



Improved germination and vigour of Sweet Pepper (*Capsicum annuum* L.) seeds by hydro- and osmopriming

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ABSTRACT

The germination of sweet pepper (*Capsicum annuum* L.) seeds by hydropriming and osmopriming was investigated. Treatments comprised immersion in different polyethylene glycol (PEG6000) solutions ranging from -1.0 to -1.5 MPa, soaking in distilled water, and unprimed (control). Seeds were soaked for 7 and 14 days at 15±2 degree Celsius, and then seed quality was tested germination in the laboratory and the greenhouse. A completely randomized design was used with three replications. When the seeds were osmoprimed in -1.5 MPa for 14 days, the highest germination percentage, plant height, number of roots and root length were obtained at both of the conditions. Furthermore, there were no abnormal seedlings in this treatment. Thus, this method is the most suitable for farmers to increase the germination percentage, germination index, and homogenous emergence of sweet pepper seeds under a wide range of environments.

INTRODUCTION

The sweet pepper is a cultivar group within the well-known chili family (*Capsicum annuum* L.). It is widely used as a vegetable and contains several metabolites that are associated with enhancing human health, including phytonutrients such as vitamin C, vitamin A, and essential minerals. (Zhuang et al. 2012). The actual nutrient and phytonutrient content of sweet pepper is impressive and also given the very low-fat nature. In terms of conventional nutrients, sweet pepper is an excellent source of vitamin C at 117 milligrams per cup. (That's more than twice the amount of vitamin C found in a typical orange.) At the same time, this vegetable is a good source of antioxidant (carotenoids, flavonoids, carotenoids, and hydroxycinnamic acids) with important anti-cancer benefits. That why the pepper is the very popular vegetable in the kitchen (Devore et al. 2010). Thus, in this paper, we focus on treatment of priming to improve the sweet pepper seed quality. The availability of high quality seeds is an important factor in crop production, and quality is

very important in determining commercial seed value. Farmers use their knowledge and production techniques, coupled with the use of good seeds, to produce high crop yields. Good seed quality ensures high germination rates and strong plants, resulting in a large number of healthy seedlings with high survival rates. Seed quality can be evaluated by testing seed germination percentage and germination rate under both laboratory and field emergence conditions (Filho 2015). Thailand is an important location of global vegetable seed production, especially for members of the cucurbitaceae such as cucumber and watermelon, and of solanaceae including pepper and tomato (Khanobdee et al. 2007; Khanobdee 2009). There are many opportunities to study various seed improvement techniques given that there can often be low seed germination during crop production. The rapid deterioration of seed during storage is also a major problem that affects seed quality (Siri et al. 2013). Priming is one technique that can be used for enhancing seed quality and for improving overall germination and seed storage in a wide range of crop species (McDonald 2000). Priming is done before sowing to improve germination and reduce the time from sowing to emergence, while also improving uniformity of seedling emergence (Brocklehurst and Dearman 1983) and stand establishment (Gupta et al. 2008). Priming can be defined as: "controlling the hydration level within seeds so that the metabolic activity necessary for germination can occur but radical emergence is

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prevented. Different physiological activities within the seed occur at different moisture levels (Cramer et al. 1985).” Priming efficiency is affected by various factors including plant species, water potential, priming duration, temperature, vigor, dehydration, and primed seed storage conditions (Parera and Cantliffe 1994; Mubshar et al. 2006). Various kinds of priming methods include: i) hydropriming, the use of water to double the volume of the seed - this is a simple low-cost method of seed priming that requires no sophisticated equipment (Afzal et al. 2005; Basra et al. 2006; Foti et al. 2008); ii) halopriming, the use of salt solutions such as KNO₃ and NaCl; and iii) osmopriming, the use of osmotic solutions involving imbibition of seeds under controlled conditions.

Immersion of seeds in solutions of different osmotic potentials can initiate early events associated with germination, after which the seed is then dried back to its initial moisture content (Harris et al. 2001; Chiu et al. 2002). Polyethylene glycol (PEG) and sand matrix priming using moist sand are other techniques which can be used (Windauer et al. 2007; Maiti and Pramanik 2013). The aim of this study was to evaluate the beneficial effects of hydropriming and osmopriming on improving seed germination and the growth of sweet pepper seedlings.

MATERIALS AND METHODS

This experiment was conducted at the Department of Agriculture, Faculty of Technology, Maharakham University, Thailand between October 2014 and August 2015. Hybrid sweet pepper seeds (*Capsicum annuum* L.) were obtained from ETG Company, Khon Kaen, Thailand. The initial seed moisture content (MC) at 8 percentages in plastic bag and kept it in storage room condition [12±2 degree Celsius (°C) with 60 percentage of relative humidity (RH)] for one year.

In a preliminary study, seeds were treated with solutions containing different concentrations of Polyethylene glycol 6000 (PEG6000) for different time periods in the laboratory to identify the most appropriate treatments for further study. The water potential of the germination substrates (-1.0 and -1.5 MPa; 1 bar = 0.1 MPa) was determined using PEG6000 solution, prepared as described by Michel and Kaufman (Michel and Kaufmann, 1973). PEG6000 was dissolved in water and placed in a shaker bed (25 °C) for 16 h.

Before priming treatments, the seeds were surface sterilized in 10%clorox (sodium hypochlorite 8.25%) solution for one minute. After applying sterilization, the seeds were washed several times with distilled water. In the main study, seeds were primed using seven different

treatments: T1 = dried seeds; T2 = seeds treated with distilled water for 7 days; T3 = seeds treated with distilled water for 14 days; T4 = seeds treated with -1 MPa PEG6000 for 7 days; T5 = seeds treated with -1 MPa PEG6000 for 14 days; T6 = seeds treated with -1.5 MPa PEG6000 for 7 days; and T7 = seeds treated with -1.5 MPa PEG6000 for 14 days. Priming treatments were performed in an incubator at 15±2 °C under dark conditions. The treatments with PEG6000 solutions and distilled water were renewed every 48 h under sterile conditions to ensure relatively constant in the treatments.

After priming, seed samples were removed, rinsed three times in distilled water and then dried to the original moisture content.

Laboratory germination: three replicates of 50 seeds from each treatment were germinated on top of tree layers' moist paper within 12x12 cm closed plastic boxes (to avoid moisture loss). Seeds were germinated at 25±2 °C for 14 days. The number of germinated seeds was recorded daily.

Greenhouse germination: three replicates of 50 seeds from each treatment were sown in peat moss in a seed tray and then kept at 30±2 °C for 14 days in a greenhouse. The number of emergent seedling was recorded each day over the 14-day period.

Germination percentage (GP): GP was calculated as described by the International Seed Testing Association (ISTA 2008). The first count was at 5 days and then every day until the final count at 14 days and normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds were separately counted (Boonsiri et al. 2007).

Germination index (GI) or speed of germination: GI was calculated by the following formula (Ellis and Roberts, 1981):

$$GI = \left(\frac{\text{No. of normal seedlings}}{\text{days to first count}} + \dots + \frac{\text{No. of normal seedlings}}{\text{days of final count}} \right)$$

The initial growth of sweet pepper seeds grown in laboratory were recorded: shoot height, root number and root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. At the end of the germination test (i.e., after 14 days in the laboratory and 14 days in greenhouse), radicles and shoots were cut from the cotyledons and then dried at 60 °C for 48 h. The mean radicle and shoot dry weights, and mean seedling dry weights, were determined.

Note: Shoot height (seedling length) is the vertical distance from the ground line to the tip of the terminal leader. The ground line is obvious in the nursery bed but must be established on harvested stock by close observation. Nurseries

measure height either 1 cm above the uppermost lateral root (Hodgson and Donald 1980).

The experiment was a completely randomized design with three replications. Data were statistically analyzed by ANOVA and the significance of the differences between means at $p < 0.01$ was estimated by Duncan's new multiple range test (DMRT).

RESULTS AND DISCUSSION

The preliminary screening results showed significant effects hydropriming and osmopriming on GP, GI, and seedling growth (including shoot height, root number, and root length) compared with the unprimed controls. The highest GP (89.33%) and GI (11.33) in the laboratory were seeds osmoprimed with -1.5 MPa PEG6000 for 14 days but it did not had statistically significant with -1.5 MPa PEG6000 for 7 days and -1.0 MPa PEG6000 for 14 days seed osmoprimed. The results of this study provided the highest initial growth of sweet pepper seedling were osmoprimed with -1.5 MPa PEG6000 for 14 days: the highest shoot height (2.37 cm), root number (4.67 root/plant), and root length (3.93 cm) were significant differences with other treatments, However, the effects on seedling fresh weight and dry weight were not significant (Table 1).

Seed storage can cause a decrease in protein content which may be related to oxidation of amino

acids due to an increase in respiratory activity and the advance in deterioration of the stored seeds (Manomani et al. 2014). Distilled water has zero osmotic potential and some seeds showed lower GP in those treatments. It is believed that the decrease in GP results from decreased water potential and decreased seed accessibility to water (Rdhan and Yanah 1982).

The osmotic potential that is used for osmopriming is lower than the critical potential required to complete the initial stages of germination prior to the protrusion of radicle (Dezfuli et al. 2008). Osmopriming can activate processes related to germination by affecting oxidative metabolism and by increasing the activities of superoxide dismutase and peroxidase (Jie et al. 2002) or by activating ATPases as well as acid phosphatase and RNA synthesis (Fu et al. 1988).

In the main study, seeds were osmoprimed with -1.5 MPa PEG6000 for either 7 or 14 days, compared with unprimed and hydroprimed seeds. In both the laboratory and greenhouse germination tests, osmoprimed seeds had significantly higher values for GP, GI and seedling growth (shoot height, root number, and root length) but not for fresh weight and dry weight where there were no significant differences amongst the treatments (Table 2). GP for seed osmoprimed with -1.5 MPa PEG6000 for 14 days was higher and faster than

Table 1. Comparison of priming methods on germination and initial growth of sweet pepper seeds grown for 14 days in laboratory.

| Treatments | GP (%) | GI | Shoot height (cm) | Root no. (root/plant) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) |
|---|---------|--------|-------------------|-----------------------|------------------|------------------------|-----------------------|----------------------|---------------------|
| 1. Unprimed (control) | 77.33c | 6.67b | 1.27e | 2.67c | 2.77c | 0.073 | 0.072 | 0.053 | 0.052 |
| 2. Hydropriming (distilled water for 7 days) | 77.33c | 6.67b | 1.33e | 3.00bc | 2.73d | 0.073 | 0.072 | 0.053 | 0.052 |
| 3. Hydropriming (distilled water for 14 days) | 78.33c | 8.67a | 1.37de | 3.00bc | 2.73d | 0.074 | 0.073 | 0.054 | 0.052 |
| 4. Osmopriming (-1.0 MPa PEG6000 for 7 days) | 80.00b | 7.33b | 1.47d | 3.00bc | 2.87bc | 0.074 | 0.073 | 0.053 | 0.052 |
| 5. Osmopriming (-1.0 MPa PEG6000 for 14 days) | 84.00ab | 10.00a | 1.67c | 3.00bc | 2.97bc | 0.074 | 0.073 | 0.054 | 0.052 |
| 6. Osmopriming (-1.5 MPa PEG6000 for 7 days) | 86.67a | 10.67a | 1.93b | 3.33b | 3.10b | 0.075 | 0.073 | 0.054 | 0.053 |
| 7. Osmopriming (-1.5 MPa PEG6000 for 14 days) | 89.33a | 11.33a | 2.37a | 4.67a | 3.93a | 0.075 | 0.074 | 0.055 | 0.053 |
| F-test | ** | ** | ** | ** | ** | ns | ns | ns | ns |
| CV (%) | 3.63 | 2.35 | 3.55 | 11.67 | 2.80 | 0.11 | 0.12 | 0.23 | 0.13 |

ns= non significant, ** = significant difference at $p < 0.01$, respectively. Means within a column followed by the same letter do not differ significantly according to DMRT. GP = Germination percentage, GI = Germination index.

Table 2. Comparison of priming treatments on seed of sweet pepper seeds subsequently grown for 14 days in either the laboratory of the greenhouse.

| Treatments | GP (%) | GI | Shoot height (cm) | Root no. (root/plant) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) |
|---|---------|--------|-------------------|-----------------------|------------------|------------------------|-----------------------|----------------------|---------------------|
| Laboratory conditions | | | | | | | | | |
| 1. Unprimed (control) | 85.33c | 7.33d | 1.53d | 2.67d | 2.07e | 0.0406 | 0.0204 | 0.0034 | 0.0020 |
| 2. Hydropriming (distilled water for 7 days) | 86.67bc | 9.00cd | 1.60d | 3.00cd | 2.23d | 0.0406 | 0.0204 | 0.0034 | 0.0023 |
| 3. Hydropriming (distilled water for 14 days) | 88.67b | 10.33c | 1.83c | 3.68c | 2.56c | 0.0408 | 0.0205 | 0.0035 | 0.0023 |
| 4. Osmopriming (-1.5 MPa PEG6000 for 7 days) | 88.00bc | 13.00b | 2.17b | 4.33b | 2.86b | 0.0409 | 0.0205 | 0.0036 | 0.0024 |
| 5. Osmopriming (-1.5 MPa PEG6000 for 14 days) | 93.33a | 16.67a | 2.43a | 5.33a | 3.17a | 0.0409 | 0.0205 | 0.0036 | 0.0024 |
| F-test | ** | ** | ** | ** | ** | ns | ns | ns | ns |
| CV (%) | 1.85 | 1.23 | 5.40 | 13.59 | 3.32 | 1.97 | 0.20 | 2.21 | 1.48 |
| Greenhouse conditions | | | | | | | | | |
| 1. Unprimed (control) | 78.67c | 4.33d | 4.43d | 4.33d | 3.70d | 0.0810 | 0.0409 | 0.0052 | 0.0043 |
| 2. Hydropriming (distilled water for 7 days) | 80.00c | 5.33cd | 4.86cd | 5.66c | 3.73d | 0.0811 | 0.0409 | 0.0053 | 0.0043 |
| 3. Hydropriming (distilled water For 14 days) | 88.00b | 5.66c | 5.13c | 6.33bc | 4.53c | 0.0811 | 0.0409 | 0.0053 | 0.0043 |
| 4. Osmopriming (-1.5 MPa PEG6000 for 7 days) | 89.33b | 7.67b | 5.76b | 7.33b | 5.03b | 0.0814 | 0.0412 | 0.0055 | 0.0044 |
| 5. Osmopriming (-1.5 MPa PEG6000 for 14 days) | 94.67a | 10.33a | 6.80a | 8.33a | 5.33a | 0.0820 | 0.0415 | 0.0057 | 0.0046 |
| F-test | ** | ** | ** | ** | ** | ns | ns | ns | ns |
| CV (%) | 2.08 | 0.67 | 5.51 | 11.41 | 2.52 | 0.19 | 0.31 | 1.86 | 2.00 |

ns= non significant, ** = significant difference at $p < 0.01$, respectively. Means within a column followed by the same letter do not differ significantly according to DMRT. GP = Germination percentage, GI = Germination index

that in the other primed and unprimed seed treatments.

Osmopriming of seeds has been shown to promote germination by repair of damaged proteins, RNA and DNA (Koehler et al. 1997) and can reduce the damage caused by aging in a number of crops (Farooq et al. 2009). Davison and

Bray (1991) observed that five polypeptides were synthesized in the embryonic tissue of leek seeds after priming in -1.0 MPa PEG solution. Lanteri et al. (1994) found that osmopriming of pepper and tomato seed in -1.1, -1.3, and -1.5 MPa PEG for 14 days reduced the mean time of germination because two amino acids were incorporated in proteins

during the first 24 h of imbibition of sweet pepper seeds in PEG solution (Khan 1992).

CONCLUSION

This study demonstrated that different priming techniques can enhance the germination of sweet pepper seeds. Osmoprimed seeds germinated better than hydroprimed seeds and seeds osmoprimed with -1.5 MPa PEG6000 for 14 days increased in germination percentage and induced germination index. The osmoprimed seeds had more seedling growth (shoot height, root number, and root length) than other seed treatments. The sweet pepper seeds growing from this treatment will, therefore, establish more rapidly and be less susceptible to a range of stresses.

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