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Ohmic Heating Pretreatment of Mung Bean Seeds: Effects of Voltage Gradient on Seed Germination and Growth of Mung Bean Sprouts

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Abstract

Mung bean (*Vigna radiata* L.) sprouts are agricultural products with many health benefits and are typically harvested within four–five days. In this study, a new pretreatment technique using ohmic heating (OH) technology was applied for the first time prior to seed germination. This study aimed to investigate the effects of OH-based soaking treatment on mung bean germination and growth. The treatments consisted of three OH treatments at a temperature of 35 °C, non-OH at 35 °C, and two control treatments, i.e. at normal temperature (26°C) and no soaking treatment. In OH treatments, three voltage gradients (VG) were used, i.e. 6 V/cm, 10 V/cm, and 14 V/cm. Parameters examined included moisture content (M_c), seed hardness (S_h), seed mass (M_s), surface morphology, percentage of germination (P_{oG}), germination index (G_i), vigor index (V_i), hypocotyl diameter (D_h), sprout length (L_s), and mass of sprouts (M_{os}), and percentage of sprout with leaf (P_{sl}). Results revealed that OH pretreatment positively affected almost all parameters. In addition, VG also impacted on the observed parameters. The OH treatment increased the absorption of water by the seeds, especially OH at VG 14 V/cm, which produced the highest M_c and M_s, and the lowest S_h. OH at VG 14 V/cm exhibited the highest P_{oG}, the highest of G_i and V_i, and P_{sl}; consistently promoted greater seedling growth and biomass accumulation. Principal component analysis found strong positive correlation among M_s, M_c, P_{oG}, G_i, V_i, M_{os}, and P_{sl}. These findings are expected to be used to increase and speed up the production of bean sprouts in industrial applications.

Keywords: electrothermal treatment; seed germination; mung bean; ohmic pretreatment

1. Introduction

Mung bean (*Vigna radiata* L.) is a legume plant belonging to the Fabaceae family that is widely cultivated and used in various types of cuisine in Asia and parts of South America, North America, and Australia (Peñas *et al.*, 2010; Dahiya *et al.*, 2015). Mung beans are rich in nutrients such as protein and iron (Zhou *et al.*, 2019). Mung beans can be consumed in the form of sprouts due to having many nutritional contents such as minerals, antioxidants, and various vitamins. In addition, these sprouts contain high phenolics, flavonoids, organic acids, amino acids, and total antioxidant activity compared to the seeds (Wei *et al.*, 2019). Mung bean and its sprouts can be processed into food ingredients by frying, boiling, sautéing, and others (Salvador and Bucu, 2021).

In recent years, there has been a growing interest in enhancing the germination and growth of mung bean sprouts to meet the increasing global demand for nutritious and sustainable food sources. Seed germination is usually defined as the period when a plant grows from a seed (Vrancheva *et al.*, 2020). Germination is the initial process of plant seed embryo growth and development, which becomes active after the process of imbibition or absorption of water by the seed. Usually, mung bean sprouts can grow for about four to five days after imbibition or even faster (Salvador and Bucu, 2021). The germination rate of seeds depends on the moisture available during the germination stage.

Seed germination is influenced by several external and internal factors. External factors such as light, water, salinity, temperature, pH, and growth hormones (Muttaqin *et al.*, 2019; Saberali and Moradi, 2019; Núñez-Gastélum *et al.*, 2023). High temperature is another external factor that reduces seed dormancy. Internal factors include seed health and genetic factors (Muttaqin *et al.*, 2019).

Traditional seed soaking methods have been widely employed to promote germination, but advancements in agricultural technologies have led to the exploration of alternative techniques to further improve seedling establishment. In the last decade, several intervention strategies have been implemented to stimulate seeds germination such as gamma irradiation (Majeed *et al.*, 2018), ultrasound (Porto *et al.*, 2018), X-irradiation (Al-Enezi *et al.*, 2012), atmospheric-pressure plasma (Zhou *et al.*, 2019), pulse electric fields (Dymek *et al.*, 2012), magnetic field (Harb *et al.*, 2021), and pesticides (Shakir *et al.*, 2016). However, these technologies have several major drawbacks, including expensive costs, lengthy

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development times, environmental contamination from chemical residues, and heightened ecological concerns (Fan *et al.*, 2020). Therefore, it is still necessary to develop cost-efficient and environment-friendly technologies to improve the production of sprouts.

Ohmic heating (OH) is a modern and innovative technology that has gained significant attention in various food and agricultural applications due to its ability to generate rapid and uniform heating within biological materials (Patel and Singh, 2018; Sagita *et al.*, 2023). OH works based on the passage of an electrical current through the material, converting electrical energy into heat energy directly. This unique approach offers numerous advantages, including precise temperature control (Sagita *et al.*, 2022), reduced processing time (Sakr and Liu, 2014), high energy efficiency (Sagita *et al.*, 2020), and preserved nutritional attributes (Kaur *et al.*, 2016), which make it an attractive candidate for seed pretreatment. The OH system is highly dependent on the electrical conductivity (EC) of the material. Thus, it is important to determine the EC of each ingredient used. EC refers to the extent to which a substance can conduct electric current (Sakr and Liu, 2014; Patel and Singh, 2018). Some literature recommends that the EC value of materials should be above 0.05 siemens per meter (S/m) (Sagita et al., 2022). For particulate or grain materials that will be heated with OH, a medium with a sufficient EC value is needed, one of which is mineral water (Sagita *et al.*, 2021). Pretreatment with OH-based seed soaking with mineral water as a medium is expected to increase seed germination, where the imbibition process is expected to occur more quickly due to the movement of water at the molecular and even ionic levels (Kaur and Singh, 2016). To the best of our knowledge, no research has been conducted on the application of OH as a pretreatment in the germination process. Therefore, in this study, seed pretreatment was performed by soaking the seeds using OH technology for the first time. This study aimed to determine the effects of soaking OH-based mung bean seed on the germination and growth of mung bean sprouts.

2. Materials and Methods

2.1. Mung bean sample

In this study, Mung bean seeds (*Vigna radiate* L.) was obtained from a local market at Subang, West Java, Indonesia. Prior to experiments, mung bean seed samples were stored in the vacuum plastic chamber at 20-25°C. The initial moisture content of mung bean was 12.3 ± 0.2 % wet basis.

2.2. Ohmic heating apparatus

In this research, an OH apparatus developed by Sagita *et al.* (2024) was used to control the temperature of water for soaking the seed (Figure 1). The heating chamber was a cylindrical OH chamber with a maximum capacity of 1000 cm^3, featuring an outer diameter of 100 mm and a wall thickness of 5 mm. The distance between the electrodes was 17 cm. The OH system was equipped with a data recording system using a microcontroller Arduino Mega 2560 (Shenzhen, Guangdong, China). Some of the parameters that can be recorded by the OH system are temperature, voltage, amperage, and time. Temperature was measured using a DS18b20 sensor (Maxim Integrated Products, Inc., California, US), which has an accuracy of ±0.5°C and a precision of ±0.1°C. For monitoring electrical current, an ACS712 sensor (Allegro Microsystems Inc., Manchester, US) was used, with an accuracy and precision of ±0.01 A. The system used AC power at a frequency of 50 Hz and a single phase. Data capture and storage were managed by a real-time clock (RTC) module and an SD card shield. Additionally, a variable transformer (0-250 V) (Focus Technology Co., Ltd., China) was included for voltage regulation. The schematic of the OH device is presented in Figure 2.

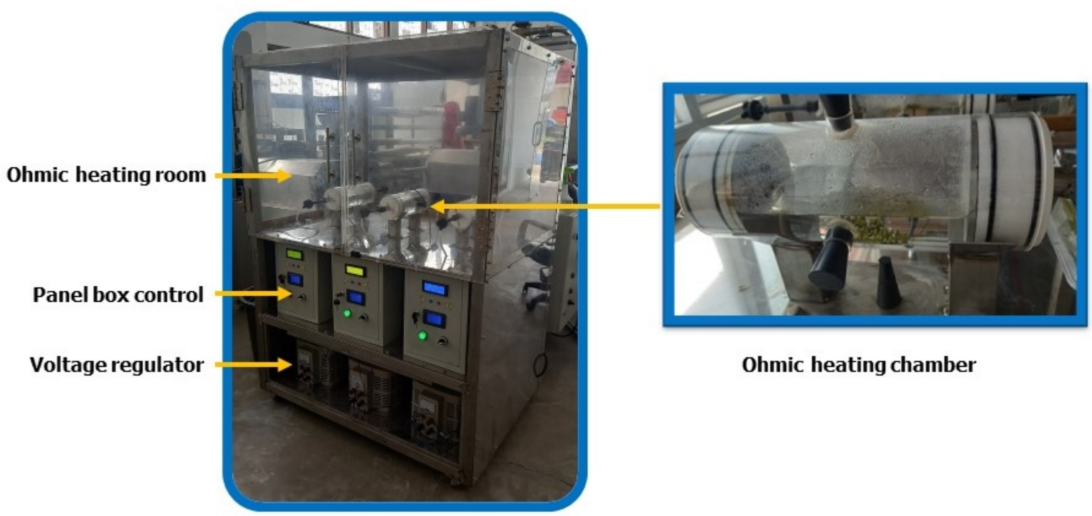

Figure 1. Ohmic heating apparatus used for experiment

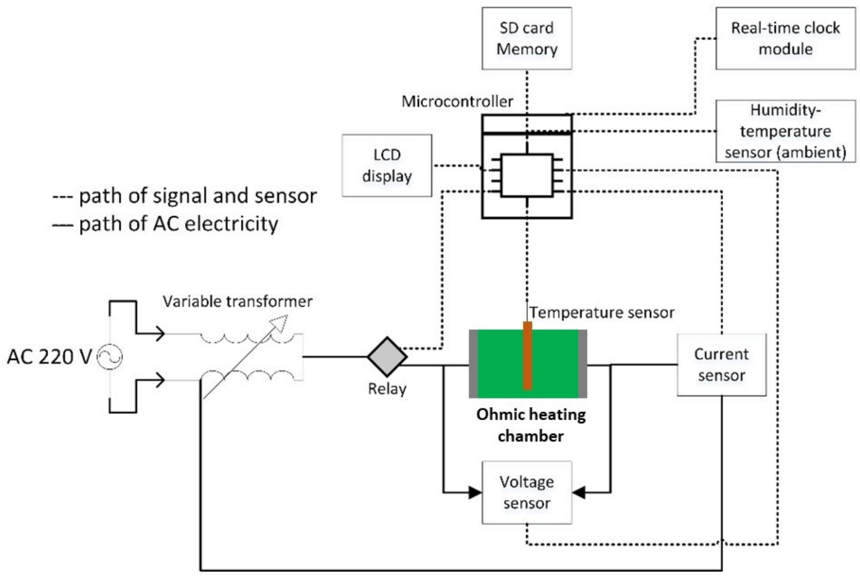

Figure 2. Schematic diagram of ohmic heating system integrated with control system

2.3. Experimental procedures

This study used a completely randomized design (CRD) method with one factor (soaking treatment) with a total of 6 treatments consisting of P1 = OH 6V/cm at 35°C; P2 = OH 10V/cm at 35°C; P3 = OH 14V/cm at 35°C; P4 = non-OH at 35°C; C1 = non-OH at ambient temperature (26°C) (Control 1); C2 = no soaking treatment (Control 2). This study focused on the investigation of voltage gradient effect; thus, all treatments were performed in the same temperature and time, except control 1 and 2.

In the first stage, 500 mL of water was prepared for each of the 5 treatments (P1-P4 and C1). For OH treatment (P1-P3), water was heated using OH based on voltage treatment (6, 10 and 14 V/cm) until it reached a temperature of 35°C. The temperature of 35°C was chosen following Chen *et al.* (2012) which produced the best seed vigor. Conversa and Elia (2009) reported that secondary dormancy in seeds can be alleviated by soaking them at 35°C for several hours. After the temperature reached 35°C, 50 grams of mung bean seeds was put into each OH chamber and the temperature was maintained at 35°C. For P4, water was heated to 35°C using an electric water heater (Q2, Jaya Utama Santikah Ltd., Indonesia) before adding the mung beans. For C1 treatment, water with a temperature of 26°C was directly used for soaking. This water temperature is the temperature in equilibrium with the environmental temperature at Subang Regency, Indonesia. The water used has an initial electrical conductivity of 26.2 mS/m. Soaking treatment was performed for 2 hours. For the C2 treatment, the seed was not given any soaking treatment. Each treatment was carried out with 4 replications.

2.4. Seed monitoring and observation

After soaking stage, the seeds (25 seeds per treatment) were placed in petri dishes which contain 2 pieces of tissue as the planting medium. Observations were carried out for 7 days. To keep the medium still in the moist condition, 5 mL of water was added daily to all samples including C2 which did not undergo soaking treatment. The parameters observed were divided into three stages, viz. after soaking, during germination, and at the end of the germination process. For the post-soaking stage, the following parameters measured were moisture content (M_c), seed hardness (S_h), seed mass (M_s), and surface morphology. During the germination process, the parameters observed included percentage of germination (P_{oG}), percentage of sprout with leaf (P_{sl}), germination index (G_i), and vigor index (V_i) (Zhou *et al.*, 2019). For the 7th DAP, the parameters measured were hypocotyl diameter (D_h), sprout length (L_s), and mass of sprouts (M_{os}).

2.5. Determination of moisture content

The moisture content measurement followed the method of AOAC (1995). Initially, a 2-gram sample (W) was weighed and placed into a ceramic cup. This cup with the sample (W_1) was then heated in an oven at 105°C for 3 hours. After heating, the sample was transferred to a desiccator for 15 minutes and weighed repeatedly until a constant weight (W_2) was achieved. The moisture content was determined using Equation 1. Sample measurements were carried out in 4 repetitions.

$$M_c = \frac{W_1 - W_2}{W} \times 100\%$$

Where M_c is moisture content (%wb), W_1 is mass of cup containing the sample before drying (g), W_2 is mass of cup and sample after drying (g), and W is initial mass of sample (g).

2.6. Determination of seed hardness and mass of single seed

Single seed mass was measured using a digital balance (MH-200, Shenzhen Zime Technology Co., Ltd, China) with an accuracy of 0.01 g. Meanwhile, the seed hardness measurement was performed by using a texture analyzer model TA-XT2 (Stable Microsystems Ltd. Surrey, UK). Measurements were carried out by using 10 seeds per treatment.

2.7. Evaluation of the internal morphology of the seed

The internal morphology of the seeds was observed by using a digital microscope model VHX-7000 (KEYENCE Corp., USA). The magnification used was 50x. Prior to analysis, the top and bottom part of the seed were cut, so that the seed could stand in the sample holder. Observations were randomly carried out on a single seed from each treatment.

2.8. Measurement of growth characteristics of mung bean seeds

Measurement of mung bean growth was carried out from day 1 to day 7 after the seeds were placed in the growing medium, i.e. a Petri dish containing 2 pieces of tissue paper made from 100% of natural fiber (virgin plantation pulp). Parameters measured included percentage of germination (PoG), percentage of sprout with leaf (P_{sl}), hypocotyl diameter (D_h), sprout length (L_s), and mass of sprouts (M_{os}). P_{oG} was observed every day, while P_{sl} was calculated on the third day. D_h measurements were carried out using a caliper with a precision of 0.01 mm for 3 seed samples in each treatment. Measurements of L_s were carried out using a ruler with a precision of 1 mm for 3 samples in each treatment. To measure the mass of sprouts, an analytical balance (MH-200, Shenzhen Zime Technology Co., Ltd, China) with a precision of 0.01 g was used.

2.9. Determination of germination index and vigor index

Several parameters utilized to describe the statistical characteristics of seeds including percentage of germination (P_{oG}), germination index (G_i) and vigor index (V_i) were calculated using Equation 2-4 (Song *et al.*, 2022; Zhou *et al.*, 2019).

$$P_{oG} = \frac{N_t}{N_{TS}} \times 100 \quad (2)$$

$$G_i = \sum \frac{N_{Dn}}{D_n} = \frac{N_{D1}}{D_1} + \frac{N_{D2}}{D_2} \dots \frac{N_{Dn}}{D_n} \quad (3)$$

$$V_i = G_i \times L_s \quad (4)$$

Where N_t is number of germinated seeds, N_{TS} is total number of seeds, N_{Dn} is percentage of seeds grown on day n (%), D_n is day at the time of measurement, and L_s is length of sprouts on the day 7 (cm).

2.10. Data analysis

Experimental tests were replicated for four times at each treatment, and averages were reported with standard deviation. All data for each parameter were analyzed statistically using ANOVA and Duncan post-hoc test at a significance level of 95% using SPSS ver. 21 (IBM Corp., USA). Furthermore, in this study, multivariate analysis was employed viz. principal component analysis (PCA) and hierarchical clustering analysis (HCA). Both were used to evaluate the treatment group and the correlation between dependent variables observed from all treatments. PCA analysis was performed by using FactoMineR package (Lê *et al.*, 2008) in open-source platform (R 4.2.2 statistical software). Prior to PCA and HCA, the data was pre-processed using normalization with the range between 0 and 1.

3. Results

3.1. Moisture content, mass and hardness of mung bean seed

The moisture content of the seeds demonstrated significant variability among the treatments (Table 1). Treatments P1, P2, P3, and P4 displayed higher moisture content compared to the control groups (C1 and C2). Moisture content is closely related to the mass of the seed, where treatments P1, P2, P3, and P4 also resulted in significantly higher seed masses compared to the control groups (C1 and C2) (Table 1). Seed hardness was also found significantly different among the treatments. Notably, treatments P1, P2, P3 exhibited lower seed hardness values compared to the control groups (C1 and C2) and P4 (non-ohmic at 35°C). Control 2 (C2), which received no soaking treatment, displayed the highest seed hardness.

Table 1: Analysis result of mass, moisture content and hardness of seed

Treatment	Single seed mass (g)	Seed hardness (kgf)	Moisture content (%)
P1	0.75 ± 0.04^{b}	16.79 ± 4.79^{a}	23.20 ± 0.54^{c}
P2	0.79 ± 0.03^{bc}	15.87 ± 2.32^{a}	24.61 ± 0.26^{d}
P3	0.80 ± 0.05^{c}	12.90 ± 3.79^{a}	25.62 ± 0.17^{e}
P4	0.77 ± 0.04^{bc}	28.85 ± 6.47^{b}	22.83 ± 0.39^{c}
C1	0.70 ± 0.06^{a}	36.20 ± 3.19^{c}	16.61 ± 0.07^{b}
C2	0.67 ± 0.03^{a}	38.39 ± 5.48^{c}	12.31 ± 0.15^{a}

Note: Values are expressed as mean ± standard deviation. Means with different letters were significantly different at $p < 0.05$ by Duncan Multiple Range Test. P1 = OH 6V/cm at 35°C; P2 = OH 10V/cm at 35°C; P3 = OH 14V/cm at 35°C; P4 = non-OH at 35°C; C1 = non-OH at ambient temperature (Control 1); C2 = No soaking treatment (Control 2)

3.2. Internal morphology of mung bean seeds after pretreatment

Based on the results obtained from a digital microscope with a magnification of 50×, the results of the observations are presented in Figure 3. Visually, the P1, P2, and P3 treatments showed a darker color appearance (from surface to core) which is indicated by the presence of more water absorbed by the seeds.

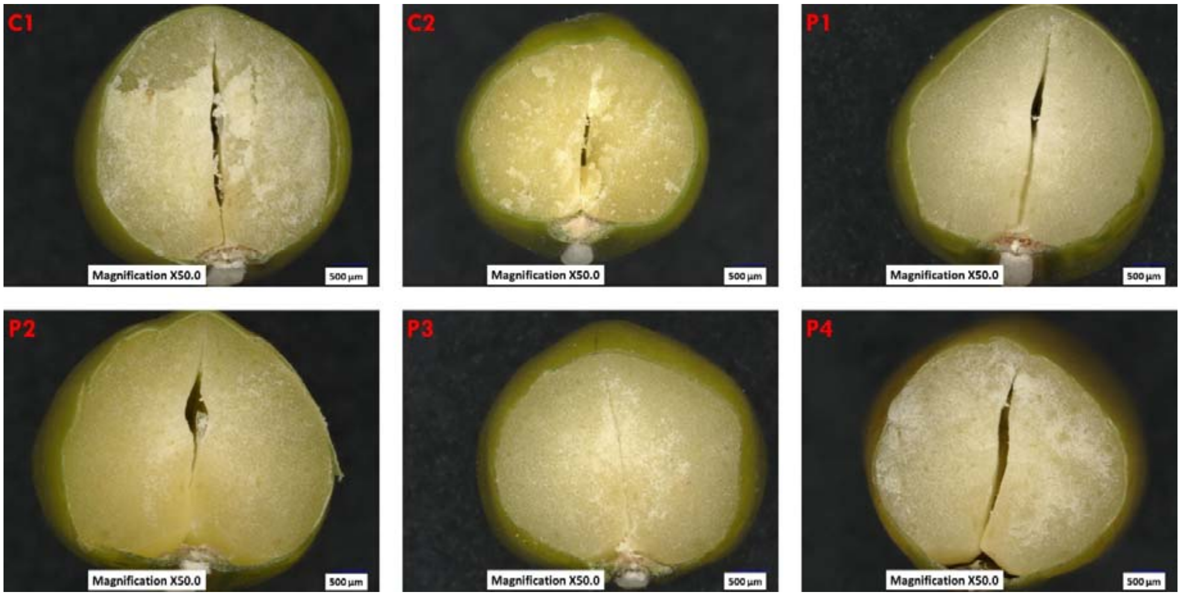

Figure 3. Visual appearance of cross-sectional area of mung bean seed

3.3. Effect of pretreatment on seed germination of mung bean

The results of the seed germination for 7 days are shown in Figure 4. The results showed a significant difference in P_{oG} occurring on day 1 (24 hours after seeding). The highest P_{oG} were found in treatments P3 and P2 and followed by P1, P4, C1 and C2, respectively. This shows that soaking treatment (P1-P4 and C1) generally increases the percentage of seed germination compared to C2 (without soaking treatment). Treatment C2 showed the lowest P_{oG}, while treatments C1 (soaked in water at room temperature) and P4 (non-OH at 35°C) showed higher P_{oG} than C2. An interesting fact from these results is that the OH treatment (P1-P3) showed higher values than C1, C2, and P4, which was most likely caused by faster water absorption by the seeds due to the presence of an electric current in the water. On the next days, all seeds began to grow with no significant change on the germination percentage among treatments, and the seeds were germinated at a percentage of 96 to 100%.

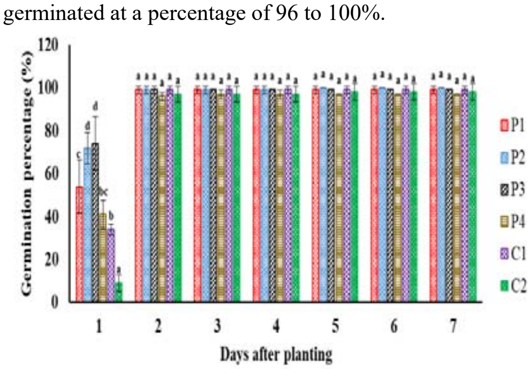

Figure 4. Seed germination percentage of mung bean under different treatment: Bar with different letters in the same day were significantly different at p < :05 by Duncan Multiple Range Test: P1 = OH 6V/cm at 35°C; P2 = OH 10V/cm at 35°C; P3 = OH 14V/cm at 35°C; P4 = non-OH at 35°C; C1 = non-OH at ambient temperature (Control 1); C2 = No soaking treatment (Control 2).

3.4. Germination index and vigor index

The results of the calculation of G_i and V_i parameters are presented in Table 2. It was found that the treatment of mung bean seeds involving OH had a significant positive effect on G_i and V_i compared to the control treatment and without OH. The G_i represents the relative speed and uniformity of seed germination. Treatments P2 and P3 exhibited significantly higher G_i compared to the control groups (C1 and C2). The vigor index is a measure of seedling strength and robustness, reflecting the overall health and vitality of the seedlings. Treatments P2 and P3 demonstrated significantly higher V_i compared to the control groups (C1 and C2).

Table 2. Germination index and vigor index of mung bean sprouts under different pretreatment

Treatment	Germination index	Vigor index
P1	52.92 ± 3.88^{c}	651.65 ± 92.66^{ab}
P2	57.55 ± 2.25^{d}	892.39 ± 190.47^{d}
P3	58.20 ± 2.87^{d}	865.78 ± 161.44^{cd}
P4	48.75 ± 2.53^{b}	751.69 ± 26.06^{bcd}
C1	47.92 ± 0.66^{b}	677.13 ± 146.15^{abc}
C2	41.00 ± 1.58^{a}	539.50 ± 13.23^{a}

Note: Values are expressed as mean ± standard deviation. Means with different letters were significantly different at p < .05 by Duncan Multiple Range Test. P1 = OH 6V/cm at 35°C; P2 = OH 10V/cm at 35°C; P3 = OH 14V/cm at 35°C; P4 = non-OH at 35°C; C1 = non-OH at ambient temperature (Control 1); C2 = No soaking treatment (Control 2)

3.5. Effect of soaking pretreatment on seedling growth of mung bean

Table 3 presents the results of experiments examining the effects of different treatments on the hypocotyl diameter, length of sprout, mass of total sprout, and percentage of sprouts with leaves of mung bean plants.

These parameters are critical indicators of seedling growth and overall plant development.

Table 3. Analysis result of hypocotyl diameter, length of sprout, mass of total sprout, and percentage of sprout with leaf

Treatments	Hypocotyl diameter (mm)	Length of sprout(cm)	Mass of total sprout (g)	Percentage of sprout with leaf at 3rd day (%)
P1	1.1 ± 0.2^{b}	12.4 ± 2.0^{a}	5.67 ± 0.59^{a}	44.0 ± 13.5^{bc}
P2	0.5 ± 0.2^{a}	15.5 ± 3.1^{a}	5.88 ± 0.78^{a}	43.0 ± 5.0^{bc}
P3	1.2 ± 0.0^{b}	15.0 ± 3.4^{a}	6.17 ± 0.57^{a}	56.0 ± 5.7^{c}
P4	1.0 ± 0.1^{b}	15.5 ± 1.3^{a}	5.91 ± 0.22^{a}	47.0 ± 13.2^{bc}
C1	0.9 ± 0.2^{b}	14.1 ± 3.0^{a}	5.60 ± 0.49^{a}	39.0 ± 11.9^{b}
C2	0.9 ± 0.2^{b}	13.2 ± 0.4^{a}	5.86 ± 1.02^{a}	23.0 ± 3.8^{a}

Note: Values are expressed as mean ± standard deviation. Means with different letters were significantly different at $p < .05$ by Duncan Multiple Range Test. P1 = OH 6V/cm at 35°C; P2 = OH 10V/cm at 35°C; P3 = OH 14V/cm at 35°C; P4 = non-OH at 35°C; C1 = non-OH at ambient temperature (Control 1); C2 = No soaking treatment (Control 2)

The hypocotyl diameter represents the width of the seedling stem just above the roots. Treatments P1, P3, and P4 demonstrated larger hypocotyl diameters (average) compared to the control groups (C1 and C2), although the values were not significantly different while treatment P2 produced the lowest value even compared to the control treatments.

The length of the sprouts showed insignificant results at the p-value level <0.05. However, treatments P2, P3 and P4 gave average results above 15 cm, while P1 produced the lowest value even compared to the control treatments. Based on these findings, there is a trade-off phenomenon between P1 and P2 where P1 gives a larger diameter but lower sprout length than P2. This indicates that there is an effect of voltage intensity on germination, where P3 (voltage intensity 14 V/cm) was found to provide optimal hypocotyl diameter and length of sprouts.

The mass of the total sprout represents the weight of the entire seedling. Overall, the total mass was not significantly different at 95% confidence interval. However, the average value showed that the highest value was obtained in the P3 treatment.

The percentage of sprouts with leaves on the third day indicates the rate of early leaf development. Treatment P3 significantly displayed the highest percentage of sprouts with leaves at the third day, followed closely by treatments P1, P2, and P4.

3.6. Multivariate analysis result

The results of PCA are presented in Figure 5, which displays the loadings plot and score plot of each treatment and response variable on the principal components analysis diagram. The loading plot of principal component 1 (PC1) and principal component 2 (PC2) provides a comprehensive representation of the relationships observed among the dependent variables. Together, PC1 and PC2 account for a substantial portion of the total variance, explaining 84.12% of the dataset. PC1 contributes significantly, explaining 69.55% of the variance, while PC2 contributes 14.57%.

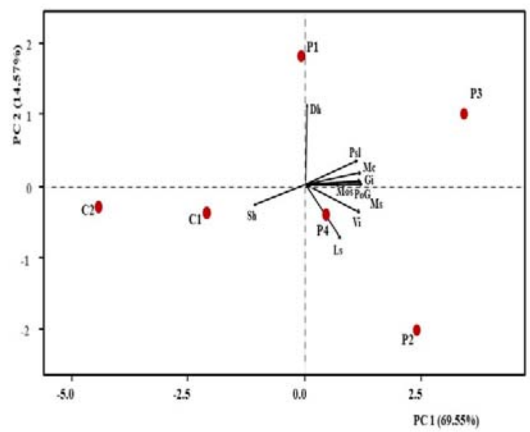

Figure 5. Biplot obtained from PCA of variables comprising mung bean sprout characteristics

Furthermore, in the biplot diagram, the observed samples were scattered in each quadrant. Thus, for better understanding about the treatment group, HCA was performed which is shown in Figure 6. It was found that the treatments with OH and non-OH at 35°C (P1-P4) were in the same cluster and the other cluster was the control group (C1 and C2). P2, P3 and P4 were found to have identical characteristics, indicated by the same cluster and the same colour pattern.

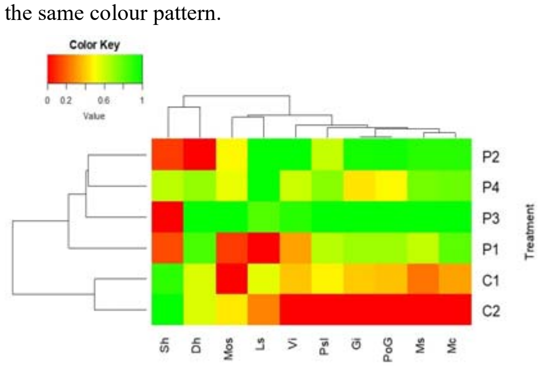

Figure 6. The relationship between treatments and response variables including their cluster; the color of the heat map represents the trend of the treatment impact from the lowest (red) to the highest (green).

4. Discussion

The application of OH pretreatment on mung bean seeds has shown significant and promising results on the germination and growth of mung bean sprouts, as demonstrated in this study where several key findings shed light on the potential benefits of OH pretreatment on speeding up seed germination and sprout growth.

OH pretreatment, particularly at VG 14 V/cm (P3) positively influenced seed development by enhancing water imbibition, resulting in heavier seeds. This shows that OH at a certain VG (14 V/cm) enhanced the ability of seeds to uptake water. In contrast, the seeds subjected to Control 1 (C1), soaked at ambient temperature, exhibited lower mass, possibly due to limited water uptake during soaking. Moreover, the C2 treatment produced the lowest seed mass because it was not given any soaking treatment so there was no water absorption. Other evidence was found on seed hardness, where OH reduced seed hardness, making the seeds more conducive to germination. This finding indicated that the application of OH can potentially alter seed structure and soften the seed coat. Another study reported that OH clearly damaged (weakened) the cell wall network and tissue structure due to the heat and electroporation effects (Allali *et al.*, 2010). This OH effect is similar to the soaking technique with Ammonium gluconate, which can accelerate water uptake in seeds (Chen *et al.*, 2022).

More evidence through moisture content analysis shows the superiority of OH at VG 14/cm, with the highest water content value. This shows that electric current in water with a certain intensity (in this case 14 V/cm) during immersion had a positive influence in terms of faster water absorption. This is in line with the result of Allali *et al.* (2010), where OH weakens cell walls and tissue structures more quickly due to the combined effects of heat and electroporation. Thus, the same soaking temperature (35 °C) as treatment P3 and P4 gives different water contents where P3 (OH at 14 V/cm) shows a higher value than P4 (without OH). Furthermore, both treatments (P3 and P4) provided higher moisture content than the control (C1) because C1 used a lower water temperature (26 °C). This finding was in line with the research conducted by Coffigniez *et al.* (2019), where higher water uptake occurs at higher temperatures because cell wall weakening occurs more quickly at higher temperatures, allowing water to enter the cells more quickly. Another study reported that moisture content of soaked seed increased with the increase in temperature and time (Chen *et al.*, 2012), but it should be noted that a higher temperature was not always effective at enhancing germination. According to Chen *et al.* (2012), the optimum temperature for stimulating seed germination and vigor (e.g. switchgrass seeds) was in the range 35-45°C.

Based on the overall result presented in Table 1, in the P3 treatment, characterized by the application of the highest voltage (14V/cm), the seed exhibited the highest value of mass and moisture content, and the lowest of seed hardness. This could be attributed to the increased collision of electron charges, resulting in higher molecular kinetic energy production so that water molecules can more quickly enter the seed. Another study reported that an electric field may change physiology and improve water absorption due to dipole-dipole interactions (Rifna *et al.*, 2019).

Regarding the visual appearance of the seed cross-sectional area, the P4 treatment showed that many dry crystal grains (indicated by white color) were still present, similar to the control treatments (C1 and C2). This was due to minimal or even no water absorption. These results are consistent with the data in Table 1 where the moisture content of the seeds with the OH treatment at VG 14 V/cm (P3) was the highest compared to other treatments. According to Dymek *et al.* (2012), electric field treatment such as high pulse electric field resulted in the opening of the cavities within the plasma membrane, which increased the level of inward and outward movement of polar molecules. Numerous studies have demonstrated that the application of a low-intensity, low-frequency electric field can alter the behavior of biosystems (organisms, tissue and cell cultures), including effects on the processes of proliferation, growth, and differentiation (Berg, 1993).

OH pretreatment can increase the germination rate. In addition, it was also revealed that the applied voltage also had an effect, in which the higher the voltage significantly increased the germination rate, where the highest value was the P3 treatment (OH 14 V/cm). This finding was consistent with data on the moisture content of the seeds after soaking, where P3 was the treatment that produced the highest water absorption. Previous study which used pulse electric field also found the same phenomenon, where application of pulse electric field about 12 V/cm increased seed germination percentage (Khotimah *et al.*, 2016). Furthermore, compared to previous studies using the PAW treatment, results of this study were slightly higher. The PAW treatment of seeds that had been soaked 6 hours previously resulted in a range of germination percentages of 60-70% after 24 hours (Fan *et al.*, 2020), while OH treatment at a certain voltage can produce an average germination rate of 74%.

According to Choudhury & Karmakar (2020), germination goes through three phases, viz. the seed imbibition phase, the activation phase, and the germination phase. In the seed imbibition phase, a process of water absorption occurs which involves the apoplast space through the force pushed by the seed. In the activation phase, plant metabolic activity occurs where protein synthesis is in this phase. Then it enters the germination phase, where cell elongation occurs and the appearance of the radicle where the sprouts will grow and develop under suitable conditions. With the intervention of OH treatment, the imbibition and the activation phases might occur faster than without OH treatment.

The implication is that the acceleration of seed emergence and faster seedling growth means that bean sprouts can be harvested more quickly. Specific voltages (P2 and P3) positively influenced the germination process, leading to faster and more synchronized seedling emergence. Conversely, Control 2 (C2) displayed the lowest germination index, suggesting that without any treatment, seed germination might be slower and less consistent.

Regarding vigor index, OH treatments at specific voltages (P2 and P3) contributed to the development of more vigorous and robust seedlings. The enhanced molecular activity due to OH might have promoted seedling growth and vigor. On the other hand, Control 2

(C2) which was soaked in lower temperature showed the lowest vigor index, indicating that untreated seeds might produce weaker and less vigorous seedlings. The G_i and V_i are closely related to the germination rate observed for 7 days, where G_i and Vi increased with the increase of germination speed as presented in Figure 4.

Results of this study when compared with previous studies by Zhou *et al.* (2019) which used plasma treated water (PTW) showed the same pattern, where the V_i of the control treatment was below 400, while the vigor index of the PTW treatment depended on the gas used. N_2-PTW, He-PTW and O_2-PTW provided V_i values ranging from 477.84 – 719.28 which were lower than the optimal results in this study, but not higher than the air-PTW treatment in Zhou *et al.* (2019). This opens opportunities for further research in terms of optimizing the temperature and soaking time of OH-based seeds in order to obtain better results.

Furthermore, this study was in line with previous study which found that germination rate index increased with the increase in temperature (Chen *et al.*, 2012; Goussous *et al.*, 2010). However, it should be noted that there is an upper limit temperature as higher temperatures can damage cell membranes. The optimal temperature for switchgrass seed germination was found to be about 38°C and a higher temperature was may not effective (Chen *et al.*, 2012). In the present study, soaking at 35°C produced higher G_i and V_i compared to the control, which adds to the evidence that this range of temperature values was effective for pretreatment.

OH treatments (VG 14 V/cm) also promoted the early growth and development of the seedling stems, resulting in larger hypocotyls. On the other hand, C1 and C2 displayed the smallest hypocotyl diameter, indicating this treatment might have slower initial stem growth due to lateness in water absorption. Additionally, a high electric field may cause the seed coat to crack, which can enhance its ability to absorb water and nutrients. This improvement could lead to a higher germination rate of mung beans and support the growth of both the hypocotyl and radicle (Zhou *et al.*, 2019). Several relevant previous studies have been conducted by Khotimah *et al.* (2016) and Kiatgamjorn *et al.* (2003), but the mechanism of electric current was different, where they used a static electric field that uses DC current without a heating effect. The results of their study showed that the bean sprouts grown from the treated seeds had heavier in fresh weight, longer roots, stems and leaves in comparison to the control.

The length of the sprout is a key indicator of early seedling growth. Treatments P2, P3, and P4 exhibited significantly longer sprouts compared to the control groups (C1 and C2) and P1. This implies that OH at specific voltages positively influenced the elongation of the seedling shoots, leading to enhanced early growth. Also, it was revealed that OH at 14 V/cm consistently promoted greater seedling growth and biomass accumulation. C2 exhibited the lowest total sprout mass, indicating that untreated seeds might produce weaker and less substantial seedlings. Regarding leaves development, OH treatments, particularly P3, significantly accelerated early leaf development, leading to more advanced and healthier seedlings. Control 2 (C2) exhibited the lowest percentage of sprouts with leaves, indicating that untreated seeds might have delayed leaf emergence.

As comprehensive information, PCA shows robustness in capturing underlying patterns and interdependencies among measured variables, thus providing valuable insight into the factors that influence bean sprout characteristics. PC 1 shows strong positive loadings for seed mass, moisture content, percentage of germination, germination index, vigor index, mass of sprouts, and percentage of sprouts with leaves. It suggests that these variables are highly correlated and contribute most significantly to the variance explained by PC 1. Thus, PC 1 could be interpreted as an overall indicator of seed and sprout quality and vigor. PC 2 has only one strong positive loading which is hypocotyl diameter. The study's findings could be instrumental in optimizing mung bean sprout cultivation practices. By understanding the key variables that influence seed germination, seedling growth, and overall sprout quality, farmers and researchers can focus on specific factors to enhance crop productivity and ensure better-quality sprouts.

5. Conclusion

The experimental results reveal the promising potential of OH as a highly effective seed pretreatment for mung bean sprout production. The application of specific voltages (i.e., 14 V/cm) consistently resulted in accelerated seedling growth, larger hypocotyl diameter, longer sprout length, increased total sprout mass, and advanced early leaf development. P3 treatment (OH at VG 14V/cm) gave a significant effect and produced almost all the optimal values for the parameters measured such as water absorption, total mass of sprouts, seed moisture content, germination rate, germination index, vigor index, and percentage of sprouts with leaves. These findings hold significant implications for industrial practices seeking to optimize mung bean sprout production. Apart from that, these findings also reveal the potential for using OH as a pre-sowing treatment, so that farmers can increase the speed of seedling establishment and foster healthier and more vigorous plants.

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