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Emilio B. Jacinto II
Silliman University College of Agriculture

Owen Lloyd A. Dumago
Silliman University College of Agriculture

Reiki Elijah L. Varquez
Silliman University College of Agriculture

Rehel A. Diaz
Silliman University College of Agriculture

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Enhancing Germination, Growth, and Vigor of Upland Kangkong Seeds through Ultrasonic Treatment

Emilio B. Jacinto II[1*], Owen Lloyd A. Dumago[1], Reiki Elijah L. Varquez[1], Rehel A. Diaz[1], Jose Edwin C. Cubelo[1], Arsenio D. Bulfa Jr.[1,2]

[1]Silliman University College of Agriculture, Bantayan, Dumaguete City, Negros Oriental 6200, Philippines
[2]Graduate School, University of the Philippines Los Baños (UPLB), College 4031, Laguna Philippines
emiliobjacintoii@su.edu.ph

Abstract. *Seed germination is vital for agricultural productivity and ecological sustainability. Orthodox seeds, while resilient, often exhibit dormancy and reduced germination rates, necessitating innovative enhancement techniques. This study examines and evaluates ultrasonic treatment's effectiveness (40 kHz) in enhancing the germination rate and early seedling growth of upland kangkong (Ipomoea reptans). Seeds underwent ultrasonic exposure for 0, 5, 10, and 15 minutes, and germination percentage, root and shoot lengths, root-shoot ratio, and seedling vigor index (SVI) were evaluated. Results revealed that ultrasonic treatment significantly improved germination, root elongation, and SVI in upland kangkong. These findings highlight ultrasonic treatment as a promising, non-chemical approach for enhancing germination, though optimization is necessary for broader application. Further research should explore the physiological mechanisms underlying these effects.*

Keywords: orthodox seeds, dormancy, ultrasonic, germination, vigor

1. INTRODUCTION

Seed germination is a critical phase in the plant life cycle, determining its ability to establish and reproduce [1]. It is a complex physiological process initiated by the absorption of water, oxygen availability, and exposure to light, leading to enzymatic and metabolic changes that enable the embryo to grow [2]. Germination success directly influences agricultural productivity, ecosystem restoration, and biodiversity conservation [3]. Consequently, understanding the mechanisms and factors affecting germination is vital for enhancing crop production and ecological sustainability. Orthodox seeds, known for their desiccation tolerance, can endure extreme drying and freezing, making them suitable for long-term storage in seed banks [4]. Despite their resilience, these seeds often face dormancy challenges that impede germination, especially under suboptimal environmental conditions [5]. Research by Solberg et al. (2020) showed that while orthodox seeds can maintain viability for extended periods, factors like seed aging, loss of vigor, and dormancy limit their germination capacity. Similarly, Liu et al. (2016) highlighted that aged orthodox seeds experience a decline in metabolic activity, reducing germination rates. These findings emphasize the need to explore effective strategies to enhance germination rates of orthodox seeds [6]. One of the pressing issues is the difficulty in breaking dormancy, particularly in aged or environmentally stressed orthodox seeds. With this, there is a need to strengthen science, technology, and innovation contributions to economic growth and align new innovations with emerging development needs [7]. Studies have demonstrated that standard methods like stratification and priming may not be consistently effective across different species [8]. Additionally, improper handling during storage can lead to seed deterioration, further complicating germination efforts [9]. These challenges necessitate innovative and sustainable techniques to optimize germination outcomes. Recent advancements have explored ultrasonic treatment as a promising method to improve germination by increasing water uptake, oxygen availability, and enzymatic activity through acoustic cavitation [10]. Ultrasonic treatment has shown potential in overcoming dormancy and enhancing germination rates in various species, as evidenced by its positive effects on aged seeds of tall fescue and rice [6][11]. However, the effectiveness of ultrasonic treatment in different orthodox species, particularly those with strong dormancy mechanisms, remains underexplored. Therefore, this study aims to evaluate the effectiveness of ultrasonic treatment in enhancing the germination rate and early seedling growth of upland kangkong (*Ipomoea reptans*). Specifically, it investigates whether exposure to ultrasonic waves can break dormancy and improve seed vigor, particularly in dormant orthodox seeds. The research hypothesizes that ultrasonic treatment, through its effects on water absorption, oxygen diffusion, and enzymatic activation, will significantly enhance germination performance and early growth of upland kangkong.

2. MATERIALS AND METHODS

2.1 Seed Materials
The orthodox seed samples, such as upland kangkong, were sourced from National Seed Foundation Institute of Plant Breeding Crop Science Cluster University of the Philippines Los Baños.

2.2 Seed Ultrasonic Treatments
The seeds were surface sterilized for ten minutes in a 30-ppm hypochlorite solution before the sonication treatment. The seeds were then given a final rinse with distilled water after being rinsed with running water. After rinsing, the seeds were divided into 4 groups: (a) control samples, which were soaked in distilled water for ten minutes without being subjected to any ultrasonic treatment [T1]; (b) samples exposed to 40 kHz for five minutes [T2]; (c) samples exposed to 40 kHz for ten minutes [T3]; (d) samples exposed to 40 kHz for fifteen minutes [T4] based on the protocol of Alvarado et al. (2024) and Machikowa et al. (2013) [8][12].The identified samples were exposed to a bath-type ultrasonic machine running at a fixed frequency 40 kHz and at a temperature of 25°C for the specified durations [8].

2.3 Experiments on Germination
In order to test for germination after the ultrasonic treatment, the seeds were sown in three (3) replications, each of which contained three subsamples of ten seeds. The seeds were sown in a petri dish lined with tissue paper (ideal condition). The petri dishes were set up outside the SU-George K. Young Feed Laboratory. Germination counts were performed daily during the first week of the sowing period and every two (2) days the following week. When seeds were believed to have germinated, radicles that were about 2 mm long were observed to emerge. The percentage germination (PG) was calculated using Equation 1 [8] .

Equation 1. $PG = \frac{germinated\ seeds}{total\ seeds\ sown} \times 100\%$

2.4 Seedling root and shoot length measurement and root-to-shoot ratio.
Throughout the first week of the germination phase, the seedlings were measured daily. The lengths of the seedlings' roots and shoots were measured using a digital caliper. Equation 2 was also used to determine the root-shoot ratio [8].

Equation 2. $Root - shoot\ ratio = \frac{root\ length}{shoot\ length}$

2.5 Seedling Vigor Index
The seedling vigor index (SVI) was calculated by Equation 3 [8].

Equation 3. $SVI = (root\ length + shoot\ length)(PG)$

2.6 Statistical Analysis
The Statistical Tool for Agricultural Research (STAR) 2.0 software, developed by the International Rice Research Institute, was used to analyze the data collected in this study, which was set up in a completely randomized design (CRD), using one-way analysis of variance and least significant difference (LSD) to determine the differences between treatment means at a 5% significance level.

3. RESULTS & DISCUSSIONS

3.1 Germination Rate
The results demonstrate that ultrasonic treatment at 40 kHz for 5 to 15 minutes significantly enhanced the germination percentage of upland kangkong on Day 1 compared to the control, with seeds treated for 15 minutes consistently exhibiting numerically higher germination rates than other treatments. These effects can be attributed to the increased cell membrane permeability induced by ultrasonic waves, which facilitates more efficient water uptake and nutrient absorption. According to Gong et al. (2024) ultrasonic treatment has been shown to significantly enhance seed germination rates across various plant species compared to untreated controls or other methods [13]. Additionally, ultrasonic treatment likely activates metabolic processes by enhancing enzyme activity and breaking seed dormancy, leading to quicker imbibition and germination. However, despite significant improvements on Day 1, no further significant differences in germination rates were observed from Day 2 to Day 5, suggesting that the effects of sonication may be more prominent during the initial stages of seed imbibition. This aligns with findings from Alvarado et al. (2024), who noted that the effectiveness of ultrasonic treatment varies among species, underscoring the importance of optimizing exposure time and frequency for different seed types [8]. The findings also support the view that ultrasonic treatments can offer significant benefits for germination but may require species-specific adjustments for optimal results.

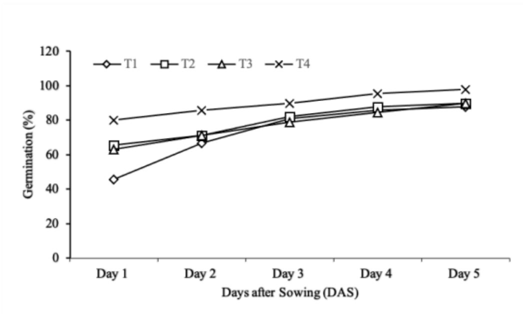

Figure 1. Germination (%) of upland kangkong after ultrasonic treatment.

3.2 Root Length

Ultrasonic exposure for 5-15 minutes led to a significant increase in root length in upland kangkong. This finding aligns with the results of Wang et al. (2012), who observed enhanced root elongation in switchgrass following ultrasonic pretreatment. The underlying mechanisms behind this effect likely involve the enhancement of cell membrane permeability, allowing for more efficient water and nutrient uptake. Additionally, ultrasonic treatment may stimulate enzymatic activity, particularly amylase, which plays a crucial role in breaking down stored starches into simpler sugars, thereby supporting nutrient mobilization during early seedling growth [6]. Ultrasound also induces cavitation within plant cells, which can generate microjets and shear forces that promote the release of growth hormones, particularly auxins, known to enhance root development [14] [15]. Furthermore, ultrasonic exposure has been shown to reduce seed coat resistance, facilitating better water absorption and activation of metabolic pathways required for root growth [16]. These mechanisms combined suggest that ultrasonic treatment optimizes the initial phases of root development by improving both nutrient availability and hormonal regulation. Similarly, the improved root growth observed in this study could be attributed to a synergistic effect of enhanced water uptake, enzyme activation, and hormonal stimulation.

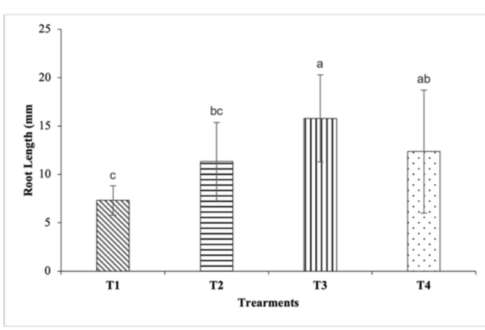

Figure 2. Root length (mm) ± SD of upland kangkong after ultrasonic treatment.

3.3 Shoot Length

The trend in shoot length closely mirrors the results observed for root length, with the 5 to 15-minute ultrasonic treatments producing the most significant growth. Alvarado et al. (2024) reported similar findings in bell pepper seeds, where controlled ultrasonic exposure significantly enhanced seedling vigor, including increased shoot growth [8]. The enhanced shoot growth observed in this study may be attributed to improved nutrient absorption resulting from the more extensive and efficient root systems stimulated by ultrasonic treatment. The mechanism behind this improvement in root development involves the ultrasonic waves' ability to increase cell membrane permeability, allowing for better water and nutrient uptake [10]. This improved root function directly supports shoot development, as the roots are responsible for acquiring the essential nutrients required for optimal shoot growth.

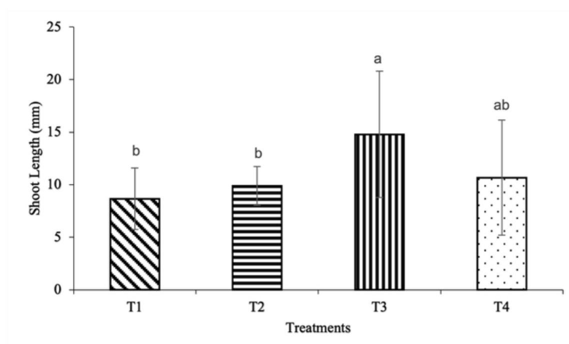

Figure 3. Shoot length (mm) ± SD of upland kangkong after ultrasonic treatment.

3.4 Root-Shoot (R/S) Ratio

Although no significant differences were observed in the root-to-shoot (R/S) ratio, seeds exposed to 15 minutes of 40 kHz sonication showed a relatively higher R/S ratio (Figure 4). A balanced R/S ratio is essential for seedling stability, efficient nutrient allocation, and water absorption, all of which support optimal growth and survival [17]. Research on the effects of sonication on the R/S ratio is limited, but studies suggest ultrasonic treatments influence root and shoot development. For example, Machikowa (2013) found that 40 kHz sonication improved germination, seed vigor index (SVI), and root and shoot growth in synthetic sunflower seeds, indicating a balanced stimulation of these components [12]. Similarly, López-Ribera and Vicient (2017) reported that short sonication periods enhanced germination rates in aged Arabidopsis seeds, suggesting potential downstream effects on seedling development [18].

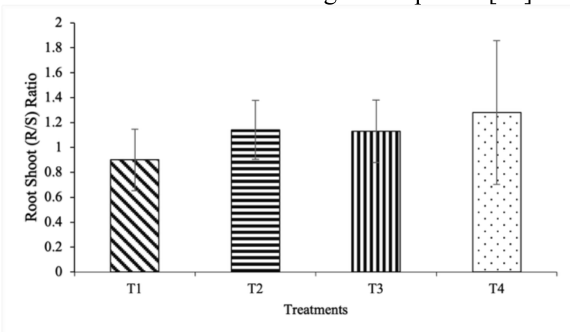

Figure 4. Root-Shoot (R/S) Ratio ± SD of upland kangkong after ultrasonic treatment.

3.5 Seedling Vigor Index

Ultrasonic seed treatment enhances Seedling Vigor Index (SVI) by improving germination and seedling development. In upland kangkong (Ipomoea aquatica), 10-minute sonication yielded the highest SVI, followed by 15-minute and 5-minute treatments (Figure 5).

The cavitation effect generated by ultrasound creates micropores in seed coats, facilitating water uptake and gas exchange, which accelerates germination and early growth [19] [20]. Sonication also enhances root and shoot growth, seedling dry weight, and vigor index while upregulating antioxidant genes, improving stress tolerance and metabolism [21]. Additionally, increased γ-aminobutyric acid (GABA) levels support carbon and nitrogen balance, further promoting seedling vigor, particularly under stress [22].

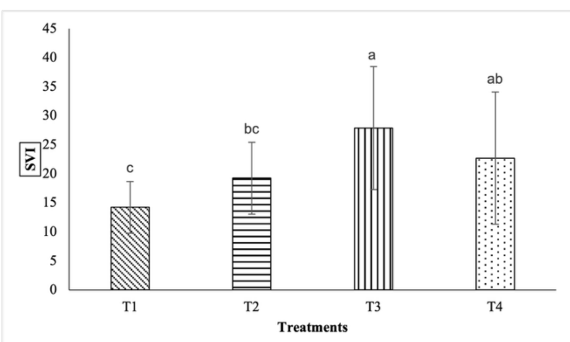

Figure 5. Seedling vigor index ± SD of upland kangkong

A higher Seedling Vigor Index (SVI) signifies greater germination rates, faster seedling development, improved stress tolerance, and greater storage potential, all of which contribute to more reliable crop establishment, higher yields, and reduced risks of crop failure. These characteristics are essential for plants to successfully establish themselves under a variety of environmental conditions and stressors, ensuring better growth, higher productivity, and resilience to adverse factors in the field [10].

Improved seedling vigor index (SVI) enhances plant yield by promoting faster germination, uniform establishment, and robust early growth, which collectively optimize resource use efficiency and stress resilience. Firstly, seeds with SVI exhibit greater germination rates and faster seedling emergence, ensuring a higher number of established plants per unit area [23]. Secondly, vigorous seedlings better tolerate abiotic stresses and biotic challenges, reducing seedling mortality [24]. Next, seedlings with higher vigor develop longer roots and shoots faster, enabling earlier nutrient uptake and photosynthesis. This accelerates vegetative growth, which correlates with higher reproductive output [24]. Also, high-vigor seeds enable farmers to use higher sowing densities without compromising establishment [25].

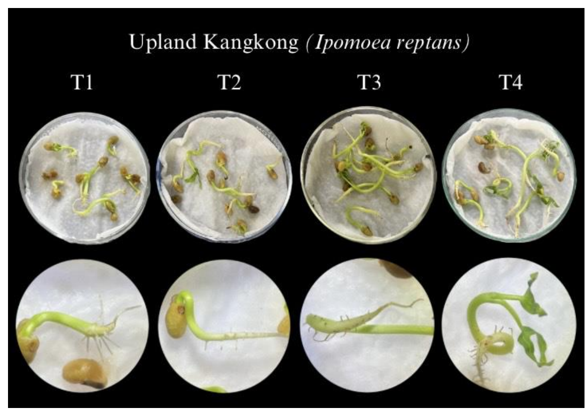

Figure 6. Germination and early emergence of kangkong seeds.

4. CONCLUSIONS

The results demonstrated that ultrasonic exposure at 40 kHz influenced germination rates, root and shoot lengths, and seedling vigor. In upland kangkong, ultrasonic treatment enhanced germination rates, root and shoot elongation, and seedling vigor, particularly at longer exposure times. Overall, ultrasonic treatment presents a promising, non-chemical approach to improving seed germination and early seedling growth, with potential applications in sustainable agriculture and seed technology.

5. RECOMMENDATIONS

These findings align with previous studies indicating that ultrasonic treatment can enhance water uptake, enzymatic activity, and seed metabolism, thereby improving germination and seedling vigor especially when applied silica nanoparticles [26]. However, the varied responses among species highlight the need for further optimization of frequency, duration, and treatment conditions to maximize benefits. Future research should explore the underlying physiological and molecular mechanisms driving these effects and assess long-term impacts on plant development and yield.

6. ACKNOWLEDGEMENT

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