

# Seed Treatment to Improve Germination and Seedling Establishment of Citrus Species

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**Abstract:** This experiment was conducted in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. The aim was to investigate if seed priming with 10% sodium hypochlorite (bleach) for 20 minutes and water for 30 minutes would improve germination and seedling vigour in the citrus species: rough lemon (*Citrus jambhiri* Lush.), smooth lemon (*Citrus limon* L. Osbeck), grapefruit (*Citrus paradisi* L.) and sweet orange (*Citrus sinensis* L. Osbeck) compared to unprimed seeds. The experimental design was a Completely Randomized Design with 4 replications with 12 treatments: unprimed seeds of the four citrus species (controls), seeds primed with 10% bleach for 20 minutes, and seeds primed with water for 30 minutes. Data collected included percent germination, seedling height, number of leaves and the seedling vigour index was calculated. Data was subjected to analyses of variance and tested at 5% significance. Seed priming with 10% bleach solution for 20 minutes improved the germination percentage of all the citrus species and seedling vigour index. There was a significant correlation between seedling height and seedling vigour index indicating that seedling height should be considered in choice of citrus seedlings to transplant to the field.

**Keywords:** Seed priming; germination; bleach; seedling vigour index, seedling height, seedling establishment

## INTRODUCTION

Germination and seedling establishment are undoubtedly critical aspects that have a huge impact on plant growth and development and ultimately are one of the factors that determine crop yield (Aryal *et al.*, 2020). Seeds planted in a field that fail to germinate and establish are a waste of the farmer's time, energy and resources. This is because optimum plant stands are imperative for crop production to be resource efficient (Finch-Savage and Bassel 2016). Optimum plant populations and high seedling vigour not only improve competitive advantage of crops over weeds, but also maximize the use of crop growth resources such as light, water, nutrients and carbon dioxide (Donohue *et al.*, 2015; ISTA, 2015). Rapid and uniform germination and seedling establishment resulting from seed priming are highly beneficial for increasing the yield and quality of plantation crops that require seedling nurseries like citrus species (Paparella *et al.*, 2015; Ibrahim, 2016). Successful plantations require the growth of healthy, well-developed seedlings, and one of the main goals of seedling production is to produce a healthy plant from every single seed. The most remarkable seed quality and seed performance tests are the germination test and the seedling vigour test (Singh *et al.*, 2016; Silva *et al.*, 2019). Good seedling establishment and seedling vigour are essential for sustainable and profitable crop production and are therefore considered the most critical stage of a developing crop. Low seed vigour greatly influences both the number of emerging seedlings, and the timing and uniformity of seedling emergence. High percentage germination and seedling vigour index are indicative of competitive ability of crops over weeds impacting yield by improving plant population density (Wilson, 2022). Priming of seeds is one of the easiest and cheapest ways for farmers to achieve better germination uniformity, and vigorous growth, help crops in overcoming abiotic stress, promote early flowering and higher yields (Szopinska *et al.*, 2014; Hussain *et al.*, 2017). Various methods of priming seeds such as hydropriming, osmo-priming halo-priming and hormonal priming etc, have been enumerated by Aryal *et al.*, (2020) and Wilson (2022). The use of bleach - sodium hypochlorite as a seed primer has been found to be beneficial for improving germination and promoting early seedling establishment in crops including some species of citrus (Akbari *et al.*, 2012; Derso and Feyissa, 2015; Wilson, 2022). This experiment was therefore set up to find out if bleach priming will enhance germination and seedling vigour in the following citrus species, rough lemon (*Citrus jambhiri* Lush), smooth lemon (*Citrus limon* L. Osbeck.), grapefruit (*Citrus paradisi* L.) and sweet orange (*Citrus sinensis* L. Osbeck).

## MATERIALS AND METHODS

### *Experimental Site*

The experiment was carried out in the screen house of the Department of Plant Science and Biotechnology, at the Rivers State University, Port Harcourt, Rivers State, Nigeria.

### *Experimental Materials*

Experimental materials were rough lemon (*Citrus jambhiri* Lush.), smooth lemon (*Citrus limon* L. Osbeck.) grapefruit (*Citrus paradisi* L.) and sweet orange (*Citrus sinensis* L. Osbeck.).

#### **Treatment Applications and Experimental Design**

The seed primer was ten percent bleach, sodium hypochlorite (NaOCl) solution prepared by diluting 10ml of Hypo™ bleach in 90ml of water to obtain the 10% bleach solution. Matured and ripened fruits of the citrus species were obtained and seeds extracted manually, washed and air-dried. A quick potential viability test was conducted by placing the seeds in a bowl of water and only the seeds that sank to the bottom of the bowl (potentially viable seeds) were used for this experiment. Seeds of each citrus species were primed in ten percent bleach solution for 20 minutes, primed in water for 30 minutes and planted after drying for 24 hours. Unprimed seeds of each citrus species were also planted to serve as appropriate controls. Five seeds each were sown at a depth of 0.5cm in rectangular transparent plastic containers (size = 16 x 10 x 5cm) filled with loamy soil. The containers were perforated to allow for drainage of excess water. Each treatment was replicated 3 times. Every day, morning and evening, 20ml of water was used in watering each experimental unit.

The experimental design was a Completely Randomized Design (CRD) with 4 replications and 12 treatments as follows:

Rough Lemon unprimed (control)

Rough Lemon primed with water for 30 minutes

Rough Lemon primed with 10% bleach for 20 minutes

Smooth Lemon unprimed (control)

Smooth Lemon primed with water for 30 minutes

Smooth Lemon primed with 10% bleach for 20 minutes

Grapefruit unprimed (control)

Grapefruit primed with water for 30 minutes

Grapefruit primed with 10% bleach for 20 minutes

Sweet Orange unprimed (control)

Sweet Orange primed with water for 30 minutes

Sweet Orange primed with 10% bleach for 20 minutes

#### **Data Collection and Statistical Analyses**

The following data were collected from first germination to 8 weeks after planting

1. Percent (%) germination,
2. Seedling height
3. Number of leaves
4. Seedling vigour index (SVI)

SVI was calculated according to Luna *et al.* (1994)

$$SVI = \frac{\text{Seedling height} \times \text{Percentage germination}}{100}$$

The treatment effects were subjected to Analysis of variance (ANOVA) using the GLM procedure of Statistical Analyses Software (SAS) version 9.3 and any effects found to be significant were tested at a significance level of 5% while means were compared using Duncan's Multiple Range Test (DMRT) at  $P = 0.05$ . Correlation analyses was conducted to determine the relationship between percentage germination, seedling height, number of leaves and seedling vigour index.

## **RESULTS**

### **Percentage Germination**

There were significant differences ( $P = 0.05$ ) in the percentage germination of the citrus species (Table 1). Percentage germination of grapefruit seeds primed with 10% bleach was significantly higher than all other treatments. Whereas on average, percentage germination of all the citrus seeds primed with water did not differ significantly from the unprimed seeds (control), those primed with 10% bleach differed significantly from both treatments (Table 2). On average, germination of grapefruit seeds was significantly higher than smooth lemon but did not differ significantly from those of rough lemon and sweet orange.

### **Seedling height**

Smooth lemon seedlings on average were significantly taller ( $P = 0.05$ ) than those of other citrus species whose heights did not differ significantly from each other (Table 1). Moreover, smooth lemon primed with water for 30 minutes had the tallest seedlings. Averaging all the treatments, unprimed seeds were the shortest and those primed with 10% bleach the tallest but none of the treatments differed significantly in seedling height (Table 2).

### **Number of leaves**

There were significant differences ( $P = 0.05$ ) in the number of leaves of the citrus species (Table 1). Rough Lemon primed with 10% bleach for 20 minutes had significantly more leaves than 7 other treatments. In Table 2, the main effects of treatments and the

responses of citrus species are presented. Overall, grapefruit and smooth lemon had significantly less leaves than rough lemon and sweet orange, whilst there were overall no significant differences between primed and unprimed seeds with respect to number of leaves.

### Seedling Vigour Index

In Table 1, treatments with the highest seedling vigour index (SVI) were smooth lemon primed with 10% bleach for 20 minutes, smooth lemon primed with water for 30 minutes and grapefruit primed with 10% bleach for 20 minutes. On the average, priming with 10% bleach gave the highest SVI of 3.64 and unprimed (control) gave the lowest SVI of 2.65 (Table 2). Smooth lemon had the highest SVI and sweet orange the lowest.

### Correlations

In Table 3, the correlation coefficients are presented showing the relationships between the various parameters measured. Most of them showed weak or negative correlations except for seedling height and seedling vigour index (SVI).

## DISCUSSION

There were no distinct patterns in the effects of priming with 10% bleach and water on the various parameters as they affected the different citrus species. Seed priming with 10% bleach solution for 20 minutes improved the germination percentage of all the citrus species (Nejad 2013; Wilson, 2022). Grapefruit seeds primed with 10% bleach gave the highest percentage germination, smooth lemon seeds primed with water had the tallest seedlings, whereas rough lemon had the highest number of leaves and smooth lemon primed with bleach had the highest seedling vigour index (SVI) (Rehman *et al.*, 2015). However, on average seeds primed with 10% bleach and water generally performed better than unprimed seeds in all the citrus species especially with respect to seedling vigour. Unprimed seeds of rough lemon were the only citrus species that had higher SVI than its primed seeds, indicating perhaps that for rough lemon the priming levels used in this experiment were not beneficial to it in terms of treatment duration and or concentrations used (Jesus *et al.*, 2016). Seedling vigour is critical to plant establishment in the field (Szopinska *et al.*, 2014; Hussain *et al.*, 2017) and there was a significant correlation between SVI and seedling height indicating that seedling height could be one of the important parameters to look out for when choosing seedlings to transplant from the nursery to the field in order to ensure optimum plant population.

## CONCLUSION

Seed priming with 10% bleach solution for 20 minutes improved the germination percentage of all the citrus species. Seedling height is an important parameter to help in choice of citrus seedlings to transplant from nursery to the field to ensure optimum plant population. The implication of this study is that by priming their seeds with 10% bleach solution when raising seedlings in the nursery, farmers can improve the speed of establishment and achieve optimum plant population of these citrus seedlings especially of smooth lemon and grapefruit in the plantation and consequently their productivity and yield. This is because application of 10% bleach solution as seed primer improves seed germination, seedling height and seedling vigour which will enhance seedling establishment on the field. A concomitant benefit is that the bleach can also act as a disinfectant against phytopathogens on the seeds. It is therefore recommended that farmers should prime their seeds for 20 minutes with 10% bleach solution when raising these citrus seedlings in the field.

**Table 1.** Effect of seed priming with bleach and water on germination, seedling height, number of leaves and seedling vigour index (SVI) of four citrus species

Treatments	Germination (%)	Seedling Height (cm)	No of Leaves	Seedling Vigour Index
Rough Lemon unprimed (control)	60.6±3.7 <sup>b</sup>	4.1±0.5 <sup>a</sup>	7.9±1.4 <sup>b</sup>	3.28
Rough Lemon primed with water for 30 minutes	54.5±1.9 <sup>ab</sup>	4.5±0.4 <sup>a</sup>	7.8±0.9 <sup>b</sup>	2.43
Rough Lemon primed with 10% bleach for 20 minutes	66.8±3.4 <sup>b</sup>	4.4±0.1 <sup>a</sup>	8.6±2.1 <sup>b</sup>	2.9
Smooth Lemon unprimed (control)	40.6±2.2 <sup>a</sup>	7.5±1.6 <sup>c</sup>	3.9±0.6 <sup>a</sup>	3.05
Smooth Lemon primed with water for 30 minutes	49.2±2.6 <sup>a</sup>	8.4±1.8 <sup>cd</sup>	4.2±1.0 <sup>a</sup>	4.12
Smooth Lemon primed with 10% bleach for 20 minutes	63.4±1.2 <sup>b</sup>	7.9±0.9 <sup>c</sup>	3.7±0.7 <sup>a</sup>	4.19
Grapefruit unprimed (control)	60.5±2.6 <sup>b</sup>	3.8±0.1 <sup>a</sup>	3.4±0.4 <sup>a</sup>	2.68

Grapefruit primed with water for 30 minutes	63.5±1.5 <sup>b</sup>	3.9±0.4 <sup>a</sup>	3.4±0.1 <sup>a</sup>	2.36
Grapefruit primed with 10% bleach for 20 minutes	80.2±1.1 <sup>c</sup>	5.0±0.7 <sup>ab</sup>	3.6±1.0 <sup>a</sup>	4
Sweet Orange unprimed (control)	53.3±1.6 <sup>ab</sup>	4.0±1.1 <sup>a</sup>	7.5±1.6 <sup>b</sup>	1.6
Sweet Orange primed with water for 30 minutes	60.2±2.2 <sup>b</sup>	4.8±0.8 <sup>ab</sup>	6.1±1.3 <sup>a</sup>	2.88
Sweet Orange primed with 10% bleach for 20 minutes	67.8±3.1 <sup>b</sup>	6.0±0.5 <sup>bc</sup>	7.9±1.5 <sup>b</sup>	3.47

(± Standard Errors)

Values with same superscripted alphabets on same column are not significantly different ( $P = 0.05$ ) DMRT

**Table 2.** Main effects of seed priming with bleach and water and the responses of four citrus species

Main effects of Bleach and Water priming	Germination (%)	Seedling height (cm)	Number of leaves	Seedling Vigour Index
Unprimed seeds (Control)	53.75	4.85	5.7	2.65
Priming with water for 30 minutes	56.85	5.4	5.4	2.95
Priming with 10% bleach for 20 minutes	69.55	5.8	6	3.64
Main responses of Citrus Species				
Rough Lemon	60.6	4.3	8.1	2.87
Smooth Lemon	51.1	7.9	3.9	3.79
Grapefruit	68.1	4.2	3.5	3.01
Sweet Orange	60.4	4.9	7.2	2.65

**Table 3.** Correlation coefficients between germination, seedling height, number of leaves and Seedling Vigour Index

	Seedling Height	Number of Leaves	Seedling Vigour Index (SVI)
Germination	-0.365	0.031	0.283
Seedling Height		-0.366	0.703*

Number of Leaves

-0.349

\*Significant correlation (*r*) at 5% level of significance**REFERENCES**

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