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Pigmented and white rice wastewater improves germinability, vigor and seedling characteristics of Papaya (*Carica papaya*) cv. 'Cavite Special' seeds

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Abstract Rice wastewater improved germination of *Carica papaya* cv. 'Cavite Special' seeds when compared to hydroprimed seeds. Earlier germination was also observed in seeds treated with white and black rice wastewater. Seeds soaked in white and black rice wastewater had the highest germination rate (75-85%), seed vigor (4.6-4.2), and seedling dry weight (0.034-0.029 g), following them are seeds with soaked in brown and red rice wastewater. Embryo sizes are compared across treatments which may be due to the early data gathering. The positive effect of rice wastewater in the seed germination rate, vigor, seedling dry weight and earliness in germination are provided evidence of its effectivity as a priming agent for *C. papaya* cv. 'Cavite Special' seeds.

Keywords: Hugas-bigas, Pawpaw, Rice wash, Seed enhancement, Seed priming

Introduction

Papaya (*Carica papaya* L.) is an economically important crop in the Philippines. Additionally, it is a monotypic species under the *Caricaceae* family cultivated for its sweet fruits that may be produced year-round (San Pascual *et al.,* 2023). When papaya plants receive proper cultural care, these plants are expected to produce numerous fruits with good horticultural characteristics and produce productive yield (Magdalita and San Pascual, 2019). In the Philippines, 92% of the total harvested fruits were locally consumed whilwe the remaining 8% were intended for industrial use.

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One perennial issue of papaya production is non-uniform and low germination of seeds due to multiple causes such as the presence of sacrotesta, genetic variability, improper seed storage and storage conditions, dormancy and immaturity of seeds, seed-borne diseases among others (Hazarika *et al*., 2023; Magdalita and San Pascual, 2019, Sinchana Jain *et al*., 2020).

In the Philippines, papaya growers sow seeds either directly in the soil or produce seedlings in seedling trays in nurseries and then transplant them in the field after 1- 2 months of seedling growth. In papaya, it would take 10- 21 days for papaya seeds to germinate and may continue intermittently for up to 35–40d (Bhadarka *et al*., 2022). Naturally, papaya seeds had sacrotesta. This is the outer covering of the seed that is described to be mucilaginous or gelatinous contanining growth inhibitors or compounds that attract fungi and bacteria which have negative effect on the germination of the seeds hence must be removed (Jotava *et al*., 2023). Sacrotesta may be removed by rubbing seeds in wire mesh and soak and wash them in tap water followed by air-drying for a week (Rodriguez *et al.*, 2020). Another factor that may affect seed germination is genetic variability. Papaya is highly heterozygous most especially those from open pollinated plants therefore had variable performance and vigor which can be observed even in non-uniform germination of seeds and seedling vigor. Additionally, imporper storate conditions affect seed variability Zulhisyam *et al*. (2013) stated that papay seeds have short survival periosd when grown in ambiewnt conditions and rapidly deteriorate in higher storage temperature. Small hold farmers keep seeds in non-conducive conditons making seeds deteriorate rapidly. (Magdalita and San Pascual, 2019). Dormancy and immaturity, on the other hand, are important factors. If seeds are not harvested at the proper maturity level, seeds do not germinate properly.

Given these problems, several approaches were done to improve germination and vigor of seeds like priming. Seed priming is a seed invigorating technique into which there is 'controlled hydration of seeds to a level that permits pre-germinative metabolic activity to proceed but prevents the actual emergence of the radicle' (Raj and Sheeja, 2019 as cited by San Pascual *et al*., 2024). It is done by farmers and horticulturists to improve germinatison and promote uniform germination of seeds by soaking seeds to different priming agents, dry them and subject them to germination. Numerous priming studies have been done using water (hydropriming), solutions (osmopriming) and even growth regulators (hormopriming). This technique may be used to synchronize germination ofg seeds and overcomelow and non-uniform germination.

Maneesha and Priya Devi (2019) prove that potassium solutions improved seed germination capacity and seedling growth of Papaya. The different potassium solutions used are Potassium sulphate, Potassium nitrate and

Potassium hydroxide. The same author reported that germination was shorten using Calcium solutions. Priming reduced mean time for seedling emergence using Calcium solutions and therefore recommended that pretreating papaya seeds be done to enhance seed germination and plant emergence.

Farmers use different ways to improve growth in plants, to increase its yield, resistance to diseases among others. Use of organic materials such as bioorganic (Fermented juices of plants and fruits, fish amino acids among others). Many studies have contributed to the proof that these are effective. Like for example the use of Lactic Acid Bacterial Serum which uses waste Rice wash as starter with muscovado sugar. Despite the many studies that utilize these materials, no studies have been reported to improve seed germinability and vigor when they are used for Seed Invigoration. In the past, only anecdotal reports show effectivity of Rice wash in plant growth promotion as in flowering of Orchids and other vegetables. For papaya, seeds germinate slowly, approximately within 7 to 21 days, while some relatives germinate after 10 months. Hence, it is essential to improve its speed and different materials may be used and one of this is Rice Wastewater or *Hugas bigas*.

The study aimed to test on the efficacy of rice wash as a priming agent to enhance germination in papaya and to determine the germination rate, days to germination and seed vigor of papaya seeds across different rice types.

Materials and methods

Plant materials

Seeds of papaya used in the study came from a hermaphrodite fruit of Cavite Special bought in Silang Cavite Market. The seeds were first cleaned to remove the sacrotesta, air-dried and then kept inside glass jar with desiccator maintained in an air-conditioned room.

Locale and duration of the study

The germination experiments were conducted in the Seed Science Laboratory of the Institute of Crop Science, College of Agriculture and Food Science, UPLB. The duration of the experiment was one and a half month.

Rice types used in the study

Four types of rice were used in the study, the well-milled rice (WR), the brown rice (BR), the red rice (RR) and the black rice (BlR). A simple characterization of the types of rice was stated below (Table 1).

Germination experiments for C. papaya cv. 'Cavite Special'

About 100 g of each of the rice type (well-milled, brown, redπ, and black) was washed with 300 mL water. The washings were used for the preparation of treatments. The treatments can be summarized below (Table 1). The experiment was laid out in Completely Randomized design with 5 treatments in three replicates.

Table 1. Summary of treatments used in the study

Treatments	Description
T1	White Rice wash (100 WR)
T2	100% Brown Rice wash (BrW)
T3	100% Black Rice wash (BlRW)
T4	100% Red Rice Wash (RRW)
T5	100% Distilled water (Control)

The seeds were soaked for 12 hours. Treatments were replicated thrice. A total of 100 seeds per replicate were soaked. Soaking was performed using a glass beaker. After 12 hours of soaking, seeds were air dried for 24 hours before germinating them on a Petri plate with moist filter paper as in top-of-paper method. The setup was placed in an ambient temperature with 70%-80% humidity placed in shade. Germinated seeds were counted daily until day 14.

Germinated papaya seeds were sown based on the recommendations of Magdalita and San Pascual (2019). "Papaya seeds were sown in a sterile potting medium (garden soil, coir dust and compost (1:1:1, v/v)). The soil medium was placed in germination trays laid on metal benches in the screenhouse and provided with a black net as partial shade".

Data collection

Germination percentage (%), which was computed using the following formula: *% Germination: No. of germinated seeds / Total No. of seeds) × 100*

Seed vigor (VI), which was computed using the formula:

VI = No. of normal seedling/ day of first count + … + No. of normal seedlings / days to final count

Embryo size (mm) - Embryo of papaya was excised from treated seeds after seed soaking. Embryos were extracted and viewed under the compound microscope at 40X. Measurements were done using ToupTek® imaging software.

Seedling dry weight (g) - Seedlings per subsample were collected and oven dried at 70^{o}C for 3 days. The weight of the dried seedlings was measured using an analytical balance.

Statistical analysis

The experiments on the testing of seed germination rate, days to germination, and seed vigor of papaya was conducted in CRD with three replications and two subsample per replication. Study was subjected to one-way analysis of variance for CRD using F-test after it has passed assumptions tests of Normality (Shapiro-Wilk test) and Homoscedasticity (Levene's test). Statistical analysis will also be performed using STAR.

Results

Germination rate

Rice wastewater (*Hugas Bigas*) was made for each of the rice type using 100g of rice and 300mL of water. Seeds were pretreated by soaking seeds with different rice wastewater (Hugas bigas). Seeds were then air dried and subjected to germination tests.

Result showed the mean germination rate of papaya after they were primed with Rice wastewater (*Hugas bigas*) of different rice types (Figure 1). Significant differences were observed on the mean percent germination of papaya seeds primed to rice wash from different types of rice ($p = < 0.001$). The highest germination rate was observed on seeds primed with white rice wash (84.17%) followed by seeds primed with red rice wash and then seeds primed with Black rice wash (75%).

First day of germination

Germination also was observed 4 days after sowing for seeds soaked in white and black rice wastewater, but germination was observed 6-7 days after sowing on seeds of the control and those treated with red and brown rice wastewater.

Seed vigor

Result showed the average vigor of seeds treated with different rice wash (Figure 2). In this graph, highest vigor was observed on white rice wash and black rice wash, and red rice wash followed by brown rice wash. The control had the

least vigor. It indicated that white, black, and red rice wash treated seeds had faster and more uniform germination compared to the other treatments. More than vigor, observable indicators than lower vigor in brown rice wash treated seeds was the presence of molds in brown rice wash treated seeds at 3rd day which may be due to the high oil content of Brown rice wash that promoted growth of molds.

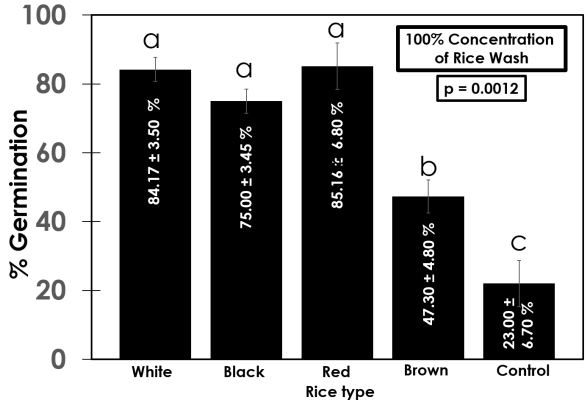


Figure 1. Germination rate of Papaya seeds soaked/ treated with rice wash from different rice types

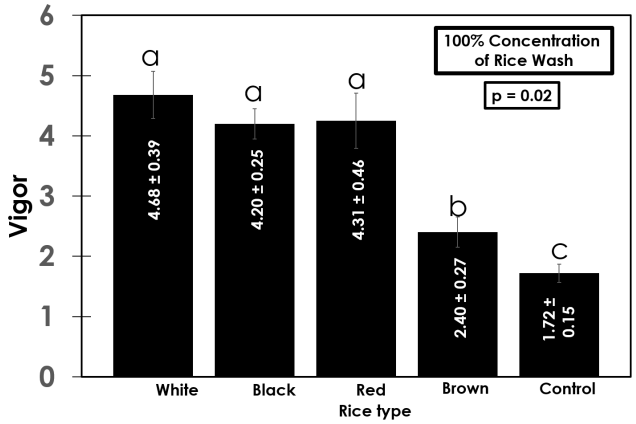


Figure 2. Effect of rice wash from different rice types on vigor of *C. papaya* cv. 'Cavite Special' seeds

Embryo size

Embryo size was determined a day after soaking the seeds to the different treatments. No significant differences were observed on the sizes (as to length (p=0.3018) and width (p=0.567)) of embryo size (Table 2).

This maybe the same mechanism to improve germination but no significant differences were observed among the treatments. This can be due to the early dissection and data gathering of the embryo. The researchers dissected the embryo a day after it has been soaked in treatments and then air dried. The researchers may have found treatment effects if they have dissected the embryo few days after (Figure 3).

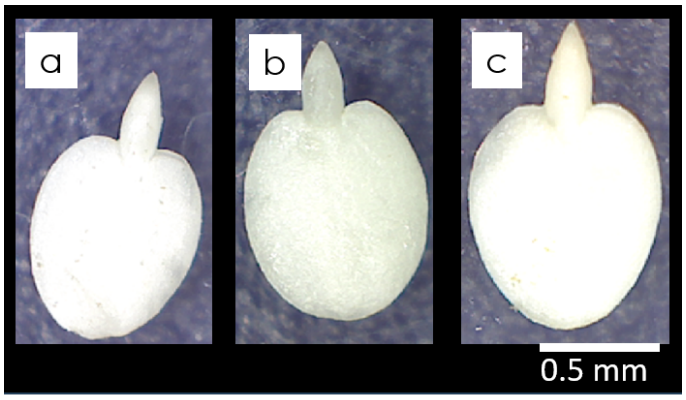


Figure 3. Embryo samples dissected from seeds soaked in a) white, b) black, and c) brown rice wash viewed under the compound microscope at 40X

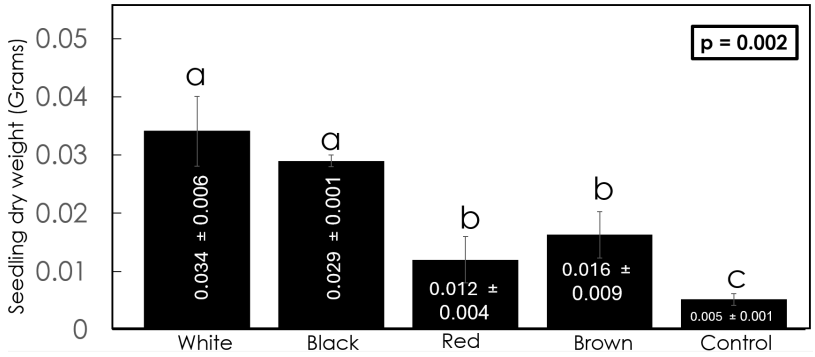


Figure 4. Seedling dry weight of papaya seeds (g) soaked in different rice wash from various types of rice

Effect of priming using different rice wastewater types on seedling dry weight of C. papaya

There was significantly differed on the seedling dry weight of papaya seeds soaked in different rice wash (p=0.0002) as seen in Figure 4. It was observed that seeds soaked in white rice wash and those seeds soaked in black

rice wash produced more seedlings as evidenced by the higher seeding dry weight. Seeds had the highest seedling dry weight of 0.0426g. Very high compared to the other seedling dry weights.

Table 2. Mean embryo length and width (in mm) dissected from Papaya seeds primed with different Rice wash type

Treatment/ Rice wash type	Embryo length (n=20)	Embryo width (n=20)
White Rice Wash	1.00 ± 0.03	0.630 ± 0.02
Black Rice Wash	1.00 ± 0.066	0.647 ± 0.037
Red Rice Wash	1.027 ± 0.01	0.643 ± 0.021
Brown Rice Wash	1.023 ± 0.02	0.633 ± 0.04
Control	0.947 ± 0.07	0.587 ± 0.05
	p= 0.302	p=0.567

[1] *Mean and standard deviation (μ±σ) with different letters are statistically significant at α=5%.*

Discussion

Improved germination was observed on seeds primed with Rice wash when compared to the control. This can be attributed to the additional nutrient found in waste rice wash. This positive effect was also observed when rice wash was used as fertilizer for tomato, pepper and eggplant (Sairi *et al*., 2018, Wijiyanti, 2019). Further, it also promoted early germination. Further, Marthandan *et al*. (2020) also discussed that priming of seeds with different agents enhanced germination rate and seedling growth through shortened imbibition time and 'increasing pre-germinative enzyme activation and and regulating osmosis.

In tomatoes, plants treated with waste rice water had higher number of developed leaves with higher fresh weight also. This is at par with the liquid NPK fertilizer being applied. Further, Evidiente *et al*. (2023) explored the use of rice wash in the growth of Okra. They observed that Rice wash treated plants are taller compared to the control. They also observed that rice wash treated had similar growth rates that those plants treated with artificial fertilizer. They concluded that rice wash can be an alternative to artificial fertilizer in Okra plants. Garcia (n.d.) explored the use of rice water in the growth of Pechay. In her paper, she determined the effect of varying concentrations of white rice wash when it is used as foliar fertilizer for pechay. In their study, it also revealed that waste rice water had Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sulfur. Further, plants watered with WRW had access to the leached nutrients found in this wastewater most importantly Nitrogen.

Further, intermediate concentrations of rice water provided better performance in pechay. The results of the study of Garcia (n.d.) are related to the results of this study where it established that waste rice water had nutrients that seeds can have access to and utilize during seed germination. In terms of the

response, like the study of Garcia (n.d.) where the treatment provided better performance in pechay. white Rice Wash priming provided higher germination rate of seeds of papaya.

In Rice water, nutrient use efficiency is high as stated by Shahane *et al*. (2019). This is due to the dissolved form of nutrients found in rice wash making it readily available for plants to adsorb making it really a viable material for Nutri priming for seeds or fertilizing plants. Sulisetijono *et al*. (2024) also reported that in Rice wash, higher amount of vitamin B which is an intermediary to Auxin and Gibberellins were observed. Auxins are phytohormones that are responsible for cell division which is vital for organogenesis and plant development in germination. Further, Gibberellins is vital for germination as it breaks dormancy and commences degradation of sugars in the seed through alpha amylase activity making the carbohydrate sources available to the embryo. The improved germination due to treatment of rice wash is established. This can be shown in the increased germination percentage of all treated seeds compared to the control.

Further, Priming promotes amylase production and enhances metabolism thus resulting in higher vigor (Guo, 2022). Increased nutrient absorption may cause faster growth and dry matter accumulation (Adhikari *et al*., 2021). More than the information on germination improvement, another target of farmers in utilizing seed invigoration techniques or priming is the uniformity and rapid emergence of seedlings from seeds. Basu and Groot (2023) indicated that seed vigor is an important measure that determines 'uniformity of seed germination, seedling growth, emergence ability under unfavorable environments, and post-storage performance'. Ideally, higher synchronous germination should be observed on healthy, viable seeds. Priming or seed invigoration techniques involve not just improving seed germinability but also the uniformity and synchrony of germination. Hence, vigor is also being measured. A higher vigor index indicates that seeds germinate uniformly. It was then compared to the control to assess if the different Rice Wastewater (hugas bigas) priming were effective in improving the germinability and vigor of seeds.

Magdalita and San Pascual (2019) observed germination of hybrid papaya seeds 7 days after sowing. However, Kumawat *et al.* (2014) observed germination of Solo papaya varieties, an inbred line, at the range of 15-24 days. In this experiment, Observable radicle emergence, which is the determinant of germination in this paper, is already observed 4 days after sowing. This indicates a faster seed germination than the ones reported in literatures. Normally, papaya seeds germinate 7-15 days (Andra Farm, n.d.). Asaduzzaman *et al*. (2021) reported that priming agents enhances seed germination and even early seedling growth in different wheat cultivars.

Rice types differ in terms of their nutrient component and even in their affinity to harbor microbes. Brown rice, according to Abba *et al*. (2021) is white rice with its bran intact. The same researcher also reported that it had higher nutrient contents than white rice. Pascual *et al*. (2013) reported that brown rice wash has iron, manganese, vitamin E, dietary fiber, protein, and carbohydrates due to the presence of the unremoved bran. Rathna-Priya *et al.* (2019) stated that oil in rice is more concentrated and found in the bran. Therefore, brown rice had higher oil content than well milled white rice. The higher oil content of the bran promotes lipid peroxidation which may cause seed deterioration. In the results, the high oil content of the brown rice wash may have promoted seed deterioration among seeds treated with brown rice hence the low germination rate and vigor.

However, Devi and Badwaik (2022) stated that pigmented rice (black or red) are rich sources of bioactive compounds including flavonoids and phenolic acids. Black rice is rich in anthocyanins while red and purple rice is rich in flavonoids. These rice types had high anti-oxidative and radical scavenging properties (Rathna-Priya *et al.*, 2019). These antioxidant properties may prevent reactive oxygen species to propagate hence preventing lipid peroxidation and seed deterioration.

Rice washes improved vigor of seeds when it is compared to seeds only primed using water. Seed germination was indicated by radicle emergence and root development (Wolny *et al*., 2018). Root growth and cell division can be promoted by different hormones such as auxins and gibberellins. Nabayi *et al*. (2023) reported that Rice windole-3-acetic acid (IAA), a hormone that promotes root growth. Further, the high carbohydrate content of white rice wash may have promoted breaking of dormancy and faster germination rate. Adhikari *et al*. (2021) stated that priming increased seedling vigor index because of reduced imbibition. Similar results have been reported in some vegetable plants of germination and field performance of vegetables like faba beans, and other vegetable crops.

Like the present study is the study of Kumar *et al.* (2013). They discussed that primed seeds had more vigorous uptake of water at the first day of priming. Further, water uptake was observed until the 3rd day of hydropriming. Hydropriming also increased the diameter of banana embryos from 948-1142 µm, approximately 20% increase. Specifically, there was an increase in talk length and haustorium length also. The study of Adhikari *et al*. (2021) is similar with the present study. Adhikari *et al*. (2021) explored the use of pre-sowing hydropriming in improving the germination and seedling vigor of bitter gourd var. 'Palee'. In their study, higher germination was observed on hydro primed seeds compared to non-hydro primed seeds. Further, vigorous seedlings were observed on hydro primed seeds as observed on high seedling vigor index.

Initially, seeds have a low moisture content because of which they are inactive. Even so, pre-sowing hydro-priming enhances water imbibition leading to enzyme activation, translocation, and utilization of reserved food materials: accordingly, the findings revealed that the germination and seedling growth is enhanced by hydro-priming.

Nawaz *et al*. (2013) elaborated on the way of mechanism of increased or early emergence in seedling. They stated that pre-softening hydropriming softens hard seed coat facilitating biological proves for germination resulting in early germination of seeds. Due to rapid and earlier germination and emergence of seeds, rapid growth of seedlings happen which may be due to better root development and increase nutrient usage capacity that promoted 'higher relative growth rate or better water status regulation of the plant (Adhikari *et al*., 2021 and Ahmed *et al*., 2016).

As a conclusion, rice wash, despite being a by product in Filipino household, has the potential for use as an agent to invigorate seeds thus increasing uniformity of germination and increasing seed germination per se. It is recommended that further studies may be done across varieties and even across different plant species in tandem with elemental analysis of the rice wash water.

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Conflicts of interest

The authors declare no conflict of interest.

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