Vol. 14(2): 439-453, May 2026
DOI: https://doi.org/10.23960/jsl.v14i2.1446

Jurnal Sylva Lestari

P-ISSN: 2339-0913
E-ISSN: 2549-5747

Journal homepage: https://sylvalestari.fp.unila.ac.id

Full Length Research Article

Improving Seed Viability and Vigor of *Acacia crassicarpa* A. Cunn. Ex Benth. Using Ultrafine Bubbles and Biopriming Based on Dark Septate Endophytes

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ARTICLE HISTORY:

Received: 19 January 2026
Peer review completed: 27 April 2026
Received in revised form: 18 May 2026
Accepted: 3 June 2026

KEYWORDS:

Acacia crassicarpa
Biopriming
Dark septate endophyte
Seed germination
Ultrafine bubbles

ABSTRACT

Acacia crassicarpa is a fast-growing tree species that has been a mainstay in the establishment of industrial plantation forests in Indonesia. The need for *A. crassicarpa* seedlings for large-scale planting programs is often hampered by poor seed germination due to strong dormancy and low seed vigor. This study aims to analyze the effects of ultrafine bubbles (UFB) and biopriming with dark septate endophytes (DSE) on the viability and vigor of *A. crassicarpa* seeds. Before treatment, all seeds were soaked in 98% H_2SO_4 for 15 minutes as a field procedure for *A. crassicarpa* seed germination, serving as the positive control in this study. A completely randomized factorial design with two factors, i.e., UFB water (0, 8, and 20 ppm) and DSE isolates (without DSE, MM15 (DSE MM.15), *Cladosporium teunissimum* (DSE KSP.1), and *Dendrothyrium* sp. (DSE CPP.114), was used to test the effects of the treatments on seed germination parameters (germination capacity, germination speed, germination value, hypocotyl and radicle length, and vigor index). The germination parameters were analyzed using analysis of variance and Duncan's Multiple Range Test at the 95% confidence level. The interaction between UFB and DSE treatments significantly affected germination capacity, germination speed, germination value, and vigor index. The optimal result was achieved by soaking seeds in 8 ppm UFB water for 24 hours, combined with soaking in DSE CPP.114 for 24 hours with a germination capacity of 78.5% (increase of 32.5% from control), germination speed of 8.04% day^{-1} (increase of 38% from control), germination value of 15.39 (increase of 188.7% from control), and vigor index of 11.29 (increase of 80.3% from control). Thus, the UFB water and DSE *Dendrothyrium* sp. (DSE CPP.114) can increase seed viability and vigor after scarification, thereby directly improving the quality and quantity of *A. crassicarpa* seedlings for planting programs.

1. Introduction

Acacia crassicarpa A. Cunn. ex Benth. is one of the species commonly found growing in tropical areas, both in dry and wet (swampy) areas (McKinnon et al. 2018; Sumawinata et al. 2019), and *Acacia* species are classified as fast-growing tree species (Baskara et al. 2022; Prayogo et al. 2023), including *A. crassicarpa*. Generally, wood from *A. crassicarpa* is used as raw material for paper (Martins et al. 2020), furniture, perfume, decoration, and street shade (Amelia et al. 2024; Ishio et al. 2024). *A. crassicarpa* is widely planted in industrial plantations for the pulp and paper industry (Zul et al. 2021), including mineral areas and wetlands (swamps) (Nirsatmanto and Sunarti 2019; Taufik et al. 2019; Sudrajat et al. 2024). To date, large-scale planting of *A. crassicarpa* still relies on generative seeds to produce large numbers of seedlings. *A. crassicarpa* seed is an orthodox seed with strong physical dormancy because it has thick and hard seed coats (Sudrajat et al. 2024). Operationally, physical seed dormancy is overcome by soaking the seeds in 98% H_2SO_4 for 15 minutes (Sinar Mas Forestry 2020). However, this technique has not yet produced optimal seed germination. Sudrajat et al. (2024) reported that pre-germination treatment by soaking *A. crassicarpa* seeds that had been stored for a long time (more than 2 years) in 98% H_2SO_4 for 15 minutes resulted in a germination capacity of 20.6%. This result may be due to the concentration and duration of immersion in H_2SO_4 solution, which is not optimal, and the role of H_2SO_4 itself is greater in breaking physical dormancy (Diokhane et al. 2025), so that other treatments are needed, which are physiologically capable of increasing the viability and vigor of *A. crassicarpa* seeds. Several relatively new technologies can be used to increase seed viability and vigor, including ultrafine bubbles (UFB) and bio-priming technologies (Sudrajat et al. 2022; Syamsuwida et al. 2020; Yuniarti et al. 2026).

UFB are very small bubbles with a diameter of less than 1 µm (10^{-6} m) (Ahmed et al. 2023). The fine bubbles in UFB can trigger the formation of reactive oxygen species (ROS), which can weaken seed cell walls, thereby affecting cell elongation (Liu et al. 2017; Ha et al. 2025). At certain concentrations, ROS accelerate seed germination (Castro-Cadamba et al. 2022; Farooq et al. 2021) and increase gibberellin hormone content (Liu et al. 2016a; Sudrajat et al. 2022). Gibberellin plays an important role in the seed germination process by activating various hydrolytic enzymes, such as α-amylase, protease, α-gluconase, phosphatase, and ribonuclease, where these enzymes play a role in the mobilization of food reserves during seed germination (Damaris et al. 2019; Cornea-Cipcigan et al. 2020; Li et al. 2019a; Shah et al. 2023). Several studies have reported that UFB application is effective in breaking dormancy and increasing seed viability and vigor in *Gmelina arborea* (Siregar et al. 2020), *Albizia procera* (Sudrajat et al. 2022), and *Vigna angularis* (Ha et al. 2025). Additionally, UFB water has been reported to support plant growth by stimulating the production of growth hormones and enhancing the regulation of genes related to nutrient absorption (Oshita et al. 2023; Wang et al. 2021). However, information on the effects of UFB on seed viability remains relatively limited, especially for tropical forest tree species. Therefore, further research is needed to evaluate the potential of UFB in improving the viability and vigor of tropical forest tree seeds, including *A. crassicarpa* seeds.

Bio-priming is a method of seed invigoration to improve seed germination, growth, and plant adaptation. Various bio-inoculants, such as beneficial microbes (bacteria, algae, fungal extracts, seaweed extracts) or biological products (humic acid, fulvic acid, chitosan), are used as biostimulating agents in seed biopriming to promote plant growth or increase tolerance to stress (Malik et al. 2021; Ugena et al. 2018). Several fungal groups, such as mycorrhizae, also positively affect seed germination (Fochi et al. 2017; Tsulsiyah et al. 2021). In addition, a group of fungi that has potential for use in seed invigoration is DSE (dark septate endophytes), which is known to have the benefit of increasing seed germination (Azmi et al. 2022; Priatna et al. 2026) and enhancing plant growth and adaptation (He et al. 2019; Putri et al. 2026). Several studies have reported that DSE positively affects the germination of *Falcataria moluccana* (Surono et al. 2024) and *Agathis borneensis* (Yuniarti et al. 2026) seeds. DSE belongs to the group of facultative biotrophic Ascomycetes, characterized by dark-pigmented, septate hyphae containing melanin and the ability to form microsclerotia in plant root tissues. It is known that DSE can colonize root tissues, both intracellularly and intercellularly, in more than 600 plant species without causing pathogenic symptoms (He et al. 2019; Santos et al. 2021). This study aims to evaluate the effect of the combination treatment of UFB and dark septate endophytes (DSE) on the viability and vigor of *A. crassicarpa* seeds.

2. Materials and Methods

2.1. Materials

The *A. crassicarpa* seeds used in this study were obtained from the seed production area at the Parung Panjang Forest Research Station (6°22′45.800″ S; 106°30′58.568″ E; 52 m above sea level). The seeds obtained were then processed and stored for 2 years at the Seed Testing Laboratory of the Institute for Implementation of Environmental and Forestry Standards and Instruments (IIEFSI), Bogor. Seeds were stored in airtight plastic containers in an air-conditioned (AC) room at 18–20 °C and 60–70% relative humidity. The dark septate endophytes (DSE) used in this study were *Dendrothyrium* sp. (DSE CPP.114) isolated from the roots of lowland rice, *Cladosporium teunissimum* (DSE KSP.1) was isolated from lowland rice roots, and DSE MM.15 was isolated from chili roots. The selection of DSE was based on previous studies that reported its positive effect on germination and seedling growth, such as *Dendrothyrium* sp. (DSE CPP.114) on *Falcataria moluccana* (Surono et al. 2024) and *Leucaena leucocephala* seeds (Widyani et al. 2024), *C. teunissimum* (DSE KSP.1) on *Gmelina arborea* seeds (Priatna et al. 2026), and DSE MM.15 on *Ceiba pentandra* and *L. leucocephala* seeds (Widyani et al. 2024).

2.2. Ultrafine Bubbles and Dark Septate Endophytes Isolate Preparation

Ultrafine bubbles (UFB) were produced using a UFB generator (FZ1N-10, IDEC) at the Biosystem and Environmental Engineering Laboratory, IPB University. UFB water was made by operating a UFB generator filled with distilled water for 55 minutes in two ways: without oxygen injection to obtain UFB water of 8–10 ppm and with oxygen injection to obtain UFB water of 20 ppm. Oxygen was injected into the UFB generator tube at 300–350 kPa. Two replicates were performed at each UFB concentration (8 ppm and 20 ppm). The UFB water produced was placed in the refrigerator. After reaching room temperature, the dissolved oxygen (DO) concentration was measured with a dissolved oxygen meter (DO-5512SD, Lutron).

The DSE isolates were prepared at the Soil Microbiology Laboratory of the Ministry of Agriculture. The three isolates were cultured in a single strain using a formula of sawdust, bran, and coarse corn flour. Preparing 500 g of DSE carrier required 300 g of sawdust, 100 g of bran, and 100 g of coarse corn starch. The ingredients were mixed evenly, distilled water was added to achieve a moisture content of ± 60%, the mixture was placed in an autoclave-resistant plastic package, and the package was then placed in the autoclave. The materials were autoclaved again and cooled for 24 hours after the first autoclaving process. The carrier material was inoculated with DSE fungal inoculum, i.e, *Dendrothyrium* sp. (DSE CPP.114), *C. teunissimum* (DSE KSP.1), and DSE MM 15 isolate, as much as 1% of the weight or volume of the carrier material, and then stored at room temperature so that DSE grew and developed in the carrier material and was ready for use in the experiment.

2.3. Experimental Design

A completely randomized factorial design was used to test the effects of seed soaking in UFB water (5 treatments) and DSE solution (4 treatments) on seed viability and vigor of *A. crassicarpa*. The UFB water application factor consisted of 5 treatments, i.e., A0 = control, A1 = soaked in 8 ppm UFB for 12 hours, A2 = soaked in 8 ppm UFB for 24 hours, A3 = soaked in 20 ppm UFB for 12 hours, and A4 = soaked in 20 ppm UFB for 24 hours. The DSE immersion factor consisted of 4 treatments, i.e., B0 = seeds with no treatment (control), B1 = seeds soaked with DSE isolate MM.15 (DSE MM.15) for 24 hours, B2 = seeds soaked with DSE *Cladosporium teunissimum* (DSE KSP.1) for 24 hours, and B3 = seeds soaked with DSE *Dendrothyrium* sp. (DSE CPP.114) for 24 hours. In this study, the positive control was *A. crassicarpa* seeds treated with 98% H_2SO_4 immersion for 15 minutes. This is the standard treatment used by several Industrial Forest Plantation Companies (Sinar Mas Forestry 2020). Each treatment had 4 replicates, with each replicate consisting of 100 seeds.

2.4. Seed Treatment and Germination Parameter Measurement

Seed testing was carried out at the Seed Testing Laboratory, IIEFSI, Bogor. Before being treated according to the design, all seeds were chemically scarified by immersing them in 98% H_2SO_4 for 15 minutes. After soaking in H_2SO_4, the seeds were washed with running water and then treated as specified in the design. Seed soaking was conducted using 8 ppm UFB water, 20 ppm UFB water, and soaking using DSE isolate MM.15 (DSE MM.15), *C. teunissimum* (DSE KSP.1), and *Dendrothyrium* sp. (DSE CPP.114). Seed soaking in UFB water was carried out for 12 and 24 hours (Siregar et al. 2020), while seed soaking in DSE was carried out for 24 hours (Priatna et al. 2026). For the combination treatment, seeds were first soaked in UFB water, then soaked in DSE solution for 24 hours. Seeds must be completely immersed in UFB water or DSE solution, with 100 seeds soaked in 50 ml of UFB water or DSE solution. After seed-soaking treatments, the seeds were removed and drained, and then *A. crassicarpa* seeds were germinated. Seeds were sown in a sand:soil mixture (1:1, v/v) in a germination tray measuring 33 cm long, 27 cm wide and 10 cm high in a greenhouse (28–34 °C and 60–75% relative humidity). Humidity in the germination tray was maintained by covering it with transparent plastic to prevent evaporation. Observations were made daily to assess the progress and pattern of germination. The observed seed germination parameters were germination capacity, germination speed, germination value, hypocotyl and radicle length, and vigor index (Sudrajat et al. 2022). These parameters were calculated using Equations 1–6.

$$Germination\ capacity = \frac{Number\ of\ normal\ seedling}{Number\ of\ sown\ seed} \times 100 \tag{1}$$

$$Germination\ speed = \sum_{0}^{tn} \frac{N}{t} \tag{2}$$

where N is the number of normal seedlings, and t is the germination time (day^{-t}).

$$Germination\ value = PV \times MDG \tag{3}$$

where PV is the peak value, and MDG is the mean daily germination.

$$PV = \frac{Highest\ seed\ germination}{Number\ of\ days} \tag{4}$$

$$MDG = \frac{Total\ number\ of\ germination}{Total\ number\ of\ days} \quad (5)$$

$$Vigor\ index = germination\ capacity\ x\ (radicle\ lenght + hypocotyl\ length) \quad (6)$$

2.5. Data Analysis

The data were tested for normality before the analysis of variance test. The Shapiro-Wilk test showed that most of the data were not normally distributed, namely germination capacity (p = 0.003), germination speed (p = 0.001), mean germination time (p = 0.041), germination value (p = 0.002), and vigor index (p = 0.012), except for radicle and hypocotyl length which had normally distributed data with p-values of 0.170 and 0.241, respectively. Data that were not normally distributed were transformed using a logarithmic transformation (Priatna et al. 2026). Normality test for germination capacity, germination speed, mean germination time, germination value, and vigor index data ($p > 0.05$). Analysis of variance with F-test was conducted to determine the effect of seed invigoration treatment (seed soaking in UFB water and DSE) on several parameters of *A. crassicarpa* seed germination. If the p-value > α (0.05), the treatment did not significantly affect the germination parameters; if the p-value < α (0.05), the treatment did. Duncan's multiple-range test (DMRT) was used to assess differences in germination parameter responses among treatments.

3. Results and Discussion

3.1. Results

The interaction between seed soaking in ultrafine bubbles (UFB) and dark septate endophytes (DSE) after 15 minutes of H_2SO_4 scarification significantly affected the germination capacity, germination speed, germination value, and vigor index of *Acacia crassicarpa* seeds (p < 0.05). Meanwhile, seed immersion in UFB water, DSE, and their interactions did not significantly affect hypocotyl and radicle length parameters (**Table 1**).

Fig. 1 shows that the highest germination capacity was achieved by soaking seeds in UFB water at 8 ppm for 24 hours, followed by soaking in DSE CPP.114 for 24 hours, resulting in a germination capacity of 78.5%. This result increased by 32.5% compared to the control (seeds treated with 98% H_2SO_4 for 15 minutes). The same thing happened to the germination speed; the treatment of soaking the seeds in UFB water at 8 ppm for 24 hours and DSE CPP.114 gave the highest germination speed (8.04% day^{-1}). In contrast, in the control treatment, the germination speed was only 5.8% day^{-1}, so the treatment of soaking the seeds in UFB water at 8 ppm for 24 hours and DSE CPP.114 increased germination by 38% over the control. Likewise, with other germination parameters, i.e., germination value and vigor index, treatment with UFB water at 8 ppm for 24 hours and DSE CPP.114 yielded the highest germination value and vigor index of 15.39 (increased 188.7% from the control) and 11.29 (increased 80.3% from the control), respectively. Observations of hypocotyl and radicle lengths until day 21 after sowing showed no significant differences between treatments (**Table 2**; **Fig. 2**).

The treatment with the worst results was soaking the seeds in UFB water at 20 ppm for 24 hours. This treatment reduced all germination parameters of *A. crassicarpa* seeds. Germination capacity in this treatment was only 14.5% with a germination speed of 1.13% day^{-1}. Seed soaking treatments in DSE gave different responses on several germination parameters. DSE CCP.114 tended to increase germination, but seed soaking in DSE KSP.1 resulted in lower germination than the control in most germination parameters. For DSE MM.15, the effect on seed germination was more moderate, tending to increase germination, but the effect was not significantly different from the control (**Fig. 1–2**; **Table 2**).

Table 1. Summary of analysis of variance of the effect of invigoration treatments by soaking in ultrafine bubbles (UFB) and dark septate endophytes (DSE) on the germination parameters of *Acacia crassicarpa* seeds

Germination Parameter	Source of variation	Degree of freedom	F-count	Sig.
Germination capacity (%)	UFB treatment (A)	4	0.376	0.771 ns
	DSE treatment (B)	3	12.812	0.000 **
	Interaction A x B	12	2.670	0.006 **
Germination speed (% day^{-1})	UFB treatment (A)	4	13.603	0.000 **
	DSE treatment (B)	3	0.474	0.702 ns
	Interaction A x B	12	2.547	0.009 **
Germination value	UFB treatment (A)	4	5.740	0.001 **
	DSE treatment (B)	3	1.191	0.321 ns
	Interaction A x B	12	2.749	0.029 *
Hypocotyl length (mm)	UFB treatment (A)	4	1.253	0.410 ns
	DSE treatment (B)	3	0.567	0.415 ns
	Interaction A x B	12	1.873	0.426 ns
Radicle length (mm)	UFB treatment (A)	4	1.653	0.584 ns
	DSE treatment (B)	3	0.875	0.547 ns
Vigor index	Interaction A x B	12	0.965	0.682 ns
	UFB treatment (A)	4	12.281	0.000 **
	DSE treatment (B)	3	0.440	0.725 ns
	Interaction A x B	12	2.252	0.020 *

Notes: ** = significant at 99% confidence level, * = significant at 95% confidence level, ns = not significant at 95% confidence level.

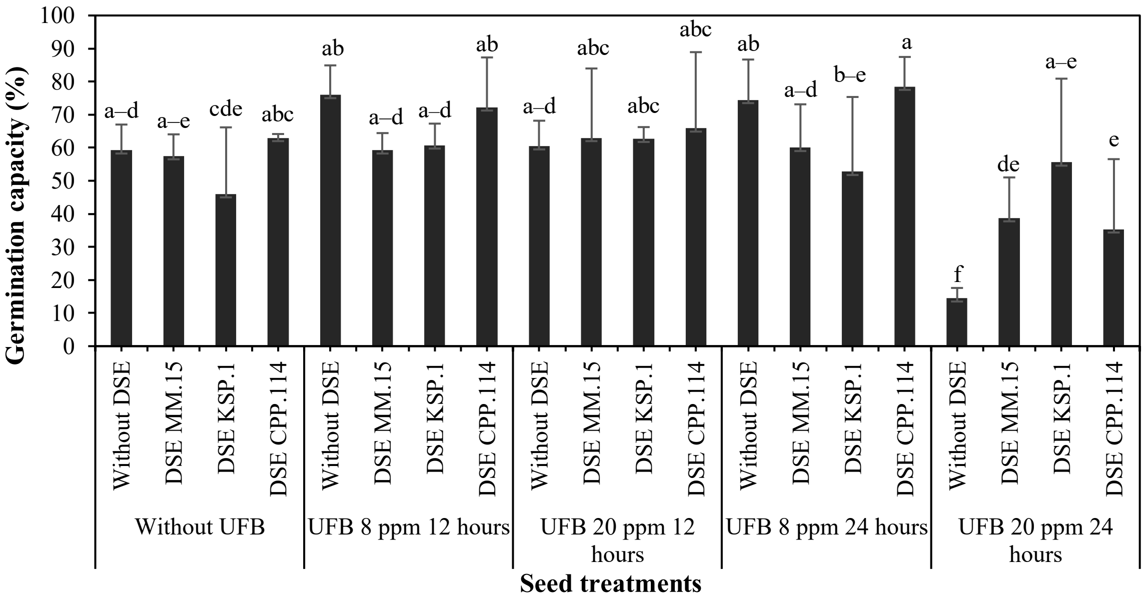

Fig. 1. Germination capacity of *Acacia crassicarpa* seeds treated by soaking in ultrafine bubbles (UFB) and dark septate endophytes (DSE). All seeds were pretreated with 98% H_2SO_4 for 15 minutes before the treatments. The same letters on the bar graph indicate no significant difference at the 95% confidence level.

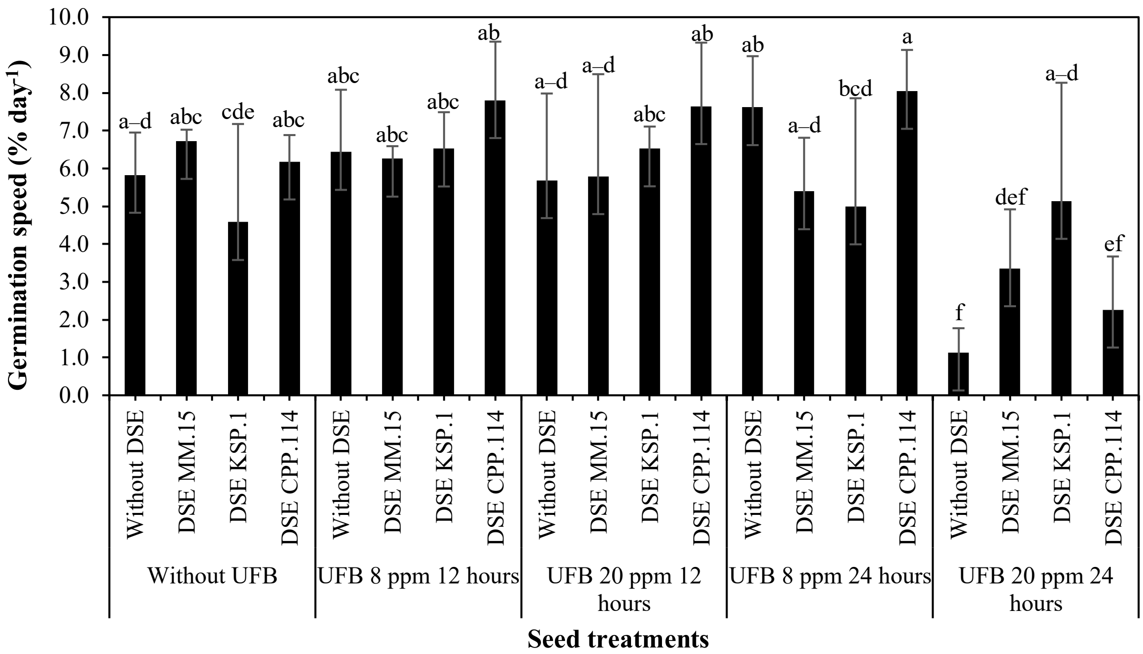

Fig. 2. Germination speed of *Acacia crassicarpa* seeds treated by soaking in ultrafine bubbles (UFB) and dark septate endophytes (DSE). All seeds were pretreated with 98% H_2SO_4 for 15 minutes before the treatments. The same letters on the bar graph indicate no significant difference at the 95% confidence level.

3.2. Discussion

A. crassicarpa seeds have hard and relatively thick skins, so their dormancy is very strong. Apart from strong dormancy, the *A. crassicarpa* seeds have been stored for 2 years, so they have undergone physiological decline (seed deterioration). The longer seeds are stored, the more their viability and vigor decrease. Even though the seeds had been treated with H_2SO_4 for 15 minutes to induce scarification, the germination rate was only 46%. In addition, the concentration and duration of the H_2SO_4 soak may not have been optimal. According to Sudrajat et al. (2024), scarification of *A. crassicarpa* seeds with 96% H_2SO_4 is more effective for 25 minutes. In this study, after H_2SO_4 scarification, the invigorating treatment with UFB water and DSE was applied. Seeds treated with UFB water 8 ppm for 24 hours with DSE *Dendrothyrium* sp. Overall, CPP.114 treatment showed the best germination capacity (78.5%), germination speed (8.04% day^{-1}), germination value (15.39), and vigor index (11.29). Germination capacity is a standard quality value that reflects the quality of a seed lot (Sudrajat et al. 2016), while high seed germination speed indicates the ability of the seed to overcome seed inhibition (Bentekhici et al. 2023; van Steijn et al. 2026). The results of Liu et al. (2016a) showed that soaking barley seeds in UFB resulted in an average germination capacity of 58%, twice that of seeds soaked in distilled water. A study of the effectiveness of UFB water was also conducted on *A. chinensis* seeds. Soaking *A. chinensis* seeds in UFB water for 30 minutes can increase germination capacity from 11.7% to 51.3% (Sudrajat et al. 2022), while in *Gmelina arborea* seeds, soaking in UFB water increased germination capacity by 12.5% compared to soaking in distilled water (Siregar et al. 2020). Increased germination in response to seed soaking treatment in nanobubble water was also reported for *Hordeum vulgare* seeds (Liu et al. 2013), increasing seed germination capacity by 15%–25% compared to seeds soaked in distilled water at the same dissolved oxygen concentration.

Table 2. Germination value, hypocotyl length, radicle length, and vigor index of *Acacia crassicarpa* seed treated by ultrafine bubbles (UFB) and dark septate endophytes (DSE)

Treatment	Germination value	Hypocotyl length (cm)	Radicle length (cm)	Vigor index
Control	5.33 ± 4.94[b–e]	8.20 ± 0.23	4.07 ± 0.69	6.26 ± 1.28[cde]
DSE MM.15	9.91 ± 1.19[abc]	8.57 ± 0.45	5.00 ± 0.72	7.97 ± 0.73[a–e]
DSE KSP.1	7.82 ± 5.85[bcd]	8.62 ± 0.89	4.95 ± 1.04	7.84 ± 2.76[a–e]
DSE CCP.114	8.79 ± 3.49[a–d]	9.05 ± 0.63	5.35 ± 0.26	8.30 ± 1.21[a–e]
UFB 8 ppm for 12 h	9.20 ± 5.77[a–d]	8.95 ± 0.71	4.82 ± 0.27	9.35 ± 0.87[a–d]
UFB 8 ppm for 12 h and DSE MM.15	9.95 ± 1.69[abc]	8.87 ± 0.70	4.90 ± 0.25	8.17 ± 1.01[a–e]
UFB 8 ppm for 12 h and DSE KSP.1	9.51 ± 2.65[abc]	9.72 ± 0.70	5.15 ± 0.38	8.36 ± 1.51[a–d]
UFB 8 ppm for 12 h and DSE CCP.114	12.61 ± 6.86[ab]	9.65 ± 0.78	5.77 ± 0.96	10.74 ± 2.65[ab]
UFB 20 ppm for 12 h	6.82 ± 4.67[b–e]	8.90 ± 0.60	4.87 ± 0.62	10.03 ± 2.92[abc]
UFB 20 ppm for 12 h and DSE MM.15	6.70 ± 4.26[b–e]	8.22 ± 1.18	5.27 ± 0.81	8.52 ± 1.67[a–d]
UFB 20 ppm for 12 h and DSE KSP.1	9.55 ± 2.14[abc]	8.87 ± 0.62	6.21 ± 0.62	9.52 ± 2.93[abc]
UFB 20 ppm for 12 h and DSE CCP.114	10.88 ± 4.35[abc]	9.05 ± 1.42	4.60 ± 1.13	10.72 ± 3.48[ab]
UFB 8 ppm for 24 h	7.75 ± 5.31[bcd]	8.85 ± 1.09	4.75 ± 0.23	10.03 ± 1.85[abc]
UFB 8 ppm for 24 h and DSE MM.15	4.54 ± 2.84[b–e]	8.65 ± 0.19	5.05 ± 0.75	8.81 ± 1.55[a–d]
UFB 8 ppm for 24 h and DSE KSP.1	4.96 ± 3.05[b–e]	9.12 ± 0.38	5.25 ± 0.70	7.30 ± 3.48[b–e]
UFB 8 ppm for 24 h and DSE CCP.114	15.39 ± 4.08[a]	9.77 ± 1.16	5.00 ± 0.37	11.29 ± 1.84[a]
UFB 20 ppm for 12 h	0.30 ± 0.26[e]	7.21 ± 1.09	4.30 ± 1.34	1.69 ± 0.54[f]
UFB 20 ppm for 24 h and DSE MM.15	2.89 ± 1.95[cde]	9.48 ± 0.43	4.78 ± 1.62	5.66 ± 2.42[de]
UFB 20 ppm for 24 h and DSE KSP.1	1.48 ± 0.91[de]	8.82 ± 0.77	4.00 ± 1.06	4.62 ± 2.16[ef]
UFB 20 ppm for 24 h and DSE CCP.114	6.95 ± 2.07[b–e]	8.42 ± 0.23	5.05 ± 0.75	7.42 ± 2.65[b–e]

Notes: The control consisted of seeds soaked in 98% H_2SO_4 for 15 minutes, and this pretreatment was applied to all seeds before treatment, as designed. The seeds were soaked in the DSE solutions for 24 hours. Different letters a and b in the same column denote significant differences ($p \leq 0.05$) between treatments based on Duncan's multiple range test (DMRT).

UFB water has been reported to increase gibberellin hormone levels in seeds (Liu et al. 2016a; Wang et al. 2021). Gibberellin is a key hormone in the seed germination process (Cornea-Cipcigan et al. 2020; Li et al. 2019a), as it plays a role in inducing the activity of various hydrolytic enzymes, such as α-amylase, protease, α-gluconase, phosphatase, and ribonuclease, which assist in the germination process (Damaris et al. 2019; Li et al. 2019a; Shah et al. 2023). These enzymes diffuse into the endosperm tissue and catalyze the breakdown of food reserves into simple compounds, mainly sugars and amino acids, which the developing embryo can utilize. In addition, soaking seeds in UFB water increases the solubility of gases, particularly oxygen, in the liquid medium (Liu et al. 2016b). Oxygen availability plays an important role in respiration, especially during the early stages of germination, by supporting the conversion of food reserves into energy. The increased germination rate of seeds treated with UFB water is also associated with the presence of ROS produced in UFB water, which, at certain levels, acts as a physiological signal in the germination process (Liu et al. 2016a; Liu et al. 2017; Ha et al. 2025). At a certain level, reactive oxygen species (ROS) can be key to successful seed germination (Oracz and Karpiński 2016; Singh et al. 2016). ROS can promote seed dormancy release through oxidation of biomolecules, weakening of the testa, and endosperm decay. In addition, reactive oxygen species modulate metabolic and hormonal signaling pathways that induce germination (Farooq et al. 2021). ROS in UFB cause the cell wall to loosen, allowing water, gas, and embryo structures to penetrate the seed coat. This loosening of the cell wall occurs because UFB can induce gene expression in this process (Liu et al. 2017). UFB are small bubbles that have a diameter of less than 1 mm or 10^{-6} m, so that it is quite easy to enter and penetrate the bio-membrane and diffuse into the cytosol (Ahmed et al. 2023; Sudrajat et al. 2022).

The *Dendrothyrium* sp. CPP.114 treatment, combined with all UFB water treatments, resulted in increased seed germination compared to the control. It gave better results than the other DSE treatments in increasing the viability and vigor of *A. crassicarpa* seeds. The positive effect of *Dendrothyrium* sp. CPP.114 on seedling germination and growth was also reported by Widyani et al. (2024) in a study of direct seeding of *L. leucocephala*, which provided the best seedling survival and seedling diameter growth. According to Surono et al. (2024), seed treatment by soaking in *Dendrothyrium* sp. DSE can increase the seed germination of *F. moluccana*. DSEs are a group of fungi that are symbiotic with plant roots and have the potential to act as promoters (seed germination and plant growth) and as biological controls (increased disease resistance) (Sharma et al. 2023; Shi et al. 2025; Surono et al. 2024). Soaking seeds in microbes, such as DSE, can be categorized as biopriming, a seed treatment that combines physiological and biological factors to improve seed viability and vigor, as well as plant growth (Dhawal et al. 2016; Priatna et al. 2026). Seed hydration occurs during DSE seed soaking. The amount of DSE solution used to maintain seed moisture potential and the duration of soaking can control seed metabolism and promote pre-germination processes (cell repair and protein synthesis) (Devika et al. 2021). According to Sarkar et al. (2017) and Singh et al. (2023), biopriming can actively influence plant cellular mechanisms, reconfiguring metabolism and enhancing plant growth. Endophytic fungi can synthesize indole-3-acetic acid (IAA) and gibberellic acid (GA) through their metabolic activity, which is considered to play an important role in their interactions with host plants and in stimulating seed germination (He et al. 2017). In addition, DSE symbiotic with *F. moluccana* roots is known to produce various secondary metabolites, including antibacterial, antifungal, and antioxidant compounds, which have the potential to protect seeds from pathogen attack during germination (Surono et al. 2024).

The application of DSE showed a tendency to increase the radicle and hypocotyl of *A. crassicarpa* seedlings, although not significantly. DSE is known to dissolve phosphate, provide a carbon source and increase root and stem biomass (He et al. 2019; Xie et al. 2021). Several previous studies have also reported that the use of bio-inoculants such as DSE as priming agents was able to accumulate stimulus in the rhizosphere, facilitate biochemical nutrient cycling, promote enzymatic activity, aid in better translocation, and therefore improve plant performance under changing climatic conditions (Adhikary et al. 2021; Sarkar et al. 2021). DSE has been reported to increase plant growth and adaptation to various environmental conditions (He et al. 2019; Putri et al. 2026) and to support seed germination (Priatna et al. 2026; Widyani et al. 2024; Yuniarti et al. 2026).

Soaking seeds in UFB water at 20 ppm for 24 hours reduced germination across all observed parameters. This condition is thought to cause seed damage due to the high concentration of UFB combined with excessive soaking duration. In the context of germination physiology, the oxidative window states that germination can occur optimally only within a specific range of ROS levels. Meanwhile, not all DSE treatments gave positive results on *A. crassicarpa* seed germination parameters. DSE CPP.114 and MM.15 tended to have a positive effect on germination, whereas DSE KSP.1 showed results below the control for several germination parameters, such as germination capacity and germination speed. Previous studies also reported that DSE strains are incompatible with increasing seed germination in plant species, as reported by Azmi et al. (2022) on *Allium ascalonicum* seeds, Suita et al. (2024) on *Pongamia pinnata* seeds, and Priatna et al. (2026) on *Gmelina arborea* seeds. Li et al. (2019b) stated that plant responses to DSE treatment ranged from neutral to beneficial, depending on the plant species and the inoculated fungus. Similar to the statements of He et al. (2020) and Harsonowati et al. (2026), the effect of DSE treatment on plant status can be positive, neutral, or negative. In contrast, in *G. arborea* seeds, DSE KSP.1 increased germination capacity and germination speed (Priatna et al. 2026). Thus, the compatibility of host plants with DSE strains is key to the success of DSE treatment, alongside environmental factors.

4. Conclusions

The interaction treatment between ultrafine bubbles (UFB) and dark septate endophytes (DSE) significantly affected the germination capacity, germination speed, germination value, and vigor index of *A. crassicarpa* seeds. The optimum combination of UFB water and DSE treatments after seed scarification in 98% H_2SO_4 for 15 minutes was to soak seeds in UFB at 8 ppm for 24 hours, followed by soaking in DSE *Dendrothyrium* sp. CPP.114 for 24 hours with a germination capacity of 78.5% (an increase of 32.5% from control), germination speed of 8.04% day^{-1} (an increase of 38% from control), germination value of 15.39 (an increase of 188.7% from control), and vigor index of 11.29 (an increase of 80.3% from control). After UFB water treatment (20 ppm) for 24 hours, seed germination decreased across all germination parameters. This treatment caused seed damage due to high UFB levels and prolonged soaking.

Acknowledgments

The authors thank the Biosystems Environmental Engineering Laboratory at IPB University and the Tree Seed Testing Laboratory at the Institute for Implementation of Environmental and Forestry Standards and Instruments in Bogor for facilitating this research.

Author Contributions

D.J.S.: Conceptualization, Supervision, Writing – Original Draft Preparation, Data Curation; E.R.: Formal Analysis, Investigation, Data Curation, Software, Writing – Review and Editing; M.A.S.: Writing – Original Draft Preparation, Writing – Review and Editing; Y.: Data Curation, Writing – Original Draft Preparation; N.: Data Curation, Writing – Original Draft Preparation; N.Y.: Formal Analysis, Investigation, Data Curation, Software; S.: Methodology, Writing – Review and Editing; Y.A.S.: Methodology, Data Curation.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI And AI-Assisted Technologies in the Manuscript Preparation

Not applicable.

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