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Impact of Surface Discharge Cold Plasma on Seed Germination and Seedling Vigour in Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT

Background: Cold plasma is a non-thermal technology that has growing attention for its potential application in agriculture, particularly for seed quality enhancement and establishment of early crops. Cold plasma seed treatment is increasingly being identified as a potential alternative to conventional seed treatment practices.

Methods: The present study evaluated the effect of surface discharge cold plasma treatment on seed germination and seedling vigour in rice. Three popular rice varieties, IR-64, MTU-1010 and BPT-5204 and two traditional varieties, Chitrakar and KaruppuKavuni, were subjected to different plasma treatment times to evaluate the differences among genotypes. Surface discharge cold plasma was generated by a dielectric barrier discharge system under atmospheric air conditions. The variables measured were seed germination percen tage, root length, shoot length, fresh weight, dry weight, seedling vigour index I and II.

Result: The plasma treatment showed increased physiological quality parameters over the untreated control, with maximum responses in the cultivated varieties after 10 min of plasma treatment and in traditional varieties after 40 min of plasma treatment. The results clearly determine the effectiveness of cold plasma seed enhancement technology for seed germination and vigour improvement in rice, thus establishing its potential application in seed technology and crop improvement.

Key words: Cold plasma, Rice, Seed germination, Seed vigour, Surface discharge plasma.

INTRODUCTION

The rate of population growth, land area not suitable for cultivation and recurrent events of climate-related stresses are putting immense pressure on the global agricultural productivity system, and hence there is an urgent need for sustainable approaches to enhance crop establishment and yield stability (Bai *et al.*, 2025). In cereal crops such as rice, successful crop establishment is highly reliant on seed quality and seedling vigour (Benabderrahim *et al.*, 2025). Hence, the need to improve seed germination and early seedling establishment remains a vital component of sustainable rice production systems.

Seed quality enhancement techniques such as seed priming have been widely practiced to improve seed germination and seedling vigor (Singh *et al*., 2020; Vinothini *et al*., 2026). However, these techniques are often faced with limitations related to chemical residues, environmental concerns, instability of storage and scalability for large-scale application (Shelar *et al.*, 2022). In recent years, cold plasma, also known as non-thermal atmospheric plasma, has been identified as a new and eco-friendly approach for seed treatment. Cold plasma is a partially ionized gas that consists of electrons, ions, reactive species and UV photons, produced at near-ambient temperatures, allowing it to be applied to biological materials without causing thermal injury (Cesniene *et al.*, 2024).

Cold plasma seed treatment has been shown to increase germination percentage, root and shoot length and seedling vigor in various field and vegetable crops such as maize, wheat, mung bean and rice (Jiang *et al.*, 2014). These beneficial effects can be attributed mainly to the plasma induced changes in seed coat surface characteristics, such as increased surface roughness and improved wettability, which facilitate faster water imbibition and activation of metabolism during germination process

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(Holc *et al.*, 2021). Additionally, reactive oxygen species generated during plasma treatment act as signaling molecules that regulate early physiological and metabolic processes associated with seed germination (Sayahi *et al.*, 2024).

Optimisation of surface discharge plasma treatments across genetically diverse rice genotypes has received limited systematic investigation in recent years (Bian *et al.*, 2024). Surface discharge plasma involves direct exposure of seeds to plasma generated along a dielectric surface and represents a simple and scalable configuration with potential applicability in the seed industry (Suwannarat *et al.*, 2025). Current evidence indicates that treatment efficacy is strongly influenced by exposure duration,genetic background and genotype-specific responses, particularly in rice, are not yet well characterised (Shilpa *et al.*, 2024).

The focus of the current study was to assess how surface discharge cold plasma treatment affected rice seed germination and seedling vigour. Assessing variations in seed germination percent, root length and shoot length and seedling vigour index among genetically varied rice genotypes and clarifying the potential of surface discharge plasma as a non-chemical rice seed enhancement.

MATERIALS AND METHODS

Experimental site and plant material

Genotypes of rice were collected from Tamil Nadu region. Three cultivated cultivars, IR-64, MTU-1010, BPT-5204 along with two traditional landraces, Chitrakar and KaruppuKavuni were selected. While the landraces were included to reflect genetic diversity and differing seed coat characteristics that may influence responses to seed enhancement treatments, the cultivated types were chosen for their commercial significance.

Applying a dielectric barrier discharge system operating in atmospheric air, surface discharge cold plasma treatments were carried out at the High Voltage Engineering Laboratory, Indian Institute of Science (IISc), Bengaluru, India, in accordance with protocols documented for agricultural seed applications. For 0 minutes (untreated control), 1 minute, 3 minutes, 10 minutes and 20 minutes, the cultivars IR-64, MTU-1010 and BPT-5204 were exposed to cold plasma. To assess their reaction to prolonged plasma exposure, seeds of the traditional landraces Chitrakar and KaruppuKavuni were treated for 0 min (control), 10 min, 20 min, 30 min and 40 min.

Experimental set up

Surface discharge cold plasma treatment, the rice seeds were uniformly placed in a single layer on a dielectric barrier discharge (DBD) platform as shown in (Fig 1a). The barrier was then positioned inside the plasma reactor. The reactor setup was further connected to two electrodes, which included a high-voltage electrode (red connection) and a grounded electrode (green connection), to produce surface discharge plasma on the seed surface (Fig 1b).

Atmospheric air was employed as the working gas and was supplied into the reactor chamber through an external air pump (Fig 1c). After confirming a closed and stable operating environment, the air supply was controlled and then maintained throughout the plasma process. The high voltage alternating current was connected to the electrode system and the working voltage was set to approximately 100V using the control panel. The time for plasma treatment was regulated using a digital timer depending on the respective treatment procedures.

Once the working voltage and time for treatment had been set, the plasma system was switched on using the main power switch and the motor switch on the control panel (Fig 1d). After the completion of the plasma treatment, the voltage supply was switched off and the system was grounded (Fig 1e). Finally, the treated seeds were removed from the reactor chamber for further germination tests.

Parameters observed

Germination (%)

Testing for seed germination was done in compliance with the ISTA (International Seed Testing Association, 2012). four hundred seeds were used for each treatment and germination was observed under carefully controlled

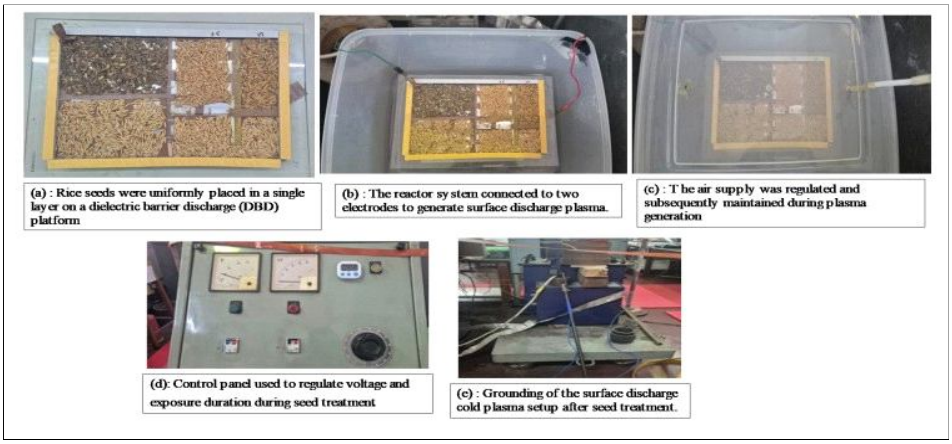


Fig 1: Surface discharge cold plasma experimental setup used for seed treatment.

environments. On the fourteenth day from sowing, the final germination counts were recorded. Germination percentage was calculated as:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Root length (cm)

After 14 days of sowing, the root length was measured. A standard method was used to measure the distance in centimeters (cm) between the point of seed attachment and the tip of the primary root. For statistical analysis, mean root length values were computed for every treatment.

Shoot length (cm)

On the fourteenth day following seeding, the length of the shoots was measured. Using a standard method, measurements were made in centimetres (cm) from the place of seed attachment to the tip of the longest leaf. For statistical analysis, the mean shoot length values for each treatment were determined.

Fresh weight (mg 10 seedlings^{-1})

The seedling biomass, fresh weight was measured on the fourteenth day. After root and shoot lengths were measured the seedlings were weighed using an analytical balance. The fresh weight was measured in milligrams per ten seedlings (mg 10 seedlings^{-1}).

Dry matter production (mg 10 seedlings^{-1})

The collected seedlings were first shade-dried for 24 hours and then oven-dried at 80^{o}C for 16 hours until a constant weight was reached in order to determine the amount of dry matter produced. An analytical balance was used to record the dry weight, which was then reported as milligrams per ten seedlings (mg 10 seedlings^{-1}).

Vigour Index I

Vigour Index I was calculated using germination percentage and mean seedling length as per the standard formula.

Vigour index I =

$$\text{Germination percentage} \times \text{Total seedling length (cm)}$$

Vigour Index II

Vigour index II was computed using germination percentage and total dry matter production following the standard formula.

Vigour index II =

$$\text{Germination percentage} \times \text{Total dry matter production}$$

Experimental design

The experiment was laid out in a factorial completely randomised design (CRD) with genotype and plasma exposure duration as factors. Plasma treatments were conducted using four independent biological replications, each performed on separate seed batches. Allthe treatments were applied using the same equipment and operator to minimise procedural variability, following standard experimental design principles for seed physiology experiments.

Statistical analysis

Analysis of variance (ANOVA) was conducted on the data to determine the significance of treatment differences. In the comparison of means, Duncan's multiple range test (DMRT) was used and differences were considered significant at P≤0.05. All statistical analysis were carried out in R studio using *agricolae*, *dplyr, tidyr, gglot2, metan, tidyverse, facto MineR, factoextra* packages. Results are presented as mean±standard error (SE) of four replications and statistically significant differences among treatments within each genotype are indicated by different superscript letters in the bar graphs. Pearson's correlation analysis was performed to examine relationships among germination and seedling vigour traits. Principal component analysis (PCA) was also conducted to identify major traits contributing to variability among treatments and genotypes.

RESULTS AND DISCUSSION

Germination (%)

Surface discharge cold plasma treatment showed a significant impact on germination (Fig 2). In cultivar varieties, IR-64 (96%) and MTU-1010 (94%) and BPT-5204 (74%) had maximum germination at T3 (10 min), which gradually reduced at T4 (20 min). The maximum germination was recorded in landraces at T4 (40 min), Karuppu Kavuni (94%) and Chitrakar (100%).

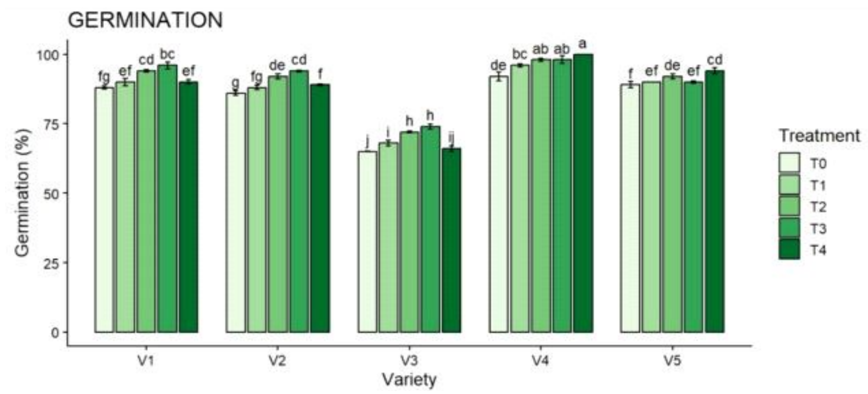
According to De Groot *et al.* (2018), cold plasma is a non-chemical and sustainable approach to seed treatment that changes the surface of the seed without compromising its genetic purity. The enhanced rate of germination indicates that plasma treatment has enhanced physiological response. In the early stages of germination, plasma treatment enhances rapid water imbibition by increasing the wettability and roughness of the seed coat (Bormashenko *et al*., 2015). Rapid hydration enhances efficient reserve mobilization and activation of metabolic enzymes in the early stages (Singh *et al.,* 2015). The genotype-dependent response shows that the plasma seed interactions are affected by the thickness of the seed coat and its biochemical components. Longer exposure times may become necessary for landraces with thicker or darker seed coats, which require greater surface modification. Thus, maximizing the improvement in germination as a function of genetic background requires optimizing exposure time.

Root length and shoot length

The effect of plasma exposure on the root and shoot length was significant (Fig 3 and 4). Although there was an increase in landraces up to T4, there was an increase up to T3 and a decrease at T4 in improved varieties for both traits. Root length was more sensitive than shoot length. Greater response to the plasma treatment was observed in root growth, indicating that there was a significant stimulation of early root growth . During early establishment, greater root growth improves the ability to uptake nutrients and water (Ongrak *et al*., 2023; Rout *et al*., 2025). Water uptake, availability of soluble sugars and cell division in meristematic cells may be attributed to the stimulation by plasma. To a relatively lesser extent, shoot growth was also stimulated. plasma exposure may induce slight stress responses, as shown by the reduction in cultivars after optimal treatment. The genotype-specific sensitivity to plasma is further supported by the varied responses among cultivars and landraces (Mildaziene *et al*., 2019).

Fresh weight and dry matter production

There were large variations in fresh weight and dry matter values among the treatments (Fig 5 and 6). In landraces, both values gradually increased up to T4 (40 min), whereas in cultivars, they increased up to T3 (10 min) and then reduced at T4 (20 min). Landraces had higher biomass production values than cultivars for longer exposure times.The enhanced water uptake and higher cell growth in plasma treated seedlings results in the enhanced fresh weight. The enhanced production of dry matter indicates better photosynthetic allocation and uptake. Plasma treatment enhances metabolic activation, which is beneficial for resource utilization. After plasma treatment, wheat exhibited a similar response in biomass production (Kucerova *et al*., 2019). The reduced biomass in cultivars at higher exposure doses indicates a short time for optimal treatment. The improvements caused by the plasma impact subsequent growth performance, besides germination. Moreover, the genotype response highlights the importance of optimal exposure time for maximum benefit. Taking everything into account, biomass production at an early stage is enhanced by plasma treatment due to improved metabolic efficiency (Perez-Piza *et al*., 2022).



Different letters above bars indicate significant differences among treatments within each genotype (DMRT, p≤0.05). Treatment details are T0- control,T1-3 min,T2-5 min,T3-10 min,T4-20 min. V1-V5 represent rice genotypes, where V1-MTU-1010, V2-IR-64,V3-BPT-5204,V4-Chitrakar,V5-KaruppuKavuni.

Fig 2: Effect of surface discharge cold plasma treatment on germination percentage of rice genotypes.

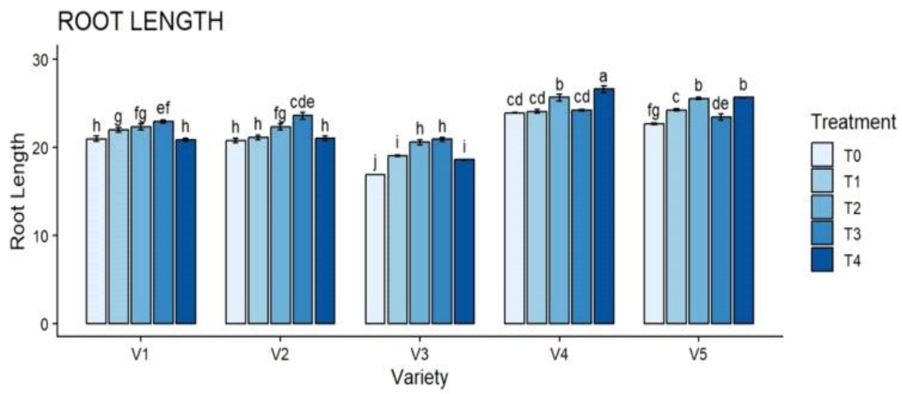


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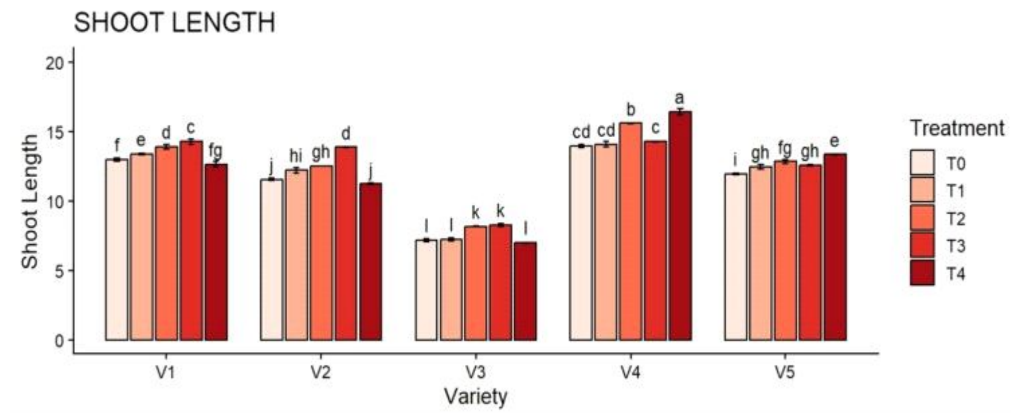
Fig 3: Effect of surface discharge cold plasma treatment on root length of rice genotypes.



Different letters above bars indicate significant differences among treatments within each genotype (DMRT, p≤0.05). Treatment details are T0- control, T1-3 min, T2-5 min, T3-10 min, T4-20 min. V1-V5 represent rice genotypes, where V1-MTU-1010,V2-IR-64,V3-BPT-5204,V4- Chitrakar,V5- KaruppuKavuni.

Fig 4: Effect of surface discharge cold plasma treatment on shoot length of rice genotypes.

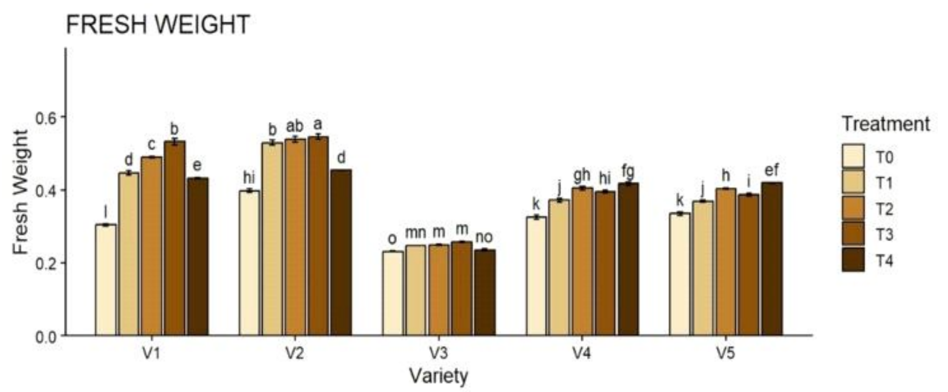


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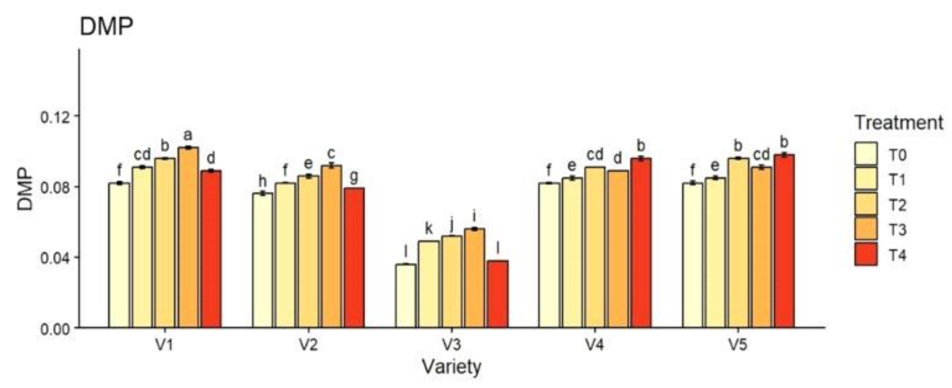
Fig 5: Effect of surface discharge cold plasma treatment on fresh weight of rice genotypes.

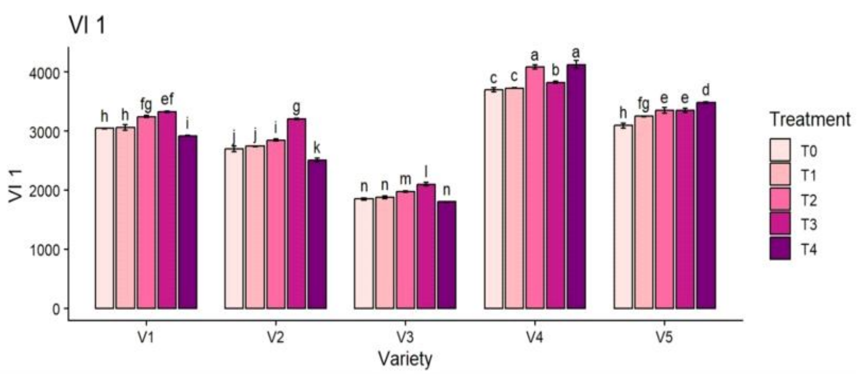


Different letters above bars indicate significant differences among treatments within each genotype (DMRT, p≤0.05). Treatment details are T0- control, T1-3 min, T2-5 min, T3-10 min, T4-20 min. V1-V5 represent rice genotypes, where V1-MTU-1010,V2-IR-64,V3-BPT-5204,V4- Chitrakar,V5- KaruppuKavuni.

Fig 6: Effect of surface discharge cold plasma treatment on DMP (dry matter production) of rice genotypes.

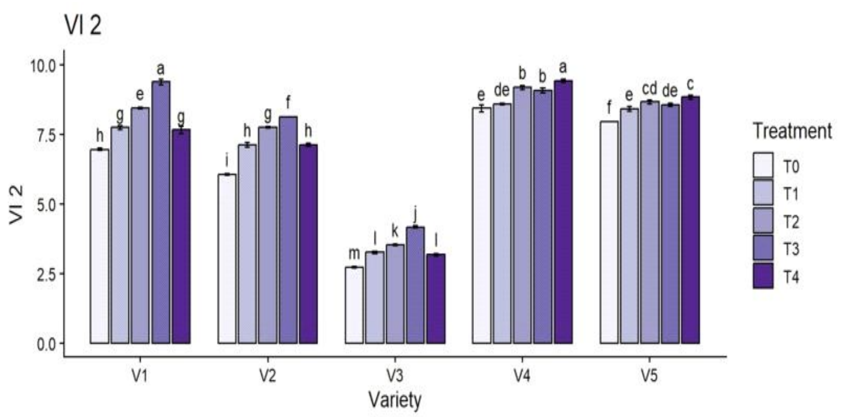
Vigour index I and vigour index II

Surface discharge cold plasma treatment showed a major impact on seedling vigour Index I and II (Fig 7 and 8). As IR-64 had the highest value of VI-I (3331.20) and VI-II (9.79) in the cultivated variety, T3 (10 min) proved to be the best treatment. MTU-1010 followed with VI-I (3210.19) and VI-II (8.65). VI-I (2102.49) and VI-II (4.14) at T3 for BPT-5204 also showed its highest vigour. Both values then decreased at T4 (20 minutes). Chitrakar showed the highest overall vigour with VI-I (4129.92) and VI-II (9.60), whereas KaruppuKavunishowed VI-I (3484.35) and VI-II (9.21). However, landraces showed their optimum value at T4 (40 minutes). Hence, T4 proved to be the best for landraces, whereas T3 proved to be the best for developed varieties. A complete indication of establishment potential, seedling vigour is measured by biomass, length and germination percentage. The higher VI-I and VI-II values at the optimal exposure times indicate the fact that plasma treatment enhances growth and dry matter accumulation simul-taneously. Higher metabolic activation, reserve mobilization and seedling growth are all signs of improved vigour. The evidence for this simultaneous improvement is also provided by the strong positive correlations between the indicators of growth and germination. Without inducing oxidative stress, the reactive oxygen and nitrogen species produced by plasma are likely to act as signalling molecules regulating hormonal and antioxidant systems (Sayahi *et al*., 2024). Cereals and legumes have also been found to have similar plasma induced increases in vigour index (Li *et al*., 2024). Genetic background differences in plasma sensitivity are suggested by the genotype-specific optimum exposure. In general, maximizing vigour increase in rice genotypes would require optimizing treatment duration.



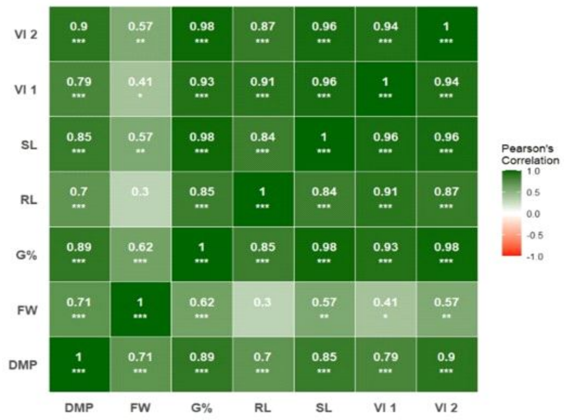
Different letters above bars indicate significant differences among treatments within each genotype (DMRT, $p \leq 0.05$). Treatment details are T0- control, T1-3 min, T2-5 min, T3-10 min, T4-20 min. V1-V5 represent rice genotypes, where V1-MTU-1010,V2-IR-64,V3-BPT-5204,V4- Chitrakar,V5-KaruppuKavuni.

Fig 7: Effect of surface discharge cold plasma treatment on (VI I) vigour index I of rice genotypes.



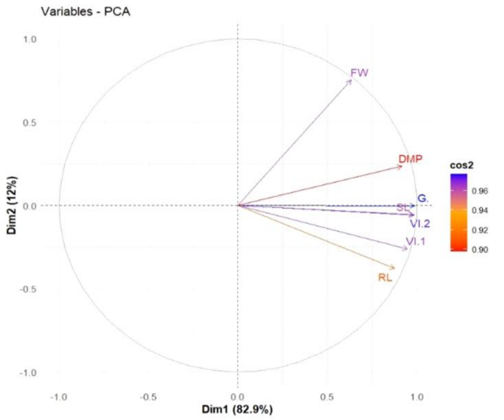
Different letters above bars indicate significant differences among treatments within each genotype (DMRT, $p \leq 0.05$). Treatment details are T0- control, T1-3 min, T2-5 min, T3-10 min, T4-20 min. V1-V5 represent rice genotypes, where V1-MTU-1010,V2-IR-64,V3-BPT-5204,V4- Chitrakar,V5-KaruppuKavuni.

Fig 8: Effect of surface discharge cold plasma treatment on (VI II) vigour index II of rice genotypes.



Colour intensity represents the strength of correlation, with darker green indicating stronger positive associations. Numerical values within the cells indicate correlation coefficients.

Fig 9: Pearson's correlation analysis among germination percentage (G%), root length (RL), shoot length (SL), vigour Index I (VI I), vigour index II (VI II), fresh weight (FW), dry matter production (DMP).



Dim1 and Dim2 explain 82.9% and 12.0% of the total variation, respectively. The position and magnitude of the vectors show the contribution and correlation of the variables, whereas the intensity of the colours shows the values of squared cosine (cos^2).

Fig 10: The PCA biplot shows the relationship between dry matter production (DMP), fresh weight (FW), germination percentage (G%), root length (RL), shoot length (SL), seedling vigour Index I (VI-I) and seedling vigour index II (VI-II).

Correlation analysis

Pearson's correlation analysis (Fig 9) revealed strong positive associations between germination and seedling growth traits. Germination showed highly significant correlations with root length (r = 0.85), shoot length (r = 0.98), VI-I (r = 0.93) and VI-II (r = 0.98), indicating that enhanced germination directly contributed to improved seedling vigour.

Principal component analysis (PCA)

Principal component analysis further supported these relationships, as shown in Fig 10. PC1 accounted for 93.6% of total variation, with strong positive loadings from germination, shoot length, root length and vigour indices, while PC2 (4.2%) was primarily influenced by root length. The clustering pattern confirmed the strong association among germination and vigour traits.

CONCLUSION

The surface discharge cold plasma has a great potential to be used as an effective non-chemical technique for seed improvement. Although the effectiveness of the cultivated varieties can be measured by treating them for a moderate period, the traditional varieties can be treated for a longer period, thus emphasizing the importance of optimizing the duration of plasma treatment. The effectiveness of plasma seed treatment in improving early seed quality can be measured by increasing the indices of germination, root and shoot growth, biomass production and vigour. In addition to its effectiveness, the cold plasma has a great potential to be used as an effective alternative to the conventional seed priming treatment. The broad application potential of cold plasma seed treatment is indicated by the uniform positive response of different genotypes of seeds. climate resilient crop protection and sustainable agriculture could greatly benefit from the integration of cold plasma into seed technology and seed enhancement strategies.

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Disclaimers

The opinions and findings presented in this article are the writers' own and may not necessarily reflect those of the organizations with which they are affiliated. The writers disclaim any liability for any direct or indirect losses resulting from the use of this content, however they are accountable for the accuracy and completeness of the information presented.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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