

Enhancement of Germination and Emergence of Hot Pepper Seeds by Priming with Acetyl Salicylic Acid

ELISA M. AGONCILLO
Agriculture and Allied Courses Department, Mindoro State College of Agriculture and Technology-Main Campus, Alcate, Victoria, Oriental Mindoro, Philippines

Abstract
The effect of priming using priming solution supplemented with acetyl salicylic acid was investigated in terms of germination and seedling emergence performance of hot pepper (*Capsicum frutescens* var. Exotica) seeds. Seeds were primed in priming solution with KNO_3 only, KNO_3 + 0.1mM ASA, KNO_3 + 0.5 mM ASA and KNO3 + 1 mM ASA for 6 days in a dark cabinet under ordinary room condition. Following priming, seeds were immediately subjected to germination test for 14 days and emergence test for 27 days. Primed seeds significantly improved the initial germination percentage, the speed of germination, the height of seedlings, shoot fresh and dry weight and the number of leaves compared to unprimed seeds. Priming seeds with KNO_3 solution only had significantly highest initial germination percentage of 97%, the speed of germination of 49.28, and final germination percentage of 100% in the germination test conducted. In the seedling emergence test, significantly, the highest final germination percentage of 99% was observed in seeds primed in KNO_3 and 0.5 ASA solution. As to speed of germination, height of seedlings, and shoot fresh weight, seeds primed in KNO_3 only had significantly got the highest results of 5.91, 11. 67 cm. and 16.93 grams, respectively. In terms of survival capacity of seedlings, seeds primed in KNO_3 + 0.1mM ASA had the highest seedling survival of 98.67 % and highest shoot dry weight of 1.50 grams. The highest number of leaves was from seeds primed in KNO_3 only and in KNO_3 +1 mM ASA. These results show the important role of priming in the germination attributes of hot pepper seeds. Although priming with KNO_3 only had the most significant results among the parameters measured, an addition of 0.1 mM ASA and 0.5 mM ASA to the priming solution improved the survival ability of the seedlings by 5.67% and 6% as well enhanced the final germination by 3% from seeds primed with KNO3 alone and by 23% on unprimed seeds, respectively.
Keywords: hot pepper, priming, acetyl salicylic acid, germination, speed of germination, initial germination percentage, final germination percentage.

1. Introduction

Hot pepper (*Capsicum frutescens* L.) is a perennial plant with small, tapering fruits, often two-three, at a node. The fruits of most varieties are red; some are yellow purple or black and the fruits are very pungent. The flowers are greenish white or yellowish white. As to germination ability of pepper seeds, depending on the variety; seeds may germinate in about 7-14 days, but hot peppers can take as long as a month or more to come up. Pepper seeds are notorious for taking their time to germinate, or germinating at different times, and it is unusual for some of them surprisingly sprouting several weeks after the first ones germinate. The inconsistent germination of hot pepper seeds is one of the main problems encountered by growers since these seeds usually show low germination rate and low vigor (Freitas, et al.2010).

Seed germination and seedling establishment are important stages in the field performance of crop plants. In the field, seed germination is controlled by many environmental factors and innate conditions, seeds are often placed to specific conditions unfavorable for germination, which subsequently affect seedling establishment (Sang In Shim, 2008). Many types of research have been conducted to investigate and to develop technologies to enhance the germination and uniformity of emergence of vegetable seeds including hot pepper. Priming seeds are one of those technologies that are being tried by researchers so as to impart new technologies to farmers, among others. Priming is the collective term for pre-germination hydration treatments which aim to produce seedlots that germinate uniformly faster or at higher optimal- and maximum-temperatures, so that more seedlings can emerge over a wider range of environmental stresses (Halmer, 2010). One of the priming technologies is the so-called Osmopriming or Osmo-conditioning where seeds are placed in contact with aerated solutions of low water potential and rinsed upon completion of priming. Mannitol, inorganic salts [KNO3, KCL, Ca (NO3)2, etc.] are now used extensively because of its small molecule size and possible uptake (Halmer, 2010). In addition to those mentioned inorganic salts like KNO3, plant growth regulators such as Salicylic Acid is also used.

Salicylic acid (SA) is a phenolic compound naturally occurring in plants in very low amounts. Phenolics contribute in some way on auxin metabolism by regulating IAA degradation or by regulating the formation of IAA conjugate (El-Mergawi et al., 2007). The sustained level of salicylic acid may be a requirement for the synthesis of auxin and/or cytokinin (Metwally et al., 2003). SA is a common plant product signal molecule which is responsible for inducing resistance to a number of biotic and abiotic stresses (Karlidag et al., 2009). SA is involved in creating the local and systemic disease resistance response of plants after pathogen attack (Kachroo et al., 2005).

Priming treatments combined with substances such as SA and KNO_3 improved seeds by the stimulation of germination and resistant to stress - germination that can enhancement components of seedling growth and seed germination (Sang In Shim 2012).This research study was conducted to determine the significance of priming in enhancing germination of hot pepper seeds, to investigate the effects of acetyl salicylic acid on the rate of germination and emergence and in improving the survival ability of hot pepper seedlings.

1.1 Methodology

Twenty-five (25) grams seeds of hot pepper (Exotica 22 F1 Hybrid variety) with eighty-five (85) % germination (as indicated in its packaging) were disinfested with 1% (active ingredient) Sodium Hypochlorite solution for 15 minutes to eliminate seed-borne microorganisms. Following disinfestation, seeds were rinsed in running tap water for one (1) minute and surface-dried by placing them between tissue papers for 30 minutes at room temperature.

Priming solution was prepared by dissolving 60 grams Potassium Nitrate (KNO_3) in two (2) liters of distilled water. The solution was then divided into four (500 ml each) and placed in separate bottles. Five (5) tablets of Aspilets (80mg.a.i.ASA) were then dissolved in a few drops of 99% ethanol and distilled water was added to make a 1 liter ASA stock solution. From the ASA stock solution, 0.1 mM, 0.5 mM, and 1 M were added to each of the 500 ml priming solution. Five (5) Petri dishes lined with two (2) layers of tissue papers saturated with ten (10) ml priming solution with ASA were placed with 240 seeds each. Priming was done for six (6) days by putting the petri dishes in dark cabinet with ordinary room temperature. The priming solutions were changed every other day to maintain a constant osmotic potential. Following priming, seeds from each petri dish were placed in a sieve and washed with running tap water for one minute and left to surface-dry in between layers of tissue paper for two (2) hours to make separation of seeds easier. Seeds from each petri dish were then used in the germination and seedling emergence tests.

Seed Germination Test

Seeds were placed in Petri dishes lined with two layers of tissue paper moistened with 5 ml distilled water. Seeds primed in 3% KNO3 priming solution containing no ASA and unprimed seeds were also included in the germination test. Treatments were as follow: Control (unprimed seeds), seeds primed with KNO_3 only, seeds primed with KNO_3 + 0.1 mM ASA, seeds primed with KNO_3 + 0.5 mM ASA and seeds primed with KNO_3 + 1 mM ASA. Treatments were arranged in Completely Randomized Design (CRD) with three (3) replications having fifty (50) seeds per replicate. Perti dishes were then placed in a dark cabinet under ordinary room conditions. Germination was monitored and recorded daily until the number stabilized for 14 days. Radicle protrusion to 1 mm was recorded as germination. From the total number of seeds germinated; initial germination percentage, speed of germination and final germination percentage were determined.

Seedling Emergence Test

From the previously primed and unprimed seeds, seedling emergence test was also carried out. Twenty-five (25) seeds per replicate (with 3 replications) per treatment were planted in eight (8) 50-holes seedling trays filled with well-watered 1:1:1 carbonized rice hull, vermicompost, and garden soil mixture. Seedling trays were then arranged using Completely Randomized Design. Emergence counts were done daily. Visible hypocotyls were considered as emergence. From the total emergence, final germination percentage and speed of germination were calculated. Twenty-seven (27) days after seed sowing, when the percentage of emergence had stabilized in all treatments; percentage seedling survival, shoot fresh and dry weight, number of leaves, height and shoot/ root ratio were determined.

1.1.1. Results and Discussion

Seed Germination Test

Initial germination percentage (IGP***)*** was determined one day after the germination test; primed seeds significantly had higher IGP compared to unprimed seeds. Seeds primed in KNO_3 only got the highest IGP of 97%, followed by primed seeds with KNO_3 + 0.5 mM ASA with 89%, primed seeds with KNO_3 + 0.1 mM ASA had 83% , and primed seeds with KNO_3 + 1 mM ASA had 68% while unprimed seeds had 0% (Fig.1 and 2.a).

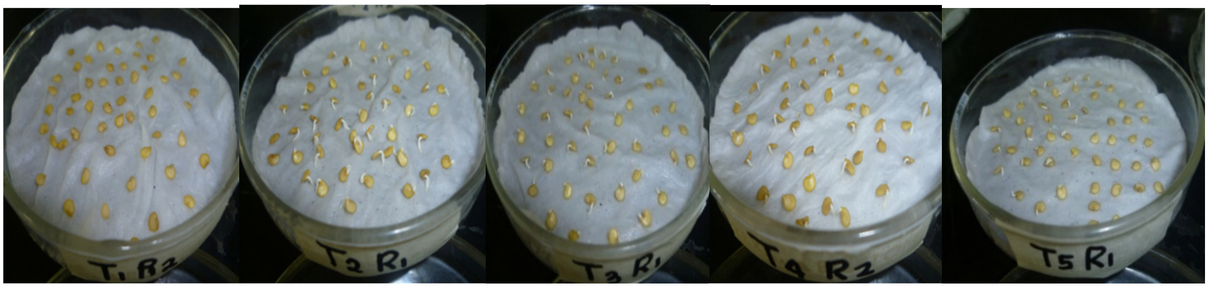

Fig. 1. Initial germination of hot pepper "Exotica" seeds 1 day after germination test. (From L-R). Control (unprimed seeds), seeds primed with KNO_3 only, seeds primed with KNO_3 + 0.1 mM ASA, seeds primed with KNO_3 + 0.5 mM ASA and seeds primed with KNO_3 + 1 mM ASA

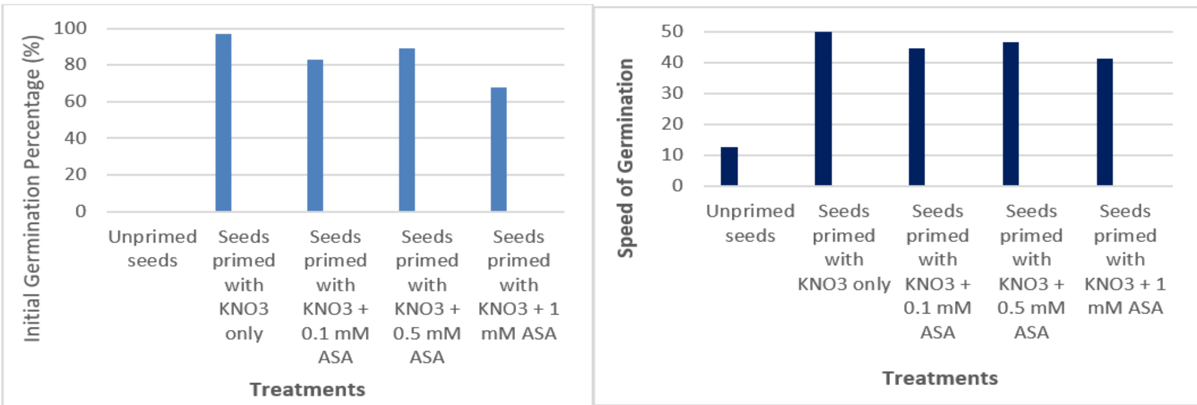

Fig.2. a (L) Initial germination percentage b. (R) speed of germination of hot pepper "Exotica" seeds primed with different levels of ASA priming solution

As to Speed of Germination (SG) which ***is*** the maximum daily germination reached at any time. Significant differences were observed between primed seeds and unprimed seeds. Unprimed seeds had mean SG counts of 12.72 while primed seeds have mean SG counts of 45.43. Highest SG counts was observed in seeds primed in KNO_3 only with SG counts of 49.28, followed by primed seeds with KNO_3 + 0.5 mM ASA with\ SG counts of 46.61, primed seeds with KNO_3 + 0.1 mM ASA with SG counts of 44.53 and primed seeds with KNO_3 + 1 mM ASA with SG counts of 41.31, respectively (Fig.2b).

Table 1. Seed Germination: Initial germination %, Final germination %, and Speed of germination of Hot pepper "Exotica" seeds primed with different priming solutions

Treatments	Initial Germination %	Speed of Germination	Final Germination %
Unprimed seeds	0d	12.72 d	97
Seeds primed with KNO3 only	97 a	49.27a	100
Seeds primed with KNO3 + 0.1 mM ASA	83 b	44.53b	99
Seeds primed with KNO3 + 0.5 mM ASA	89 ab	46.61b	99
Seeds primed with KNO_3 + 1 mM ASA	68 c	41.31c	99

Means with the same letter are not significantly different at 5 % level

As to final germination percentage (FG), 100% germination was obtained from seeds primed in KNO_3 only while the lowest FG percentage was recorded from unprimed seeds (97%) (Table 1). Priming hot pepper seeds regardless of levels of priming solution significantly enhanced seed germination. During priming, seeds are able to imbibe or partially imbibe water and achieve elevated seed moisture content. Since primed seeds have completed Phase I (hydration) and Phase II (lag phase) of germination, they only require a favorable water potential gradient for water uptake to begin growth (Korkmaz, 2005).

Primed hot pepper seeds treated with KNO_3 had the highest initial germination percentage, the speed of germination, and highest FGP and highest SFW, the number of leaves and tallest seedlings. Potassium nitrate solution has long been known as a suitable chemical method for promoting germination in numerous plant species and generally as a priming agent or germination media (Argerich, 1989; Bush et al., 2000; Madakadze et al., 1993; McDonald, 2000). Potassium nitrate (KNO_3) has been associated with an osmotic effect that enhances water uptake by the embryo and a nutritional effect on protein synthesis (McIntyre et al., 1996; McIntyre, 1997), and has also been linked to increased O2 uptake (Adkins et al., 1984; Hilton et al., 1986). The duration of priming with KNO_3 influenced germination percentage differentially, i.e., prolonged priming had a more positive effect on germination.

Seedling Emergence Test

As to speed of germination, shoot fresh weight, height of seedlings, number of leaves, highest means were observed in seeds primed with KNO_3 only with SG of 5.73, shoot fresh weight of 16.93 grams (Table 2) and the tallest height with 11.67 cm (Fig. 3.b). It was found that priming with KNO3 greatly influenced the plant height as nitrogen supplied by KNO3 is an indispensable constituent of numerous organic compounds such as amino acids, proteins, and nucleic acids. Moreover, it plays role in the formation of protoplasm and new cells, as well as encourages plant elongation. Also, potassium is major essential element required for the physiological mechanism of plant growth (Aisha et al., 2007).It may also be due to the availability of nitrogen and potassium from priming solution. The enhanced seedling length in primed seed may be due to the improved and faster plant's seedling emergence and plant length may be due to the efficiency of the plant for utilization of nitrogen which is essential for plant growth and as well as other processes related to nitrogen metabolism (El-Bassiony, 2006).

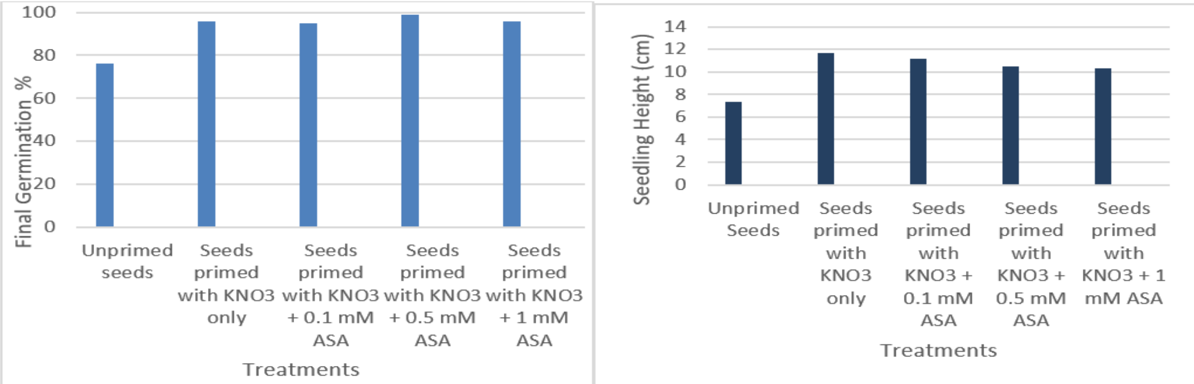

Fig 3. a (L) Final germination percentage b. (R) height of seedlings of hot pepper "Exotica" seeds primed with different levels of ASA priming solution

In terms of survival percentage, seeds primed with KNO_3 and 0.1, 0.5 and 1 mM ASA had the higher survival % compared to seeds primed with KNO3 only (Table 2). Salicylic acid (SA) in plants that are under environmental stress has a protective role. SA, a naturally occurring plant hormone, acts as an important signaling molecule in plants and has diverse effects on tolerance to abiotic stress (Chaudhuri, 2008).

The role of SA at a certain level with moderate and severe abiotic stress may be different and can be attributed to redox regulations in plant cells and protection of the cell structure under stress. SA shows important roles in response to external stimulation and activating defense system in plants. Activation of phospholipase D is an early response to low temperature, involved in the accumulation of free SA and the development of thermotolerance induced by low temperature acclimation in grape berries. Pretreatment with 20 µ g/ml SA significantly improved germination potential and growth criteria of maize seedlings (Gharib et al. 2010).

Table 2. **Seedling Emergence.** Speed of germination, final germination percentage, shoot fresh weight, shoot dry weight, number of leaves, height of seedlings and % of survival of Hot pepper "exotica" primed with different priming solutions

Treatments	Speed of germination	FGP (%)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	No. of leaves	Height (cm)	Survival (%)
Unprimed seeds	2.41c	76 b	9.75 b	0.88b	7.33 b	7.33 b	92.67
Seeds primed with KNO3 only	5.73a	96 a	16.93 a	1.44a	9.00 a	11.67 a	93
Seeds primed with KNO3 + 0.1 mM ASA	5.16ab	95a	16.40 a	1.50a	8.33ab	11.17a	98.67
Seeds primed with KNO3 + 0.5 mM ASA	5.50a	99a	15.38 a	1.48a	8.67ab	10.5a	96
seeds primed with KNO3 + 1 mM ASA (T5)	4.96ab	96a	15.13 a	1.41ab	9.00a	10.33a	96

Means with the same letter are not significantly different at 5 % level

Pepper seed treated with 10-4 M SA and sulfosalicylic acid could induce better growth recovery demonstrated by tall seedlings and high plant fresh and dry weights in the seedlings subjected to low temperature (Benavides et al., 2002). Application of 3-5 mM SA increased growth criteria in faba bean but reduced growth in maize plants (El-Mergawi et al., 2007). Moreover, SA could lessen the injury initiated by low temperature in maize (Farooq et al., 2009), strawberry (Karlidag et al., 2009) and cucumber through the alteration of antioxidant- enzyme activities (Cao et al., 2009; Tao et al., 2010). In recent years, application of exogenous SA at non-toxic concentrations to plants has been shown to be effective in the regulation number of processes, such as biotic and abiotic stresses (Ananieva et al., 2004; Eraslan et al., 2007; Janda et al., 2007; Xu and Tian, 2008).

1.1.2 Conclusion and Recommendation

Priming is an important production practice in enhancing speed and uniformity of germination of hot pepper seeds. Priming solution (KNO_3 + ASA) improves final germination percentage of seedling emergence, shoot dry weight and survival ability of the seedlings.

Priming of seeds should be done to enhance the speed and uniformity of hot pepper germination. Follow-up study should be conducted to compare the separate effect of priming with potassium nitrate and acetyl salicylic acid (ASA) on seed germination. Since the main role of ASA displays when plants are subjected to stresses, study on the effect of ASA on seedling survival under field conditions is recommended.

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