity of cucumber seed.

As reported by Amritphale et al. (2000), the membrane fluidity of cucumber seed increased during the transition of germination status from dormant to germinable stage; the slight increase of electrical conductivity of 'HB128' after hydropriming is thus accordingly due to the increase of membrane permeability. In addition to inducing membrane transition, priming also accelerates the degradation of the perisperm envelope enclosing embryo, allowing easier air and water movement into the embryo, resulting in faster embryo growth (Aroonrungsikul, 2001).

Effects of electric field treatments on membrane leakage: The influences of EF treatments on membrane leakage depend on both field strength and exposing time. The variation of seed lot also played an important role.

'HB128': When seeds were exposed to the EF for less than 3 min. and the field strength in the range of 1 kV/cm to 7 kV/cm, the leakage increased gradually with the increase of field strength, though the increase was very small (≤ 1.09 µS.cm-

$^{1}g^{-1}$); when the exposure time extended to 5 min., the consequence became unsteady (Figure 9).

'Bingo I': When seeds were exposed to the EF less than 3 min. and the field strength in the range of 1 kV/cm to 7 kV/cm, the higher the voltage applied, the lower the leakage was observed; when the exposing time extended to 5 min., the leakage became unsteady, no tendency of correlation among the field strength, exposing time and the leakage was found (Figure 10).

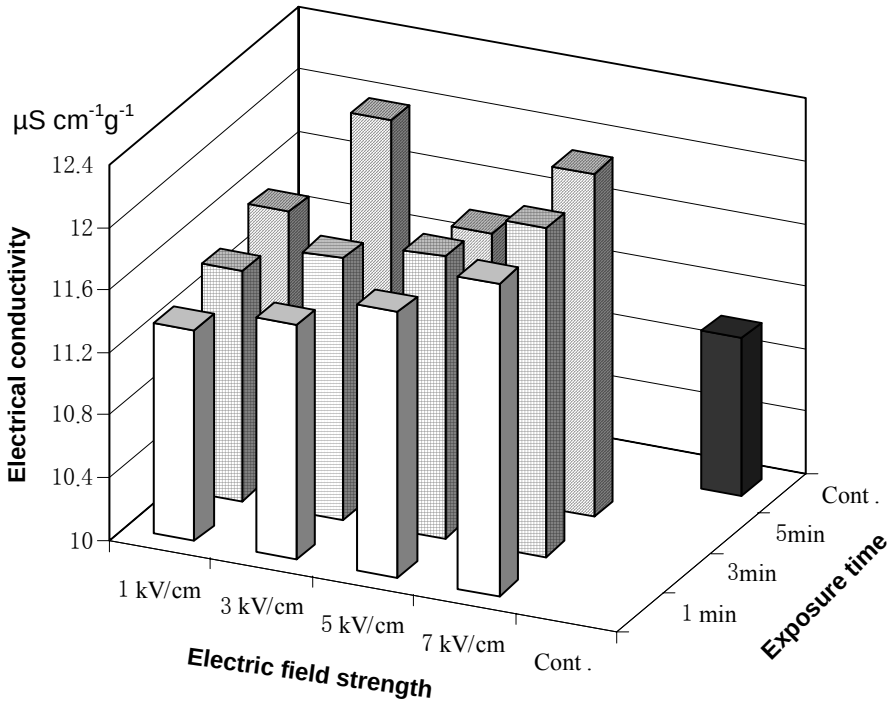

Figure 9 Changes in electrical conductivity of cucumber seed 'HB128' after exposure to electric field (field strength in the range of 1 to 7 kV/cm and exposure time in the range of 1 min. to 5 min.).

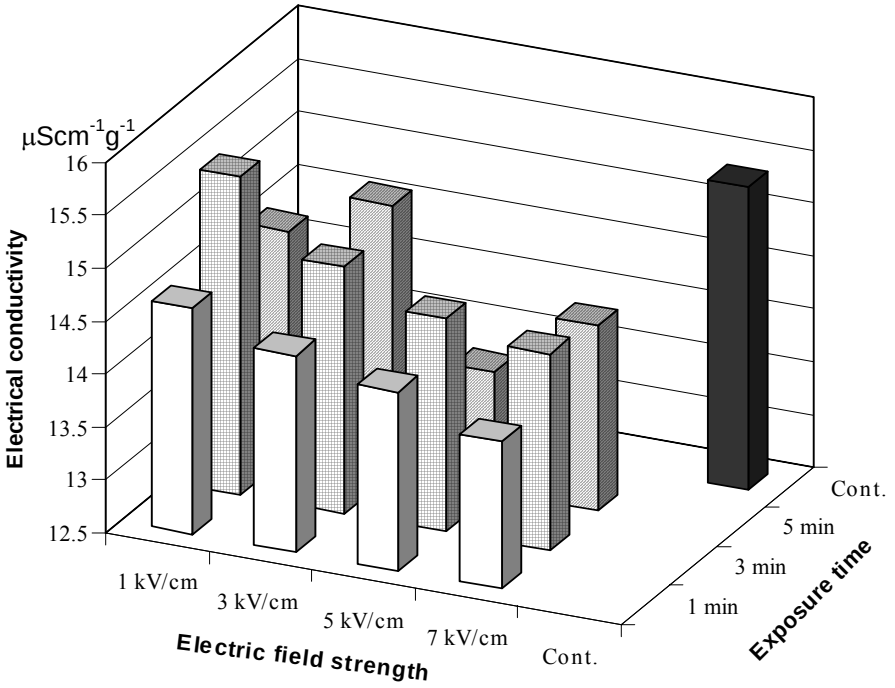

Figure 10 Changes in electrical conductivity of 'Bingo I' cucumber seeds after exposure to electric field (field strength in the range of 1 kV/cm to 7 kV/cm, and exposure time in the range of 1 min. to 5 min.)**.**

'Bingo II': When seeds were exposed for 1 min. to the EF, the EC decreased as the field strength increased from 1 kV/cm to 5 kV/cm. When seeds were exposed fro 3 min. to the EF, the lowest rate of EC was observed at 1 kV/cm, then increased gradually thereafter. When seeds were exposed for 5 min. to the EF, similar trend in the changes of EC in 5 min. exposure was found similar as that of 3 min. exposure, except in 1 kV/cm, where an unexpected high leakage was found (Figure 11).

The changes in EC after the EF treatments indicated the alteration of membrane permeability, which might be due to reorientation of the cellular membrane. Similar results were also observed in soybean, cucumber and pumpkin (Zhao *et al.,* 1995, 1997; Zhu *et al.,* 2000 and Wu *et al.,* 2004). However, the mechanism of EF treatment that enhances alternating membrane permeability is likely alternative to that of priming, since the hydration status of seed has not been changed. In the view of physics, the explanation to such phenomenon might be attributed to the polarization

of electric field as general occurring to all dielectric substances. At subcellular level, the polarization can occur on all of the ultrastructural elements, such as proteins and membranes. The polarization may induce lateral movement of the phospholipid molecules, resulting in reconfiguration within the phopholipid bilayer of membrane; the polarization may also induce reorientation of proteins and other complex macromolecules, contributing to the recovery of membrane functions, and consequently improved the semipermeability (Chen *et al.,* 2003).

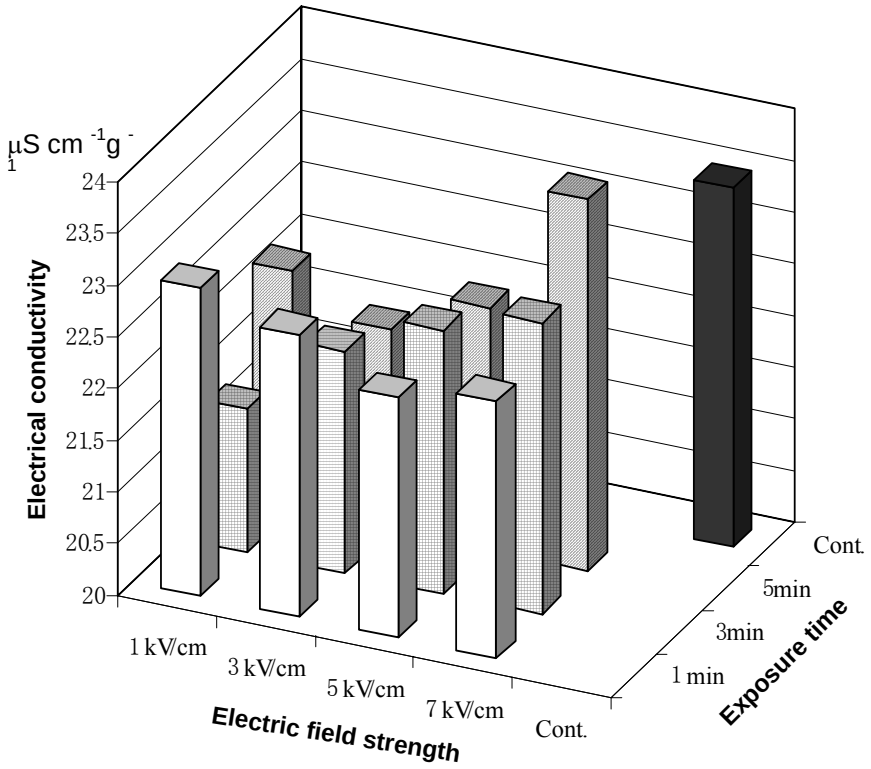

Figure 11 Changes in electrical conductivity of cucumber 'Bingo II' seeds after exposure to electric field (field strength in the range of 1 kV/cm to 7 kV/cm, and exposure time in the range of 1 min. to 5

The polarization effect on cell membrane was also discussed by Isobe *et al.* (1999), who investigated a pernicious effect on morning glory seeds after exposing to EF of 5 kV/cm for 60 min., and they attributed this to the electric polarization that induced an unusual accumulation of water and hydration of stored macromolecules during the imbibition process, resulting in the disruption of membrane systems and

irregular organization of tissue structures. A previous study by Moon and Chung (2000) on the electric field treated tomato seed germination also found that when the exposure time lasted within a certain range, the germination of the treated seeds was improved, however, when the exposure time was prolonged to some extent, the germination percentage declined. Thus, based on the present study and some literatures, it might be stated that the cellular membrane reorientation mechanism responsible for the changes of electrical conductivity in seeds after electric field treatment is a consequence of EF polarization, which could be beneficial or harmful to the seed, depending on the strength of the EF and the time of exposure.

2.3 Antioxidant enzyme activities and lipid peroxidation

The enzyme activity 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 as coincided with the enzyme activity (Table 6 and 7). 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.

Seed deterioration has been discussed widely and free radicals had drawn much attention (Priestley, 1986). In dry seed, free radicals can be produced by autoxidation; the highly aggressive free radicals can react with the majority of biomolecules, causing cellular damage; membrane dysfunction can be a consequence when the free radicals attack the phospholipid bilayers; enzymes are also easily inactivated when amino acids essential for, or close to the active sites are degraded by free radicals (McDonald, 2000; Bailly, 2004).

The advantage of priming on germination performance could be attributed partly to the elevation of antioxidant enzyme activity, as indicated by McDonald (2000), many previously latent enzymes become active immediately upon hydration of seeds, including the antioxidant enzymes, which eliminating the free radicals and

preventing them from damaging the cellular organelles. A review of Priestley (1986) summarized several reactions to which lipid peroxidation could be reduced as seed moisture content increases: first, water slowed down the access of oxygen to the sensitive sites; second, by hydrating metal ions, it lowers their catalytic effectiveness; third, it renders antioxidants more efficiently by increasing their rate of diffusion; and fourth, by hydrogen bonding, it interferes with the decomposition of hydroperoxides

A previous research conducted by Zhu *et al.*(2000) investigated the influence of EF on aged cucumber seeds, found that the activities of antioxidant enzymes SOD, CAT and APX were decreased further as the time of aging was prolonged; and increased significantly after the aged seeds were treated in an electric field (1.67 kV/cm, 1 min. to 5 min.). Whereas the accumulation of MDA presented in a reverse manner, its content increased with the time of aging and decreased after the aged seeds were treated by electric field. The elevation in the activity of antioxidant enzymes and the reduction of lipid peroxidation after the EF treatment were also observed in other crops such as soybean (Zhao *et al.*, 1995, 1998), pumpkin (Wu *et al.*, 2004) *Oenothera biennes* (Wang *et al.*, 1997), and in the present study (Table 6 and 7).

High voltage EF can cause the hydrogen bonding in water to become bent or broken (Chaplin, 2005), hence it is reasonable to assume that when seeds were located in high voltage EF, the hydrogen bonding in various macromolecules, such as enzymes, within the cells could also be bent or broken, resulting in structural alteration of enzyme, which in turn causes the increasing of enzyme activity when the external EF strength is proper, or enzyme denaturizing when the external electric field strength is too high. On the other hand, the EF force might also induce movement of free radicals and metal ions, which bare some kind of electrical charge, toward the electrodes, two results might yield from such movement: radical-radical recombination might occur when anions collide with cations, which consequently reduce the free radical level; replacement of the metal ions, reduce their availability to trigger the reaction of lipid peroxidation, as known that in isolated oils, the reaction of lipid peroxydation is often initiated by metal ions by reacting with oxygen to form the superoxide anion (Frankel, 1980).

Table 6 Changes in antioxidant enzyme activities and MDA accumulation in response to hydropriming and electric field treatments in 'Bingo I' cucumber seeds.

Treatments	SOD activity (unit g^{-1} FW)	CAT activity (μmol H_2O_2 g^{-1} $FWmin^{-1}$)	APX activity (μmol ascorbate. g^{-1} $FWmin^{-1}$)	MDA content (μmol g FW^{-1})	Soluble protein content (μg g^{-1} 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;
Different letters within column indicate statistical difference at level of $p< 0.01$.

Table 7 Changes in antioxidant enzyme activity and MDA accumulation in response to hydropriming and electric field treatments in 'Bingo II' cucumber seeds.

Treatments	SOD activity (unit g^{-1} FW)	CAT activity (μmol H_2O_2 g^{-1} $FWmin^{-1}$)	APX activity (μmol ascorbate. g^{-1} $FWmin^{-1}$)	MDA content (μmol g^{-1} FW)	Soluble protein content (μg g^{-1} 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 [b]
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;
Different letters within column indicate statistical difference at level of $p< 0.01$.

2.2 Seedling emergence vigour

Seedling emergence under optimal temperature and high temperature stress: When 'Bingo I' and 'Bingo II' were germinated in sand at 25°C and in growing media at the ambient temperature of the net house, they responded differently to HP and EF treatment as data showed in Table 8.

Table 8 Germination of 'Bingo I' and 'Bingo II' cucumber seeds under 25°C and the ambient temperature of net house after electric field and hydropriming treatments.

Treatments	Germination temperature (°C)	Germination (%)		Mean emergence time (day)	
		'Bingo I'	'Bingo II'	'Bingo I'	'Bingo II'
Priming	25	94.0 ± 2.3 [a]	71.0 ± 6.6 [a]	3.0 ± 0.0 [c]	3.3 ± 0.1 [d]
	Ambient	94.3 ± 2.4 [a]	54.2 ± 1.8 [b]	4.5 ± 0.3 [b]	4.5 ± 0.1 [c]
Electric field	25	93.0 ± 6.0 [a]	67.0 ± 2.0 [a]	4.2 ± 0.1 [b]	5.3 ± 0.2 [b]
	Ambient	89.6 ± 6.9 [a]	51.6 ± 6.3 [b]	5.5 ± 0.3 [a]	6.5 ± 0.2 [a]
Control	25	91.0 ± 4.2 [a]	45.0 ± 3.2 [b]	4.1 ± 0.1 [b]	5.3 ± 0.1 [b]
	Ambient	88.5 ± 2.7 [a]	47.4 ± 11.0 [b]	5.4 ± 0.1 [a]	6.2 ± 0.2 [a]

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p < 0.01$.
The interaction of treatment with temperature is significant (a two-way ANOVA showing the interaction between treatment and the temperature was presented in Appendix Table 5).

'Bingo I': Although no differences on the percentage of germination were evident under 25°C and the ambient temperature, the primed seeds emerged faster with greater uniformity. The EF treated seeds showed no obvious difference in both

germination percentage and the mean emergence time as compared to the control. Further more, in all treatments, seedling emerged faster at 25°C than that at the ambient temperature (Table 8).

'Bingo II': Both HP and EF treatments significantly improved germination at 25°C by 26% and 21%, respectively, as compared to the control. However, the percentage of germination at ambient temperature did not increased by HP and EF as compared to the control. The primed seeds emerged faster and the mean emergence times were reduced by 36.95% (2.36 d) at 25°C and 28.69% (1.79 d) at ambient condition over the control (Table 8).

2.3 Effects of Hydropriming and Electric Field Treatments on Seedling Growth in Seedling Nursery

Cucumber cv. 'Bingo' is an early mature variety, which starts flowering in approx. 35 days after sowing.

'Bingo I': Seedlings of primed seeds grew faster and achieved larger plants at 30 DAS, coinciding with the greater plant height, stem diameter, and dry weight as showed in Figure 12; EF treatment also improved the seedling growth, particularly in the parameters of root dry weight, but the effects were less significant than that of the priming (Figure 12)

'Bingo II': Both HP and EF treatments showed enhancement on seedling growth to some extent, though it was less remarkable than that of 'Bingo I'; seedlings of HP and EF treated seeds had similar growth rates in the development of plant height, stem diameter and plant dry weight (Figure 13).

The faster germination of hydroprimed seed could result in farther seedling emergence and seedling growth. Similar observations were reported in other cucumber cultivar, okra, and corn (Passam *et al.*, 1989; Conway *et al.*, 2001; Subedi and Ma, 2005). Additionally, the percentage of seedling emergence were not

improved in 'Bingo II' under net house condition (Table 8), which suggesting that this low vigour seed lot contained some badly deteriorated seeds, which, even though were invigorated to some extent after HP and EF treatments, and can germinate under favorable condition (25^{o}C), could not survive the high temperature stress (32-38^{o}C) in the net house.

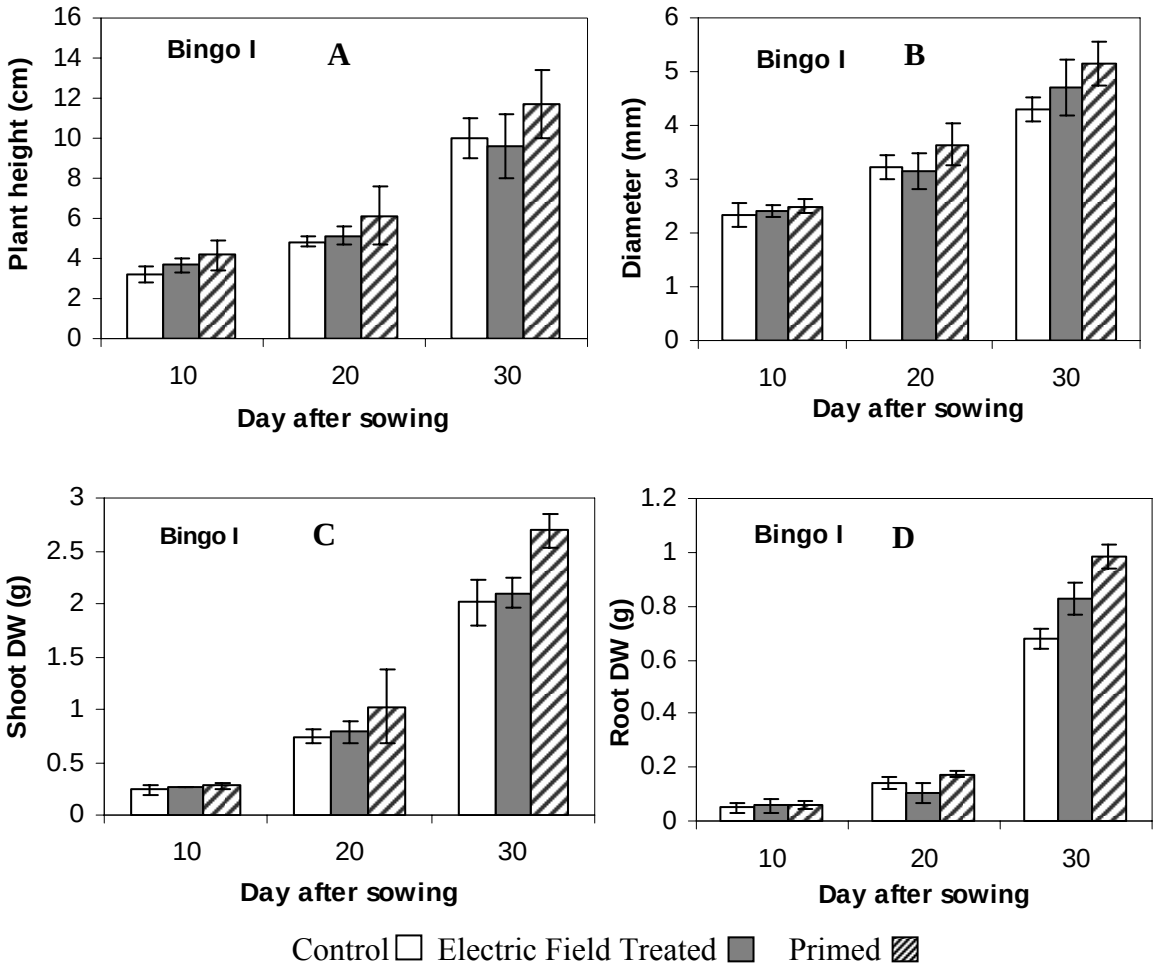

Figure 12 Changes in seedling growth of cucumber 'Bingo I' in response to hydropriming and electric field treatments.
Vertical bar indicated standard error.

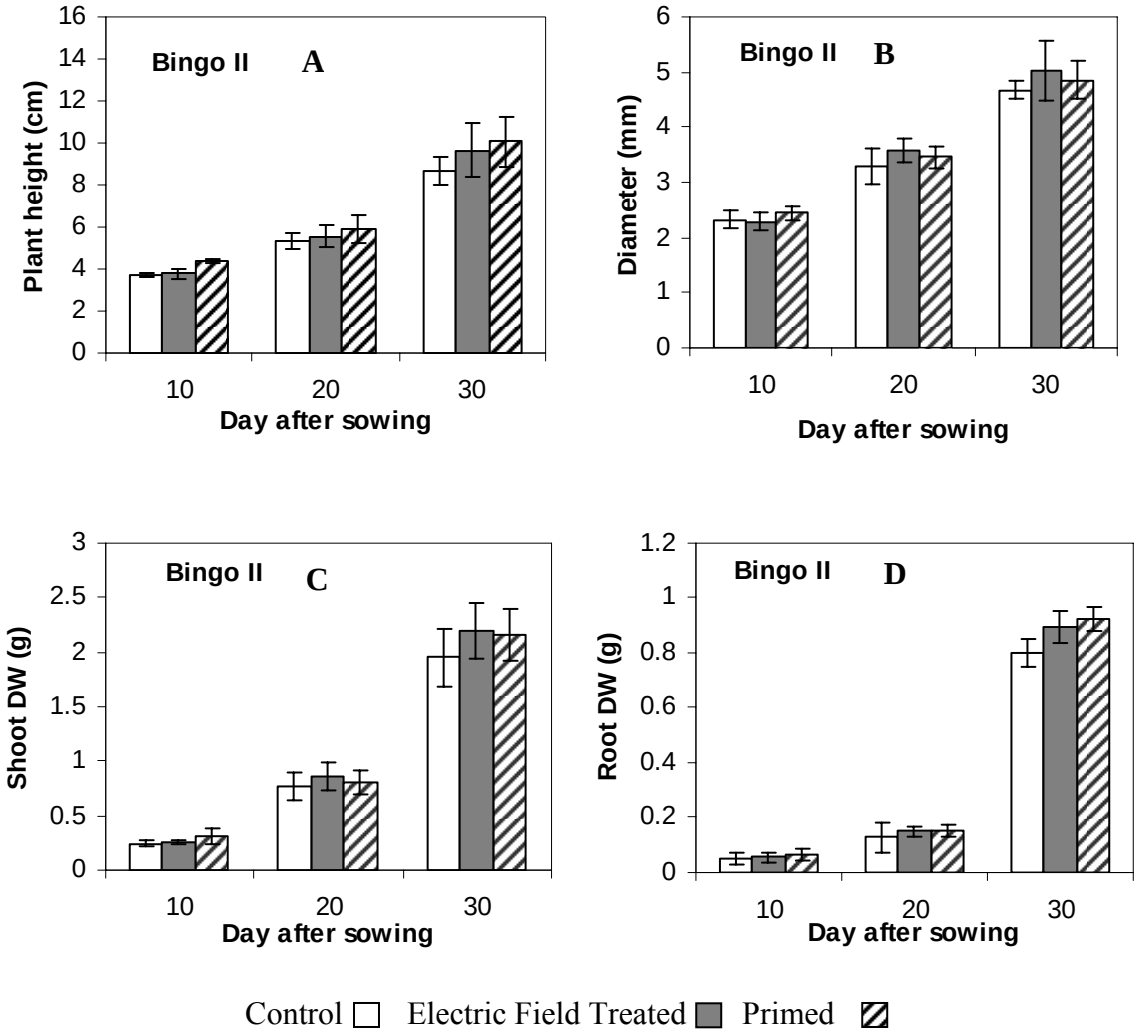

Figure 13 Changes in seedling growth cucumber 'Bingo II' in response to hydropriming and electric field treatments.
Vertical bar indicated standard error.

3. Effects of Hydropriming and Electric Field Treatments on Storability of Cucumber Seeds

3.1 Accelerated Aging (AA) Test

Accelerated aging at 41°C and saturated RH for 72 h significantly reduced germination of both high and low vigour seed lots, and the seed treatments of HP and EF showed different impacts on different seed lot.

Table 9 Germination of 'Bingo I' and 'Bingo II' cucumber seeds after hydropriming and electric field treatments followed by accelerated aging.

Accelerated aging	Treatments	Germination (%)		Mean germination time (day)	
		'Bingo I'	'Bingo II'	'Bingo I'	'Bingo II'
Aging	Priming	86 ± 4.6 ^{b}	22 ± 4.6 ^{c}	2.3 ± 0.0 ^{cd}	2.6 ± 0.3 ^{bc}
	Electric field	78 ± 2.3 ^{c}	13 ± 4.0 ^{c}	2.5 ± 0.1 ^{bc}	2.3 ± 0.2 ^{c}
	Control	80 ± 9.2 ^{c}	13 ± 4.0 ^{c}	2.4 ± 0.0 ^{ab}	2.7 ± 0.2 ^{b}
Non-aging	Priming	93 ± 2.0 ^{ab}	73 ± 4.6 ^{a}	2.2 ± 0.1 ^{d}	2.2 ± 0.0 ^{c}
	Electric field	98 ± 2.3 ^{a}	78 ±12.2 ^{a}	2.7 ± 0.2 ^{a}	3.4 ± 0.1 ^{a}
	Control	95 ± 4.0 ^{ab}	48 ± 10.1 ^{b}	2.6 ± 0.2 ^{bc}	2.8 ± 0.1 ^{b}

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$.
The interaction of treatment with temperature is significant (a two-way ANOVA showing the interaction between treatment and the accelerated aging was presented in Appendix Table 6).

'Bingo I': priming improved the vigour and the tolerance to AA of the sample, the germination of primed seeds was not affected by aging treatment. On the other hand, the EF treatment did not alter the tolerance to aging, both EF treated seeds and the control seeds showed significant reduction in the percentage of germination to a similar level. Priming reduced 14.8% of the MGT over control, and the advantage persisted after accelerated aging. EF treatment, however, showed the increase in MGT to some extent. A reduction of MGT after aging was observed, this was evidently due to the loss of germinability of some lower vigour seeds after AA, which could otherwise germinate slowly and prolong the duration of germination (Table 9).

'Bingo II': HP and EF treatments significantly increased the germination by 52.1% and 62.5% over control, respectively; however, the tolerance to AA was not

improved by both treatments, the germination of both HP and EF treated seeds decreased to the same level as the control seeds after AA. The impact of the seed treatments and the aging on the MGT of 'Bingo II' existed with the same pattern as that of 'Bingo I' (Table 9).

3.2 Post storage germination performance of hydroprimed and electric field treated seeds

3.2.1 Changes in seed germinability

'Bingo I': The germination of the HP and EF treated seeds and the control seeds were retained up to six months at 15^{o}C (Figure 14 A); while decreased at ambient condition (Figure 14 C). The reduction of germinability during ambient storage became significant at the third month of storage then proceeding further with the time prolonged. Even though seeds of all three treatments decreased in germination, the primed seeds proceeded at a faster rate, which, after six month storage, resulted in 51.1% dropped of the germination over the germination at the starting point (Figure 14 C). On the other hand, the reduction in germination of the EF treated seeds and the control seeds appeared in the same rate, indicating that no prejudicial effect on storability by the EF treatment was presented (Figure 14 C).

'Bingo II': The germination of the EF treated and the control seeds were retained at 15^{o}C for up to six months, while that of the primed seeds decreased gradually throughout the storage period (Figure 14 B). At ambient condition, the storage performance of 'Bingo II' was similar to that of 'Bingo I', which the germination of the primed seeds decreased gradually throughout the six month storage, and 65.7% reduced in germination compared to that of the starting point was observed. Still, no negative effect on storability by EF treatment was discovered (Figure 14 D).

3.2.2 Changes in mean germination time (MGT)

A considerable reduction in the MGT was observed following priming (Figure 5), this suggests that metabolic events required for germination were activated by the HP treatment and retained in the seed during dehydration. The initial advantage gained due to priming was retained after six months of storage at 15^{o}C, even though the MGT increased with time in storage, this increase proceeded at essentially the same rate for both primed and control seeds (Figure 15 A, C). The gradual increase in MGT may be the result of normal deterioration, which would require a longer imbibition period prior to germination for repair and reconfiguration of membranes, organelles and enzymes (Berjak and Villiers, 1972).

Substantial increases in MGT of primed seeds were associated with extended storage periods at ambient condition (Figure 15 B, D). The detrimental effect on the primed seeds was reflected not only in delayed germination, but also in a considerable drop of the viability (Figure 14 B, D). These results are in agreement with reports that priming treatments reduce seed longevity (McDonald, 2000), especially at adverse storage conditions. Delaying seed deterioration by storage at low temperature appears to be required to maintain the quality of primed cucumber seeds for extended storage period.

EF treatment did not significantly affect the MGT of seed lots, this is not in agreement with the report of Zhu *et al.*, (2000), in which seed germination speed was accelerated by EF treatment. The changes in MGT of EF treated seed during storage in both cool and ambient temperature conditions appeared similarly to that of the control seeds.

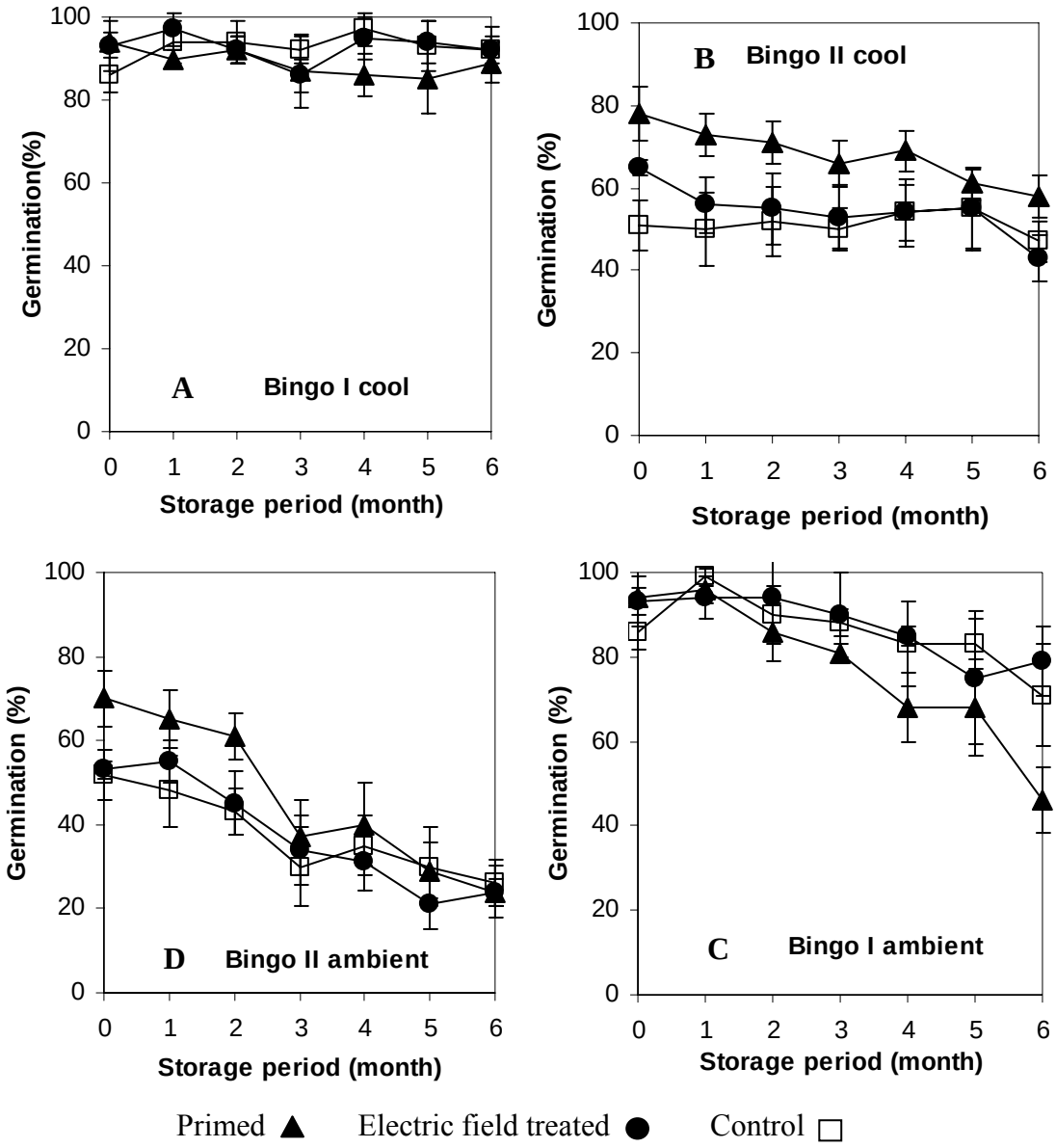

Figure 14 Effects of storage conditions on cucumber seed germination after hydropriming and electric field treatments.
Vertical bar indicated standard error.

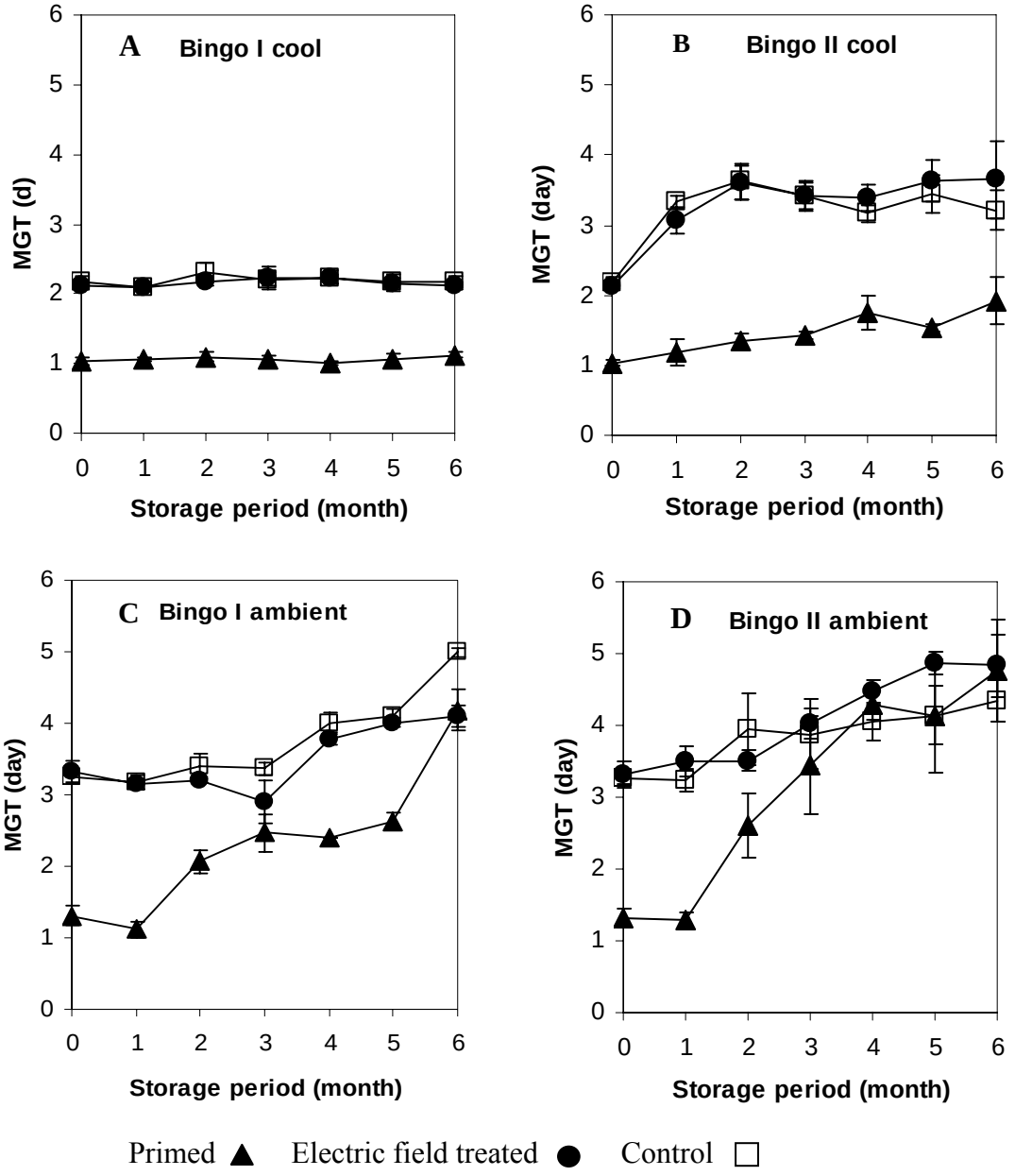

Figure 15 Effects of storage conditions on mean germination time of cucumber seeds after hydropriming and electric field treatments. Vertical bar indicated standard error.

When the primed seeds were germinated after stored for a period of time, the number of fungi infected seed (Figure 16) and abnormal seedlings increased noticeably with time, particularly the seedlings with damaged root system (Figure 17). This phenomenon may be explained as the dehydration stress caused physiological

injuries without immediate effect on viability, during redrying of primed seeds, thus when primed seeds were germinated immediately after the treatment, no increase of abnormal seedlings were found. Seed storage might have accelerated the deterioration of these injured seeds, the delayed effects of mechanical injuries can consequently be seen after storage, where the injured areas serve as infection centers and promote the weakening and early death of surrounding normal tissues (Roberts, 1973; Alvarado and Bradford, 1988).

Storage condition plays an important role in preserving germinability of cucumber seeds (Figure 16). Chiu *et al.* (2003) found that vacuum storage can extend longevity of primed sweet corn *sh*-2 seeds, and they observed that the activities in the ROS-scavenging systems decreased in non-vacuum stored primed sh-2 seeds, which, they attributed to aging-enhanced protein degradation and protein modification (browning products). Similar result was found in bitter gourd (Yeh *et al.*, 2005). Storing primed seeds in low temperature was found to be able to retain the benefit of priming longer than in high temperature (Alvarado and Bradford, 1988; Huang *et al*., 2002). In the present study, the detrimental effect of storage on primed seed was more evident at adverse temperature.

No negative impact of electric field on storability of cucumber seeds was observed in the present study.

Primed seeds ambient storage **Primed seeds cool storage**

Control seeds cool storage **Control seeds ambient storage**

Figure 16 **Germination test of primed 'Bingo I' after six month storing at cool and ambient condition.**
Red circle indicating fungi grew and spread about the seeds.

Figure 17 **Abnormal seedlings in the germination test of 'Bingo I' cucumber after hydropriming and six month storage, showing primary root injury**

CONCLUSION

The influences of hydropriming and electric field treatments on cucumber seeds germination performance were compared at some physiological and biochemical points in this study. Conclusions might be drawn upon the results:

(1) Both hydropriming and electric field treatments can improve cucumber seeds germination, however, the optimum conditions of the treatment are seed lot dependent, greater benefit was achieved in seed lots that inhibited low vigour or dormancy.

(2) The improvement on membrane permeability and some antioxidant enzyme activities after hydropriming and electric field treatments are likely the consequent of different mechanisms. The beneficial effect of hydropriming might due to the advancement of metabolic activities, while the enhancement of electric field might be caused by the phenomenon of polarization.

(3) The benefits gained from hydropriming and electric field treatments could be retained during seedling growth.

(4) Hydroprimed cucumber seeds cv. ‘Bingo’ can be stored over a period of six months without lost of germinability, however, low temperature is required to maintain the quality of primed cucumber seeds for extended storage life. The electric field showed no effects on the storability of cucumber cv. ‘Bingo’ seeds.

In summery, both hydropriming and electric field treatments can be applied for seed enhancement to cucumber seeds, the beneficial effects of hydropriming are more pronounce than that of the electric field. Alternatively, the application of hydropriming is more time consuming and labor costing than that of the electric field. In addition, hydropriming reduced the storability of cucumber seeds, especially in advert storage condition. Therefore, in terms of large-scale application, seed lot condition, purpose of the treatment, the situation of laboring and timing should be considered when selecting the technique of seed enhancement.

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Appendix

Appendix Table 1 Germination of cucumber seeds after electric field treatments (Tow-Way ANOVA).

Fac.	Germination (%)			MGT (day)		
	HB128	Bingo I	Bingo II	HB128	Bingo I	Bingo II
S (kV/cm)						
1	92.7 ± 5.1 [a]	92.0 ± 4.8	67.1 ± 7.4 [ab]	2.7 ± 0.3[a]	2.1 ± 0.1	3.4 ± 0.3
3	93.0 ± 6.2 [a]	92.0 ± 3.0	69.8 ± 10.4[a]	2.4 ± 0.4[b]	2.1 ± 0.1	3.5 ± 0.4
5	86.3 ± 6.0 [b]	91.0 ± 5.2	75.6 ± 6.2 [a]	2.4 ± 0.2[b]	2.1 ± 0.1	3.5 ± 0.2
7	90.0 ± 7.7 [ab]	95.3 ± 3.3	60.4 ± 13.5 [b]	2.3 ± 0.3[b]	2.1 ± 0.1	3.3 ± 0.2
T (min.)						
1	92.8 ± 4.9	90.5 ± 4.1	64.3 ± 11.5	2.5 ± 0.3[a]	2.1 ± 0.1	3.3 ± 0.2
3	91.0 ± 6.3	93.3 ± 5.2	71.3 ± 8.8	2.5 ± 0.3[a]	2.1 ± 0.1	3.5 ± 0.3
5	87.8 ± 7.9	94.0 ± 2.9	69.0 ± 11.7	2.3 ± 0.3[b]	2.1 ± 0.1	3.5 ± 0.3
Cont.	84.0 ± 9.5	94.0 ± 5.2	61.5 ± 7.2	2.3 ± 0.1	2.1 ± 0.1	3.1 ± 0.3
F-test						
Fac. vs.						
Cont.	*	ns	*	*	ns	ns
S	*	ns	*	**	ns	ns
T	ns	ns	ns	*	ns	ns
S × T	ns	ns	ns	ns	ns	ns

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$.
*, ** = significant difference at the level of $p<0.05$ and $p<0.01$, respectively; ns = no significant difference; S = strength of electric field; T = time of exposing.

Appendix Table 2 **Influence of different seed moisture contents on the electric field treatments to enhance cucumber seed germination (Three-Way ANOVA).**

Factor	Germination (%)	
	Bingo I	Bingo II
SMC (%)		
8.5	95.2 ± 3.8	76.1 ± 6.3
6.5	95.6 ± 3.8	75.3 ± 9.4
Strength (kV/cm)		
3	95.0 ± 4.5	75.0 ± 6.8 [ab]
4	94.2 ± 3.8	80.2 ± 5.3 [a]
5	95.5 ± 3.2	70.0 ± 10.5 [b]
Time (min.)		
1	94.7 ± 4.9	74.7 ± 9.0
3	94.2 ± 3.1	77.7 ± 6.8
5	95.8 ± 3.5	74.8 ± 7.6
Cont. (6.5 % SMC)	97.0 ± 3.8	62.0 ± 4.5
Cont. (8.5 % SMC)	94.0 ± 4.0	69.0 ± 3.8
F-test		
Fac. vs. Cont.	ns	*
MC	ns	ns
Strength (S)	ns	*
Time (T)	ns	ns
MC*S	ns	ns
MC*T	ns	ns
S*T	ns	ns
MC*S*T	ns	ns

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p < 0.05$.
ns = no significant difference; * = significantly different ($p < 0.05$)
MC = seed moisture content; Strength = the strength of electric field; Time = duration of seed exposed to electric field.

Appendix Table 3 Effects of chemical disinfection on hydropriming of cucumber seed priming (Two-Way ANOVA)

Factor	HB128		Bingo I		Bingo II	
	Germination (%)	Infection (%)	Germination (%)	Infection (%)	Germination (%)	Infection (%)
Chemical disinfection (Ch)						
Non chem.	93.8^{b}	71.5^{a}	89.8^{a}	64.5^{a}	60.5^{b}	75.3^{a}
Chem.	96.8^{a}	6.0^{b}	93.0^{a}	5.5^{b}	70.8^{a}	7.8^{b}
Time of priming incubation (T)						
Non primed	87.0^{c}	1.5^{b}	92.0^{a}	0 ^{d}	74.5^{a}	10.0^{b}
1 d	94.0^{b}	50.0^{a}	92.5^{a}	39.0^{c}	57.0^{b}	51.5^{a}
2 d	100.0^{c}	50.0^{a}	92.0^{a}	47.0^{b}	63.5^{b}	51.5^{a}
3 d	100.0^{c}	53.5^{a}	89.0^{a}	54.0^{a}	67.5^{ab}	53.0^{b}
Ch × T	ns	**	ns	**	ns	**

Data were presented as mean ± standard error;

Different letters within column indicate statistical difference at level of $p< 0.05$.

** = significantly different ($p<0.01$); ns = no significant difference.

Appendix Table 4 Disinfection effects of electric field treatments on hydroprimed cucumber seeds (Two-Way ANOVA).

Factors	Bingo I		Bingo II	
	Germination (%)	Infection (%)	Germination (%)	Infection (%)
Strength (kV/cm)				
1	94.0 ± 2.0	47.5 ± 10.0	69.3 ± 8.6 [a]	70.3 ± 13.3 [b]
2	92.8 ± 3.8	64.9 ± 6.5	66.3 ± 8.3 [a]	77.5 ± 14.7 [a]
3	95.0 ± 3.8	61.9 ± 9.8	57.3 ± 8.6 [b]	81.3 ± 6.0 [a]
Time (s)				
15	93.3 ± 3.8	57.7 ± 12.4	65.3 ± 6.0	72.7 ± 2.0
30	92.0 ± 4.0	59.7 ± 8.3	67.0 ± 5.2	76.0 ± 10.8
45	95.7 ± 3.3	56.1 ± 13.3	63.0 ± 4.6	78.0 ± 13.5
60	94.7 ± 3.8	58.9 ± 5.2	61.7 ± 8.0	78.7 ± 14.8
Cont. (primed)	88.0 ± 3.3	92.0 ± 8.6	62.0 ± 8.3	98.0 ± 4.0
F- test				
Fac. vs. Cont.	*	**	**	**
Strength (S)	ns	ns	**	*
Time (T)	ns	ns	ns	ns
S × T	ns	ns	ns	ns

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$.
*, ** = significant difference at the level of $p<0.05$ and $p<0.01$, respectively; ns = no significant difference. Strength = the strength of electric field; time = duration of seed exposed to electric field.

Appendix Table 5 Germination of 'Bingo I' and 'Bingo II' cucumber seeds at 25°C and the ambient temperature of the net house after electric field and hydropriming treatments (Two-Way ANOVA).

Factors	Germination (%)		Mean Emergence Time (day)	
	Bingo I	Bingo II	Bingo I	Bingo II
Temperature (Temp.)				
25 °C	91.5 ± 1.9[a]	61.0 ± 2.6[a]	4.7 ± 0.01[a]	5.0 ± 0.0[b]
Ambient	91.9 ± 6.2[a]	51.2 ± 11.0[b]	4.2 ± 0.3[b]	5.3 ± 0.2[a]
Treatment (Treat.)				
Cont.	89.1 ± 4.2[a]	46.3 ± 6.6[b]	5.4 ± 0.3[a]	6.4 ± 0.1[a]
EF	92.6 ± 5.1[a]	59.3 ± 6.0[a]	4.3 ± 0.1[b]	4.9 ± 0.1[a]
HP	93.5 ± 3.4[a]	62.8 ± 2.3[a]	3.6 ± 0.1[c]	4.3 ± 0.1[b]
F- test				
Temp.	ns	**	**	**
Treat.	ns	ns	**	**
Cond. × Treat.	ns	*	**	**

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$.
*, ** = significant difference at the level of $p<0.05$ and $p<0.01$, respectively; ns = no significant difference.

Appendix Table 6 Germination of 'Bingo I' and 'Bingo II' cucumber seeds after electric field and hydropriming treatments followed by accelerated aging (Two-Way ANOWA).

Factors	Germination (%)		Mean Emergence Time (day)	
	Bingo I	Bingo II	Bingo I	Bingo II
Accelerated Aging (AA)				
Aging	81.3 ± 6.9^{b}	16.7 ± 12.6^{b}	2.4 ± 0.3^{a}	2.5 ± 0.1^{b}
Non-aging	95.3 ± 1.2^{a}	66.3 ± 2.1^{a}	2.5 ± 0.1^{b}	2.8 ± 0.2^{a}
Treatment (Treat.)				
Cont.	87.6 ± 5.2^{a}	31.5 ± 6.0^{b}	2.5 ± 0.3^{a}	2.8 ± 0.0^{ab}
EF	98.5 ± 4.1^{a}	47.5 ± 6.7^{a}	2.3 ± 0.1^{b}	2.4 ± 0.2^{b}
HP	88.0 ± 2.5^{a}	45.5 ± 2.3^{a}	2.6 ± 0.1^{a}	2.8 ± 0.1^{a}
F- test				
AA	**	**	ns	*
Treat.	ns	**	**	*
AA × Treat.	ns	**	ns	**

Data were presented as mean ± standard error;
Different letters within column indicate statistical difference at level of $p< 0.05$.
*, ** = significant difference at the level of $p<0.05$ and $p<0.01$, respectively; ns = no significant difference.