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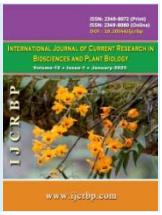

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Impact of hydration duration and UV-C treatment on germination of a local variety of mung bean seed of Nayagarh district of Odisha

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Abstract

In the present study the effects of duration of hydration and time of exposure to UV-C radiation on seeds of *Vigna radiata* of Nayagarh local B variety were investigated. It was determined on the basis of seed germination percentage, rate of seed germination, length of radicle and plumule, height of the seedling and seedling vigour index. Hydration duration and UV-C exposure had no significant effect on the percentage of seed germination or the rate of seed germination. Seeds were considered germinated if 1mm of radicle emerges from the seed coat. Germination initiated within 24 h and completed within 48 h across all treatments. 6-8 h of hydration was sufficient to initiate germination. The radicle length of mung bean seeds was significantly affected by hydration and/ or the UV-C radiation treatments. Radicle length was significantly affected by hydration and UV-C treatments, with the longest radicle observed at 3 h of hydration coupled with 2 h of UV-C exposure (14.38 cm), which was 0.77 cm longer than the radicle of the control. The plumule length of mung bean seeds was not significantly affected by hydration and/ or the UV-C radiation treatments. The longest plumule was observed with 4 h of UV-C exposure (1.39 cm), which was 0.167 cm longer than the radicle of the control. The height of seedlings of mung bean seeds was significantly affected by hydration and/ or the UV-C radiation treatments, with the tallest seedlings resulting from 3 h of hydration and 2 h of UV-C exposure (15.57 cm), which was 0.78 cm longer than the seedling of the control. Maximum seedling vigour index was found at 3 h of hydration and 2 h of UV-C exposure. UV-C radiation positively influenced radicle length, seedling height, and seedling vigor, demonstrating its potential to enhance the growth of mung bean seeds.

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Introduction

Ultraviolet (UV) radiation is a form of electromagnetic radiation with wavelengths shorter than visible light but longer than X-rays. It is categorized into three types: UV-A (380-315 nm) called long wave or dark light; UV-B (315-280 nm) called medium wave and UV-C (280-10nm) called short wave or germicidal. Due to climate change and variations in the ozone layer, more harmful UV radiation is reaching the Earth's surface.

Factors such as the use of chlorofluorocarbons (CFCs) contribute to the depletion of the ozone layer, influencing the amount of UV radiation that penetrates the atmosphere (Krupa, 2000). Radiation affects various physiological and biochemical processes in both animals and plants (Solomon, 1999). Elevated UV radiation causes changes in higher plants (Björn and Vogelmann, 1996; Caldwell et al., 1998; Greenberg et al., 1997; Rozema et al., 1997), with variations observed among species (Barnes et al., 1990; Day, 1993) and even within varieties of the same species (C.M et al., 1998; Corlett et al., 1997; Ziska et al., 1992). High radiation levels can inhibit growth and affect processes like protein synthesis, water exchange, enzyme activity, and leaf-gas exchange (Madronich et al., 1998). UV-C is the most energetic UV radiation and is not naturally found in the biosphere. It is used for its bactericidal and germicidal properties. When UV-C interacts with plant tissues, it can cause DNA damage, dimerization of pyrimidines in DNA molecules, decrease protein synthesis, and alter protein structures (Rastogi et al., 2010; Pournavab et al., 2019). However, sub-lethal doses can induce stress responses, leading to the accumulation of protective compounds like flavonoids and phenolics (Rastogi et al., 2010). The accumulation of a series of phyto-alexins has also been observed, as well as the typical adaptations of the induction of resistance, such as the modification of cell walls and even cell death. Higher doses of UV-B and UV-C can cause cellular damage (Nawkar et al., 2013). Strong UV-C irradiation has negative effects on photosynthetic tissues, fruits, vegetables, and post-harvest products. Seeds are particularly susceptible to radiation when they have high water content, as the presence of oxygen increases free radical production (Arvind Kumar and Purohit, 1998). The role of water content influencing the effects of physical and chemical mutagens is well established (Conger et al., 1968). Water content appears to facilitate the mobility and action of free radicles and oxygen with physical mutagens (Ehrenberg, 1961). The information regarding irradiation of seeds with UV-C is very scarce (Kobzar et al., 1998).

Mung bean, a well-known pulse crop, plays a crucial role in nitrogen fixation. It thrives on marginal lands where other crops often fail, making it ideal for green manure use (Dainavizadeh and Mehranzadeh, 2013). In Nayagarh district, mung beans are alternately cultivated with rice, likely due to their ability to enrich the soil with nitrogen, benefiting subsequent rice cultivation. This study focused on Nayagarh local B variety (Tripathy et al., 2016) mung bean seeds, characterized by their dull green seed coat. The primary objective was to examine the effects of UV-C irradiation at different doses and hydration durations on: Germination percentage, Germination rate, Plumule length, Radicle length, Seedling length and Seedling vigor index. By analyzing these parameters, the study aimed to better understand how UV-C radiation and hydration influence the germination and growth of mung bean seedlings.

Materials and methods

The study was conducted at the Department of Botany, Nayagarh Autonomous College, Nayagarh during the year 2023. Whole mung bean seeds (Nayagarh local B variety) with dull green seed coats (Fig. 1a) were collected from Sidhamula village, Khandapada block. To ensure uniformity, the seeds were handpicked by visual inspection. The experimental set up was prepared with 0, 3, 6 and 9 h of hydration and / or 0, 2, 4 and 6 h of UV-C radiation (254 nm) exposure (Table 1). Exposure was done in a laminar airflow cabinet (Fig. 1b and 1c). For each treatment, three replicates of 14 seeds each were used. Post-treatment, seeds were sterilized with 1% NaOH solution for 10 minutes, followed by multiple rinses with sterile tap water. They were then inoculated into sterilized petri dishes containing tissue paper saturated with 5ml of sterile tap water (Fig. 1d) and placed in the dark for germination.

Table 1. Details of the hydration and UV-C radiation exposure treatments.

Code	Hours of hydration	Hours of UV-C exposure
C	-	-
T1	-	2
T2	-	4
T3	-	6
T4	3	-
T5	3	2
T6	3	4
T7	3	6
T8	6	-
T9	6	2
T10	6	4
T11	6	6
T12	9	-
T13	9	2
T14	9	4
T15	9	6

Evaluated variables

Data were collected for germination %, germination rate, radicle and plumule length, seedling height and seedling vigour. The germination percentage was calculated using the following formula:

$$\text{Germination } \% = \frac{\text{number of seeds germinated x 100}}{\text{total number of seeds}} \quad (1)$$

The germination rate was calculated every 24 h interval using the following formula:

$$\text{Germination rate} = \frac{X1}{Y1} + \frac{X2}{Y2} + \cdots + \frac{Xn}{Yn} \quad (2)$$

Where, X= number of seeds germinated
Y= hours from showing

A precise ruler was used to measure the radicle, plumule and seedling length of the seeds. The lengths of the radicle, plumule and the seedling were measured after 7 days of inoculation for each treatment. The seedling vigour index was calculated using following formula

$$\text{Seedling Vigour Index} = \text{average seed gemination \% x average seedling height} \quad (3)$$

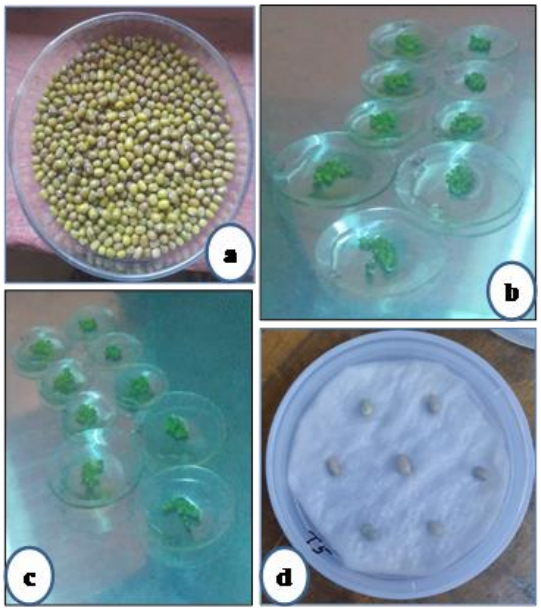

Fig. 1: (a) Nayagarh Local B variety of mung bean with dull green seed coat, **(b) and (c).** Exposure of seeds to UV-C radiation in the laminar airflow cabinet, **(d).** Inoculation of treated seeds in petridishes on filter papers saturated with 5 ml of sterile tap water.

Data analysis

The data was analysed statistically using Microsoft excel for determination of mean, standard deviation etc. Two-way ANOVA was done to find whether the difference or deviations between the samples are significant from the replicate and treatment point of view.

Results

In this study, we investigated the effects of hydration duration and UV-C radiation exposure on seeds of the local variety B of *Vigna radiata* (mung bean). The following parameters were examined to assess the impact such as the proportion of seeds that successfully germinated, rate of seeds germination, the length of the emerging root, the length of the emerging shoot, overall height of the seedlings and Seedling Vigour Index. Through these parameters, we aimed to understand how different hydration durations and UV-C exposure times influence the germination and growth of mung bean seedlings.

Germination percentage and rate of seed germination

The duration of hydration and the exposure time to UV-C radiation had no significant effect on the percentage of seed germination or the rate of seed germination (Table 2). Seeds were considered germinated if 1mm of radicle emerges from the seed coat. Across all treatments, germination began within 24 h. All seeds had germinated within 48 h of being placed in petri dishes with saturated tissue papers (Fig. 2a and 2b). This consistent germination pattern suggested that hydration and UV-C radiation did not notably affect the initial stages of mung bean seed germination in terms of timing or success rate.

It was found that 6-8 h of hydration is enough to initiate germination (Fig. 2c and d). From the table 2 it is evident that, 6 h of hydration coupled with 2 or 4 h of UV-C treatment or 9 h of hydration alone is sufficient for all the seeds to germinate within 24 h, similar to control.

Length of radicle

The data on radicle length (Table 3) reveal that both hydration duration and UV-C radiation treatments

significantly impacted the radicle length of mung bean seeds. This conclusion is supported by the two-way ANOVA results (Table 4). The 3 h hydration and 2 h exposure to UV-C radiation treatment resulted in the longest radicle (14.38 cm), which was 0.77 cm longer than the radicle of the control. This was followed by the treatment of 3 h hydration alone, which resulted in the second longest radicle (13.79 cm), which was 0.18 cm longer than the control. The seeds in the control showed the third longest (13.61cm) radicles. These results indicated that a combination of 3 h of hydration and 2 h of UV-C exposure significantly enhanced radicle length, surpassing both the control and other treatments.

Table 2. Germination percentage and rate of seed germination per treatment.

Treatments	Number of seeds germinated on day 1	Number of seeds germinated on day 2	Germination %	Germination rate
Control	14	0	100	0.58
T1	13	1	100	0.56
T2	14	0	100	0.58
T3	13	1	100	0.56
T4	12	2	100	0.54
T5	13	1	100	0.56
T6	13	1	100	0.56
T7	13	1	100	0.56
T8	13	1	100	0.56
T9	12	2	100	0.54
T10	14	0	100	0.58
T11	14	0	100	0.58
T12	14	0	100	0.58
T13	13	1	100	0.56
T14	14	0	100	0.58
T15	13	1	100	0.56

Fig. 2: (a and b) Germinated seeds after 24 h of inoculation, **(c and d)** Emergence of radicle after 6-8 h of hydration.

Length of plumule

The data on plumule length (Table 3) indicates that hydration and UV-C radiation treatments did not significantly affect the plumule length of mung bean seeds. This is supported by the two-way ANOVA results (Table 5). The 4 h exposure to UV-C radiation alone followed by inoculation in saturated filter papers resulted in the largest plumule (1.39 cm), which was 0.167 cm larger than the radicle of the control. This was followed by the treatment of 2 h UV-C radiation alone followed by inoculation in saturated filter papers, which resulted in the second largest plumule (1.36 cm), which was 0.13 cm larger than the control. The third largest (1.35 cm) plumule was found in seeds treated with 6 h of hydration and 6 h of UV-C. Despite these specific treatments showing slightly increased plumule lengths, the overall impact of hydration and UV-C treatments on plumule length was not significant.

Height of the seedling

The data on seedling height (Table 3) indicate that hydration and UV-C radiation treatments significantly affected the height of mung bean seedlings. This

conclusion is supported by the two-way ANOVA results (Table 6). The 3 h hydration and 2 h exposure to UV-C radiation treatment resulted in the largest seedlings (15.57 cm), which was 0.78 cm larger than the seedling of the control. This was followed by the treatment of 3 h hydration alone, which resulted in the second largest seedling (15.07 cm), which was 0.28 cm larger than the control. The seeds in the control showed the third largest (14.79 cm) seedlings. These results suggest that combining 3 h of hydration with 2 h of UV-C exposure significantly enhances seedling height, outperforming both the control and other treatments.

Seedling Vigour Index

The seedling vigour index, calculated by multiplying the germination percentage by the height of the seedling, revealed some notable findings (Table 7). Maximum seedling vigour index was found at 3 h of hydration and 2 h of UV-C exposure (1557), followed by at 3 h of hydration alone (1507 and at control (1479). These results indicate that a combination of 3 h of hydration and 2 h of UV-C exposure significantly enhances seedling vigour, outperforming both the control and other treatments.

Discussion

The study found that UV-C (100–280 nm) photons, which have higher energy than visible light photons (> 400 nm), had some positive effects on radicle length, overall seedling height, and the seedling vigour index. These effects might be due to several factors (Kovács and Keresztes, 2002).

Seed Coat Breakdown: UV-C radiation may have broken down the seed coat, leading to higher and faster oxygen and water imbibition by the seeds. This process could help alleviate seed dormancy.

Table 3. Length of radicles, plumules and seedlings after 7 days of inoculation of the seeds.

Treatments	**Length of radicle (in cm)** Mean±STDEV	**Length of plumule (in cm)** Mean±STDEV	**Length of seedling (in cm)** Mean±STDEV
Control	13.61±5.68	1.23 ± 0.3	14.79 ± 5.74
T1	11.84±5.26	1.36 ± 0.15	13.19 ± 5.29
T2	10.59±4.9	**1.39 ± 0.14**	11.98 ± 4.96
T3	8.31±4.52	1.26 ± 0.19	9.56 ± 4.6
T4	13.79±2.57	1.29 ± 0.12	15.07 ± 2.58
T5	**14.38±4.47**	1.19 ± 0.34	**15.57 ± 4.43**
T6	12.36±5.95	1.29 ± 0.17	13.66 ± 6.01
T7	8.51±3.21	1.29 ± 0.14	9.79 ± 3.22
T8	9.16±5.02	1.28 ± 0.13	10.44 ± 5
T9	8.01±5.21	1.31 ± 0.13	9.32 ± 5.26
T10	6.65±4.02	1.23 ± 0.28	7.88 ± 4.12
T11	8.26±3.8	1.35 ± 0.19	9.61 ± 3.87
T12	6.06±4.48	1.1 ± 0.36	7.16 ± 4.93
T13	7.55±4.5	1.18 ± 0.23	8.73 ± 4.66
T14	8.43±3.65	1.29 ± 0.21	9.72 ± 3.73
T15	7.64±5.58	1.24 ± 0.18	8.89 ± 5.61

Table 4. Details of two-way ANOVA of radicle length.

Source of variation	**Sum of squares**	**Degree of freedom**	**Mean squares**	**F-value (Calculated)**	**P-value**	**F-value (Tabulated)**
Between Treatments	1510.72	15	100.71	5.51	0.001	2.78
Between Replicates	1194.67	13	91.9	5.03	0.001	2.99
Residual	3566.04	195	18.29			
Total	6271.44	223				

Table 5. Details of two-way ANOVA of plumule length.

Source of variation	Sum of squares	Degree of freedom	Mean squares	F-value (Calculated)	P-value	F-value (Tabulated)
Between Treatments	1.09	15	0.07	0.05	0.001	2.78
Between Replicates	1.4	13	0.11	0.08	0.001	2.99
Residual	278.08	195	1.43			
Total	280.57	223				

Table 6. Details of two-way ANOVA of Height of seedling.

Source of variation	Sum of squares	Degree of freedom	Mean squares	F-value (Calculated)	P-value	F-value (Tabulated)
Between Treatments	1526.16	15	101.74	5.84	0.001	2.78
Between Replicates	1265.37	13	97.34	5.59	0.001	2.99
Residual	3395.2	195	17.41			
Total	6186.73	223				

Table 7. Seeding Vigour Index of the seeds hydrated for different durations and different time of exposure to UV-C radiation.

Treatments	Seedling Vigour Index
Control	1479
T1	1319
T2	1198
T3	956
T4	1507
T5	**1557**
T6	1366
T7	979
T8	1044
T9	932
T10	788
T11	961
T12	716
T13	873
T14	972
T15	889

Temperature Increase: UV-C radiation likely increased the temperature, accelerating the achievement of optimum temperatures required for germination.

Enhanced Respiration and Mitochondrial Activities: The increase in temperature from UV-C radiation could have boosted seed respiration and mitochondrial activities. These factors combined could explain the observed positive effects of UV-C radiation on the growth parameters of mung bean seedlings.

Germination percentage and rate of seed germination

In the present study, the duration of hydration and exposure to UV-C radiation were found to have no significant effect on the percentage or rate of seed germination of *Vigna radiata* (mung bean). However, it was determined that 6-8 h of hydration is sufficient to initiate germination. 6 h of hydration combined with 2 or 4 h of UV-C treatment, or 9 h of hydration alone, resulted in all seeds germinating within 24 h, similar to the control. In contrary to our findings Hamid and Jawaid (2011) found an increased mean rate of germination in UV-C irradiated mung bean seeds compared to the control. Neelamegam and Sutha (2015) concluded that UV-C irradiation promoted seed germination in groundnut seeds, with germination increasing with up to 60 minutes of UV-C exposure compared to dry and soaked seed control. Gandhi et al (2019) found that UV light had a positive effect on the seed germination percentage of Bengal gram (*Cicer arietinum* L.) at lower exposure periods (13-17 minutes), with significant changes observed. In contrast, horse gram (*Macrotyloma uniflorum* L.) showed mixed effects, with germination percentages decreasing progressively with increased UV exposure time. Horse gram was more affected by UV light compared to Bengal gram. Flores et al (2024) found that germination rate was higher in seeds of black beans irradiated with UV-C radiation than non-irradiated or control seeds.

Length of radicle, plumule and height of seedling

The radicle length of mung bean seeds was significantly affected by hydration and UV-C radiation treatments. The longest radicle was observed with 3 h of hydration combined with 2 h of UV-C exposure, followed by 3 hs of hydration alone, and then the control group. The length of radicles was generally greater than that of plumules, possibly because the seedlings were maintained in dark rather than light conditions. Neelamegam and Sutha (2015) found that increasing exposure to UV-C irradiation gradually increased the growth of groundnut seedlings (both root and shoot). Seedling growth was more pronounced in UV-C irradiated, water-soaked seeds compared to dry seeds. Contrary to our findings, they observed greater shoot growth than root growth, with a decreasing root/shoot ratio as the seedlings aged. Gandhi et al (2019) observed that the control treatment (no exposure) significantly differed from other studied exposure periods. Bengal gram (*Cicer arietinum* L.) seeds showed almost similar shoot length at shorter UV-C exposure periods, with significant changes at higher exposure times. Horse gram (*Macrotyloma uniflorum* L.) seeds showed decreased shoot length with increased UV-C exposure. UV exposure promoted root growth in both Bengal gram and horse gram, with root length significantly higher than the control. (Adiga, 2021) reported that UV-C radiation has a dose-dependent inhibition on plant growth and development. Gandhi et al (2019) concluded that bengal gram seeds were the most tolerant to UV light exposure, whereas horse gram seeds were the most sensitive.

Seedling Vigour Index

In the present study maximum seedling vigour index was found at 3 h of hydration and 2 h of UV-C exposure, followed by at 3 h of hydration alone and at control. Pournavab et al (2019) studied the responses in terms of germination and growth of four common North Mexican species such as *Glycine max*, *Triticum aestivum*, *Helianthus annuus* and *Pinus maximartinezii* exposed to different doses of UV-C and UV-B radiation. They found that the sunflower and pine species were the most affected by UV-C radiation, decreasing their vigour and germinative power.

Conclusions

This present study investigated the duration of hydration and time of exposure to UV-C radiation on seeds of *Vigna radiata*. It was determined on the basis of seed germination percentage, rate of seed germination, length of radicle and plumule, height of the seedling and seedling vigour index. UV-C radiation was indicated to positively affect the radicle length, height of seedling and seedling vigour. These positive effects may be attributed to factors like breakdown in seed coat, mobilization of reserve foods and an increase in temperature by hydration and UV-C radiation. our research offered valuable insights into optimizing seed germination and seedling growth conditions, particularly for mung beans. The results are crucial for optimizing growth conditions and understanding species-specific responses to UV-C radiation.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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