



Impact of Priming with UV Radiation on Seed Germination and Seedling Growth of Chakhao Rice Cultivar

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The development of eco-friendly technology with innovative ideas is always essential to enhance plant growth and improve yield in a sustainable manner. Seed priming methods such as conventional and physical priming are well-known pre-sowing techniques that are considered to be effective in improving the growth and yield of a crop. In view of this, an experiment was conducted to study the impact of physical priming with non-ionizing radiation like UV-C on seed germination and early seedling growth of Chakhao cultivars of rice (*Oryza sativa*). Three Chakhao rice cultivars namely Black (Amubi) - V₁, Red (Angangbi) - V₂, and White (Angoubi) - V₃ were used, and seeds were initially exposed to UV-C (8.32 KJm⁻²) radiation for 3 hours. Then seeds were soaked in water at 28 ± 2 °C for 2 days and the emerging seedlings were again treated with UV-C (8.32 KJm⁻²) for different durations i.e. 30 mins (T₃), 45 mins (T₄) and 60 mins (T₅). Seeds without any UV-C treatment (T₁) and only seed treatment for three hours (T₂) were taken as control. The data on

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germination rate, speed of germination, root length, shoot length, and seedling vigor index were recorded as per standard procedures. The study found that all three types of rice, V₁, V₂, and V₃, had the highest germination percentage (71.07%, 73.63%, and 69.71%), speed of germination (9.53 days, 9.40 days, and 9.320 days), shoot length (9.83 cm, 8.43 cm, and 10.70 cm), root length (9.03 cm, 6.40 cm, and 8.97 cm), and seedling vigor index (1688.50, 1570.23, and 1729.13) when treated with UV-C for 60 minutes followed by 45 minutes of seedling treatment. This indicates that UV-C seed priming is an effective method for enhancing the growth of rice seedlings.

Keywords: Chakhao rice; UV-C; SVI; germination rate; seedling treatment; seed priming.

1. INTRODUCTION

Rice (*Oryza sativa*) is a major cereal crop that is consumed by more than half of the world's population as a primary staple food. It contributes to 21% of the world's per capita calorie intake, with over 90% of rice being produced and consumed in Asia. Rice can be categorized into pigmented and non-pigmented varieties, with "specialty rice" having unique aromas, flavors, and colors. In Manipur, India, a unique type of rice called Chakhao is consumed for its unique health benefits and flavor as well as high anthocyanin content. Chakhao rice has the potential to be used as a functional food and food colorant. However, *Chakhao* rice is less popular among farmers due to its inherent undesirable traits such as low yield, high photo-insensitivity, longer vegetative phase, and tall stature which causes its lodging. Another constraint of Chakhao rice cultivation is its long harvest time which must needs to be compensated either by introducing early maturity traits or by increasing productivity. Further, with the introduction of many high-yielding rice varieties, farmers' preferences have also been changed, and as a result, many *Chakhao* landraces are getting degraded [1]. Thus, more research needs to be done on *Chakhao* rice to strengthen sustainability by preserving the local Chakhao rice of Manipur as well as improving desirable traits in the best rice varieties.

Low-level UV seed priming is an environmentally safe technique for increasing crop production and yield as well as for inducing resistance to a variety of stresses in wheat (*Triticum aestivum*) and mung (*Vigna radiata*) [2,3]. Ultra Violet radiation priming, a novel non-ionizing physical priming method proven to be beneficial on seed germination, seedling development, plant growth, and yield contribution [4]. UV priming has been shown to have a variety of physiological effects, including changes in growth, enhancement of photosynthetic pigments, taste or quality of the fruit, increased photo-protection, upregulation of

the plant defense systems to strengthen crop production, and increased production of secondary metabolites in *Phaseolus vulgaris*, *Triticum aestivum*, and *Vigna sp.* [5-8]. According to Semenov et al. [9], UV-C irradiation stimulated seed vigor and germination in the seed quality of winter wheat cultivars. The study found UV-C irradiation, at specific optimal doses per cultivar, enhanced seed quality in terms of vigor, germination rate, and seedling development in the 6 Ukrainian wheat cultivars tested. As Maia et al. [10], suggested that the combined UV-C and 30-minute ozone exposure increased initial germination, but did not negatively affect broader seed physiological quality, across the 3 maize cultivars tested. The results suggest potential for using these non-thermal techniques to help treat organic maize seeds.

2. MATERIALS AND METHODS

The experiment was conducted in the RKVY Laboratory of Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal (26°19'86" N, 89°23'53" E) with an altitude of (43 m), India. The seeds were obtained from the local farmers with germination between 70% and 85%. The seeds were kept in a lineage bag in an ambient chamber, at temperature and relative humidity (27 ± 2 °C and 45 ± 2% respectively) until the beginning of the experiment.

To perform experiment, a completely randomized design in a 3 × 5 factorial scheme with three replications was used. The treatments consisted of three Chakhao rice cultivars i.e. Black rice (V₁), Red rice (V₂), and White Rice (V₃), submitted to one UV-C radiation dose of 8.32 KJm⁻², corresponding to 0 hours i.e. control (T₁), 3 hours seed priming (T₂), 3 hours seed priming with 30 minutes seedling treatments (T₃), 3 hours seed priming with 45 minutes seedling treatments (T₄) and 3 hours seed priming with 60 minutes seedling treatments (T₅). For each treatment, the seeds were exposed to UV-C radiation in a single batch.

The treatment with UV-C radiation was performed using a cylindrical irradiation chamber (46 cm in diameter, 90 cm in length), which has two lamps capable of emitting waves with a length of 254 nm, which corresponds to the range of UV-C radiation. The seeds were placed on a petri disc for each radiation at a distance of 20 cm from the source of radiation and were treated according to the purpose of the experiment, separately. The seeds were arranged during the periods, so that they were as scattered as possible, to avoid some seeds are on the other and not be struck by UV-C light. To transform the exposure periods of the seeds into UV-C radiation at doses of UV-C (kJm^{-2}), we used the Lux Meter for measuring the light intensity, and with the conversion factors we converted it into kJm^{-2} . As the seeds were arranged in the center of the chamber, under the influence of the two lamps, they received a dose equivalent to 2.370 Wm^{-2} ($0.0237 \text{ kJs}^{-1}\text{m}^{-2}$).

The radiation intensity was kept constant at 2.37 W/m^2 for UV-C. The applied doses were obtained by varying the exposure time to the set distance, using the equation of López-Rubira et al. (2007) :

$$D = \frac{(F \times T)}{1000}$$

Based on the exposure times and the dose emitted by the lamps, the equivalent doses were obtained at each time, by the product between these factors, resulting in the following doses: 8.32 KJ/m^2 .

After exposure of the seeds to different doses of UV-C radiation, they were subjected to physiological quality and later to sanitary quality tests.

2.1 Determination of Germination Percentage

The exposed seeds i.e. 3 hours of radiation treatment (approximately 10-12% moisture content) were kept in a desiccator. The seed germination study was done by using sterilized corning Petri dishes of 9 cm diameter. Each petri dish was layered with blotting paper and distilled water was used to moisten the blotting paper [11]. The seeds were placed after 3 hours of imbibition @ 50 seeds per Petri dish for germination. All Petri dishes were kept in the incubator maintained at $27 \pm 2^\circ\text{C}$ temperature. Seeds with radical length equal to or greater than

2 mm were considered as germinated. Germination percentage was calculated on the total no germinated seeds per treatment after three days of incubation. The experiment was done in three replicates and repeated for six months.

2.2 Speed of Germination

The speed of germination was estimated according to the following formula given by Haugland and Brandsaeter [12].

$$\bar{T} = \frac{\sum_{i=1}^k N_i}{\sum_{i=1}^k T_i}$$

Where T_i is the time from the start of the experiment to the i^{th} observation, N_i is the number of seeds germinated in the i^{th} time and k is the last time of observation.

2.3 Determination of Seedling Growth

To determine seedling growth, root length, and shoot length of different parts of seedlings were measured. Early seedling growth parameters were evaluated on 10-day-old seedlings. On the day of final measurements, seedlings were removed from the petri dish carefully and the length of shoot and root were measured separately.

2.3.1 Seedling vigor index

Based on the mean seedling length and mean seedling dry weight, the seedling vigor index (SVI) was calculated as per Abdul Baki and Anderson [13] Using the following formulae;

$$\text{SVI} = \text{Mean seedling length (cm)} \times \text{Germination (\%)}$$

The vigor index (VI) of the seedlings was estimated as per the formula given by Abdul-Baki, and Anderson (1973):

$$\text{SVI} = (\text{RL} + \text{SL}) \times \text{GP}$$

Where, RL - Root Length (cm) SL - Shoot Length (cm)
GP - Germination Percentage

2.3.2 Seedling Length (cm)

For determining seedling length, five normal seedlings from each treatment were randomly

selected for measuring the seedling length on the 12th day of germination. From the collar region to the tip of the root, the seedling length was measured. The average seedling length of five seedlings was computed and expressed in centimetres.

2.3.3 Root Length (cm)

Five normal seedlings in each treatment were randomly selected from the germination test for measuring the root length on the 12th day of germination. From the collar region to the tip of the root, the root length was measured. The average root length of five seedlings was computed and expressed in centimetres.

2.3.4 Shoot Length (cm)

The earlier five seedlings used for the root length measurement were also used for measuring shoot length. The shoot length was measured from the collar region to the point of attachment of cotyledons. The average of five seedlings was computed and expressed in centimetres.

2.4 Statistical Analysis

The data were submitted to ANOVA and the means of the qualitative treatments, when significant, were compared by the Tukey test ($p \leq 0.05$). The quantitative treatments were submitted to regression analysis ($p \leq 0.05$). The values of the parameters of the models were estimated as a function of the exposing duration of UV-C radiation. The degree of fit of the models to the experimental data was based on the magnitude and significance of the coefficient of determination, the significance of regression coefficients, the magnitude of coefficient of variation, and on verification of the fit of the statistical model to the experimental data. The data was analysed in Microsoft excel software.

3. RESULTS AND DISCUSSION

The present investigation was carried out on three different Chakhao rice cultivars collected from different parts of Manipur. The three cultivars include Black rice (chakhao amubi), Red rice (chakhao angangbi), and White rice (Chakhao angoubi). The provided data appears to represent the mean values for various parameters across different treatments (T_1 to T_5) and varieties (V_1 , V_2 , V_3), along with analysis of variance at 0.05 level.

3.1 Estimation of Germination Percentage

As depicted in Fig.1, the Mean Germination Percentage varied between 63.655% and 73.630%. Among all treatments, T_5 exhibited the highest Mean Germination Percentage for all varieties, with values ranging from 71.07% to 73.63% and 69.71%. On the other hand, T_2 displayed the lowest Mean Germination Percentage (63.655%) for V_1 . For V_2 and V_3 , the lowest germination percentage was observed at T_1 , with values of 66.85% and 67.46%, respectively. Notably, significant differences were observed in all three factors, namely treatment, variety, and their interaction (Table 1). According to Guajardo-Flores et al. [14], reactive oxygen species (ROS), which are beneficial during seed germination, are present at higher concentrations in plant tissues subjected to UV-C exposure. In line with this, Rupiasih and Vidyasagar [15] found that exposing wheat seedlings to UV-C for up to 180 minutes can enhance seed germination. Moreover, Neelamegam and Sutha [16] discovered that after 10 days of germination, groundnut seeds (*Arachis hypogaea* L.) exposed to UV-C showed a decline in germination rates, followed by an increase at 5 and 60 minutes, respectively.

Table 1. Anova table of Impact of priming with UV radiation on germination rate, speed of germination, shoot length, root length seedling length, and seed vigor index

| Source of Variation | DF | Germination % Mean Squares | Speed of germination | of Shoot Length | Root Length | Seedling length | SVI |
|---------------------|-----------|-------------------------------|----------------------|-----------------|-------------|-----------------|-------------|
| V | 2 | 23.66** | 0.032 | 0.763 | 0.543 | 0.211 | 14,071.57 |
| Treatment | 4 | 33.683** | 0.167* | 11.067** | 9.722** | 40.88** | 413492.18** |
| V X T | 8 | 8.054* | 0.165** | 2.307* | 1.908* | 5.04** | 29330.056* |
| Error | 30 | 3.338 | 0.044 | 0.996 | 0.841 | 1.569 | 11,442.24 |
| Total | 44 | | | | | | |

**,* Significant at 1% and 5% levels of probability, respectively

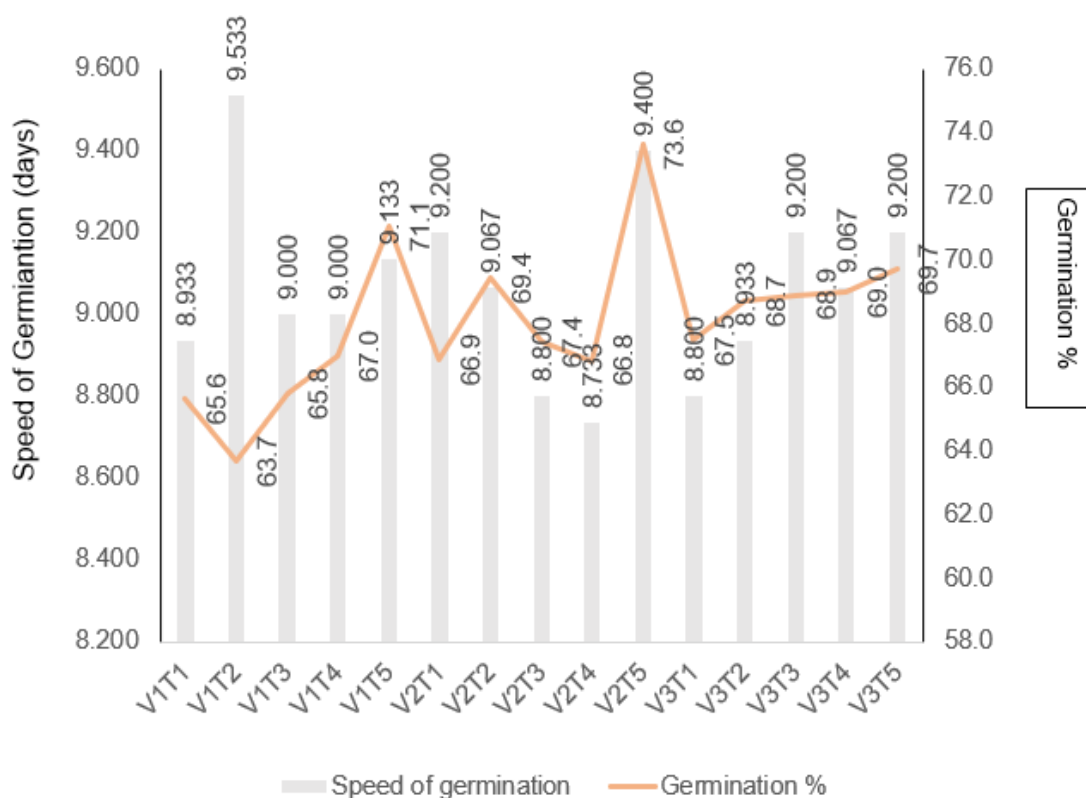


Fig. 1. Impact of priming with UV radiation on speed of germination and germination rate

3.2 Estimation of Speed of Germination

As shown in Fig. 1, The mean Speed of Germination (SOG) spans from 8.73 to 9.53 days. Specifically, the mean value of V₁ demonstrates the highest at T₂ (9.53 days) and attains its minimum at T₁ (8.93 days). In the case of V₂, T₅ manifests the highest mean SOG (9.40 days), while T₂ records the lowest (9.06 days). Concerning V₃, T₃ and T₅ display the highest mean SOG (9.20 days), whereas T₁ exhibits the lowest (8.80 days). Significant values were observed for both the treatment and the interaction between treatment and variety; however, no significance was noted for variety alone (Table. 1).

The findings of Neelamegan and Shuta [16] corroborate the present study's observations, as both report that priming or treatment of seeds with UV radiation results in the breakdown of radicles or molecules within the seed embryo, initiating metabolic processes at the earliest possible time. The supplied radiation energy triggers the breakdown of metabolic processes, which, when combined with water, leads to an increase in free radicals in the seeds, altering their ability to germinate. Kondrateva *et al.* [17]

also observed the effects of UV radiation on seed germination, reporting an increase in the average length of sprouts and roots in seeds of naked oats by 11-21%.

3.3 Estimation of Seedling Length, Shoot Length and Root Length

According to Fig. 2, the mean seedling length for V₁ and V₃ varieties ranged from 11.37 cm to 18.87 cm and 11.37 cm to 13.40 cm, respectively whereas for V₂ it ranged from 14.57 cm to 17.60 cm. T₅ showed the highest mean seedling length for both varieties V₁ and V₃ (18.87 cm and 19.67 cm), similarly while T₁ had the lowest (11.37 cm and 13.40 cm). For the V₂, T₄ had the longest seedling length (17.60 cm), and T₂ had the shortest (14.57 cm).

In terms of shoot length, the mean values ranged from 6.333 cm to 10.700 cm. T₅ has the maximum shoot length (9.83 cm and 10.70 cm) for varieties V₁ and V₃ respectively but for V₂, T₄ has the maximum (8.43 cm) similarly T₁ had the lowest (6.33 cm) for V₁, T₁, and T₂ both has the lowest (8.17 cm) for V₂. Lastly, for V₃, T₂ has the lowest (6.87 cm).

For root length, the mean values ranged from 5.033 cm to 9.033 cm, and T₅ had the highest mean root length (9.03 cm and 8.97 cm) for variety V₁ and V₃ respectively, while T₁ had the lowest (5.033 cm) for V₁, T₂ (6.40 cm) for V₂ and

T₁ (6.53 cm) for V₃. Significant values were observed in the treatment and its interaction with variety, but no significance was found for variety in any of the cases (Table 1) for seedling length, root length, and shoot length.

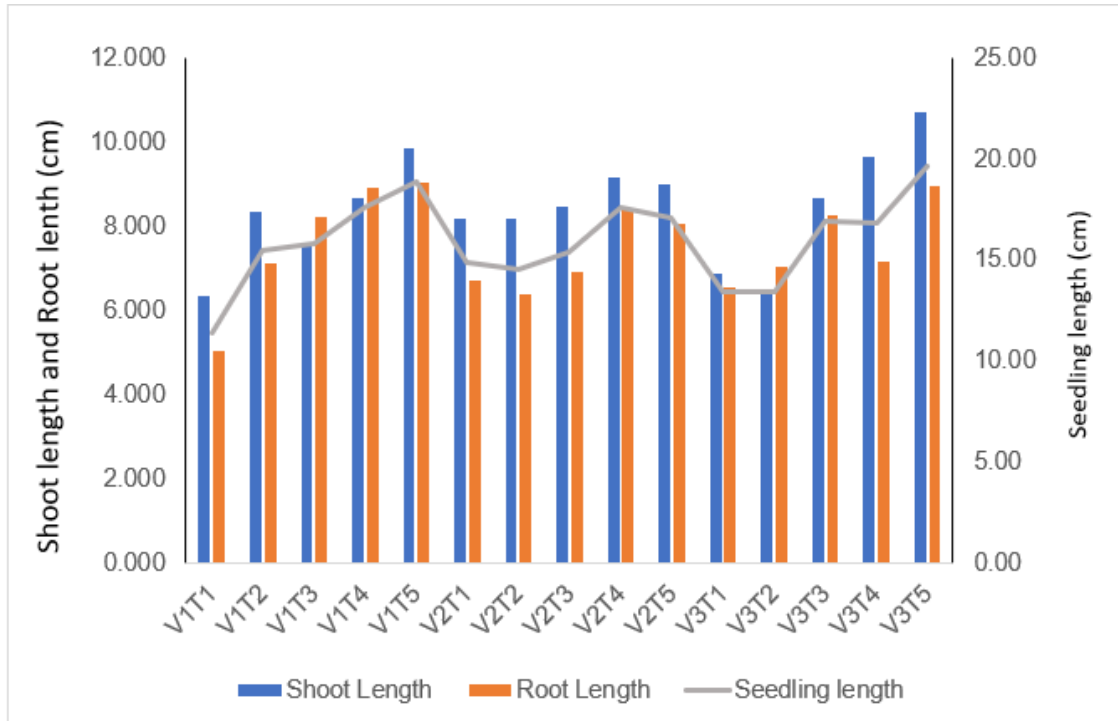


Fig. 2. Impact of priming with UV radiation on shoot length, root length, and seedling length.

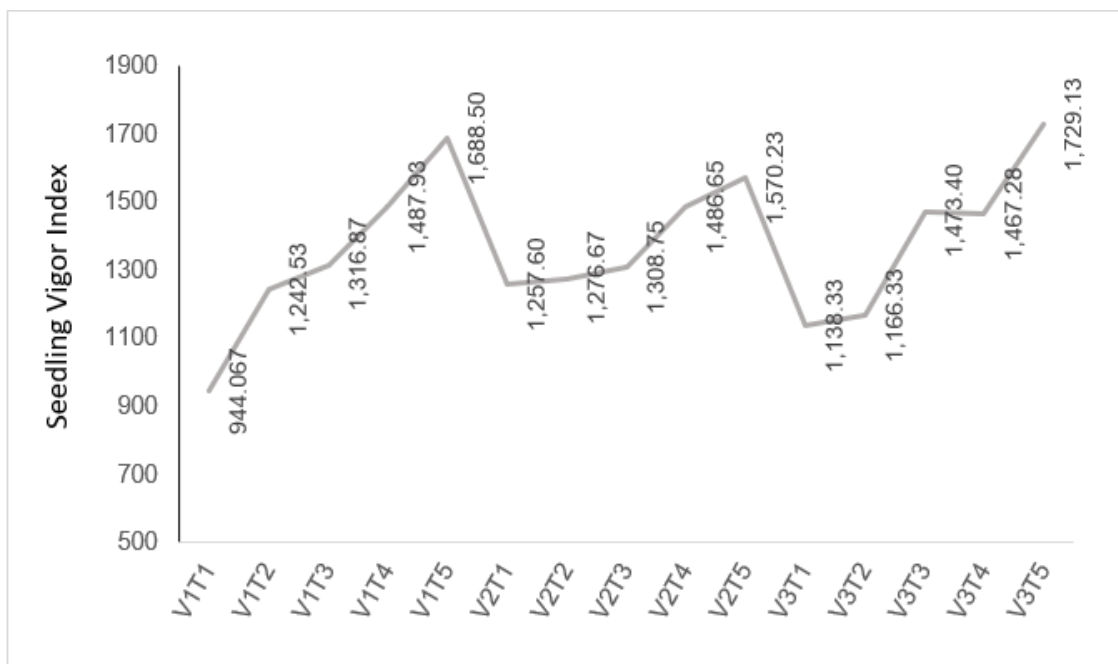


Fig. 3. Impact of priming with UV radiation on seedling vigor index.

According to Neelamegam and Shuta [16], UV-C treatment displayed favourable effects on the seedling growth rate, and it was observed that UV-C irradiation for up to 60 minutes enhanced the growth parameters and biomass production of groundnut plants. Similarly, Scott et al. [18] demonstrated that UV-C treatments on tomato seeds resulted in a reduction in disease incidence and severity without adversely affecting germination or seedling growth. Additionally, Siddiqui et al. [19] reported that UV-C exposure led to an increase in shoot weight, shoot length, root length, root weight, leaf area, and the number of nodules in groundnut plants.

3.4 Estimation of Seedling Vigor Index

Fig. 3 displays the mean values for the Seedling Vigor Index (SVI) ranging from 944.70 to 1729.13. Among the treatments, T₅ exhibited the highest mean SVI values for V₁, V₂, and V₃ (1688.50, 1570.23, and 1729.13), while T₁ displayed the lowest mean SVI for V₁, V₂, and V₃ (944.70, 1257.60, and 1138.33), respectively. The treatment and its interaction with variety were found to be significant, whereas no significant difference was observed for the variety alone. Previous research by Rupiasih and Vidyasagar [15]. has reported that exposure to UV-C radiation can yield positive results in terms of the germination and early seedling growth of groundnut plants.

Each treatment (T₁ to T₅) shows variations in mean values across different parameters. T₅ consistently shows higher mean values for speed of germination, shoot length, root length, SVI, and germination percentage. T₁ consistently shows lower mean values for these parameters.

4. CONCLUSION

The results suggest that Chakhao rice cultivars, namely black, red, and white, exhibited positive effects on seedling growth and development when exposed to UV-C radiation. According to the data, it is recommended that priming with UV radiation at 8.32 KJm⁻² for 3 hours before soaking and an additional 60 minutes of treatment after seedling emergence can significantly improve seedling growth and development, compare to the control and other treatments. Each treatment (T₁-T₅) displayed variations in mean values across different parameters. T₅ (3 hours seed priming with 60 minutes seedling treatments) consistently exhibited higher mean values for germination

speed, shoot length, root length, SVI, and germination percentage, while T₁ (Control) showed lower mean values for these parameters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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