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COLD PLASMA TREATMENT - A SUSTAINABLE APPROACH IN ENHANCING SEED QUALITY IN SOYBEAN (*Glycine max* L.)

K. Seetha Bhagya Laxmi*, K. Sukumar, M. Pallavi and P. Jagan Mohan Rao

Department of Seed Science & Technology, Genetics and Plant Breeding, SRTC, Rajendranagar, Hyderabad-30, Professor Jayashankar Telangana Agricultural University, Hyderabad, India

*Corresponding author E-mail: kseethapg@gmail.com

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ABSTRACT

In tropical seed production systems, soybean crop encounters heavy rain fall during physiological maturity resulting in rapid decline in seed germination and seedling vigour. To mitigate the viability and vigour losses during succeeding season, the present investigation evaluated the efficacy of cold plasma as a pre-sowing seed treatment for enhancing seed quality in soybean during *Kharif*, 2024. The soybean variety JS 335 was subjected to three voltage and duration combinations (10, 15 and 20 kV for 10, 15 and 20 min) and was studied in completely randomized design along with an untreated control. The Plasma treatment significantly demonstrated the highest germination (85%), speed of germination (16.52), field emergence (85%), root length (16.6 cm) and shoot length (15.9 cm) with 20 kV for 20 min (T9) and also achieved the lowest seed infection (10%), reflecting a 69.7% reduction over the control (33%). The treatment 20 kV for 15 min (T8) also produced a comparatively higher vigour (root length 15.9 cm, shoot length 15.4 cm) and reduced infection (13%).

Keywords : Cold plasma, seed quality, germination, Soybean.

Introduction

Soybean (*Glycine max* L.) is a globally important crop and it occupies an important place in world's agricultural sector being a pulse and oil seed crop. The crop is popular among farming community due to its high productivity, ease in cultivation with high profitability. It's high nutritive value assists in mitigation of food and nutritional security in developing countries therefore often called as "Golden bean". It contributes to about 25% of the global vegetable oil production and two thirds of the world's protein in livestock feeding. In India, it is cultivated in an area of 12.04 million hectares with a production of 12.58 million tons and productivity of 1063 kg ha⁻¹ (SOPA, 2024). The crop has gained importance during past few years in the state of Telangana occupying an area of 1.44 million hectares with a production of 1.49 million tons and productivity of 920 kg ha⁻¹ (SOPA, 2024). Low seed viability coupled with low seed multiplication ratio, high seed requirement and seed infestation with seed-borne pathogens like

Diaporthe/Phomopsis complex affecting seed germination are major bottlenecks in soybean cultivation.

Soybean seeds reach their maximum germination potential at physiological maturity, after which vigour declines, leading to loss of viability. The rate of seed deterioration varies among genotypes. Additionally, fungal contamination poses a threat to seed health thus reduces germination rates, ultimately lowering crop yields and it is further enhanced especially under high rainfall at maturity. Enhancing seed germination and maintaining the biochemical integrity of seeds are vital strategies to improve soybean production. Though chemical fungicides are effective, they cause environmental pollution along with development of resistance to fungi thereby increasing costs and exacerbating the issue over time (Mravlje *et al.*, 2021a).

To mitigate the aforementioned constraints, a range of alternative pre-sowing treatments have been employed to enhance seed germination and vigour.

These include physical priming techniques such as magnetic field exposure, photo priming using sunlight or ultraviolet radiation and thermal treatments like hot water soaking. Additionally, chemical priming involving the application of growth regulators, fungicidal agents and osmopriming with chemical solutions has shown promising results. Despite their efficacy in certain contexts, these methods are often labour-intensive, time-consuming and may exhibit variable reproducibility and scalability under field conditions.

Plasma treatment of seeds is a novel approach that has been proposed to facilitate seed germination and survival. Plasma is defined as a neutral ionized gas and represents the fourth and the highest energy state of matter following solids, liquids, and gas (Lee *et al.*, 2021). Cold plasma treatment is a fast, economic and eco-friendly method to improve crop performance and seed yield. Thus, seed treatment with cold plasma in soybean would enable to overcome seed deteriorative changes with enhanced crop performance. It also aids in reducing seed microbial load, altering seed coat structures, increasing seed coat permeability and stimulating seed germination and seedling growth (Sayahi *et al.*, 2024).

Materials and Methods

The cold plasma treatment was carried out in an open air multipin plasma reactor, designed by Ingenium Naturae Private Limited. It functions with open air without expensive noble gases and included a high voltage step-up transformer, an electronic control panel and a plasma reactor. It featured 88 pins in an 11 × 8 grid, with adjustable spacing between pin tips and the plane electrode, operating at a peak voltage of 35 KV and power below 200 W.

Soybean seed of the variety, JS-335 was subjected to pre-sowing cold plasma treatment with three different voltages (10 kV, 15 kV and 20 kV) and exposure durations of (10 min, 15 min and 20 minute). The treated seed along with the control was evaluated for various seed quality parameters viz. percent seed germination by between paper method as per ISTA 2024, speed of germination was determined following the method recommended by Maguire (1962). The root length (cm) and shoot length (cm) was measured in ten normal seedlings randomly collected on 8th day from germination test. Root length was measured from the tip of primary root to the base of the root at collar region, while chosen from each set. Root length was measured from the tip of primary root to the base of the root at collar region, while shoot length was measured from the tip of the first leaf to the base of the shoot at

collar region. The average root and shoot length of these ten seedlings was then determined and presented in centimetres. The same seedlings were oven dried at 100 °C for 24 hrs and the weighed to measure seedling dry weight (mg). The seedling vigour Index I and II were calculated as per the method suggested by Abdul Baki and Anderson (1973) and field emergence was measured by sowing 100 seeds in four replications and calculated as follows

$$\text{Field emergence (\%)} = \frac{\text{Number of seedlings emerged on eighth day}}{\text{Total number of seeds sown}} \times 100$$

Seedling vigour index: Seedling vigour index was calculated as per procedure given by Abdul-Baki and Anderson and expressed in whole number.

$$\text{SVI-I} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

$$\text{SVI-II} = \text{Germination (\%)} \times \text{Seedling dry weight (mg)}$$

The percent seed infection (%) of soybean seeds was determined using the blotter method in accordance with the International Rules for Seed Testing Association (ISTA, 2024) by placing seeds on moist filter paper in Petri dishes and incubated at 25°C with 12-hour light/dark cycles for seven days.

The data obtained was analyzed statistically using Fischer's analysis of variance technique and least significant difference (LSD) test at 5% probability level was applied to compare differences among treatment mean.

Results and Discussion

Seed quality parameters and seedling growth parameters are essential indicators of seed quality, as they reflect the physiological readiness of seeds to establish healthy plants. The overall response patterns across treatments provided a basis for identifying optimal combination of voltage and duration in enhancing seed vigour. The current study evaluated the comparative performance of treated seeds over the control thereby aiding in identification of potential cold plasma treatment as pre sowing tool for enhancing seed vigour and performance in soybean.

Effect of cold plasma treatment on seed quality parameters: Pre sowing cold plasma treatments recorded a statistically significant improvement in standard germination over untreated control. All plasma treated seed lots (T1-T9) demonstrated higher germination percentages compared to the untreated control (T10:72%) (Table 1). The percent increase in germination across cold plasma treatments ranged from 8-13% over the control and it can be attributed to surface etching, improved wettability, water uptake and decreased apparent contact angle which collectively promoted faster and more uniform

germination. The findings of this study are consistent with Sandra *et al.* (2023) where pre sowing cold plasma treatment significantly improved seed germination, growth and seed yield in soybean by activation of the enzymes without causing damage to the biological tissues. Likewise, Sohan *et al.* (2022) showed low frequency glow discharge (LFGD) plasma treatment significantly improved seed germination compared to untreated control in maize. Similar improvement in seed quality were observed by Li *et al.* (2016) in Peanut, Li *et al.* (2017b) in wheat, Švubová *et al.* (2020) in Pea, Sadhu *et al.* (2017) in mung beans (*Vigna radiata*).

A significant influence of cold plasma treatments on the speed of germination of soybean (var. JS 335) seeds was observed (Table 2). An increasing trend in speed of germination was recorded with higher voltage and longer exposure durations. The maximum speed of germination was observed in T9 with a value of 16.52 followed by T8 (15.99) and T7 (14.85) indicating that plasma exposure at 20 kV markedly accelerated early metabolic activation and radicle protrusion. Bormashenko *et al.* (2012) reported a marked decrease in the apparent contact angle of seeds and increased surface hydrophilicity on cold plasma treatment, resulting in accelerated germination speed and improved overall germination rate of various seeds including *Lens culinaris* (lentils), *Phaseolus vulgaris* (beans) and *Triticum* spp. (wheat, species C9). Similarly, Brust *et al.* (2021) reported that short atmospheric plasma exposures (10-60 s) markedly evidenced reduction of t50 values and higher cumulative germination speed and accelerated germination in wheat and barley by increasing seed surface hydrophilicity and water uptake.

The effect of cold plasma treatment on field emergence (%) is presented in Table 1. Cold plasma treatment exerted a significant and positive influence on field emergence of soybean seeds as indicated by the clear variation among the treatments. Across all plasma treated seeds, there was a clear cut improvement in field emergence over the untreated control (73%) with the highest value recorded in T9 (85%) followed by T8 (83%) and T6 (82%), highlighting the consistent stimulatory effect of higher voltage and longer exposure durations. In soybean plasma induced oxidation of the seed coat leading to enhanced hydrophilicity, thereby accelerating seed soil interactions and thereby promoting early seedling establishment (Zhang *et al.*, 2017). Perner *et al.* (2024) demonstrated the effect of cold plasma in millet seeds resulting in enhancement of early seedling development particularly radicle elongation.

A significant enhancement in root and shoot length was observed due to cold plasma treatment across voltage-duration combinations, with values ranging from 14.1 to 16.6 cm and 13.7 to 15.9, respectively, compared to the control (11.3 cm). The maximum root and shoot length was recorded in T9, which was significantly superior to all other treatments and clearly indicates that higher plasma intensity coupled with longer exposure duration markedly stimulated root and shoot elongation. Treatments at 15-20 kV voltages with longer exposure periods (15-20 minutes) also displayed consistently improved root and shoot development, while the lowest plasma intensity (10 kV) produced moderate increase. The substantial improvement in root and shoot length with increasing plasma intensity may be attributed to surface etching of the seed coat, which enhances water absorption, seed hydration, activation of metabolic enzymes and increased phytohormone signalling which together accelerate cell division and elongation. The effect of cold plasma treatment on root length and shoot length (cm) is presented in Table 2 and Fig 3. Similarly, Feng *et al.* (2018) reported significant improvement in germination potential, and root length parameters in *Medicago sativa* L and Motrescu *et al.* (2023) found an increase in root length by about 38% in *Brassica oleracea* on cold plasma treatment. Likewise, studies by Ling *et al.* (2014) in soybean, Ling *et al.* (2015) in oilseed rape and Zahoranova *et al.* (2018) in maize have also documented improvements in root length following plasma exposure. Karmakar *et al.* (2021) reported an increased germination rate of 15.88%, accompanied by increase in shoot length (33.42%) in maize seeds on plasma exposure.

Seedling vigour index-I & II was significantly influenced by plasma treatment with a clear, dose dependent improvement, with values ranging from 2219 to 2760 and 6453 to 7700 respectively, compared to the control (1648 and 6755) (Table 1). The maximum Seedling vigour index-I was recorded in T9, whereas, T5 recorded the highest for Seedling vigour index-II. The differences between the top performers (T9 & T5) and the control (T10) indicating that the enhancement is both statistically and biologically meaningful. Likewise, Maria *et al.* (2019) studied the impact of cold plasma where seedling vigour index-I was increased by 2.25 times on soybean seeds. Similarly, Gagan *et al.* (2023) investigated the influence of cold plasma treatment on soybean with significantly enhanced germination percentage and vigour index.

The effect of cold plasma treatment on seedling dry weight in soybean showed noticeable variation

across voltages and exposure durations (Table 2). Among the treated seeds, the maximum dry matter accumulation was recorded in seeds exposed to 15 kV for 10 minutes (939 mg), which was statistically on par with the control (937 mg), while other treatments displayed moderate improvements. Treatments such as 15 kV for 15 and 20 minutes and 20 kV at different durations also enhanced seedling biomass relative to 10 kV treatments, indicating that intermediate to high voltage plasma might have stimulated the early seed metabolic activity and biomass accumulation.

These findings are in consistent with Matra, K. 2018 who demonstrated that plasma exposure significantly enhanced seedling growth with the increase of 1.79 fold higher dry weight in sun flower. Similarly, Fadhlamawla *et al.* (2019) recorded an early seedling growth of fenugreek (*Trigonella foenum-graecum* L.) where germination rates were increased by 7 fold with increased root and shoot dry weights. Sayahi *et al.* (2024) investigated that that plasma treatment markedly enhanced seedling dry weight (1.80-36.63 g) in soybean and similarly Ji *et al.*, 2016 in Spinach.

Effect of cold plasma treatment on seed health parameter: The effect of cold plasma treatment on seed infection (%) is presented in Table 1. The data on seed infection (%) clearly indicated that cold plasma treatment had a substantial impact on reducing microbial contamination in soybean seeds. There was a significant reduction of seed infection per count for all the treatments except for T1 (30%). Among the treatments, T9 recorded the lowest infection level (10%) followed by T8 with 13% whereas, the untreated control (T10) exhibited the highest seed infection (33%). The significant reduction in pathogen load under higher voltage and longer exposure durations suggests that reactive oxygen and nitrogen species (RONS) generated during plasma exposure might have played a role in disrupting fungal and bacterial cells on the seed surface. Plasma treatment on barley grains inactivated the *Fusarium meridionale* and reduced native mycobiota highlighting its potential impact on plant health and development (Chiappim *et al.*, 2023). Likewise, Tamošiūnė *et al.* (2020) reported a decreased pathogenic fungal activity resulting in prevention of seed deterioration during storage. Plasma treated maize seeds result in improved plant resistance to common diseases leading to increased crop yield (Filatova *et al.*, 2011, 2012, 2020).

Table 1 : *Per se* performance of Germination (%), Field emergence (%), Seed Infection (%), SVI-I and SVI-II with pre-sowing cold plasma treated soybean variety, JS 335

Treatment	Germination (%)	Field emergence (%)	Seed Infection (%)	SVI-I	SVI-II
T1	80	76	30	2,219	6,453
T2	83	78	27	2,387	6,934
T3	84	81	20	2,469	7,204
T4	81	78	20	2,267	7,605
T5	84	80	20	2,461	7,700
T6	84	82	20	2,526	7,684
T7	82	80	20	2,460	7,285
T8	84	83	13	2,613	7,654
T9	85	85	10	2,760	7,693
T10 (Control)	72	73	33	1,648	6,755
C.D (5%)	3.848	4.981	5.424	278.983	767.165
SEm ±	1.295	1.677	1.826	97.622	260.057
C.V %	2.744	3.633	14.823	7.584	6.173

Note:

- T1, T2 & T3 are 10kV with 10 min, 15 min & 20 min durations respectively
- T4, T5 & T6 are 15 kV with 10 min, 15 min & 20 min durations respectively
- T7, T8 & T9 are 20 kV with 10 min, 15 min & 20 min durations respectively

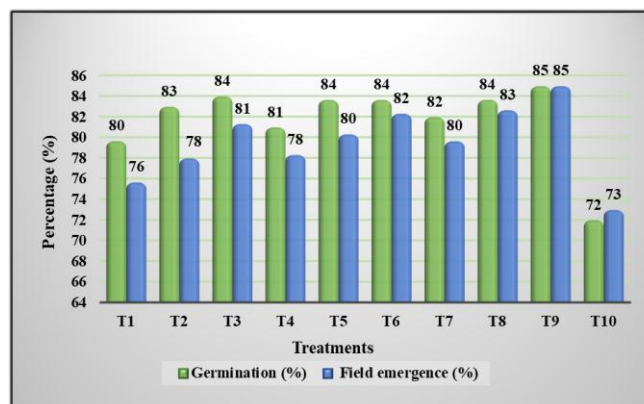


Fig. 1: *Per se* performance of Germination (%) and Field emergence (%) among different treatments

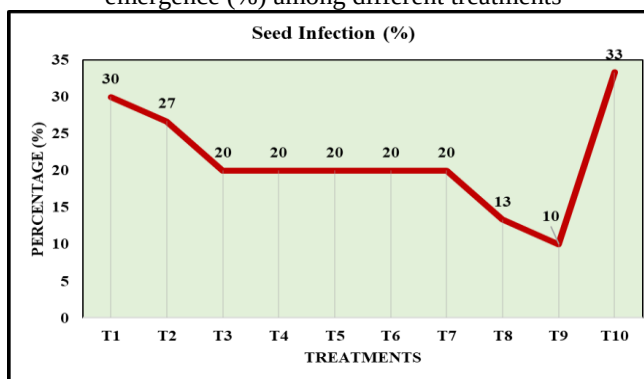


Fig. 2 : *Per se* performance of Seed infection among different treatments

Table 2 : *Per se* performance of Speed of germination, Root length (cm), Shoot length (cm) and Seedling dry weight (mg) with pre cold plasma treated soybean variety, JS 335

Treatment	Speed of germination	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)
T1	12.34	14.1	13.7	810
T2	14.00	14.8	13.9	835
T3	14.15	15.1	14.3	791
T4	13.92	14.1	13.9	939
T5	14.02	15.1	14.3	920
T6	14.35	15.3	14.8	918
T7	14.85	15.5	14.5	888
T8	15.99	15.9	15.4	914
T9	16.52	16.6	15.9	905
T10 (Control)	11.88	11.3	11.3	937
C.D (5%)	0.42	0.2	0.2	26.768
SEm ±	0.594	0.3	0.3	37.856
CV%	5.121	2.5	2.7	5.234

Note:

- T1, T2 & T3 are 10kV with 10 min, 15 min & 20 min durations respectively
- T4, T5 & T6 are 15 kV with 10 min, 15 min & 20 min durations respectively
- T7, T8 & T9 are 20 kV with 10 min, 15 min & 20 min durations respectively

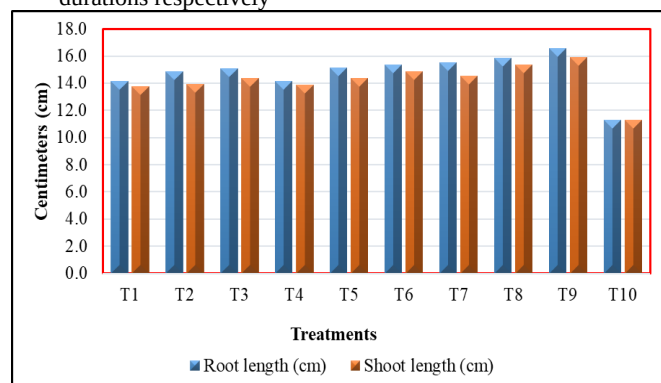


Fig. 3 : *Per se* performance of Root length(cm) and Shoot length(cm) among different treatments

Conclusion

From the above investigation, it is noted that the treatments T9 (20 kV for 20 minutes) emerged as the most effective treatment that exhibited the highest gains in germination (18.06%), speed of germination (39.03%), field emergence (16.44%), root length (46.90%), shoot length (40.71%) and seed infection (-39.70%), followed by T8 (20 kV for 15 minutes) which also exhibited the improvements of the discussed parameters in a desired direction especially with respect to speed of germination (34.60%), root length (40.71%), shoot length (36.28%) along with a major reduction in seed infection (-30.61%) indicating a strong enhancement in membrane stability, reduced

lipid peroxidation and improved seed health with cold plasma treatment.

Hence, from the above findings, it can be concluded that pre-sowing exposure of soybean seed to cold plasma at 20 kV for 20 minutes or 15 minutes proved best with respect to germination and seedling vigour indicating its strong potential as a scalable, eco-friendly seed enhancement technology for improving seed quality especially for seed prone to weather induced deterioration.

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