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# ASSESSMENT OF GROWTH REGULATOR PROHEXADIONE CALCIUM AS PRIMING AGENT FOR GERMINATION ENHANCEMENT OF PEPPER AT LOW TEMPERATURE

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## ABSTRACT

Priming is considered a promising technique to speed up germination and to improve seed performance under unfavorable conditions. The effects of incorporating Prohexadione-Calcium (Pro-Ca) into priming solutions on low temperature germination and emergence performances of sweet pepper seeds were investigated. Seeds were primed in 3% $KNO_3$, 2% $KH_2PO_4$, and 10% PEG solutions containing 0, 25, 50, and 100 mg.$L^{-1}$Pro-Ca in darkness at 25°C for three days. The total number of treatments was 12. After priming treatment, the seeds were washed with distilled water and dried at 20°C on filter paper for 24 h, and then subjected to germination and emergence tests at 15°Cingrowth chamber. Priming pepper seeds in the presence or absence of plant growth regulators improved final germination percentage (FGP), mean time to germination time (MTG), and germination index (GI), final emergence percentage (FEP), and mean time to emergence (MTE) compared to nonprimed seeds. Priming seeds in $KH_2PO_4$ solution containing 25 mg.$L^{-1}$ Pro-Ca resulted in the highest FGP (85.33%), and the lowest MTG (12.83 days) at 15°C. The highest FEP (95%) was obtained from the application of $KNO_3$ + 50 mg.$L^{-1}$ Pro-Ca treatment. Final emergence percentage was 64% in nonprimed seeds. The improvements by Pro-Ca might be related to the alterations of some ingredients of seeds, such as proline content, total carbohydrates and total soluble sugars. The results indicated that inclusion of Pro-Ca into the priming solutions can be used as an effective method to improve performance of sweet pepper seeds at low temperature.

**Keywords:** Pepper, priming, Prohexadione-Calcium, germination, emergence.

## INTRODUCTION

Seed germination is a critical step in the plant life, and is regulated by many factors. Temperature, especially low temperature, is one of the most important limiting factors in the germination of vegetable seeds. Peppers have a prolonged germination period and an optimum germination temperature of about 30°C. The rate of germination and emergence is markedly reduced at temperatures in the range of 15-20°C. Hastening the germination and emergence of pepper seeds, especially at suboptimal temperatures, would be of significant value in the production of greenhouse-raised plants (Bosland and Votava, 2000). Investigations of techniques to improve pepper germination and emergence are numerous and somewhat conflicting. One of the techniques used to accelerate the germination and seedling emergence is seed priming. This technique consists of seed hydration in a solution whose osmotic potential is sufficient to permit initial germination events, but not enough for radicle protrusion (Bradford, 1986). At the conclusion of the priming process, seeds are used immediately or re-dried to their storage moisture levels to store and use in future. Since important pre-germinative steps such as DNA and RNA synthesis are already accomplished in the seed, primed seeds are physiologically closer to germination than unprimed seeds after planting (McDonald, 2000).

Priming enables the seed to germinate and emerge faster at suboptimal temperatures (Bosland and Votava, 2000). Various priming treatments have been developed to increase the speed and synchrony of seed germination. Common priming techniques include hydropriming (soaking seed in water), osmopriming (soaking seed in osmotic solutions such as PEG), halopriming (soaking seed in salt solutions), and priming with plant growth hormones (Tian *et al.*, 2014).

Beneficial effects from priming have been reported for several vegetable seeds included pepper (Gomes *et al.*, 2012). Priming pepper seeds in PEG and $KNO_3$ solutions significantly increased emergence percentage and decreased mean time to emergence of pepper seedlings (El-Shatoury, 2010). The results of Kaya *et al.* (2010) showed that priming pepper seeds can increase germination percentage and accelerate germination at suboptimal temperatures.

Incorporation of plant growth regulators during pre-soaking and priming treatments have improved seed germination and emergence performances in many crops including peppers (Korkmaz and Korkmaz, 2009). Korkmaz (2005) reported that inclusion of acetyl salicylic acid and methyl jasmonate into the priming solution improved low-temperature germination and emergence of sweet pepper. In the same way, Tiryaki and Buyukcingil (2009) found that inclusion of 50µM benzyl adenine into priming media further enhanced germination of sorghum (*Sorghum bicolor* L. Moench) seeds at low temperature.

Prohexadione-calcium (Pro-Ca) is a plant growth regulator that inhibits the biosynthesis of gibberellin resulting in reduced internode length and vegetative growth (Kim *et al*., 2007). Pro-Ca as a bioregulator affects plant metabolism such as hormonal balance. In apple, the alteration of flavonoid biosynthesis leads to the accumulation of luteoforol, a novel molecule with phytoalexin activity responsible for increased resistance against pathogens (Spinelli *et al*.,2005). This compound is under actual development for use as a growth retardant in different crops. Recently, Aghdam (2013) reported that the treatment of tomato fruit with Pro-Ca mitigated chilling injury. To the best of our knowledge, there is no information available on the use of Pro-Ca as a priming agent on enhancing pepper seed germination and emergence at low temperatures.

The objective of this study was to investigate if incorporation of Pro-Ca into priming solution would improve sweet pepper seed germination and emergence at low temperature (15°C).

## MATERIALS AND METHODS

This study was conducted during 2014 at the Vegetable Physiology Laboratory, Department of Horticulture, Faculty of Agriculture, University of Bingol, Turkey. Pepper (*Capsicum annuum*L.) seeds of 'BT InceSivri Tatli-016' (Bursa Seed Company, Turkey) were used in the experiments. The initial seed moisture was 8 % (dry weight basis). The standard germination test was conducted on the seeds and their initial germination percentage was determined as 95%.

**Priming and Pro-Ca Treatments:** Based on results of the preliminary experiments, 3% $KNO_3$, %2 $KH_2PO_4$, and 10% polyethylene glycol 8000 (PEG) were chosen as priming agents. The priming agents were supplemented with 0, 25, 50, or 100 $mg.L^{-1}$ Pro-Ca (Regalis, BASF 125 10W containing 10% Prohexadione-Ca as the active ingredient). For all the treatments, the seeds were surface disinfested in 1% (active ingredient) sodium hypochlorite for 15 min to eliminate seed-borne microorganisms. Following disinfestations, they were rinsed under running tap water for 1 min and surface dried by placing them between sterile paper towels for 30 min at room temperature. Priming was accomplished by imbibing pepper seeds for 3 d at 25 °C in darkness in 3% $KNO_3$, %2 $KH_2PO_4$, and 10% PEG containing 0, 25, 50, or 100 $mg.L^{-1}$ Pro-Ca. The seeds were placed in covered transparent polystyrene germination boxes (10 × 10 × 4 cm) on double layers of filter paper (Whatman #1) saturated with 10 mL priming solution. Following priming, the seeds from each box were washed in a sieve and rinsed under running tap water for 1 min to remove adhering priming chemicals and left to surface dry on filter papers placed in petri plates under room conditions (20 °C and 45% relative humidity) for 24 h. Untreated dry seeds were taken as control.

**Germination and Emergence Studies in Laboratory:** Germination tests were carried out in an instrumentation specialties model growth chamber (Model ICE 256, Memmert, Germany) at 15°C in the dark. Seeds were placed on two layers of filter paper moistened with 2 mL of distilled water in sterile petri plates (60mm x 15mm). Treatments were arranged in a completely randomized design with four replications of 25 seeds. The filter papers were moistened with distilled water as needed. The numbers of seeds germinated (having radicle protrusion to 2 mm) were recorded daily until no further germination occurred and remaining seeds began to deteriorate (24 d). Germinated seeds were discarded. From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation (arcsine√FGP), mean time to germination (MTG), days between 10% and 90% of FGP ($G_{10-90}$),andgermination index (GI) were calculated (Ellis and Roberts 1981).

For emergence test, seeds were primed as described above and 25 seeds from each treatment were planted into 1.5 cm depth in 7 × 5 cm (diameter × height) round plastic cups filled with growth medium consisting of peat and per liter in the ratio of 4:1. The cups were watered and placed in the same growth chamber used in germination test. Seedling emergence was counted daily for 32 days with seedlings recorded as emerged when the hypocotyls appeared on or above the surface of the growing medium. Final emergence percentage (FEP) and its angular transformation (arcsine√FEP), mean time to emergence (MTE), days between 10% and 90% of FEP ($E_{10–90}$), and emergence index (EI) were calculated.

**Experimental Design and Statistical Analysis:** The experiment was arranged according to completely randomized design with four replicates, each replicate having 25 seeds. The entire experiment was repeated twice. Differences between the two experiments were non-significant, so the data were combined into a single data set. Data from all experiments were subjected to analysis of variance using the PROC GLM procedure of the SAS package 9.1 (SAS Institute, Cary, NC, USA). Mean separation was performed by Fisher's least significant difference (LSD) test if F test was significant at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Seed Germination at Low Temperature:** Different priming treatments, regardless of adding plant growth regulator to the priming solutions, significantly improved germination at 15°C compared to nonprimed seeds which had an FGP of 69% (Table 1). Inclusion of 25 and 50 $mg.L^{-1}$ Pro-Ca into the $KNO_3$ solution resulted in the

highest FGP (85%, 84%, respectively); however, increasing the concentration of Pro-Ca from 50 to 100 $mg.L^{-1}$ significantly reduced FGP of pepper seeds. Even though all priming treatments improved mean time to germination (MTG) of pepper seeds compared to the nonprimed seeds (MTG = 16.57 d), the fastest germination rate was obtained from the seeds primed in 25 and 0 $mg.L^{-1}$ Pro-Ca (MTG = 12.83 and 13.40 d, respectively) (Table 1). Among the priming treatments, priming in the presence of 0 mg $L^{-1}$ Pro-Ca ($G_{10–90}$ = 5 d) and 25 mg $L^{-1}$ Pro-Ca ($G_{10–90}$ = 3.67 d) were the only treatments that improved the germination synchrony compared to nonprimed seeds ($G_{10–90}$ = 7.67 d). Germination of seeds primed in $KH_2PO_4$ supplemented with 100 mg $L^{-1}$ Pro-Ca was the least synchronous ($G_{10-90}$ = 9.95 d) (Table 1). Pro-Ca concentrations (except for 100 $mg.L^{-1}$) significantly increased germination index (GI) compared to nonprimed seeds. The highest GI (1.57) was obtained from the seeds primed in $KH_2PO_4$ supplemented with 25 $mg.L^{-1}$ Pro-Ca (Table 1). Nascimento (2005) also reported primed pepper seeds had higher germination compared to unprimed seeds at low temperatures. Hence, results of present investigation are confirmatory to earlier findings.

**Seedling Emergence at Low Temperature:** The effects of priming treatments on FEP of pepper seedlings were found significant (Table 2). Priming supplemented with 25 and 50 $mg.L^{-1}$ Pro-Ca improved FEP of pepper seedlings at low temperature compared to nonprimed seeds which had an FEP of 64%. The highest FEP was obtained from the seeds primed in $KNO_3$ with 50 $mg.L^{-1}$ and PEG with 50 $mg.L^{-1}$ Pro-Ca (95% and 89%, respectively). All priming treatments improved mean time to emergence (MTE) of pepper seedlings by up to 33% compared to the nonprimed seeds (MTE = 26 d). On the other hand, all priming treatments failed to improve the emergence synchrony of pepper seedlings compared to nonprimed seedlings (Table 2). Pro-Ca concentrations (except for 100 $mg.L^{-1}$) significantly increased emergence index (EI) compared to nonprimed seeds. Interaction between priming agents and Pro-Ca concentrations had significant effect on EI (Table 2). The highest EI was obtained from the seeds primed in $KH_2PO_4$ supplemented with 25 $mg.L^{-1}$ Pro-Ca and $KNO_3$ with 50 $mg.L^{-1}$ Pro-Ca (1.25, 1.17, respectively). On the other hand, the effect of priming on low temperature performance of pepper seeds has ranged from no improvement to some advancement in germination and emergence percentages and rates. For example, field emergence percentages of pepper generally were unaffected by priming (Bradford *et al*., 1990) while some of the field emergence trials found little response to seed priming in pepper (Yaklich and Orzolek, 1977). Thus, addition of growth regulator Pro-Ca affected the process of germination and emergence positively at low temperature.

The simple correlation analysis showed that there were numerous significant correlations among variables used in this study (Table 3). The data showed highly positive correlation between FGP and GI (r = 0.85). Similarly, FEP exhibited highly positive correlation with the EI (r = 0.85). The MGT was also positively correlated with MET (r = 0.75). Similar associations between MTG and MET have been seen in previous studies (Bradford *et al*., 1990, Demir *et al*., 2008). On the other hand, results of current study revealed negative correlations between MGT and GI, also between MET and EI (r = -0.75 and -0.81, respectively). These findings are in line with those of Kausar *et al*. (2009) who have reported that strong negative correlation was found between MGT and GI of primed sunflower seeds.

The present study has investigated if incorporation of Prohexadione-Calcium (Pro-Ca) into priming solution would improve sweet pepper seed germination and emergence at low temperature (15°C). Priming supplemented with Pro-Ca solutions lead to more and faster germination than priming in the assessed osmotica alone in general. The beneficial effects of priming solution on low-temperature germination of pepper seeds found in this study confirm the findings of Korkmaz (2005), who reported that priming treatments significantly improved FGP and germination rate of pepper seeds at low temperature. Similar results have also been obtained with other vegetable species, such as tomato (Pill *et al*., 1991), watermelon (Korkmaz*et al*., 2004), muskmelon (Nascimento and Argão, 2004), asparagus (Bittencourt *et al*.,2005), carrot (Pereira *et al*., 2009), and chicory (Tzortzakis, 2009).

Kikuti *et al*., (2005) reported that sweet pepper seeds primed with PEG 6000 performed better in all the vigor tests assessed, except the germination test. Similar results have also been reported with other species of the same family, such as eggplant (Fanan and Novembre, 2007).

Our results showed that all priming treatments improved FEP and MTE of pepper seedlings (by up to 33% and 19%, respectively) compared to the nonprimed seeds. The increment in seedling performance due to seed priming treatment is in conformity with other researchers (Korkmaz *et al*., 2005; Amjad *et al*., 2007). The seed lot having greater germination and emergence index is considered to be more vigorous. The enhanced germination and emergence index found in present study agree with the findings of Ruan *et al*. (2002), who demonstrated that priming the rice seed had improved results for germination index.

Priming solutions combined with growth regulators have improved germination under stress conditions (Parera and Cantliffe, 1994). Various plant

growth regulators such as jasmonic acid (Korkmaz*et al*., 2004), polyamines (Farooq *et al*., 2008), and salicylic acid (Zhang *et al*., 2011) have also been associated with priming in order to accelerate their effects on germination and seedling growth, particularly under adverse emergence conditions. Inclusion of Pro-Ca into priming solutions further improved germination and emergence performance of pepper seedlings in our study. These findings are corroborating with the results obtained in other studies (Tiryaki, 2006). Zhang *et al*. (2011) reported that priming seeds in 3% $KNO_3$ solution supplement with 0.1 mMsalicylicacid resulted in the best priming effect compared with other priming treatments and non-priming treatment. Korkmaz *et al*. (2005) observed greater germination and emergence of muskmelon seeds at 15 °C, when seeds are primed in a solution of $KNO_3$ and methyl jasmonate. In a similar way, Tiryaki (2006) reported that inclusion of methyl jasmonate, ACC, BAP, or spermine into priming solution further improved germination of amaranth seeds. Primed seeds of pepper might have better water absorption from the growing media that enables faster metabolic activities in seeds and leads to better germination and emergence. Since primed seeds have completed phase I (hydration) and II (lag phase) of germination, they only require a favorable water potential gradient for water uptake to begin radicle growth (Pill, 1995).

Priming seed with inorganic salts may significantly alter activity of enzymes in germinating seed. Activity of certain preformed enzymes such as dehydrogenase and α-amylase are positively correlated with seed vigor. Singh *et al*., (1999) reported dehydrogenase and α-amylase were increased in the seeds of muskmelon primed with PEG and $KNO_3$ indicating the positive effect of priming in terms of enhancing seed performance at low temperature. Increased α-amylase activity and sugar contents were also reported in the primed fine rice seeds (Basra *et al*., 2005).

As previously mentioned, incorporation of Pro-Ca into priming solutions further improved germination and emergence performances of pepper seedlings in the current study. Pro-Ca as a bioregulator affects plant metabolism such as hormonal balance. The improvements by Pro-Ca may be related to the alterations of some ingredients of seeds. It has been reported that Pro-Ca increased proline content, total carbohydrates and total soluble sugars in faba bean (Bekheta *et al*., 2009). Many plants accumulate proline as a protective osmolyte under stress conditions, such as low temperature (Aloui *et al*., 2014). Proline protects plants by functioning as a cellular osmotic regulator between cytoplasm and vacuole, and by detoxifying of reactive oxygen species (ROS), thus protecting membrane integrity and stabilizing antioxidant enzymes (Bohnert and Jensen, 1996). Positive correlations between the accumulation of endogenous proline and improved cold tolerance have been found mostly in chilling-sensitive plants (Zhao *et al*., 2009). Recently, Aghdam (2013) suggested that the growth retardant Pro-Ca might mitigate chilling injury on tomato fruits by inhibiting phospholipase D (PLD) and lipoxygenase (LOX) activities and by enhancing proline content and membrane integrity. A low temperature can also increase flavonoid production (Jaakola and Hohtola, 2010). Pro-Ca also blocks flavanone 3-hydroxylase, thereby inducing a completely new spectrum of flavonoids and other phenolic plant constituents (Rademacher, 2000). It has been reported that the application of Pro-Ca also induced alteration of the flavonoid metabolism in the plant tissue compared to the control (Halbwirth *et al*., 2002). Positive effects of Pro-Ca in our study can also possibly be explained by an alteration of flavonoids and other phenolic compounds as affected by the Pro-Ca treatment, which may reduce low temperature stress by scavenging ROS.

In summary, priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. The addition of Pro-Ca into the priming solution significantly improved sweet pepper germination and emergence at low temperatures compared to uprimed seeds and the seeds primed in $KNO_3$, $KH_2PO_4$, and PEG only. These beneficial effects of priming supplemented with Pro-Ca are more evident in the lower concentrations of the growth retardant. Priming with the inclusion of Pro-Ca may be an effective way to increase germination and emergence percentages and therefore improve stand establishment in pepper at low temperatures. Rapid seedling establishment might minimize crop risk due to adverse environmental conditions or insect and disease problems during field emergence.

**Table 1. Final germination percentage [FGP and angular transformation (in brackets)], mean time to germination (MTG), days between 10% and 90% of FGP ($G_{10-90}$), and germination index (GI) of pepper seeds at 15°C as influenced by priming treatments**

| Priming treatments | | FGP | MTG | $G_{10-90}$ | GI |
|---|---|---|---|---|---|
| **Priming Agent** | **Pro-Ca (mg $L^{-1}$)** | **(%)** | **(days)** | **(days)** | |
| $KNO_3$ | 0 | 79 [63] | 14.54 | 7.67 | 1.40 |
| | 25 | 85 [67] | 14.91 | 6.67 | 1.47 |
| | 50 | 84 [66] | 15.06 | 9.67 | 1.36 |
| | 100 | 65 [54] | 15.19 | 9.00 | 1.12 |

| | | | | | |
|---|---|---|---|---|---|
| $KH_2PO_4$ | 0 | 75 [60] | 13.40 | 5.00 | 1.43 |
| | 25 | 79 [63] | 12.83 | 3.67 | 1.57 |
| | 50 | 76 [61] | 14.38 | 8.00 | 1.39 |
| | 100 | 57 [49] | 15.20 | 9.95 | 1.00 |
| PEG | 0 | 72 [58] | 15.10 | 8.67 | 1.24 |
| | 25 | 77 [61] | 13.96 | 9.33 | 1.44 |
| | 50 | 77 [61] | 14.05 | 8.00 | 1.43 |
| | 100 | 63 [53] | 15.12 | 8.67 | 1.07 |
| Nonprimed seeds | | 69 [56] | 16.57 | 7.67 | 1.07 |
| $LSD_{0.05}$ (two-way interaction) | | 5.34 | 1.02 | 1.61 | 0.17 |
| Significance | | | | | |
| Priming agent (PA) | | *** | *** | *** | *** |
| Pro-CA | | *** | *** | ** | *** |
| PA*Pro-CA | | * | * | * | *** |

*significant at P≤0.05, ** significant at P≤0.01, *** significant at P≤0.001

**Table 2. Final emergence percentage [FEP and angular transformation (in brackets)], mean time to emergence (MTE), days between 10% and 90% of FEP ($E_{10-90}$), and emergence index (EI) of pepper seeds at 15°C as influenced by priming treatments**

| Priming treatments | | FEP | MTE | $E_{10-90}$ | EI |
|---|---|---|---|---|---|
| **Priming Agent** | **Pro-Ca (mg $L^{-1}$)** | **(%)** | **(days)** | **(days)** | |
| $KNO_3$ | 0 | 65 [54] | 19.57 | 12.33 | 0.89 |
| | 25 | 83 [66] | 20.35 | 14.00 | 1.08 |
| | 50 | 95 [77] | 21.50 | 13.67 | 1.17 |
| | 100 | 76 [61] | 22.23 | 11.00 | 0.88 |
| $KH_2PO_4$ | 0 | 76 [61] | 19.72 | 11.33 | 1.00 |
| | 25 | 85 [67] | 17.92 | 10.00 | 1.25 |
| | 50 | 68 [56] | 21.20 | 11.67 | 0.85 |
| | 100 | 61 [51] | 22.29 | 11.00 | 0.72 |
| PEG | 0 | 65 [54] | 23.17 | 11.00 | 0.73 |
| | 25 | 73 [59] | 22.50 | 11.67 | 0.84 |
| | 50 | 89 [71] | 21.78 | 11.67 | 1.08 |
| | 100 | 63 [53] | 22.47 | 10.00 | 0.72 |
| Nonprimed seeds | | 64 [53] | 26.00 | 9.00 | 0.62 |
| $LSD_{0.05}$ (two-way interaction) | | 7.67 | 2.15 | 2.22 | 0.16 |
| Significance | | | | | |
| Priming agent (PA) | | *** | *** | *** | *** |
| Pro-CA | | *** | * | NS | *** |
| PA*Pro-CA | | *** | * | NS | *** |

NS Non-significant, * significant at P≤0.05, *** significant at P≤0.001

**Table 3. Pearsoncorrelationcoefficients of thegerminationandemergenceproperties**

| | FGP | MGT | $G_{1090}$ | GI | FEP | MET | $E_{1090}$ | EI |
|---|---|---|---|---|---|---|---|---|
| **FGP** | 1.00 | | | | | | | |
| **MGT** | -0.34* | 1.00 | | | | | | |
| **$G_{1090}$** | -0.28 | 0.44** | 1.00 | | | | | |
| **GI** | 0.85*** | -0.75*** | -0.42** | 1.00 | | | | |
| **FEP** | 0.58*** | -0.29* | -0.15 | 0.57*** | 1.00 | | | |
| **MET** | -0.37** | 0.73*** | 0.29* | -0.64*** | -0.45** | 1.00 | | |
| **$E_{1090}$** | 0.41** | -0.26 | 0.11 | 0.45** | 0.50*** | -0.48*** | 1.00 | |
| **EI** | 0.61*** | -0.58*** | -0.32* | 0.74*** | 0.85*** | -0.81*** | 0.56*** | 1.00 |

Numbers followed by * significant at P≤0.05, ** significant at P≤0.01, *** significant at P≤0.001

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