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A Comparison of Electric Field Treatments to Hydropriming on Cucumber Seed Germination Enhancement

Rukui Huang[1], Sutevee Sukprakarn[2]*, Lop Phavaphutanon[2], Sunanta Juntakool[3] and Chaiwat Chaikul[4]

ABSTRACT

Three cucumber seed lots: 'Bingo I', 'Bingo II' and 'HB128' were subjected to hydropriming and electric field treatments. The optimum conditions of these two treatments were seed lot dependent. Seed germination and the membrane permeability (electrical conductivity of seed leachates) were tested after the treatments. Hydropriming increased the germination speed of all three seed lots, as well as the germination percentage of 'HB128'. Electric field treatment enhanced the germination percentage in both 'Bingo II' (the low germination seed lot) and 'HB128', but had no effect on 'Bingo I' (the high germination seed lot). The electrical conductivity of 'Bingo I' and 'Bingo II' was significantly reduced by both hydropriming and electric field treatments. However, a slight increase in the electrical conductivity of 'HB128' was also observed.

Key words: hydropriming, electric field, germination, membrane permeability

INTRODUCTION

Seed priming involves a process that partly hydrates seeds to initiate the early events of germination and dries them back to stop the germination before redicle protrusion. Seed priming has been well documented to have beneficial effects on germination performance in various plant species (Welbaum *et al.*, 1998). A number of literature suggested that priming provides time and moisture for seeds to 'repair' damage from deteriorative events, e.g. mitochondrial dysfunction, enzyme inactivation, membrane perturbations and genetic damage incurring during storage and aging (McDonald, 2000). Nevertheless, the optimum treatment condition of priming is varietal dependent, and differs largely among seed lots of the same variety (McDonald, 2000). Moreover, priming is a labor costing practice in terms of large-scale seed treatment.

Another seed enhancement technique is the electric field (EF) treatment, which can be handled as simple as exposing seed to an EF with a predetermined exposure time and field strength (Moon and Chung, 2000). The EF treatment can be easily handled using conveyer belt (TYIDC, 2003), and is thus more convenient and practical than priming. EF treatments were reported to increase the germination percentage and speed in

[1] Vegetable Research Center, Guangxi Academy of Agricultural Sciences, Nanning 530007, China.
[2] Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
[3] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
[4] Department of Electrical Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: hrukui@excite.com

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soybean (Zhao *et al.*, 1995); tomato (Moon and Chung, 2000); and cucumber (Zhu *et al.*, 2000). Chiabrera and Bianco (1987) suggested that the EF treatment could cause seed invigoration by influencing the biochemical processes involving free radicals and antioxidative enzymes. However, more evidence is still needed to support this hypothesis. To provide a better understanding to the EF treatment on seed germination enhancement, this study attempted to investigate the optimum treatment conditions of both HP and EF for three cucumber seed lots with different initial seed quality, namely 'HB128', 'Bingo I' and 'Bingo II'. The effects on germination performance of these two treatments were compared. An examination on cellular membrane permeability before and after treatments by measuring seed electrolyte was also carried out.

MATERIALS AND METHODS

Seed materials

Three cucumber seed lots were obtained from Thai Seed & Agriculture Co. Ltd., Thailand with the initial germination percentage and moisture content as 84 %(99% viability) and 5.6% in 'HB128'; 94 % and 5.5 % in "Bingo I"; 61.5 % and 5.8 % in "Bingo II" respectively.

Hydropriming

Cucumber seeds were soaked for 30 min. in 0.5% carbendazim (methyl benzimidzol-2-ylcarbamate 50% w.p.) suspension for disinfection (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 meshes suspending over water in airtight plastic boxes (relative humidity ≈ 100%), and incubated at 25°C for varied durations of 1-day, 2-day, and 3-day. The incubated seeds were redried at ambient condition in the laboratory for two days to the seed moisture content ranged within 6% to 7%. The controls were the untreated dry seeds of each seed lot.

Electric field treatment

Seeds of three seed lots were exposed for 1 min., 3 min. and 5 min. to EF, with the field strength varied in the range of 1 kV/cm to 7 kV/cm, at intervals of 2 kV/cm (Zhu *et al.*, 2000).

The test cell of the electric field as shown in Figure 1 consisted of two horizontal electrodes (20×20 cm square copper plates; inter-electrode gap = 2 cm), connected to a fully adjustable high-voltage supply (0-20 kV, 50 Hz). The seeds were loaded one layer in a shallow polyethylene (transparent high-density polyethylene) tray with the cover of same material to avoid contact with the electrodes. No heating effect was noticed during the experiments, even when the maximum voltage was applied to the electrode system.

Germination test

Germination tests were carried out

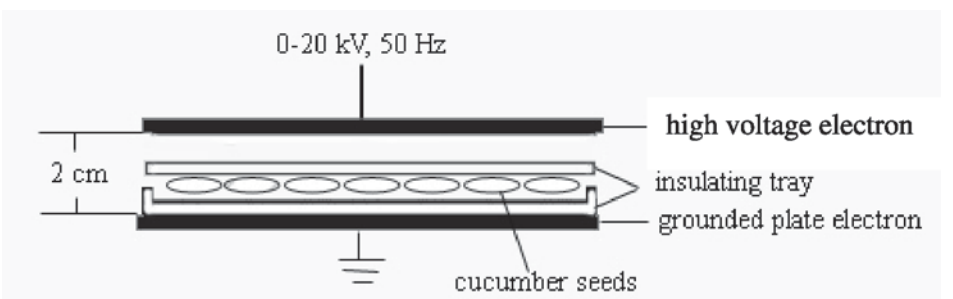

Figure 1 Schematic presentation of the experimental set up used to impose electric field treatments on cucumber seeds.

immediately after HP and EF treatments. Seeds were sown on top of moistened blotter paper in covered transparent polyethylene boxes (17×25 cm). Each box contained 25 seeds, and there were four replicated boxes per treatment. The boxes were placed in a germination chamber at 25°C (ISTA, 2003). Seeds with radicle protrusion to 4 mm were scored as germination; counts of the number of germinated seeds were made at 24 h intervals until no further germination was observed. Germination percentage was presented as the percentage of normal seedlings (ISTA, 2003). The mean germination time (MGT) was calculated from the formula proposed by Ellis and Roberts (1980):

$$MGT = \sum Ti.Ni/\sum Ni$$

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

Electrical conductivity test

The best conditions of HP and EF treatment for each seed lot obtained from the experiment were immediately tested on the electrical conductivity (EC). 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 shaken slowly by hand to ensure no seed adhered to the wall, and placed in a germination chamber at 20°C for 24 hours. Every jar contained 50 seeds, and there were four replicated 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 ($\mu S\ cm^{-1}g^{-1}$) = (recorded EC of the sample – EC of the blank)/ seed weight

Statistical analysis

Completely randomized design with four replications was used in this experiment. Data analyses were performed using Analysis of Variance (ANOVA) (SAS statistical software Version 6.12). Multiple comparison tests were performed by least significant difference test (LSD) at the level of $p< 0.05$.

RESULTS

Hydropriming

The germination percentages of both 'Bingo I' and 'Bingo II' were not affected by HP; while remarkable increases in that of 'HB128' after 2-day and 3-day incubation treatments were found (Figure 2 A). On the other hand, the mean germination time of these three seed lots were significantly reduced as compared to that of the control (Figure 2B). Therefore, the optimum duration of incubation for each seed lot was different: 3-day incubation brought the most pronounced effect to 'Bingo I' and 'Bingo II'; while 2-day incubation was sufficient to release dormancy in 'HB128'.

Electric field treatments

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' 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 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

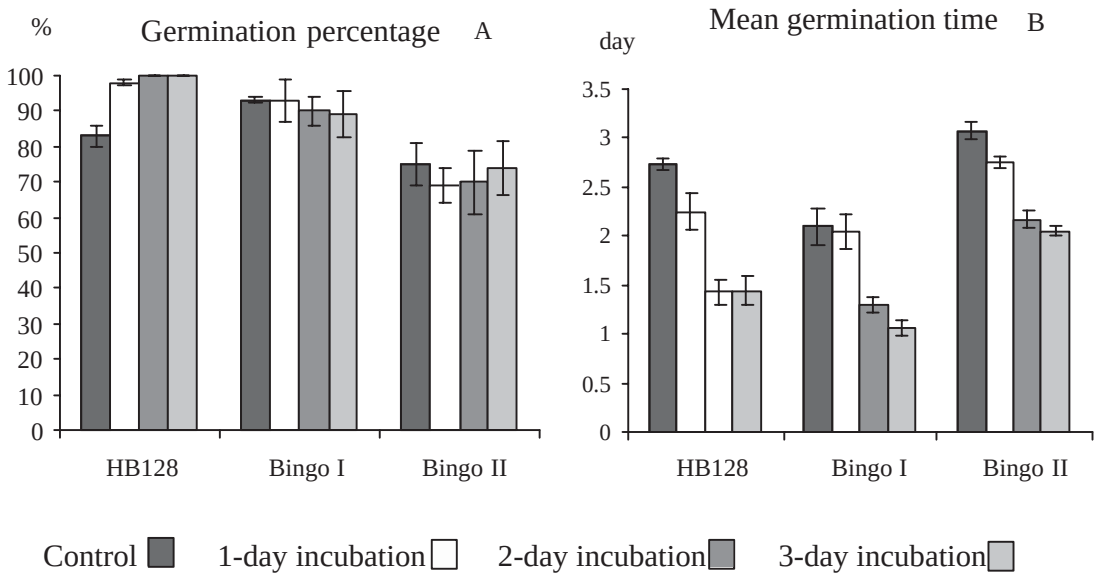

Figure 2 Changes in seed germination percentages (A) and mean germination time (B) after hydropriming (vertical bars indicate standard error).

treatments tended to increase (Table 1).

Electrical conductivity (EC)

Each seed lot was hydroprimed by incubating in saturated relative humidity for two days and redrying at ambient condition for two days (Figure 1) or electric field treated at 1KV/cm for 1 min. in "HB 128"; 5KV/cm for 3 min. in "Bingo I" and 3 KV/cm for 5 min. in "Bingo II" (Table 1).The EC of 'Bingo II' (the low germination seed lot) was higher than that of ' Bingo I' (the high germination seed lot), suggesting higher membrane leakage occurring in low vigour seed lot. Both HP and EF treatments reduced the EC of 'Bingo I' and 'Bingo II'; conversely, only slight increase in the EC of 'HB128' by both HP and EF treatments were found (Figure 3).

DISCUSSION

The most wildly accepted explanation of germination acceleration of priming 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).

Hydropriming and EF treatments with certain treatment condition could enhance germination performance and alter the membrane permeability of cucumber seeds, suggesting that both treatments have impact on subcellular level. Cellular membrane is considered 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 is 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

Table 1 Germination of cucumber seeds after electric field treatments.

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

Data presented as mean ± standard error; values within one column followed by different letters are significantly different ($p<$ 0.05).

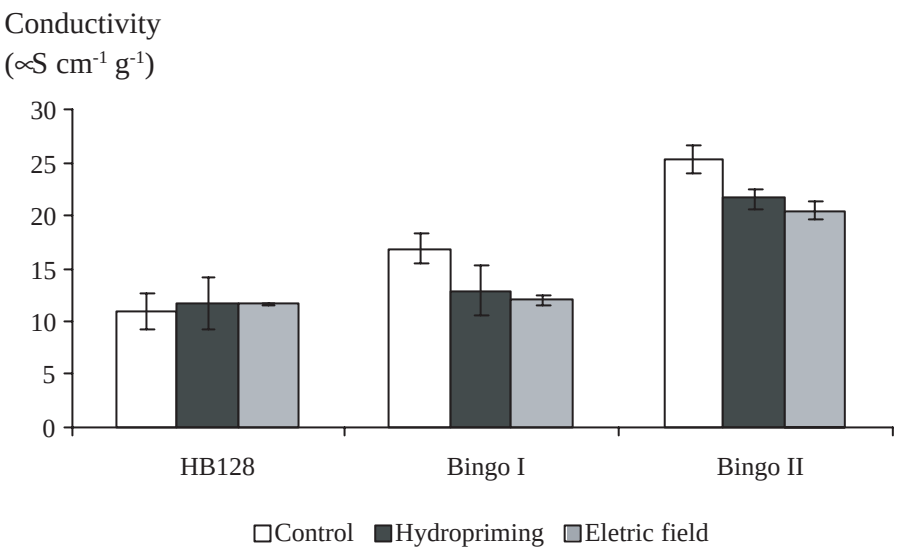

Figure 3 Changes in seed electrical conductivity after hydropriming and electric field treatments (vertical bars indicate standard error).

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.

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. Furthermore, priming is also observed to accelerate the degradation of the perisperm envelope-enclosing embryo (Aroonrungsikul, 2001), which may consequent easier air and water movement into the embryo, resulting in faster embryo growth.

EF treatments increased the germination percentage of 'Bingo II', but had no effect on 'Bingo I'. This agreed with a previous study of Zhu *et al.* (2000), in which greater benefit of EF treatment was obtained by the lower vigour seed lot of cucumber. EF treatments also reduced the electrical conductivity of 'Bingo I' and 'Bingo II', and slightly increased that of 'HB128'. However, the mechanism of EF treatment that enhanced seed germination was likely alternative to that of priming, since the hydration status of seed was not changed. In the view of physics, the explanation to such phenomenon might be attributed to the polarization of electric field as generally 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 phospholipid bilayer of membrane; the polarization may also induce reorientation of proteins and other complex macromolecules, contributing to the recovery of membrane functions, and consequently improve the semipermeability (Chen *et al.*, 2003).

Aside from the membrane permeability, other metabolic activities might also be influenced by hydropriming and electric field treatments, thus further study on the biochemical changes, such as enzyme and free radical activities, might provide better understanding on the mechanism of these two seed enhancement techniques.

CONCLUSION

The influences of hydropriming and electric field treatments on cucumber seed germination and seed membrane permeability were compared and the results indicated that both hydropriming and electric field treatments could improve cucumber seeds germination. However, the optimum conditions were seed lot dependent. Greater benefit was achieved in seed lots exhibiting low germination or dormancy. The effects of hydropriming and electric field treatments on seed performance were different. Hydropriming tended to increase the speed of germination, whereas electric field had the potential to increase the percentage of germination. Both hydropriming and electric field treatments could reduce electrolyte leakage in both high and low germination of 'Bingo' seed lots, and slightly increased that of the dormant seed lot of 'HB128'.

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