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Effect of Cold Plasma Seed Treatment on Physiological and Biochemical Changes in Aged Mustard Seed Variety-RH0749

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Authors' contributions

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

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ABSTRACT

The low rate of seed germination is a major problem in modern agriculture. Cold-activated air plasma has been tested as a pre-treatment method to improve the rate and uniformity of the germination. In view of the widespread cultivation of mustard in India and other parts of the world, This study was conducted to determine alternative non-destructive seed treatment method based on cold plasma chemistry would offer a more viable alternative over traditional seed coating technologies Seed physiological characteristics as was modified in aged low vigour mustard seeds

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by coating the surface of the seeds with cold plasma process using a rotating plasma reactor during 2023-24 at CCS HAU, College of Agriculture Bawal. The source of atmospheric oxygen gas, nitrogen and heliun gas entering the plasma chamber during the reaction process determined the type of coating. Among the different treatments imposed, seed treatment with 300V at 10 Min significantly enhanced seed quality attributes *viz*., germination (92 %), root length (4.37 cm), shoot length (6.98 cm), mean seedling length (11.0 cm), Seedling dry weight (2.67 mg) and SVI-I (1115), SVI-II (254), TDH (0.78 A480 nm) with lower electrical conductivity (14.20 dsm^{-1}). The inhibitory effect noticed with a higher dose of plasma of 400v at 10 min suggested the need for judicious usage of these plasma frequencies in such applications. The first-ever report on the effect of new plasma state on seed germination and establishment of mustard revealed the positive influence that could be used as a seed treatment to enhance seed yield and quality.

Keywords: Cold plasma; mustard; seed germination; seed vigour.

1. INTRODUCTION

"In the last few decades, seed companies invested a lot of effort in the development of seed enhancement treatments aimed at the improvement of both germination rate and uniformity. These two factors have a major impact on final yield and quality. Even though these treatments are generally effective in the enhancement of germination rates and uniformity, they involve the use of expensive materials and bulky procedures, which are sometimes health hazardous. The plasma treatment of seeds has been investigated as an alternative to traditional pre-sowing seed treatment in agriculture, such as physical scratching (scarification), heat treatment, chemical treatment with various acids, *etc"* [1]. "Multiple studies have investigated the possibility of controlling germination by exposure of seeds to various types of plasma, including atmospheric and low-pressure plasma discharges" [2,3,4].

"Indian mustard (*Brassica juncea* (Linn.) is one of the important oilseed crops contributing 25 per cent of the oilseed production of the country. It occupies a prominent place next to groundnut in meeting the oil requirement of about 50 per cent of the population. Physiological deterioration of seeds during storage is considered to be one of the major factors preventing seeds from normal germination and vigorous growth" [5]. "In recent years, low-temperature plasma (LTP), also known as Non-Thermal Plasma (NTP) or Cold Atmospheric Plasma (CAP), has been widely applied in biology. It has broad applications in the field of biology, including seed germination, cultivation, surface sterilization, microorganism decontamination, food manufacturing and processing, wound healing, and food storage. In the field of agriculture, plasma agriculture or plasma farming involves the comprehensive application of plasma to process from pre-cultivation until the product reaches the kitchen table. In plant sciences, studies on plasma treatment have been focused on exploring the possible applications, standardization of treatment, and characterization of plasma effects in terms of plant biochemistry" [6]. "Presently molecular mechanisms underlying the effects of plasma on seed germination and plant growth have been explored at the cellular level, including gene expression analysis, transcriptome profiling, protein expression analysis, and epigenetics" [7,8].

"Cold plasma treatment is a fast, economical and pollution-free method to improve seed performance and crop yield" [9]. "It has essential roles in a broad spectrum of developmental and physiological processes in plants, including reducing the bacterial bearing rate of seeds, changing seed coat structures, increasing the permeability of seed coats, and stimulating seed germination and seedling growth" [10,11].

2. MATERIALS AND METHODS

Seed lots of medium vigour (less germinable than standards) were collected from the Regional research station, Bawal, and cold plasma frequencies (300v and 400v) were treated to seeds at different durations (Control, 3min, 5 min and 10 min). Seed and seedling quality parameters were analysed as per ISTA [12].

2.1 Germination (%)

Four replications of 100 seeds from each treatment were kept for germination at 25±1°C for 10 days using the between-paper (BP) method. The germination percentage was expressed based on normal seedlings as described in ISTA Rules [12].

2.2 Root Length (cm)

From the standard germination test, ten normal seedlings were selected at random from each replication on the 7th day and the length of the root was measured from the collar region to the tip of the root to the base of hypocotyl and the average root length was expressed in centimetre. Dhayala et al. 2006 indicated that the effects of a short low-pressure plasma treatment on safflower seed germination was much more effective than a long high-pressure plasma treatment.

2.3 Shoot Length (cm)

From the standard germination test, ten normal seedlings were selected at random from each replication on the 10th day and the length of the shoot was measured from the collar region to the tip of the coleoptile and the average shoot length was expressed in centimetre.

2.4 Seedling Vigour Index-I (SVI-I)

“The germinated seedlings were evaluated on the 5th and 7th day as first and final count, respectively. The percentage of germination was expressed based on the normal seedlings present in the test. Ten normal and healthy seedlings from each replication were selected randomly on the 10th day and mean seedling length (shoot and root) was measured in centimetres. Then the Seedling Vigour Index-I was determined by multiplying standard germination (%) and mean seedling length (cm) and expressed in number” (Abdul-Baki and Anderson, 1973) [13].

SVI-I = Germination (%) × Mean seedling length (cm)

2.5 Seedling Vigour Index-II

The seedlings selected for calculating the seedling vigour index-I were oven-dried at 80^{0}c for 24 hour after removing the cotyledon (remnant seed) and the mean seedling dry weight of these seedlings was used for calculating the Seedling Vigour Index-II by using the formula given by Abdul Baki and Anderson [13] as indicated below:

Seedling vigour index II =
Germination (%) x Mean seedling dry weight (mg)

2.6 Mean Seedling Dry Weight (mg)

The seedlings used for measuring the seedling length after removing cotyledons (remnant seed) were dried in a hot air oven at 80 ± $1^{\circ C}$ for 24 hours and mean seedling dry weight was expressed in milligrams.


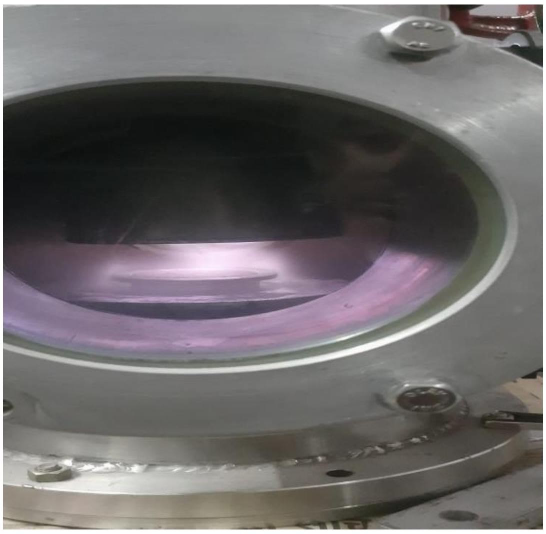

Fig. 1. A. plasma discharge treatment chamber (left) B. mustard seeds undergoing in a plasma reactor (right)

2.7 Electrical Conductivity (dSm^{-1})

"Three replicates of 50 seeds each were taken, pre-washed and soaked in 50 ml of distilled water for 8 hours at room temperature. The seed leachate was collected by decanting and Electrical Conductivity (EC) was measured in a digital model conductivity meter (Elicotype Cm-82) possessing an electrode at a cell constant of 1.1 with calibration on EC mode. The mean value was expressed as dSm^{-1}" [14].

2.8 Dehydrogenase Activity (OD value)

The dehydrogenase activity of the seeds was estimated according to procedure developed by [15]. 25 seeds from each treatment were pre-conditioned for 6 hours. From that, five embryonic axes were separated and incubated in darkness with 5 ml of 0.1 per cent Tetrazolium Chloride solution in glass vials for 2 hrs at 40ºC. After incubation, the Tetrazolium Chloride solution was decanted and the embryos were thoroughly washed with distilled water and surface dried with blotters. The Formazan was eluted by soaking the stained embryo in 5 ml of methyl cellosolve (2 methoxy ethanol) overnight and the optical density was measured using a Spectrophotometer model at 470 nm and methyl cellosolve alone was used as a blank. The dehydrogenase activity was expressed as optical density.

3. RESULTS AND DISCUSSION

The results obtained from the laboratory experiments on the physiological and biochemical characteristics of aged seeds of mustard seeds.

3.1 Germination (%)

The data on germination percentage of mustard seeds treated with plasma revealed highly significant differences among the treatments, period and their dosage, T_3: 300 volts with 10 min (92 %) followed by T_3: 300 volts with 5 min (86.67 %) compared to control (84 %) (Table 1). Immediately after the treatment, it was observed that germination was not influenced much by plasma treatment. During seed germination, many biochemical pathways are activated inside the seed such as hydrolysis of starch by amylolytic enzymes, elevated expression of amylolytic enzyme genes, and increase in GA_3 (gibberrellin) level (Chrispeels and Varma1967., S. Miyata et al., 1981., Chandler et al., 1984., Deikman et al., 1985 and Muthukrishnan et al., 1983).

Numerous studies found that cold plasma significantly increased seed germination (Dhayal et al*,* Zhou et al., Yin, M. Q et al., For example, Selcuk et al. [16,9,17,18], found that a plasma treatment significantly increased tomato seed germination. Dhayala et al. indicated that the effects of a short low-pressure plasma treatment on safflower seed germination were much more effective than a long high-pressure plasma treatment. The appearance of CO molecules (bands of the Angstrem, the Herzberg and the third positive systems) and ionized O_2 + molecules (the second negative (2-) system) in spectra during seeds treatment confirmed that the plasma chemical etching of seed surface plays an important role in the stimulation of biochemical processes that influence on seed germination [19,20,17].

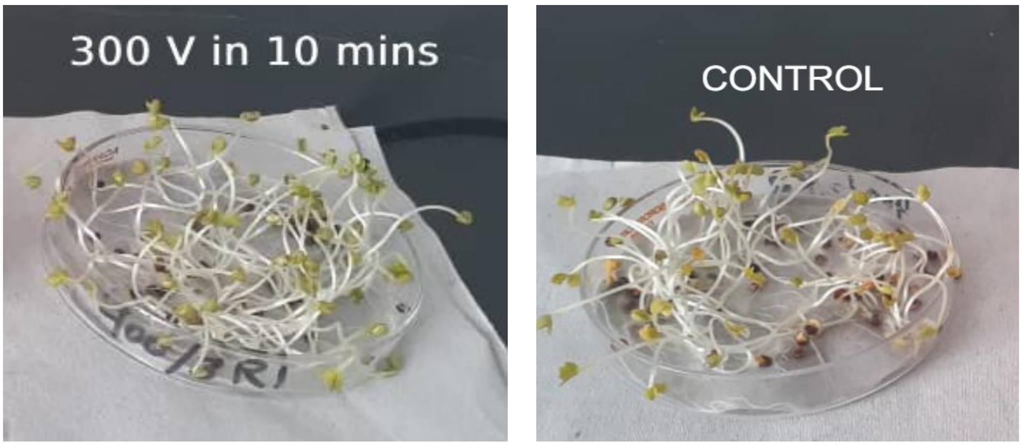

Fig. 2. A. Seed germination at 300v in 10 min (Left) B. Control(Right)

Table 1. Effect of cold plasma seed treatment on Seed germination, root length, shoot length, and dry weight of mustard under lab condition

Treatments	Seed germination (%)	Root length (cm)	Shoot Length (cm)	Mean seedling length (cm)	Seedling dry weight (mg)
T_1: Control	84.33	3.61	5.39	9.0	1.97
T_2: 300 volt with 3 min	86.33	4.03	6.40	10.0	2.30
T_3: 300 volt with 5 min	86.67	4.37	6.98	11.0	2.67
T_4: 300 volt with 10 min	92.00	5.27	7.60	13.0	2.93
T_5: 400 volt with 3 min	84.33	5.55	6.90	12.0	2.93
T_6: 400 volt with 5 min	79.33	3.21	4.58	8.0	1.98
T_7:400 volt with 10 min	73.67	2.99	4.48	7	1.90
Mean	**83.80**	**4.14**	**6.04**	**10.19**	**2.38**
S.Em±	1.5886	0.1314	0.3202	0.39	0.1
CD (0.05)	6.68804	0.5532	1.3484	1.62	0.6
CV (%)	3.28318	5.4878	9.1725	6.55	10.3

3.2 Shoot Length and Root Length (cm)

The data on shoot length and root length of mustard seeds treated with plasma revealed highly significant differences among the treatments, time and their dosage, T_3: 300 volts with 10 min (7.6 cm and 5.27 cm) followed by T_3: 300 volts with 5 min (6.90 cm and 5.55cm) compared to control (5.39 cm and 3.61 cm) (Table 1). Immediately after the treatment, it was observed that germination was not influenced much by plasma treatment. plasma induces water uptake of seeds, which increases seed germination and accelerated seedling growth [21,22,23] Our results are in line with findings on the influence of the cold plasma treatment on the oilseed rape (*Brassica napus L.*) seed germination under drought stress [24,25,26]. Changed seed surface in plasma treated seeds could probably enhance and accelerate water uptake. The seeds can also change their dormancy and germination processes [27].

3.3 Dry Matter Production (mg seedling^{-10})

The data on dry matter production of mustard seeds treated with plasma revealed highly significant differences among the treatments, time and their dosage, T_3: 300 volts with 10 min (2.93 mg) followed by T_3: 400 volts with 5 min (2.93 mg) compared to control (1.97mg). Variations among the quantities of dry matter produced by the seedlings were highly significant due to the carry-over period, cold plasma seed treatment and its dosages as well as time (Table 1).

3.4 Vigour Index

Significant variation was observed for vigour index-I and II due to activated plasma treatment, and its frequency as well as duration of seed exposure (Table 2). The Vigour index value was not influenced significantly by plasma treatments immediately after treatment. However treatment T_4: 300 volts with 10 min recorded higher vigour index-I value (1115) and seedling vigour index -II (256). Followed by T_5:400 volt with 3 min (1049, 247)compared to values recorded for control was (760, 165).

3.4.1 Electrical conductivity (dSm^{-1})

The Electrical Conductivity of the seed leachate was significantly influenced due to cold plasma (Table 2). However, T_4: 300 volts with 10 min treatment recorded the minimum Electrical Conductivity (14.2 dSm^{-1}) compared to control (17.73 dSm^{-1}). Among the treatments, recorded the lowest Electrical Conductivity of 16.50 and 16.70 dSm^{-1} respectively. T_7: 400 volts with 10 min treatment**.** Seed recorded the highest Electrical Conductivity of 18.37 dSm^{-1} followed by control (17.73) Zivkoviproposed and discussed “three possible plasma treatment effects on seed outside: etching, surfacefunctionalization, and deposition of small

Table 2. Effect of cold plasma seed treatment on Seedling Vigour index-I, Seedling vigour index-II, Electrical conductivity, and Total dehydrogena

Treatments	Seedling vigour index -I	Seedling Vigour Index –II	Electrical Conductivity (dSm^{-1})	Total Dehydrogenase Activity (OD value)
T_1: Control	760	165	17.73	0.67
T_2: 300 volt with 3 min	900	198	15.70	0.70
T_3: 300 volt with 5min	1044	246	15.87	0.70
T_4: 300 volt with 10 min	1115	254	14.20	0.78
T_5:400 volt with 3 min	1049	247	14.53	0.73
T_6: 400 volt with 5 min	615	157	16.80	0.70
T_7: 400 volt with 10 min	551	139	18.37	0.65
Mean	**862**	**201**	**16.17**	**0.70**
S.Em±	36.197	14.87	0.272	0.021
CD (0.05)	152.38	62.63	1.148	0.090
CV (%)	7.268	12.79	2.922	5.282

bioactive molecules. We focused on the mechanical outside changes in the seed coat. We confirmed that the plasma-treated seeds of Chenopodium album had a changed surface".

3.4.2 Dehydrogenase activity (OD value)

Cold plasma seed treatments, dosages, and periods resulted in significant differences in dehydrogenase enzyme activity (Table 2). it was observed that control seeds recordedthe lowest OD value(0.67) for dehydrogenase activity and T_4: 300 volts with 10 min recorded the highest OD. value (0.78) the values recorded by all other treatments were on par with control.

"The quality and vigour of seeds are very often based on the estimation of the viability of seeds with the Tetrazolium test using dehydrogenase systems" [28,29]. Generally, the higher dehydrogenase activity was determined in the embryo, than in the root, which might depend on the respiratory or enzymatic activity at an early stage of the germination process [30-34].

4. CONCLUSION

The current research introduces the development of a technology which is based on cold radiofrequency plasma treatment of seeds that can enhance both the rate and uniformity of germination of seeds. The methodology demonstrates a great impact since it proposes an inexpensive and effective solution for seeds as a pre-germination treatment, enabling their permeability increase; replacing and even totally avoiding the need for hazardous acids and/or costly scarification treatments. Despite recent investigations, the relation between the change in the wettability of seeds and the parameters of germination (time and rate) and the effects of plasma treatment on morphology, phenology and quality of plants and fruits after plasma treatment remains obscure. In laboratory germination, the treatments by different currents did present an obvious advantage in comparison to the control on the first day of emergence. However, the sprouting percentage of the pretreated seeds improved by about 6 % higher than the control in the present plasma experiment

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Be clear that Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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