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# Priming Methods: Alternative Strategy to Improve Seed and Seedling Performance of Soursop (*Annona muricata*)

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors designed the study. Author DJAN gathered and consolidated the data. Author RJGR performed the statistical analysis and interpreted analyzed the data with author DJAN. Both authors searched for related studies and literature, read and approved the final manuscript.*

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## ABSTRACT

The evaluation of seed priming methods on the seed and seedling performance of soursop was conducted January 9 to April 3, 2019 in San Nicolas, Ilocos Norte, Philippines. The study was conducted to investigate the effect of seed priming methods capable of breaking dormancy; improve germination rate; determine the seed vigor of soursop; and identify the best seed priming method that provide better seedling performance. The experimental treatments (unprimed and three priming methods, hydropriming, halopriming and hormonal priming) were laid out in Completely Randomized Design with three replications. A total of 20 polyethylene bags were used per treatment per replication with one seed sown in every bag.

Alternative way to improve seed and seedling performance is the use of these seed priming methods. The seed and seedling performance of soursop were significantly affected by priming methods. Primed seeds had higher percentage germination rate (PGR) than unprimed seeds. But numerically, the highest PGR was hydropriming. Hormonal priming produced significantly taller

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seedlings at 10 and 40 days after emergence (DAE) than unprimed seeds and more leaves per seedling at 30 to 50 DAE. Hormonal, hydropriming and halopriming produced significantly higher fresh weight of seedlings than unprimed seeds.

*Keywords: Hydropriming; halopriming; hormonal priming; seed treatment.*

## 1. INTRODUCTION

Soursop *(Annona muricata)* is one fruit crop being propagated in the Philippines particularly in Ilocos Norte. Its fruit is a good source of essential minerals and nutrients which are beneficial to human health [1]. The crop is commonly propagated by seed due to its easy procedure. However, the seeds have hard and thick seed coats which restrict the entry of moisture, thus delaying germination and resulting in uneven maturity [2]. The unprimed seeds emerge for almost two to three weeks. Because of this situation, mass propagation is done through vegetative method, which has shorter waiting time to flower. Still, not all growers have the capacity to access materials and tools to perform vegetative propagation which entails additional cost on their part; hence, seed propagation is still economical for them. An alternative way to better improve the seed and seedling performance of a crop is the utilization of seed priming methods. This is used to break seed dormancy and thus enhance the germination. Among the methods are hydropriming (soaking in water), halopriming (soaking in salt solution) and hormonal priming (soaking in gibberellic acid solution). These methods hydrate the seeds and reduce seedling emergence time [3] and the procedure is easy to perform.

The following are the effects of seed priming methods: allows some of the metabolic processes necessary for germination to occur without germination taking place; increase germination rate; uniform seedling emergence; enhance crop enzyme rate resulted to increase ration of crop development; and faster and better seedling development [4,5]. Moreover, those seeds that are different solutions with high osmotic potential prevent the seeds from absorbing in enough water for radicle protrusion which will suspend the seeds in the lag phase [4, 5]. The faster and better seedling performance is a result of reduced time between sowing and seedling performance and to syncrhonized emergence [6].

In order to extend help to the growers, avoid the delay in the germination of the seeds and longer germination period, this experiment was conducted to improve the performance of soursop at seedling stage and evaluate the seedling performance of soursop using three seed priming methods.

Specifically, this study aimed to: determine if the seed priming methods are capable of breaking dormancy; determine if it will improve germination rate; determine the seed vigor of soursop; and identify the best seed priming method that provides the best seedling performance.

The results of the study will provide further information on how to improve the seedling performance of soursop which could help interested growers. Another advantage is that if seed priming methods shorten the time to germination then transplanting can be done much earlier.

## 2. MATERIALS AND METHODS

The study was conducted in a partially shaded area in Barangay 14 San Gregorio, San Nicolas, Ilocos Norte in the Philippines. The area is shaded due to trees of sugar apple or sweetsop (Annona squamosa), drumstick tree (Moringa oleifera) and mango (Mangifera indica).

Ripe soursop fruits were bought from the public market of Laoag City, Ilocos Norte. The seeds were freshly removed from the ripe fruits. Extracted seeds were soaked in water for a minute to see if they float or not. All floating seeds which are immature were discarded. The seeds were stored in a refrigerator to avoid rapid drying of the seeds.

Seeds were scarified by removing small portion of the seed coat using a nail cutter before they were sown. All the seeds were soaked in separate treatments such as hydropriming (water), halopriming (salt solution) and hormonal priming (GA3) for 24 Hours. For GA3, the concentration used was 500 ppm [2]. For hydropriming method, tap water was used to soak the seeds. Four teaspoons or 19.7 Grams of salt was dissolved in 500 ml of water for the salt solution priming method.

Equal parts of garden soil, carbonized rice hull, and organic fertilizer were placed in polyethylene bag (2x6 inches size) used to grow the soursop seeds. One seed was sown for every polyethylene bag at 2-3 cm depth and irrigation followed after sowing. A total of 20 polyethylene bags were used per treatment per replication giving an overall of 240 bags.

Complete fertilizer (5 g per bag) was dissolved in a Liter of water and applied through fertigation after germination and at 21 days after germination [7]. Irrigation was done after fertilizer application. Follow-up irrigation was done depending on the soil and weather conditions.

## 2.1 Data Gathering Procedures

**Percent germination rate:** This was recorded at 10 days after emergence (DAE) up to 50 DAE with 10 days interval using the formula below.

Percent Germination = (Total Seeds Germinated) / (Total Number of Seeds Sown) x 100

**Days to seedling emergence:** This was taken by counting the number of days from sowing up to the time when 50% of the population of the seeds have emerged.

**Seed Vigor Index (SVI):** This was done by counting for five times starting at five days after emergence (DAE) up to 25 DAE within five days interval. The SVI was calculated using the formula below [3].

$$SVI = \frac{G1 + G2 + \cdots + GL}{D1 + D2 + \cdots + DL}$$

G1 = number of germinants (first count)
D1 = number of days to first count
G2 = number of germinants (second count)
D2 = number of days to second count
GL = number of germinants (last count)
DL = number of days to last count

**Seedling height:** The seedling height of four sample seedlings per treatment was measured at 10, 20, 30, 40 and 50 DAE. The measurement was done from ground surface to the tip of the longest leaf using a ruler.

**Number of leaves per seedling:** The same sample seedlings in the measurement of plant height were used to count the number of leaves per seedling. Counting was done at 10 up to 50 DAE with 10 days interval.

**Leaf length:** First shoot that appeared in the same sample seedling in measuring plant height was used in measuring leaf length. Measurement was done at 20 up to 50 DAE with 10 days interval.

**Shoot and root length:** Shoot and root length of four sample seedlings were measured from the collar region to the point of attachment of cotyledons and from collar region up to the tip of root, respectively using ruler. This was taken at 40 days after emergence (DAE). To facilitate easy data gathering, the media of the seedling was removed first and the roots are rinsed with water afterwards.

**Seedling length (Shoot length plus root length):** This was taken by getting the sum of the measurement of shoot and root length using the same sample seedlings used in shoot and root length measurement.

**Fresh and oven dry weight of seedling:** The sample seedlings used in the measurement of shoot and root length were used to gather fresh and oven dry weight of seedling at 40 DAE. Fresh roots and shoots of the four sample seedlings per treatment were oven dried for two days with the temperature of 65ºC – 70ºC [8]. Afterwards, these were weighed using an electronically digital weighing scale.

## 2.2 Data Analysis

All data gathered was analyzed using analysis of variance in a Completely Randomized Design. Where F-test was significant at 5% (P = .05) or 1% (P = .01), treatment mean difference was further tested using Least Significant Difference (LSD) test at (P = .05). Statistical analysis was done using STAR program.

# 3. RESULTS AND DISCUSSION

## 3.1 Percent Germination Rate

Seed priming methods significantly (P = .01) affected the percent germination rate (PGR) at 10 days after emergence (DAE). Table 1 shows that primed seeds had higher PGR irrespective of priming methods than unprimed seeds. According to Namaz et al. (2013) priming improves germination. There was no significant difference between priming methods but numerically, hydropriming (52%) had the highest PGR among the priming methods. Moreover, although germination rate increased from 20 to 50 DAE there were no significant differences between primed and unprimed seeds.

Percent germination was increased and shortened the seed germination period in Judas tree *(Cercis siliquastrum)* with the use of halopriming [9]. Furthermore, hydropriming improve improved seed germination [10] and enhanced seedling emergence of soursop [6]. However, numerically, hydropriming had the highest PGR with 10% difference from halopriming and hormonal priming. According to El-Barghathi and El-Bakosh (2005) the external application of $GA_3$ enhances the seed germination of Kermes oaks *(Quercus coccifera)*. Similar results were reported by Gonzalez et al. (2005) and Singh and Maheswari (2017) that seeds soaked in 500 PPM $GA_3$ had best and highest seed germination of soursop. Moreover, seeds of tomato soaked in 900 MG $L^{-1}$ $GA_3$ have high percentage germination [11].

Also the experiment of Armin et al. (2010) on watermelon, $KNO_3$ increased germination and germination rate.

## 3.2 Days to Seedling Emergence and Seed Vigor Index

There was no significant difference (P = .01) between primed and unprimed seeds on the days to emergence and seed vigor index of soursop (Table 2). Numerically, earliest seed emergence was observed from hormonal priming while unprimed seeds emerged last.

## 3.3 Seedling Height

At 10 DAE, seedling height was highly significantly affected (P = .01) by priming methods while at 40 DAE, seedling height was significantly (P = .05) affected by priming methods (Fig. 1). At 10 DAE, seeds primed seed with hormone (GA3) produced highly significantly taller (P = .01) seedlings than the other methods and unprimed seeds. Heights of seedlings from hydroprimed and haloprimed seeds did not differe significantly from those of unprimed seeds.

At 40 DAE, hormonal and halopriming seeds had significantly taller (P = .05) seedlings than the unprimed seeds. Prasad et al. (2002) noted that gladioli are taller with the use of hormonal priming. This means that if the seedlings are taller, it could be transplanted early in the field.

## 3.4 Number of Leaves per Seedling

The number of leaves per seedling at 30 (P = .01) and 50 DAE (P = .05) was significantly higher by the use of priming methods than unprimed (Fig. 2). Generally, hormonal priming consistently produced higher number of leaves per seedling at 30 and 50 DAE than the other priming methods. According to Prasad *et al.* (2002), hormonal priming (250 PPM $GA_3$) increased the number of leaves in gladioli. Also there was more number of leaves in soursop

**Table 1. Effects of seed priming methods on apercent germination rate of soursop at different observation periods**

| Treatment | 10 DAE$^{**}$ | 20 DAE $^{ns}$ | 30 DAE$^{ns}$ | 40 DAE$^{ns}$ | 50 DAE$^{ns}$ |
|---|---|---|---|---|---|
| Unprimed | 22$^{b}$ | 65 | 67 | 75 | 78 |
| Hydropriming (water) | 52$^{a}$ | 72 | 75 | 77 | 80 |
| Halopriming (salt solution) | 42$^{a}$ | 67 | 75 | 88 | 88 |
| Hormonal priming ($GA_3$) | 42$^{a}$ | 70 | 73 | 80 | 80 |
| CV (%) | 22.42 | 9.45 | 9.55 | 11.55 | 9.52 |

*CV – coefficient of variance; ** - significant at 1% level*

**Table 2. Effects of seed priming methods on days to seedling emergence and seed vigor index of soursop**

| Treatment | Days to seedling emergence$^{ns}$ | Seed vigor index$^{ns}$ |
|---|---|---|
| Unprimed | 32 | 2 |
| Hydropriming (water) | 30 | 3 |
| Halopriming (salt solution) | 30 | 3 |
| Hormonal priming ($GA_3$) | 29 | 2 |
| CV (%) | 4.45 | 9.97 |

*CV – coefficient of variance; ns – not significant* using $GA_3$ at 500 ppm soaking in 24 hours than soaking in water for 24 hours [2]. If there is high number of leaves, there will be higher photosynthetic activity which will result in faster growth and development.

### 3.5 Leaf Length

There was no significant difference in length of leaves produced by primed and unprimed seeds over the period (Table 3) though; hormonal priming consistently had the longest leaf length. Leaf length of gladioli is increased with the use of 500 PPM $GA_3$, hormonal priming [12].

### 3.6 Shoot Length and Root Length

Priming methods did not significantly affect the shoot and root length of soursop (Table 4). In the experiment of Singh and Maheswari (2017) soursop shoot and root length was high using 500 ppm $GA_3$ than using water. However, Matsushima and Sakagami (2013) found that the shoot length of rice increased using hydropriming due to rapid supply of nutrient required for cell growth. Balaguera-Lopez *et al* (2009) also reported that seeds of tomato soaked in 900 mg $L^{-1}$ $GA_3$ gave high root length.

### 3.7 Seedling Length

Seeds either primed or unprimed had comparable seedling length (Table 5). Numerically, hormonal priming had the lowest seedling length and seedling vigor index. $KNO_3$ has the most effective impact on the seedling growth compared with unprimed, PEG 6000 3%, HCL 0.1N, and NaCl 1.5N [13].

### 3.8 Fresh Weight and Oven Dry Weight

Priming methods had significant (P = .05) influence on the seedling fresh weight but not on dry weight compared to unprimed seeds (Table 6). Primed seeds produced significantly higher seedling fresh weight than unprimed seeds. Among priming methods, hormonal priming had the highest seedling fresh weight. High fresh weight of hormonal priming means higher biomass than the other treatments.

**Table 3. Effect of seed priming methods on leaf length (cm) of soursop at different observation periods**

| Treatment | 20 DAE$^{ns}$ | 30 DAE$^{ns}$ | 40 DAE$^{ns}$ | 50 DAE$^{ns}$ |
|---|---|---|---|---|
| Unprimed | 2.98 | 3.60 | 3.99 | 4.62 |
| Hydropriming (water) | 2.93 | 4.04 | 4.35 | 5.32 |
| Halopriming (salt solution) | 3.84 | 4.15 | 4.65 | 5.24 |
| Hormonal priming ($GA_3$) | 4.62 | 6.14 | 4.95 | 5.69 |
| CV(%) | | 28.37 | 12.32 | 8.87 |

*CV – coefficient of variance; ns – not significant*

**Table 4. Effect of seed priming methods on shoot length and root length of soursop**

| Treatment | Shoot length$^{ns}$ (cm) | Root length$^{ns}$ (cm) |
|---|---|---|
| Unprimed | 12.67 | 11.83 |
| Hydropriming (water) | 12.70 | 12.73 |
| Halopriming (salt solution) | 12.63 | 12.43 |
| Hormonal priming ($GA_3$) | 11.60 | 12.73 |
| CV(%) | 5.39 | 10.81 |

*CV – coefficient of variance; ns – not significant*

**Table 5. Effect of seed priming methods on seedling length (cm) of soursop**

| Treatment | Seedling length$^{ns}$ (cm) |
|---|---|
| Unprimed | 25 |
| Hydropriming (water) | 25 |
| Halopriming (salt solution) | 25 |
| Hormonal priming ($GA_3$) | 24 |
| CV(%) | 5.85 |

*CV – coefficient of variance; ns – not significant*

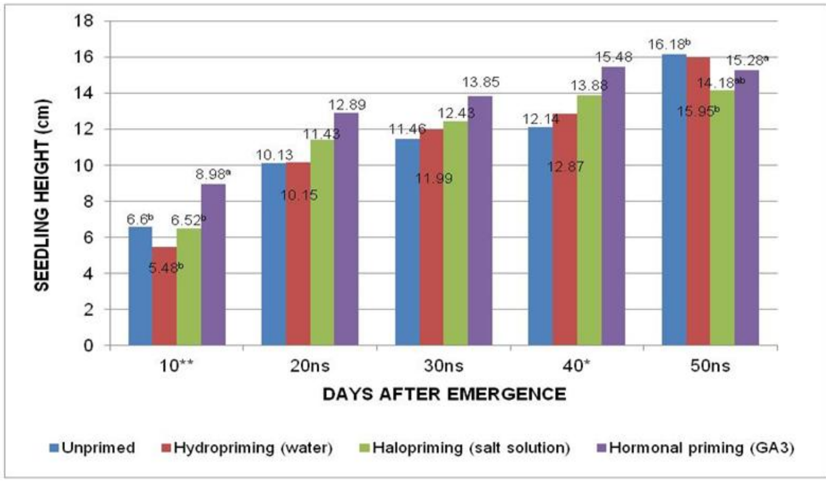


**Fig. 1. Seedling height at different observation periods of soursop**

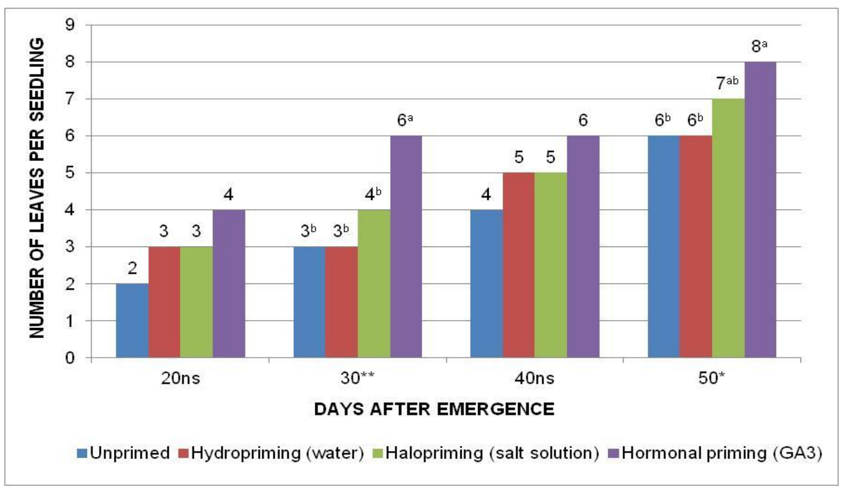


**Fig. 2. Number of leaves at different observation periods of soursop**

**Table 6. Effect of seed priming methods on fresh weight and oven dry weight of soursop**

| Treatment | Fresh weight* (g) | Oven dry weight$^{ns}$ (g) |
|---|---|---|
| Unprimed | 3.26$^{b}$ | 0.96 |
| Hydropriming (water) | 3.60$^{b}$ | 1.16 |
| Halopriming (salt solution) | 4.10$^{ab}$ | 1.36 |
| Hormonal priming ($GA_3$) | 4.93$^{a}$ | 1.10 |
| CV(%) | 15.64 | 19.92 |

*CV – coefficient of variance; * - significant at 5% level*

## 4. CONCLUSION

It can be concluded that priming methods significantly affected the seed and seedling performance of soursop. The seeds of soursop were able to break the dormancy either unprimed or primed taking 29 to 32 days to emerge. One advantage was that primed seeds produced significantly higher percent germination rate than unprimed seeds by 10 days after emergence especially with hydropriming. Both primed and unprimed seeds had the same seed vigor index. However, hormonal priming had the best effective impact due to significant influence because it consistently produced taller seedlings, more leaves per seedling, and comparable seedling fresh weight with other priming methods.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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