

**“STUDIES ON COLD PLASMA
TREATMENTS FOR VEGETATIVE
COLD TOLERANCE IN *RABI* RICE
(*Oryza sativa* L.)”**

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B.Sc. (Hons.) Ag.

**MASTER OF SCIENCE IN AGRICULTURE
(SEED SCIENCE AND TECHNOLOGY)**



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COLD TOLERANCE IN *RABI* RICE
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BY

PAVUSETTY SAIRAM

B.Sc. (Hons.) Ag.

**THESIS SUBMITTED TO THE PROFESSOR
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CHAIRPERSON: Dr. Y. BHARATHI



**DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY
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HYDERABAD- 500030
2024**

DECLARATION

I, **PAVUSETTY SAIRAM**, hereby declare that the thesis entitled **“STUDIES ON COLD PLASMA TREATMENTS FOR VEGETATIVE COLD TOLERANCE IN *RABI* RICE (*Oryza sativa* L.)”** submitted to the **Professor Jayashankar Telangana Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Hyderabad

(P. SAIRAM)

Date:

I.D. No. RAM/2022-90

CERTIFICATE

Mr. **PAVUSETTY SAIRAM** satisfactorily prosecuted the course of research and that the thesis entitled “**STUDIES ON COLD PLASMA TREATMENTS FOR VEGETATIVE COLD TOLERANCE IN *RABI* RICE (*Oryza sativa* L.)**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree from any university.

Place:

(Dr. Y. BHARATHI)

Date:

Chairperson

CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON COLD PLASMA TREATMENTS FOR VEGETATIVE COLD TOLERANCE IN *RABI* RICE (*Oryza sativa* L.)**” is submitted in partial fulfilment of the requirements for the degree of ‘**Master of Science in Agriculture**’ of the Professor Jayashankar Telangana Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. PAVUSETTY SAIRAM** under our guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part and all assistance received during the course of the investigation have been duly acknowledged by the author of the thesis.

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Date:

Place:

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
~	:	Approximately
&	:	And
±	:	Plus, or minus
@	:	At the rate of
ABA	:	Abscisic acid
ACP	:	Atmospheric Cold Plasma
ANOVA	:	Analysis of variance
APX	:	Ascorbate peroxidase
Ar	:	Argon
CAP	:	Cold atmospheric plasma
CAT	:	Catalase
CD	:	Critical difference
CFU	:	Colony forming units
CIC	:	Central Instrumentation Cell
CP	:	Cold plasma
CRD	:	Completely Randomized Design
CV	:	Coefficient of variation
DBD	:	Dielectric barrier discharge
DCSBD	:	Diffuse Coplanar Surface Barrier Discharge
df	:	Degrees of freedom
DSST	:	Department of Seed Science and Technology
EMF	:	Electromagnetic field
<i>et al.</i> ,	:	And others
etc.	:	And so on
FAO	:	Food and Agriculture Organization
Fig	:	Figure
GA	:	Gliding Arc
GA3	:	Gibberellic acid
GABA	:	Gamma-amino butyric acid

GHz	:	Gega Hegz
gm	:	Gram
h	:	Hours
H ₂ O ₂	:	Hydrogen peroxide
H ₂ SO ₄	:	Sulfuric acid
ISTA	:	International Seed Testing Association
JGL	:	Jagtial
kg	:	Kilogram
Kg/ton	:	Kilogram per ton
kg ha ⁻¹	:	Kilogram per hectare
KMNO ₄	:	Potassium permanganate
kV	:	Kilo volts
LPGAD	:	Low pressure glow air discharge
LTP	:	Low-temperature plasma
mA	:	milli ampere
mg	:	Milligram
MHz	:	Mega Hegz
min	:	Minutes
ml	:	Milli liter
ml/min	:	Milli liter per minute
mM	:	Milli molar
Mtha ⁻¹	:	Million tons per hectare
NaCl	:	Sodium chloride
NADH	:	Nicotinamide Adenine Dinucleotide Hydrogen
NBT	:	Nitro blue tetrazolium
NO	:	Nitric Oxide
NTP	:	Non-Thermal Plasma
O ₂	:	oxygen
°C	:	Degree Celsius
PAL	:	Plasma-activated ringer's lactate solution

PAW	:	Plasma activated water
PEG	:	Poly ethylene glycol
PER	:	Pin electrode reactor
PJTAU	:	Professor Jayashankar Telangana Agricultural University
POD	:	Peroxidase
ppm	:	Parts per million
RBOH1	:	Respiratory burst oxidase homologue 1
RNR	:	Rajendranagar
RONs,	:	Reactive oxygen and nitrogen species
S	:	Seconds
SCP	:	Streamer corona plasma
SEM	:	Scanning Electron Microscopy
SOD	:	Superoxide dismutase
SPAD	:	Soil Plant Analysis Development
SRTC	:	Seed Research and Technology Centre
STL	:	Seed Testing Laboratory
SVI-I	:	Seedling Vigour Index-I
SVI-II	:	Seedling Vigour Index-II
U gFW ⁻¹	:	Units per gram of fresh weight
W	:	watts
μl	:	Micro liter

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ABSTRACT

The present investigation was undertaken to assess the effect of cold plasma treatments on cold tolerance during vegetative stage in *Rabi* rice (*Oryza sativa* L.). The standardization work pertaining to cold plasma treatments were carried out at Quality Control lab and Central Instrumentation Cell, PJTSAU, Rajendranagar, Hyderabad using single variety RNR 15048. The best cold plasma treatment identified from the standardization work was imposed on three rice varieties viz., RNR 15048, JGL 24423 and Tellahamsa. The field experiment was laid out to determine the effects of cold plasma treatment during *Rabi*, 2023-24 at Seed Production Farm, Seed Research and Technology Centre, Rajendranagar, Hyderabad. The seed harvested from cold plasma treated crop was assessed for seed quality parameters at Seed Testing Laboratory of Department of Seed Science and Technology, Rajendranagar, Hyderabad.

In standardization experiment among six different cold plasma treatments imposed on RNR 15048 using both normal and sprouted seed, the normal seed performed better than the sprouted seed for majority of the seed quality parameters. Among the cold plasma treatments the seed treated with 20 kV plasma for 15 min (P3) has recorded best seed quality parameters. The seed quality parameters in the seed kept at 25°C were given best results compared to the seeds kept at 15°C. The high seed germination per cent at 25°C (91%) and 15°C (87%), seedling dry weight at 25°C (5.85 mg) and 15°C (3.70 mg) and seedling vigour index-II at 25°C (532) and 15°C (323), were recorded in normal seed treated with 20 kV plasma for 15 min (S1P3). The low seed infection per cent at 25°C (1.5%) and 15°C (1.5%), high speed of germination both at 25°C (22.86) and 15°C (16.49) and high cold tolerance index (97) was also recorded in normal seed treated with 20 kV plasma for 15 min (S1P3).

Whereas, biochemical parameters revealed non-significant difference between cold plasma treatments for superoxide dismutase activity at 25°C and 15°C, peroxidase activity at 25°C, catalase activity at 25°C and 15°C and proline content at 25°C and 15°C while high peroxidase activity at 15°C (0.207) was recorded in normal seed treated with 10 kV plasma for 20 min (S1P2). Radicle emergence at 25°C recorded non-significant difference between treatments while radicle emergence at 15°C showed significant differences and untreated control recorded low radicle emergence % at 15°C.

The effective cold plasma treatment i.e., 20 kV for 15 min was imposed on three rice varieties RNR 15048, JGL 24423 and Tellahamsa and field experiment was laid out to study the effects of cold plasma treatments on cold tolerance at vegetative stage as well as on yield contributing characters. The high germination per cent was recorded in S2V3: Tellahamsa sprouted seed treated with plasma (93%) and S3V3: Tellahamsa control (93%). High dry matter for shoot at 15 (0.092 g), 30 (1.439 g) and 45 (2.909 g) days was recorded in S1V3: Tellahamsa normal seed treated with plasma. High dry matter for root at 15 (0.031g) and 45 (2.434 g) days was recorded in S1V3: Tellahamsa normal seed treated with plasma and high dry matter for root at 30 days was recorded in S2V3: Tellahamsa sprouted seed treated with plasma (0.497g). Over all high dry matter for root at 15, 30 and 45 days was recorded in Tellahamsa. Non-significant differences were observed for chlorophyll content of leaf between varieties. RNR15048 sprouted seed treated with cold plasma (S2V1) was early to days to 50% flowering (90.67) and early to days to maturity (118.67). The parameters number of tillers/hill and number of productive tillers per m² recorded non-significant differences between varieties.

Among the varieties RNR15048 untreated control (S3V1) recorded high proline content (0.067), Tellahamsa normal seed treated with cold plasma (S1V3) recorded high plant height (99.67cm). JGL24423 sprouted seed treated with cold plasma (S2V2) recorded high seed yield per hill (58.85 g), Tellahamsa normal seed treated with cold plasma (S1V3) recorded more seed yield (12.94 t/ha), Tellahamsa sprouted seed treated with cold plasma recorded high 1000 seed weight (23.72 g).

Paddy seed harvested from three varieties viz., RNR 15048, JGL 24423 and Tellahamsa treated with cold plasma (20kV for 15 min) are used to study fresh seed quality parameters. For germination per cent non-significant difference was observed among varieties while high radicle emergence per cent was recorded in S1V1: RNR15048 normal seed treated with cold plasma (100). High seedling dry weight (10.3 mg) and seedling vigour index-II (1023) was recorded in S3V3: Tellahamsa untreated control while, high speed of germination was observed in S1V2: JGL 24423 normal seed treated with cold plasma (15.95). Low seed moisture content was recorded in S2V2: JGL 24423 sprouted seed treated with cold plasma (6.55) and low electrical conductivity of seed leachates was observed in S3V2: JGL 24423 untreated control (84.98). The overall study revealed that cold plasma treatment P3 (20 kV for 15 min) performed better in terms of seed quality parameters and among varieties treated with cold plasma grown under field conditions Tellahamsa performed better for yield contributing characters while, RNR 15048 was early to days to 50% flowering and days to maturity. The seed quality parameters of harvested produce of JGL 24423 treated with cold plasma (20 kV for 15 min) recorded good seed quality parameters.

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Rice is a staple food for over half of the world's population, especially in Asia where it is a dietary mainstay. It offers vital carbohydrates for energy and is a good source of iron, magnesium, phosphorus, zinc and several other vitamins and minerals, including vitamins B₁, B₂, B₃, B₆ and B₉. Global rice production estimate was 503.0 million tons during 2022-23 which is two per cent less than the previous year. In India rice area during *Rabi*, 2022-23 has increased by 37.89% i.e., 55.02 lakh hectares as compared to 39.90 lakh hectares during *Rabi*, 2021-22. Among different states in India, Telangana ranked first in area with 22.74 lakh ha followed by Tamil Nadu with 12.21 lakh ha, Andhra Pradesh with 5.51 lakh ha and Assam 1.84 lakh ha. Major paddy-growing districts in Telangana are Nalgonda (2.24 lakh ha), Suryapet (1.92 lakh ha), Nizamabad (1.61 lakh ha), Siddipet (1.40 lakh ha) and Jagtial (1.20 lakh ha) (PJTAU Paddy Outlook, 2023).

Abiotic stresses such as heat, cold, salt, drought and heavy metal toxicity frequently hinder crop stand and results in poor yields (Soltani *et al.*, 2006; Jabbari *et al.*, 2013). Cramer *et al.*, 2011 estimated that 96.5% of the world's rural land area is affected by abiotic stresses as per the reports given by FAO. Rice crop is exposed to various environmental stresses during its growth period both in *Kharif* and *Rabi* seasons. In *Rabi*, cold stress is the major environmental factor limiting its growth and productivity up to 40% (Zhu *et al.*, 2007). Due to its origin in tropical and subtropical regions, rice is more sensitive to cold stress compared to other cereal crops such as wheat and barley. Cold stress affects the chlorophyll content of leaf and thus interferes with photosynthesis process (Kanneganti and Gupta 2008; Kim *et al.*, 2009). Due to diverse growing locations and seasons rice cultivars face cold stress at vegetative growth stages especially in *Rabi* (Saito *et al.*, 2001).

In general, low germination, stunted seedlings, leaf yellowing, withering and decreased tillering are the outcomes of cold stress during vegetative stage. Cold stress severely affect the reproductive development in rice plants especially at the time of anthesis, leading to sterility in flowers (Jiang *et al.*, 2002). The effects of cold stress at the reproductive stage of plants delay heading and result in pollen sterility, which are the key factors responsible for the reduction in seed yield of crops (Suzuki *et al.*, 2008).

Somerville (1995) defined chilling tolerance as plant's ability to withstand low temperatures (0-15°C) without suffering any damage.

Genetic and biotechnological tools are employed by breeders to generate varieties that are resilient to climate stress. The use of non-genetic approaches such as exogenous application of hormones, plant growth regulators, magnetic and electric fields (Godoy *et al.*, 2021) and seed treatments using non thermal plasma has captured high importance in recent years as they are environmentally safe and sustainable approaches for the management of abiotic stresses in plants (Susmita *et al.*, 2022).

Cold plasma is fourth state of matter contains a mixture of an ionized gas composed of excited species of atoms, molecules and ions coexisting with the reactive species of electrons reactive oxygen species (ROS) and reactive nitrogen species (RNS), negative and positive ions, free radicals, atoms and molecules in the excited or ground state. Due to its non-equilibrium status, the gas persists at low temperatures due to the cooling of ions and uncharged molecules rather than electron energy transfer (Bourke *et al.*, 2018). It is informally considered that plasma sources operating at near ambient temperatures below 60°C were consider as cold plasmas (Misra *et al.*, 2019a and b).

Cold plasma generates reactive nitrogen and oxygen species as well as strong oxidizing elements which can enter microorganisms and oxidize the cytoplasmic membrane, alter cellular systems and inactivate the microbes (Klampfl *et al.*, 2012). Cold plasma treatments significantly improved seed germination percentage and water absorption in the seedlings when subjected to chilling temperature (De Groot *et al.*, 2018) and played an important role in reducing the frequency and intensity of diseases as since plant diseases cause 20-30% crop losses annually (Oerke, 2006). The anti-microbial activity of cold plasma has been demonstrated in many crops (Pignata *et al.*, 2017, Sakudo *et al.*, 2019). The plasma activated water is also used for the control of many plant diseases (Aktar *et al.*, 2021; Adhikari *et al.*, 2019). However, the induction of defense responses by cold plasma seed treatment, especially chilling response is still less investigated in agriculture. Due to climate change and an increase in extreme temperatures, rice crop is subjected to yield penalties (Manning and Solomon *et al.*, 2007).

At vegetative stage, rice is more sensitive to low temperatures which inhibit seed germination (Morsy *et al.*, 2007; Baruah *et al.*, 2009) and retard seedling growth resulting in leaf curving, shoot shortening and less tillering (Dashtnian *et al.*,

2014). Cold temperatures will damage the rice root tissue, results in poor uptake of water by roots and nutrient transport to the shoot, results in reduced seedlings growth (Setter and Greenway 1988; Neilson *et al.*, 2013). Non thermal plasma treatment at 15 kV for 30 seconds promotes rice seed germination and growth under low-temperature stress and increases the expression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Bian *et al.*, 2024).

Cold plasma was utilized to improve the growth and yield in rice crop (Yodpitak *et al.*, 2019; Zargarchi and Saremnezhad, 2019; Hashizume *et al.*, 2021). It also significantly improved rice cooking properties (Chen *et al.*, 2012; Thirumdas *et al.*, 2015). The cold plasma treatment of rice seed has improved the panicle length, stem length, seedling height, panicle weight as well as harvest index (Hashizume *et al.*, 2021). Further, plasma treatment is employed to increase the seed coat permeability without increasing the moisture content of seed and it has been successfully applied in agriculture for seed quality improvement, seed enhancement and pathogenic micro- organisms inactivation (Filatova *et al.*, 2013).

Since a positive effect of plasma treatment on cold tolerance, germination and seed quality aspects in various agricultural crops has been observed and in Telangana state due to cold stress ~ 30% yield losses occur in paddy. The present investigation was planned to study the effect of cold plasma treatment on cold tolerance and seed quality in rice with the following objectives:

1. Standardization of protocols for cold plasma treatments for seed quality under *in vitro*
2. Field evaluation of effective cold plasma treatments on vegetative growth and yield parameters during *Rabi* season
3. Evaluation of seed quality parameters of paddy seed harvested from cold plasma treatments

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Abiotic stresses such as heat, cold, salt, drought and heavy metal toxicity frequently hinder crop stand and results in poor yields. The decreased productivity caused by low temperatures remains as a problem especially where indica rice is cultivated. Considering the frequency of extreme temperature events as part of the general global warming phenomena and damage caused by cold temperatures may increase in future in several parts of world. The use of environmentally safe sustainable and non-genetic approaches like seed physical treatments viz., magnetic, electric fields and non-thermal plasma treatments had captured high importance in recent years to combat abiotic stresses in plants.

The present investigation was carried out to study the effects of cold plasma treatments on vegetative cold tolerance in *Rabi* rice (*Oryza sativa* L). The appropriate cold plasma treatments and their impact on seed quality parameters, biochemical parameters and yield contributing characters were reviewed here.

2.1 Effect of cold plasma treatments on seed quality parameters

Dubinov *et al.* (2000) studied the effects of continuous (25mA for 2 min) and pulsating glow discharge air plasma (40 mA for 4 min) on germination per cent and seedling growth in barley and oat and identified that plasma treatments can be used as pre-sowing seed treatments as they will provide stimulating effects in barley and oats. Among two types of plasma used, continuous plasma discharge given better results than pulsating glow discharge air plasma.

Zivkovic *et al.* (2004) investigated the treatment effects of non-equilibrium air plasma (50, 100 and 200W up to 40 min) on the germination per cent of *Paulownia tomentosa* seeds and reported that the seeds exposed to the plasma (4 to 6 minutes) showed 75% increase in germination and significant increase in light sensitivity of *P. tomentosa* seeds compared to untreated seeds.

Spatenka *et al.* (2010) investigated the effects of cold plasma treatments with the voltage of 500 W and an air gas flow of 200 ml/min for varying time periods viz., 0 to 2400 seconds in wheat and oat. Plasma treatments had no effect on per cent seed germination but it has speedup the rootlet development in oats while in wheat plasma

treatment for 180 seconds has recorded significantly high dry weight compared to other durations.

Mitra *et al.* (2014) studied the cold atmospheric plasma seed treatments in bengal gram and revealed that plasma treatment for 1 min showed improved seed germination (89.2 %), speed of germination (7.1seeds/day) and seed vigour beside a decrease in the mean germination time (2.7 days) when compared to control.

Chen *et al.* (2016) investigated the low-pressure plasma effects 1 to 3 kV for 10 minutes on brown rice of the treatments tested, 3 kV plasma exposure for 10 min produced the best results. α -amylase activity in germinating brown rice was considerably improved in plasma treated seeds compared to. The germination rate and early vigour of the seedlings in brown rice treated with plasma are probably caused by the increased enzyme activity in the rice. Gamma-amino butyric acid (GABA) levels were similarly raised by low-pressure plasma, rising from about 19 to about 28 mg/100 g.

Penado *et al.* (2017) explored the effect of atmospheric air plasma jet treatment for 15 kV for 1, 2, and 3 seconds in rice and revealed that plasma exposure reduced the number of trichomes on seed surface which leads to increase in wettability. Plasma treatments significantly affected the seedling length and did not affect the seed germination count.

Sherawat *et al.* (2017) studied the effects of cold plasma treatment 500 V for 3, 6, 9, 12 and 15 minutes in okra for seed germination and seedling vigour in two different aged seeds subjected to plasma treatments and four storage durations. Results showed that plasma treatments had positive effects on seed germination and seedling vigour for 12 min duration in one year old seed. Germination and vigour indices significantly increased by 16.42 % and 50.66 %, respectively. Characteristics of speed of germination, seedling length, seedling dry weight, seedling vigour index-II were significantly increased by 36.18, 30.60, 52.87 and 77.98 % respectively, compared to control. It was found that immediately after plasma exposure the per cent germination (91.07) and vigour (2281) were highest and was reduced to (80.00) and (1460.74) after 9 months of storage in one year old seed.

Sadhu *et al.* (2017) studied the effect of the cold plasma in mung bean by applying two plasma power levels (40 W and 60 W) and three exposure time intervals (10, 15 and 20 min) and reported that the cold plasma treatments not only significantly increased the germination rate by 36.2%, radical root length by 20% and electrical

conductivity of seeds by 102% but also increased soluble sugars, proteins, amylase, protease and phytase content compared to control.

Sheteiwiy *et al.* (2019) studied the effects of 10 kV cold plasma and 2 mM salicylic acid (SA) priming and their combinations on the physiological parameters in paddy under salinity stress (150 mM NaCl) and normal growth condition (0 mM NaCl). They reported that seed germination, seedling growth, photosynthetic pigments, photosynthetic gas exchange and chlorophyll fluorescence were enhanced by SA priming and cold plasma treatment either alone or in combination under salinity stress compared to the untreated seeds.

De Groot *et al.* (2018) studied the cold atmospheric-pressure plasma (CAP) treatments (38 kV using air for 3 and 27 minutes and 11kV using argon for 81 minutes) in cotton and revealed that CAP treatment for 27 minutes significantly increased water absorption of the seed and improved germination and chilling tolerance in cotton and concluded that CAP treatment would significantly improve plant establishment in a wider range of environmental conditions.

Amnuaysin *et al.* (2018) studied the effect of atmospheric air dielectric barrier discharge plasma on seed germination and early growth in rice. Rice seeds were exposed to 18kV plasma for 10, 30 and 60 s had positive effects on germination. Seeds exposed to plasma for 60 s had the highest germination rate. Seedling vigour index and speed of germination of plasma treated seeds were higher in comparison to untreated seeds. The photosynthetic pigments tended to increase in the seedlings of treated seeds. Further the results suggested that the exposure of rice seeds to plasma promoted seed germination and seedling growth by changing the surface morphology of rice seeds.

Los *et al.* (2019) employed atmospheric cold plasma (ACP) treatment 80 kV for 30, 60 and 180 seconds on wheat and recorded the changes in the surface chemistry and characteristics of the wheat seeds exposed to plasma. Plasma treatments of 30 and 60 seconds significantly enhanced the germination rate and showed positive effects on seedling growth. ACP treatment resulted in changes of seed surface and chemical characteristics including water uptake and contact angle values.

Volkov *et al.* (2020) subjected cold atmospheric pressure plasma (CP) He-plasma jet (CPJ), reactive oxygen and nitrogen species (RONS), UV-Vis photons and high- frequency strong electromagnetic fields on *Phaseolus vulgaris* seeds and plants. They showed that CPJ accelerated seed imbibition, germination, radicle growing rates,

induced roughness, corrugation and poration in *Phaseolus vulgaris* seed coats. CPJ produced hydrophilization of seed coats and improved the wetting properties of seed surfaces. Magnetic resonance imaging studies showed the acceleration of water uptake in *P. vulgaris* seeds.

Adhikari *et al.* (2020) studied the structural changes in tomato seed that were treated with cold air plasma jet for a different time periods (0.70 kV for 1 min, 5 min and 10 min) using Scanning Electron Microscope. Drought stress tolerance potential of cold plasma primed tomato seedling was examined by treating with 30% PEG which modulated tomato seed coat and improves the germination efficiency and augmented the reactive species at molecular level.

Rashid *et al.* (2021) treated paddy seeds with low pressure glow air discharge (LPGAD) plasma 1 to 10 kV for duration of 30, 60, 90, 120 and 150 seconds for finding the highest germination rate and also applied plasma activated water (PAW: 1 to 10 kV for 10 minutes) as foliar spray to the plants grown from the treated seeds to investigate the combined effects of LPGAD and PAW. The results revealed that the maximum paddy seed germination rate of ~7% was obtained from LPGAD plasma treatment for 90 seconds duration. The plant growth parameters and defense mechanisms of plants were improved through enhancement of enzymatic activities and concentrations of total soluble protein and sugar in paddy and finally the yield was increased by ~16.67% through combined effects.

Rongsangchaicharean *et al.* (2022) used streamer corona plasma (SCP) 7 kV for 2 min and dielectric barrier discharge (DBD) plasma 7 kV for 10 min, to enhance rice seed vigour through surface modification. The results revealed that SCP required less treatment time (~2 min) for surface activation compared to DBD plasma (~10 min). Both plasma-treated seeds recorded higher seed vigour than non-treated seeds. For 50% of the viable seeds to emerge, the SCP treated seeds took on an average of about 62 hours while untreated rice seeds took around 72 hours.

Chueauan *et al.* (2023) used air gliding arc (GA) plasma 6 to 10 kV for 15 min to generate plasma-activated water (PAW) and studied the effects of PAW on germination of rice seeds. The N_2 , NO and OH molecules in GA plasma led to NO_3^- , NO_2^- and H_2O_2 formation in the PAW. The NO_3^- , NO_2^- and H_2O_2 contents in PAW rapidly decreased after 5 days of storage. The NO_3^- and NO_2^- contents increased resulted in an increase in total nitrogen (N) and gibberellic acid (GA_3) accumulation in rice seeds. The PAW at an

airflow rate of 5 L/min was optimal for enhancing radicle emergence at 48h and 72h, germination rate, germination index, shoot length, fresh weight and dry weight of seedlings.

El-Shaer *et al.* (2023) treated rice with plasma directly in a dielectric barrier discharge (9kV for 2, 5, 10, 15 and 20 min) and indirectly by gliding arc discharge (9kV for 2, 5, 10, 15 and 20 min). After 20 min of direct and indirect plasma exposure to seed the germination potential was increased by 36.73 and 50.4%, germination rate by 26.0 and 30.0%, germination index by 25.92 and 36.53%, total shoot length increase by 42.2 and 48.5% and total root length by 15.93 and 22.42%, respectively. After 20 min for direct and indirect exposure free proline increased by 30.0 and 40.0%, total soluble carbohydrates by 49.7 and 54.6%, cellular reactive oxygen species (ROS) increase by 52.0 and 60.0%, and malondialdehyde (MDA) decreased by 68.0 and 88.0%, respectively.

Harish *et al.* (2024) tested cold-activated air plasma in mustard seeds and among the different treatments imposed (300 V and 400 V for 3,5 and 10 minutes), 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 at 480 nm) and lower electrical conductivity (14.20 dsm-1). The inhibitory effects were recorded with a higher dose of plasma i.e., 400 Vfor10 min suggesting the need for judicious usage of plasma frequencies.

Abeysingha *et al.* (2024) reported the impact of cold plasma seed treatments on nodulation, root and shoot growth in pea and lentil under controlled environmental conditions. Seeds treated with cold plasma generated by a dielectric barrier discharge (DBD) and pin electrode reactor (PER) with 0 to 34 kV for three different exposure durations (3, 6 and 12 min) exhibited notable enhancements in nodule number and dry weight, root dry weight, length, volume, surface area and shoot dry weight at 4 weeks after treatment. However, 3 and 6-min exposure using the DBD and the 3 min exposure using the PER system demonstrated the most significant increase in these traits, highlighting the intricate nature of seed plasma interactions.

2.2 Effect of cold plasma treatments on biochemical parameters

Ling *et al.* (2015) studied the effects of cold plasma treatment (100 W for 15 seconds) on seed germination, seedling growth, antioxidant enzymes, lipid peroxidation and osmotic products in oilseed rape under drought stress. They reported that under

drought stress, cold plasma treatment significantly improved the germination rate, shoot and root dry weights, shoot and root lengths and lateral root number. Cold plasma treatment markedly improved the superoxide dismutase and catalase activities by 17.71% and 16.52% in Zhongshuang 7 and by 13.00% and 13.21% in Zhongshuang 11 paddy varieties respectively and also significantly increased the soluble sugar and protein contents, but reduced the malondialdehyde content in seedlings.

Guo *et al.* (2017) applied dielectric barrier discharge (DBD) plasma (0 to 50 kV for 4 min) to improve drought resistance in wheat and reported that the DBD plasma treatment alleviated the adverse effects of drought stress; the germination potential and germination rate were increased by 27.2% and 27.6%. The root length and shoot length of the wheat seedlings were increased. Proline, abscisic acid, superoxide dismutase, catalase, peroxidase and soluble sugar levels under drought stress were improved, whereas the malondialdehyde content and ROS contents decreased after the DBD plasma treatment.

Mildaziene *et al.* (2019) studied the effects of pre-sowing seed treatment using 200 Pa vacuum for 7 min, 5.28 MHz radio-frequency cold plasma for 2, 5 and 7 min and electromagnetic field (EMF) of 17.96 kV for 5, 10, 15min) in sunflower. Cold plasma for 7 min and electromagnetic field for 15 min caused 19-24% faster germination under in vitro. Proteome analysis also showed long-term effects on gene expression in leaves, mostly stimulating the expression of proteins involved in photosynthetic processes and its regulation.

Zargarchi and Saremnezhad (2019) evaluated the effects of low-pressure plasma (50 and 100 W for 5 minutes) on biochemical activities in germinated rice. The results revealed significant increase in water uptake, germination rate and GABA content of seeds after low-pressure plasma exposure, while phenol concentration and antioxidant activity were decreased as compared to control and they concluded that low-pressure plasma can be used as a novel approach for increasing GABA content as a functional component in paddy rice.

Filatova *et al.* (2020) studied the effects of pre-sowing plasma seed treatment (200 pa and 5.28MHz) in maize and wheat. They reported that low-pressure radio frequency plasma suppressed the fungal crop diseases. Decrease in the level of seed infection, stimulation of field germination, early seedling growth and plant resistance to pathogens during the vegetation period. Grain yield was increased by 2.3% in winter wheat, 1.7%

in maize 26.8% in narrow-leaved lupine compared to untreated control. Non-enzymatic antioxidants such as proline, anthocyanins and total phenol contents were increased in roots of maize seedling indicating a significant role of plasma seed treatment in improving the plant resistance to biotic and abiotic stress during the vegetation.

Gierczik *et al.* (2020) showed that plasma-activated water (PAW: 12kV for 30 min) improved tolerance against low temperature and hypoxia stresses during germination in barley. Plasma treatments improved PAW's effect on the antioxidant system which has mitigated the reducing effect on plant growth under salt stress. PAW pre-treatment reduced the salt-stress and cysteine and γ -glutamylcysteine content and improved the plant's recovery after NaCl stress. PAW pre-treatment also modified the NaCl-induced changes in carotenoid content, especially, β -carotenes and xanthophylls and reduced salt-induced damage through the removal of excess reactive oxygen species.

Sajib *et al.* (2020) investigated the effects of plasma activated water (PAW) 3 to 6 kV for 3, 6, 9, 12 and 15 minutes in blackgram. Significant changes in H₂O₂ and reactive-oxygen-species (ROS) were observed in seeds, leaves and roots. Increase in catalase observed in roots of PAW treated seeds and up regulation of CAT activity resulted in increased germination, plant growth and development. Non-covalent binding pattern of H₂O₂ with CAT through docking strongly supported the phenomenon and increase in H₂O₂ tightly regulated by the up regulation of CAT providing optimum conditions for improved seed germination and growth in black gram.

Li *et al.* (2021) explored the effects of cold plasma treatments (25, 50, 75 and 100 W for 10 seconds) on the chilling tolerance in tomato plants. Among these treatments 75 W cold plasma treatment showed the best mitigative effect on cold- induced injury of tomato seedlings, as it has given higher maximum photochemical efficiency of PSII (Fv/Fm), lower ion leakage and chilling injury index. They also showed that cold plasma induced chilling tolerance was related to the hydrogen peroxide (H₂O₂) mediated by respiratory burst oxidase homologue 1 (RBOH1). Thus, cold plasma induced H₂O₂ and ABA cascade signal up regulated the regulatory genes (ICE1 and CBF1) of cold acclimation, which increased the osmotic adjustment substances (proline and soluble sugar) accumulation and antioxidant enzymes (SOD, APX and CAT) activities.

Holubova *et al.* (2021) reported the potential of cold atmospheric pressure plasma (CAPP) 400 W for 30, 60, 90, 120, 180 and 300 seconds in maize, and reported that short CAPP treatment of seeds had a positive effect on the vitality of seeds and young seedlings

and stimulated antioxidant and lytic enzyme activities. The prolonged treatments had a negative impact on the germination, growth and production indexes. Further, the CAPP treatment increased the expression of genes for heat shock proteins HSP101 and HSP70.

Billah *et al.* (2021) investigated the effect of LPDBD (Low Pressure Dielectric Barrier Discharge) (Ar + Air) plasma (3 to 5 kV) for 2, 4, 6, 8 and 10 min in rice. Germination rate, seedling growth, total chlorophyll content, enzymatic activity, total soluble sugar, CAT, SOD activity and protein concentration were increased in plants grown from the LPDBD (Ar + Air) plasma treated seeds and H₂O₂ level was increased significantly both in leaves and roots and no significant change was observed in Nitric Oxide (NO) concentration.

Guragain *et al.* (2021) studied the kinetics and amount of water uptake by seeds which are influenced by both seed surface properties and the surrounding environment. Non Thermal Plasma (NTP) of 12.5 kV for 1 to 4 minutes was used to investigate the pre-sowing treatment for modifying seed germination in carrot seeds. The results stated that, germination percentage, vigour index and chlorophyll content were improved by NTP treatment while, non-significant changes were seen in the carotenoid content. Similarly, the in-vitro radical scavenging activities, total phenol and total flavonoid contents in the seedlings were altered by NTP treatment.

Rashid *et al.* (2021) used atmospheric pressure multi-tube air bubble discharge plasma with 0.5 to 10 kV, 0.05 to 5 kHz bipolar power supply within a water treatment reactor. Water was treated in the reactor with an air flow rate of 5 lpm for 15 min and the chemical properties of plasma activated water (PAW) were determined. PAWs were applied 2, 4 and 6 times as foliar spray to the plants during the vegetative growth stage in potato. The results revealed that the potato plant length, stem diameter, fresh weight, total chlorophyll and carotene concentrations are enhanced by ~ 14, ~ 23, ~ 34.45, ~ 37 and ~13.2%, respectively, with respect to control. Apart from this enhancement in enzymatic activities, total soluble protein and sugar content in potato tubers with ~ 14.15% tuber yield.

Suriyasak *et al.* (2021) treated rice seeds with cold plasma (2.17 W for 3 min) and subjected them to heat stress and found that per cent germination was significantly restored. They also noticed that genes involved in ABA biosynthesis (*OsNCED2* and *OsNCED5*) were down regulated. Whereas, genes involved in ABA catabolism

(*OsABA8OH1* and *OsABA8OH3*) and α -amylase genes (*OsAmy1A*, *OsAmy1C*, *OsAmy3B*, and *OsAmy3E*) were up regulated.

Bian *et al.* (2024) reported that non thermal plasma (NTP) treatment (0, 13.9, 15 and 19.7 kV for 30s) optimally promoted rice seed germination and growth under low-temperature stress. Furthermore, NTP treatment increased the activity and expression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), under low-temperature conditions. Transcriptomic analysis revealed the involvement of various key genes in phosphotransferase activity, phosphate containing compound metabolic processes and defense responses.

2.3 Effect of cold plasma treatments on yield contributing characters

Filatova *et al.* (2010) evaluated the effects of radio frequency (5.28MHz) and microwave air plasma (2.45GHz) treatments to improve seed germination in wheat. Plasma treatments improved branching of roots especially from 7th to 10th day of seedling growth. The per cent germination, germination energy and shoot weight of radio frequency plasma treated wheat seedlings was higher compared to microwave air plasma treatments.

Henselova *et al.* (2012) subjected maize seeds to low-temperature plasma (LTP) by using diffuse coplanar surface barrier discharge of 10kV for 60 and 120 seconds and observed that post-germination growth of maize seedlings was affected. 60 seconds duration significantly increased the seedling length, fresh and dry weight of the roots while plasma treatment for 120 seconds duration had inhibitory effect on the growth parameters, whereas, non-significant changes in antioxidant enzymes were noticed.

Jiang *et al.* (2014a) investigated the effect of cold plasma treatment on seed germination, plant growth and seed yield in wheat. The 80 W cold plasma treatment significantly improved seed germination potential by 6.0% and germination rate by 6.7% when compared to control. Field studies also revealed that plasma treated wheat seeds improved plant height by 20.3%, root length by 9.0% and fresh weight by 21.8% significantly at seedling stage compared to control. At booting stage, plant height, root length, fresh weight, stem diameter, leaf area, leaf thickness and seed yield of the plasma treated plants were increased by 21.8%, 11.0%, 7.0%, 9.0%, 13.0%, 25.5% and 5.89% respectively compared to control.

Ling *et al.* (2016) explored the effects of cold plasma treatment (60, 80, 100, 120 and 140 W for 15 seconds) on seed germination, plant growth and yield in peanut.

Germination potential and germination rate were markedly increased by 150% and 21%, respectively in plasma treated seed. Seedling shoot and root dry weights increased by 11% and 9% respectively. Leaf area, leaf thickness, leaf nitrogen concentration, chlorophyll content, dry weight at the fruiting stage, plant height, stem diameter and root dry weight at the maturity stage were markedly improved by the cold plasma treatment. The cold plasma treatment enhanced yield components, such as number of branches per plant, number of pods per plant and 100 pods weight by 8%, 13% and 9%, respectively when compared to the control.

Nalwa *et al.* (2018) investigated the effect of glow discharge cold plasma treatment 100 W for 0, 3, 6, 9, 12, 15 minutes on growth, fruit and seed parameters of bell pepper. The changes in surface morphology of plasma treated seeds were studied by Scanning Electron Microscopy (SEM) and contact angle goniometer. The plasma treatment alone as well as in combination with Osmo priming up to 6 minutes was found superior for plant growth, fruit and seed yield characters. Osmo primed seeds exposed with 6 minutes plasma were found best for plant height at 30 DAT (18.97 cm), days to first mature fruit picking (75.33), number of fruits per plant (17), fruit yield per plant (1.00 kg), number of seeds per fruit (190 g) and 1000 seed weight (4.35 g).

Ling *et al.* (2018) studied the effects of low-vacuum helium cold plasma treatment (60 W, 80 W, 100 W and 120 W for 15 seconds) in oilseed rape. The cold plasma treatments increased the germination, plant growth and yield of oilseed rape where the treatment of 100 W provided the best result. The germination rate, vigour indices, and uniformity of emergence, relative conductivity and water uptake were improved by cold plasma treatment while the apparent contact angle reduced. The plant height, stem diameter, dry weight of shoot and root at the seedling, bolting and flowering stages and yield components, including pod numbers per plant and 1000 seed weight were increased by the cold plasma treatment with 28.20% increase in yield per plant. They reported that cold plasma treatment has the potential to improve the yield of oilseed rape through the enhancement of permeability, wettability and capacity of water uptake of the seeds, seed germination and plant growth.

Saberi *et al.* (2019) treated wheat seeds with cold air plasma 50W with different time intervals of 60, 120, 180 and 240 seconds. In greenhouse, haze stress test was performed and plasma treatment for 180 seconds ameliorated the photosynthetic rate, chlorophyll content and stomata conductance and seed yield by 34, 32, 93% and 75%, respectively.

Kabir *et al.* (2019) examined the Ar/O₂ (5 to 10 kV for 90 seconds) and Ar/Air (5 kV for 90 seconds) plasma techniques for cadmium detoxification in wheat. These treatments showed considerable progress in morphology and total chlorophyll synthesis compared to cadmium-treated wheat without plasma treatments, suggesting that plasma technology is effective for cadmium detoxification. Furthermore, plasma treated plants showed a significant decrease in root and shoot cadmium concentration. This cadmium inhibition is possibly accomplished by the decrease of pH reducing the bioavailability of cadmium in the rhizosphere.

Hashizume *et al.* (2021) examined the effects of plasma irradiation (9 kV for 5 min) and treatment with plasma-activated ringer's lactate solution (PAL: 9 kV for 5 min) on rice seedlings under field conditions. Irradiation during the vegetative growth period increased seedling growth, panicle number and seed yield. In contrast, treatment during the reproductive growth period had no effect.

2.4 Effect of cold plasma treatments on disease incidence

Jong *et al.* (2014) evaluated the non-thermal plasma effects (3W for 10, 30, and 60, 120 and 180 seconds) against surface disinfection treatments for seed borne pathogen, *Gibberella fujikuroi* in rice wherein plasma treatments effectively inhibited the growth of *G. fujikuroi* on nutrient growth medium and reduced the number of fungal colonies forming units (CFU) on the rice seed surface by > 92% after 120 s exposure to plasma.

Jiang *et al.* (2014b) investigated the effects of cold plasma treatment (80 W for 15 seconds) on tomato bacterial wilt and its regulatory mechanism. They identified that plasma treatment increased the resistance to *R. solanacearum* with an efficacy of 25.0% in tomato plants and H₂O₂ levels in plasma treated plants reached a higher peak at 2.579 $\mu\text{M gFW}^{-1}$ which is 140% higher than control. In addition peroxidase activity (421.3 U gFW⁻¹), polyphenol oxidase (508.8 U gFW⁻¹) and phenylalanine ammonia lyase (707.3 U gFW⁻¹) activity also increased in the plasma treated plants.

Zahoranova *et al.* (2018) investigated the effects of cold plasma (20 kV for 30 to 300 seconds) generated by Diffuse Coplanar Surface Barrier Discharge (DCSBD) for inhibition of native microbiota (*Aspergillus flavus*, *Alternaria alternata* and *Fusarium culmorum*) in maize. A complete devitalisation of native microbiota on the surface of seeds was observed after a treatment time of 60 s for bacteria and 180 s for filamentous fungi. The plasma treatment efficiency of artificially contaminated maize seeds was estimated and a reduction of 3.79 log (CFU/g) in *F. culmorum* after a 60 second plasma

treatment while, 4.21 log (CFU/g) in *A. flavus* and 3.22 log (CFU/g) in *A. alternata* after a 300 seconds plasma treatment.

Iqbal *et al.* (2019) applied plasma (600, 700, 800 and 850 V for 2 minutes) and laser treatments (10 Hz for 1800 seconds) on wheat seeds to improve growth and development. Plasma treatment enhanced the germination rate, protein concentration and also exhibited high immunity against fungus wherein, only 20% seeds were found affected by fungus as compared to the untreated control. Laser treatment lowered the germination time, increased water absorption rate and abolished disease development.

Hasan *et al.* (2020) used O₂-air glow discharge plasma 1 to 10 kV for 90 and 120 seconds and plasma activated water (PAW) for 1 to 10 kV for 9 and 12 minutes as seed treatment to control blast disease in rice. Of these treatments O₂-air glow discharge plasma for 120 sec and air-plasma activated water for 12 min successfully reduced the conidial germination of *Pyricularia oryzae*. However, O₂-air glow discharge plasma for 90 sec and PAW for 9 min has recorded 14.96% and 20.21% increased plant stand over control treatment, respectively. The blast incidence and severity were significantly reduced in the plots which transplanted with seed treated with O₂-air glow discharge plasma for 120 sec and air plasma activated water (PAW) for 12 min.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present study entitled “Studies on cold plasma treatments for vegetative cold tolerance in *Rabi* rice (*Oryza sativa* L.)” was taken up with an objective to study the effects of cold plasma treatment in rice against cold tolerance at vegetative stage during *Rabi* season.

The experiment was carried out during *Rabi*, 2023-24. The standardization work pertaining to cold plasma treatments was carried out at the Quality Control Lab, Seed Testing Laboratory and Central Instrumentation Cell, PJTAU, Rajendranagar, Hyderabad, using single variety RNR 15048. The best cold plasma treatment identified from the standardization work was imposed on three rice varieties viz., RNR 15048, JGL 24423 and Tellahamsa and field experiment was laid out to determine the effects of cold plasma treatments on paddy vegetative growth during *Rabi* 2023-24 at seed production farm of Seed Research and Technology Centre, Rajendranagar, Hyderabad. The seed harvested from cold plasma treated three paddy varieties was used to study seed quality parameters at Seed Testing Laboratory of Department of Seed Science and Technology (DSST), Rajendranagar, Hyderabad. The materials used and methods followed for the present study are depicted below.

3.1 Experimental Details

3.1.1. Location of work

The work was carried out with the facilities available at the Seed Testing Laboratory of Department of Seed Science and Technology, Seed Research and Technology Centre, PJTAU, Hyderabad, Central Instrumentation Cell, PJTAU, Rajendranagar, Hyderabad and Quality Control Lab, PJTAU for cold plasma treatments (Plate 3.1).

3.1.2. Materials

The experimental material consisting of three paddy varieties were procured from Seed Research and Technology Centre, PJTAU, Hyderabad (RNR15048), Rice Research Centre Rajendranagar (Tellahamsa) and Regional Agriculture Research Station, Jagtial (JGL 24423). The field experiment was laid out in three replications and the details of the varieties used (Table 3.1 and plate 3.2) and the field layout plan presented in Figure 3.1



Plate 3.1 Non-thermal plasma device used for treating paddy seed



Plate 3.2 Three varieties of paddy treated with cold plasma (20kV for 15 minutes) used for field studies

Table: 3.1 Details of rice varieties used in the experiment

S. No	Variety	Important character
1.	RNR10754 (Tellahamsa)	Long slender grain popular because of its cold tolerance nature and having yield potential of 6 to 6.5 tons/ha.
2.	RNR 15048 (Telangana sona)	Short slender and super fine rice variety with blast resistance having high yield (6.5 to 7 t/ha) and low glycemic index.
3.	JGL 24423 (Jagtial Rice-1)	Long slender, medium duration rice variety having non-lodging and less grain shattering nature, tolerant to brown plant hopper, blast and slightly tolerant to cold.

3.2 Methods

3.2.1 Experiment 1: Standardization of cold plasma treatments for seed quality under *in vitro* conditions.

The standardization work was carried out using cold plasma equipment (plate 3.1) available at quality control lab of PJTAU in RNR15048. Three different voltages 10 kV, 20 kV and 30 kV, two duration of exposure time *i.e.*, 15 min and 20 min by using two different types of seeds *i.e.*, normal seed and sprouted seeds were employed for plasma treatments. The best cold plasma treatment was identified based on physiological parameters *viz.*, seed germination per cent, seedling dry weight (mg), seedling vigour index-II, cold tolerance index (%), seed infection (%), speed of germination, radicle emergence (%) and biochemical parameters *viz.*, superoxide dismutase activity (units/ mg of protein), catalase activity (n moles of H₂O₂ used/ min/ g weight of sample), peroxidase activity (units/min/mg of protein) and proline content (μmoles of proline/ g). All the above observations were recorded by keeping the cold plasma treated seed at normal walk-in seed germinator condition in where the temperature was maintained at 25°C and at cold conditions in where the temperature was maintained at 15°C.

Cold -plasma Treatment details:

S.no	Type of seed (S)	Plasma treatment (P)
1.	S1(Normal seed)	P1-10 kV for 15 min P2- 10 kV for 20 min P3-20 kV for 15 min P4-20 kV for 20 min P5-30 kV for 15min P6-30 kV for 20 min P7-Untreated control
2.	S2 (Sprouted seed)	P1-10 kV for 15 min P2- 10 kV for 20 min P3-20 kV for 15 min P4-20 kV for 20 min P5-30 kV for 15min P6-30 kV for 20 min P7-Untreated control

3.2.2 Experiment 2: Field evaluation of effective cold plasma treatments (20 kV for 15 min) on vegetative growth and yield contributing characters in paddy during *Rabi*, 2023-24.

The effective cold plasma treatment identified from experiment 1 was imposed on three rice varieties RNR 15048, JGL 24423 and Tellahamsa (Plate3.2). The three varieties treated with 20 kV plasma for 15 minutes were evaluated for their performance under field conditions during *Rabi*, 2023-24 in randomized block design with three replications at seed production farm of Seed Research and Technology Centre, PJTAU, Hyderabad. Nursery was raised in the month of December, 2023 (Plate 3.3) and transplanted to the main field in the month of January,2024. A plot size of 12 m² was maintained by keeping row to row distance of 30 cm and plant to plant spacing of 10 cm in each replication. Good crop stand was maintained by following all recommended package of practices. The data pertaining to the yield and yield attributing characters *viz.*, germination per cent, dry weight of root and shoot at 15, 30 and 45 days, per cent incidence of blast, chilling injury (%), days to 50% flowering, days to maturity, chlorophyll content, plant height (cm), number of tillers/ hill, number of tillers/m², proline content (μmoles/g), 1000 seed weight (g), seed yield/hill (g), seed yield/ha (t/ha) were recorded.

3.2.3 Experiment 3: Evaluation of seed quality parameters of paddy seed harvested from cold plasma treatments.

The impact of cold plasma treatment on seed quality parameters of freshly harvested seeds obtained from *Rabi*, 2023-24 harvested produce were evaluated. The observations pertaining to per cent germination, seedling dry weight (mg), speed of germination, seedling vigour index-II, electrical conductivity of seed leachates ($\mu\text{S cm}^{-1}\text{g}^{-1}$), radicle emergence (%) and seed moisture content (%) were recorded.

3.3 Observations recorded

3.3.1 Standardization of protocols for cold plasma treatments for seed quality under *in vitro* conditions.

3.3.1.1 Germination (%)

The germination test was conducted as per ISTA, 2022. Hundred seeds each in four replications were taken from each treatment and placed on germination paper. The paper towel was then placed in seed germination chamber, maintained with temperature of $25 \pm 0.5^\circ\text{C}$ and $95 \pm 2\%$ relative humidity and also in cold chamber maintained with temperature of $15^\circ\text{C} \pm 0.5^\circ\text{C}$ and $45 \pm 2\%$ relative humidity. On the 14th day, the normal seedlings (seedlings with normal shoot and root growth and without any secondary infections) were calculated and the mean was expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds kept for germination}} \times 100$$

3.3.1.2 Seedling dry weight (mg)

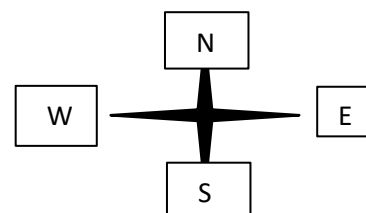
Ten normal seedlings from each replication were placed in butter paper bags and kept in an oven maintained at $80 \pm 1^\circ\text{C}$ for 24 hours. The oven dried seedlings were then placed in a desiccator for cooling. Weights of 10 dried seedlings was recorded and average of seedling weight was expressed in milligrams per seedling.

3.3.1.3 Seedling Vigour Index- II

The seedling vigour index - II was calculated as per the method suggested by Abdul Baki and Anderson (1973) as given below and expressed in whole number.

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

R1	R2	R3
S1V1	S2V2	S3V3
S2V1	S3V2	S1V3
S3V1	S1V2	S2V3
S1V2	S2V3	S3V1
S2V2	S3V3	S1V1
S3V2	S1V3	S2V1
S1V3	S2V1	S3V2
S2V3	S3V1	S1V2
S3V3	S1V1	S2V2



----- Road -----

Fig. 3.1 Field Layout of the cold plasma treated crop raised during *Rabi*, 2023-24 Treatments

1. S1V1- RNR 15048 normal seed treated with cold plasma (20 kV for 15 min)
2. S2V1- RNR 15048 sprouted seed treated with cold plasma (20 kV for 15 min)
3. S3V1- RNR 15048 untreated control both for normal seed and sprouted seed
4. S1V2- JGL 24423 normal seed treated with cold plasma (20 kV for 15 min)
5. S2V2- JGL 24423 sprouted seed treated with cold plasma (20 kV for 15 min)
6. S3V2- JGL 24423 untreated control both for normal seed and sprouted seed
7. S1V3- Tellahamsa normal seed treated with cold plasma (20 kV for 15 min)
8. S2V3- Tellahamsa sprouted seed treated with cold plasma (20 kV for 15 min)
9. S3V3- Tellahamsa untreated control both for normal seed and sprouted seed

*R1, R2 and R3 indicates replications.

3.3.1.4 Seed infection (%)

The paper towels were initially soaked in sterile distilled water and drained out excess water. Randomly 100 seeds were taken from individual seed sample in four replicates and kept at equidistance spacing on moistened paper towel, covered with another moistened towel and finally rolled. The rolled paper towels were kept in a walk-in seed germinator maintained with $25 \pm 0.5^\circ \text{C}$ constant temperatures and RH of 90 ± 3 . Data on per cent seed infection was recorded on 14th day and as calculated as below

$$\text{Per cent seed infection} = \frac{\text{Number of seeds infected with disease}}{\text{Total number of seeds}} \times 100$$

3.3.1.5 Speed of germination

Speed of germination was estimated by placing 100 seeds each in four replications in sand. The number of seedlings germinated at each day up to 14 days after setting the test were recorded regularly. Speed of germination was calculated as mentioned below (Maguire, 1962)

$$\text{speed of germination} = \sum \left(\frac{N_1}{d_1} + \frac{N_2 - N_1}{d_2} \dots \dots \dots \frac{N_n - N_{n-1}}{d_n} \right)$$

Where, N = number of seeds germinated on days (d) d = serial number of days

3.3.1.6 Cold tolerance index (%)

From each of the four replications 100 seeds were kept for germination both at 25°C (Standard temperature) and 15°C (Low temperature) for 14 days and the germination per cent was calculated as per the procedure given in 3.3.1.1. The cold tolerance index was calculated by using the formula.

$$\begin{aligned} &\text{Cold tolerance index (\%)} \\ &= \frac{\text{Per cent germination at low temperature (15}^\circ\text{C)}}{\text{Per cent germination at standard temperature (25}^\circ\text{C)}} \times 100 \end{aligned}$$

3.3.1.7 Super oxide dismutase Activity (units /mg of protein)

The super oxide dismutase activity was estimated as per the procedure given by Abedi and Pakniyat (2010). where 0.5 g of leaf sample was homogenized with 3.0 ml of potassium phosphate buffer and centrifuged at 2000 rpm for 10 min. To 0.2 ml of enzyme extract, added 1.2 ml of sodium pyrophosphate buffer (0.25 M, pH-8.3), 0.1 ml of

phenazine methosulfate (186 μ M), 0.3 ml of nitro blue tetrazolium (NBT) (300 μ M) and 2.8 ml of water. Reaction was initiated by adding 0.2 ml of NADH (780 μ M) and reaction was incubated at 30°C for 90 seconds and 1.0 ml of glacial acetic acid was added to stop the reaction. The reaction mixture was then shaken with 4.0 ml of n-butanol and then allowed to stand for 10 min and centrifuged. The intensity of chromogen in the butanol layer was read at 560 nm in a spectrophotometer. One unit of SOD enzyme activity was defined as the amount of enzyme that gave 50% inhibition of reduction of NBT (Nitro blue tetrazolium).

$$\text{S. O. D Activity} = \frac{A_0 - A_1}{A_1} \div 50\% \times \frac{\text{Total reaction volume}}{\text{volume of enzyme extract}} \times \text{Dilution factor}$$

Where,

A0 = Initial value

A1 = Final value

3.3.1.8 Peroxidase activity (units/min/mg of protein)

The peroxidase activity was estimated using the procedure given by Reddy *et al.* (1985). Using a pre-chilled mortar and pestle 200 g of leaf material was ground by adding 10 ml of 0.1 M sodium phosphate buffer having pH 7.0. The homogenate was centrifuged at 10,000 rpm at 4°C for 30 min and the supernatant was used as the source of the enzyme. Peroxidase activity was calculated by the method of Kar and Mishra (1976) by following few modifications. The assay mixture contained 2 ml of 0.1 M sodium phosphate buffer, pH 7.0, 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H₂O₂ and 1 ml of enzyme extract diluted 20 times. After incubating for 5 min at 25°C the reaction was stopped by adding 1 ml of 1.25 M H₂SO₄ to the reaction mixture, and the amount of purpurogallin formed was estimated by measuring absorbance at 420 nm. The enzyme activity was expressed as milli molar purpurogallin formed per g fresh weight per min.

3.3.1.9 Catalase activity (n moles of H₂O₂ used/ min/ g weight of sample)

The catalase activity was estimated by using H₂O₂ as given by Barber (1980). For enzyme extraction the sample (0.1g) was grinded using 0.1M phosphate buffer (pH 7.0) in a pre-chilled mortar and pestle and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant extracted was used as enzyme source 3 ml of phosphate buffer, 2 ml of H₂O₂ and 1ml of enzyme extract were added and incubated at 20°C for 1min. The reaction was stopped by adding 10ml of 0.7N H₂SO₄. The reaction mixture was titrated against 0.01N KMNO₄ to find out the residual H₂O₂ until a faint purple colour persisted at least for 15 seconds. The blank solution was prepared by adding the enzyme extract to an acidified

solution of reaction mixture at zero time and the concentration of H₂O₂ was calculated using the extinction coefficient 0.036 /μmole/ml. The activity of the enzyme was expressed in n moles of H₂O₂ used/min/g weight of sample.

Enzyme activity

$$= \text{change in time} \times \frac{1}{\text{co-efficient efficiency}} \\ \times \frac{\text{Total reaction volume}}{\text{volume of enzyme extract}} \times \frac{\text{Total volume enzyme extract}}{\text{Fresh weight of sample}} \\ \times \text{Total protein in 1ml} \times 1000$$

3.3.1.10 Proline content (μmoles of proline/g of tissue)

The proline content was estimated as per the protocol developed by Bates *et al.*(1973). Fresh plant material (0.2g) was homogenized in 5 ml of 3 % aqueous sulfosalicylic acid and centrifuged at 4000 rpm for 20 minutes. The residue was re-extracted with 5 ml of 3 per cent sulfosalicylic acid and centrifuged. The extracted supernatant was combined together and the volume was made up to 10 ml. 2 ml of the extracted aliquot was transferred in to a test tube and added 2 ml each of acid ninhydrin solution (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6M orthophosphoric acid) and glacial acetic acid. The mixture was heated on boiling water bath at 100°C for 1h after which the reaction was terminated by placing the test tubes containing mixture on ice bath. After that the reaction mixture was shaken vigorously with 6ml toluene and kept for one hour at room temperature and chromatophore thus extracted in toluene phase was separated. Absorbance was measured at 520 nm using toluene as blank. Standard curve was prepared with graded concentration of DL-proline and the quantity of proline was calculated in the sample.

$$\text{Proline} \frac{\text{g}}{\text{tissue}} = \frac{\mu\text{g proline/ml} \times \text{ml toluene}}{115.5} \times \frac{5}{\text{g/sample}}$$

3.3.1.11 Radicle emergence (%)

50 seeds were placed in petri plates containing blotter paper and were kept in walk in seed germinator at 25 ± 2°C. After 96 hrs the seeds which showed 2 mm radicle emergence were counted and was calculated as per the method suggested by the (Chueauan *et al.*, 2023).

$$\text{Radicle emergence (\%)} = \frac{\text{No. of seeds with a radicle length more than 2 mm}}{\text{Total no of seeds planted}} \times 100$$

3.3.2 Field evaluation of effective cold plasma treatments (20kV for 15 min) on vegetative growth and yield parameters during *Rabi*, 2023-24 .

3.3.2.1 Germination (%)

The germination percentage was calculated as mentioned in 3.3.1.1.

3.3.2.2 Chilling injury (%)

Chilling injury % was calculated using the 0 to 9 scale given by International Rice Research Institute (IRRI). However in the current study chilling injury was not observed during crop growth period.

3.3.2.3 Per cent Disease Index (PDI) of blast

The per cent disease index (PDI) of blast was calculated using standard formula given by Gopi *et al.*(2016).

$$\text{PDI} = \frac{\text{Sum of individual rating}}{\text{Number of plants examined} \times \text{Max disease grades in the scales}} \times 100$$

However, the incidence of blast was not observed during crop growth period in the current study.

3.3.2.4 Dry weight of shoot and root at 15, 30 and 45 days (g)

The rice plants were up rooted from nursery at 15 days, 30 days while, 45 days plants were uprooted from main field to calculate dry weight of root and shoot. The roots and shoots from the up rooted plants were separated and placed in paper bags and kept for drying in hot air oven at temperatures 100°C for 24 hours. The dry weight of root and shoot were weighed using precision weighing balance and expressed in grams.

3.3.2.5 Chlorophyll content of leaf

Readings were taken on upper most flag leaf of the rice plants by SPAD meter during heading stage.

3.3.2.6 Days to 50% flowering

The number of days taken from sowing to days when primary panicles in 50 per cent plants were emerged in each plot and in each replication was recorded as days to 50 % flowering.

3.3.2.7 Days to maturity

The number of days taken from the date of sowing up to more than 90% of plants in the plot from treatment reached maturity was recorded as days to maturity.

3.3.2.8 Number of tillers hill⁻¹

Ten randomly selected hills from each variety in each plot were selected and number of tillers per each hill were counted and the average was calculated.

3.3.2.9 Number of productive tillers per m²

The square was put at five randomly selected places in the plot and number of productive tillers present in each square were counted and the average was calculated by taking the number of productive tillers per m².

3.3.2.10 Plant height (cm)

Plant height was measured in centimeters from ground level to the tip of the panicle in each hill at the time of harvest.

3.3.2.11 Seed yield hill⁻¹ (g)

Mature panicles were collected from five randomly selected plants in each replication of variety and were sun dried, threshed and grain yield was calculated. Grain were weighed on precision balance, and averaged to estimate grain yield per plant and expressed in grams.

3.3.2.12 Seed yield (t/ha)

All the panicles from the plot were collected from each replication and were sundried threshed and seed yield was calculated. Seeds were weighed on the electronic balance to estimate seed yield per plot and was extrapolated to hectare and expressed in tons.

3.3.2.13 1000 seed weight (g)

One thousand well filled grains were counted from each variety per replication and weighed on the electronic top pan balance (precision of 0.001g) after adequate drying of grains to 13 per cent moisture.

3.3.3. Evaluation of seed quality parameters of paddy seed harvested from cold plasma treatments.

3.3.3.1 Germination per cent

The germination per cent was calculated as mentioned in 3.3.1.1.

3.3.3.2 Seedling dry weight(mg)

The seedling dry weight was calculated as mentioned in 3.3.1.2.

3.3.3.3 Seedling Vigour Index II

The seedling vigour index II was calculated as mentioned in 3.3.1.3

3.3.3.4 Radicle emergence (%)

The Radicle emergence (%) was calculated as mentioned in 3.3.1.11

3.3.3.5 Speed of germination

The speed of germination was calculated as mentioned in 3.3.1.5

3.3.3.6 Moisture content (%)

The moisture content was determined by the hot air oven method as per ISTA (2022) for the seed samples. Five grams of paddy sample was taken and grounded finely and placed in aluminium box from each treatment in three replicates. The moisture tins were placed in hot air oven and dried at 130⁰C for 2 hours. After 2 hrs, the samples were removed from the hot air oven, immediately kept in desiccators for cooling and later weighed. The moisture per cent of the seed sample was calculated by using the formula given below.

$$\text{Moisture per cent (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,

W1 - weight of empty container along with lid (g)

W2- Weight of the grounded seed sample before drying (g) kept in container along with the lid.

W3- Weight of the grounded seed sample after drying (g) kept in container along with the lid.

3.3.3.7 Electrical conductivity of seed leachates ($\mu\text{S cm}^{-1}\text{g}^{-1}$)

Electrical conductivity test was performed as per ISTA (2022) procedures by randomly taking fifty seeds from each treatment and weighed using precision balance. 50 seeds were kept in 250 ml flask and 250 ml of distilled water was added. To avoid contamination the flasks were covered with aluminium foil and kept at 20⁰C temperature for 1 day. After one day, the soaked seeds were removed with the help of nylon sieve and water was transferred into another flask. The conductivity of the deionized water which

was taken as control was measured with the help of electrical conductivity meter. The EC readings of the water in which seed were soaked was measured and the conductivity per gram of the seed was expressed in micro-siemens per cm per gram of seed. The conductance was calculated using the formula given below

$$\text{Electrical conductivity} = \frac{\text{Conductivity reading} - \text{control reading}}{\text{Weight of replicate (g)}}$$

3.4 Statistical analysis

The data recorded in all the experiments were analyzed statistically using Completely Randomized Design as per the protocols suggested by Panse and Sukhatme (1985) by maintaining four replications. In case of interaction treatments, two factorial CRD and Factorial RBD was used for analysing the data. Wherever necessary, the data was transformed using angular transformations.

ONE WAY ANOVA

Source of variation	Degrees of freedom (df)	Sum of squares	Mean sum of squares	F. cal value
Replication	r - 1	RSS	RMS=RSS/ (r-1)	RMS/ErMS
Treatments	t-1	TrSS	TrMS=TrSS/ (t-1)	TrMS/ErMS
Error	(r-1) (t-1)	ErSS	ErMS= ErSS/ r-1) (t-1)	
Total	(rt-1)	TSS		

TWO WAY ANOVA

Source of variation	Degrees of freedom (df)	Sum of squares	Mean sum of squares	F. cal value
Replication	r - 1	RSS	RMS = RSS / (r-1)	RMS/ErMS
Factor A	m-1	ASS	AMS = ASS / (m-1)	AMS / ErMS
Factor B	n-1	BSS	BMS = BSS / (n-1)	BMS/ ErMS
Interaction (A x B)	(m-1) (n-1)	ABSS	ABMS = ABSS/(m-1) (n-1)	ABMS / ErMS
Error	(r-1) (mn-1)	ErSS	ErMS = ErSS / (r-1) (mn-1)	
Total	(rmn-1)	TSS		

CHAPTER IV

RESULTS AND DISCUSSION

.CHAPTER IV

RESULTS AND DISCUSSION

Rice (*Oryza sativa* L.) is a major staple food for more than 4 billion people in world. The rice being originated in subtropical regions it experiences the negative effects from cold stress in winter. In Telangana state being the highest rice cultivated state in India during winter the rice crop is severely affected with cold stress and major loss is experienced due to cold stress. The present study entitled “Studies on cold plasma treatments for vegetative cold tolerance in *Rabi* rice (*Oryza sativa* L.)” was taken up to study the effects of cold plasma treatments against cold tolerance in paddy during *Rabi*, 2023-24. The cold plasma treatments for the rice variety RNR 15048 were carried out at MPMI-Quality Control Laboratory, PJTAU and seed quality parameters and biochemical analysis were carried out . After standardization work the effective cold plasma treatment was imposed on three paddy varieties *viz.*, RNR15048, JGL24423 and Tellahamsa to carry out field studies, for recording data on yield contributing characters while seed quality parameters of the harvested seed was studied under lab conditions. The results of the experiments conducted in the present investigation are discussed in detail under the following sections.

4.1 OBJECTIVE 1: STANDARDIZATION OF PROTOCOLS FOR COLD PLASMA TREATMENTS FOR SEED QUALITY UNDER *IN VITRO*

4.1.1 Analysis of Variance (ANOVA)

The normal and sprouted seed of paddy were subjected to plasma treatments and the seed quality parameters were recorded. The analysis of variance for the parameters *viz.*, germination per cent at 25°C, germination per cent at 15°C, seedling dry weight at 25°C, seedling dry weight at 15°C, seedling vigour index-II at 25°C and seedling vigour index - II at 15°C were presented in Table 4.1. The treatment mean sum of squares for type of seeds (S) and plasma treatments (P) showed significant variation for all the seed quality parameters mentioned above. The interaction mean sum of squares showed significant variation for per cent germination at 15°C, seedling vigour index-II at 15°C and seedling dry weight at 15°C revealed non-significant differences for per cent germination at 25°C, seedling dry weight at 25°C and seedling vigour index-II at 25°C.

The analysis of variance for the parameters per cent seed infection at 25°C, per cent seed infection at 15°C, speed of germination at 25°C, speed of germination at

15°C and cold tolerance index (%) were presented in Table 4.2. The treatment mean sum of squares for type of seeds (S) showed significant variation for all the seed quality parameters except for per cent seed infection at 15°C and speed of germination at 25°C the treatment mean sum of squares for plasma treatments (P) showed significant variation for all the above mentioned parameters. The interaction mean sum of squares showed significant variation for per cent seed infection at 25°C, per cent seed infection at 15°C and cold tolerance index (%) whereas, non-significant difference was observed for speed of germination at 25°C and 15°C.

The analysis of variance for the biochemical parameters superoxide dismutase activity at 25°C, superoxide dismutase activity at 15°C, peroxidase activity at 25°C, peroxidase activity at 15°C, catalase activity at 25°C, catalase activity at 15°C, proline content at 25°C and proline content at 15°C were presented in (Table 4.3). The treatment mean sum of squares for type of seeds (S) and plasma treatments (P) showed non-significant variation for all the biochemical parameters except peroxidase activity at 25°C and peroxidase activity at 15°C. The interaction mean sum of squares showed non-significant variation for all the parameters. The analysis of variance for the parameters radicle emergence at 25°C and radicle emergence at 15°C were presented in Table 4.4 and showed non-significant difference for radicle emergence at 25°C while significance was observed for radicle emergence at 15°C.

4.1.2 Seed Quality Parameters

The results of the seed quality parameters *viz.*, germination per cent at 25°C, germination per cent at 15°C, seedling dry weight at 25°C, seedling dry weight at 15°C, seedling vigour index-II at 25°C, seedling vigour index-II at 15°C, per cent seed infection at 25°C, per cent seed infection at 15°C, speed of germination at 25°C, speed of germination at 15°C, cold tolerance index (%), superoxide dismutase activity at 25°C, superoxide dismutase activity at 15°C, peroxidase activity at 25°C, peroxidase activity at 15°C, catalase activity at 25°C, catalase activity at 15°C, proline content at 25°C, proline content at 15°C, radicle emergence at 25°C, radicle emergence at 15°C were discussed.

Table 4.1 Analysis of variance for seed physiological parameters viz., percent germination at 25°C and 15°C, seedling dry weight (mg) at 25°C and 15°C (mg), seedling vigour index-II at 25°C and 15°C in cold plasma treated paddy (RNR 15048)

Source of variance	d.f	Germination per cent at 25°C	Germination per cent at 15°C	Seedling dry weight at 25 °C (mg)	Seedling dry weight at 15°C (mg)	Seedling vigour index-II at 25°C	Seedling vigour index -II at 15°C
S	1	925.750 ***	4152.893 ***	8.036 ***	1.509 **	139308 ***	79310.570 ***
P	6	37.810 *	136.143 ***	0.357 *	1.135 ***	5151 **	7599.465 ***
S*P	6	15.667	81.143 ***	0.034	0.436 *	361.891	2111.411 ***
Error (P)	14	11.821	7.107	0.119	3.011	1080.966	388.247
Total	27	52.300	205.782	0.446	11.141	6945.263	8.684

NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively.

S: Type of seeds, **P:** Plasma treatments

Table 4.2 Analysis of variance for seed physiological parameters viz., per cent seed infection at 25°C and 15°C, speed of germination at 25°C and 15°C and cold tolerance index (%) in paddy (RNR15048) after cold plasma treatments

Source of variance	d.f	Per cent seed infection at 25°C	Per cent seed infection at 15°C	Speed of germination at 25°C	Speed of germination at 15°C	Cold tolerance index (%)
S	1	234.321 ***	1.286	0.401	36.161 ***	2196.572 ***
P	6	4.286 *	5.810 **	71.067 ***	73.897 ***	141.536 ***
S*P	6	2.905 *	2.619 *	0.285	0.902	141.488 ***
Error (P)	14	0.964	0.857	1.287	0.422	13.429
Total	27	10.776	2.365	16.538	18.180	151.212

NOTE: *, **, * represents significance at 5%, 1% and 0.1 % probability levels, respectively.**

S: Type of seeds, P: Plasma treatments

Table 4.3 Analysis of variance for biochemical parameters viz., superoxide dismutase activity (units/mg) at 25°C and 15°C , peroxidase activity (units/ min/mg) at 25°C and 15°C, Catalase (n moles/ min/ g) at 25°C and 15°C, Proline content (µmoles of proline/g) at 25°C and 15°C in paddy (RNR15048) after cold plasma treatments

Source of variance	d. f	Superoxide dismutase activity (units/mg) at 25°C	Superoxide dismutase activity (units/ mg) at 15°C	Peroxidase activity (units/ min/mg) at 25°C	Peroxidase activity (unit/ min/ mg) at 15°C	Catalase (n moles/ min/g) at 25°C	Catalase (n moles/ min/g) at 15°C	Proline content (µ moles of proline/ g) at 25°C	Proline content (µ moles of proline/g) at 15°C
S	1	0.003	17.991	0.003*	0.030*	1287.929	11606.260	0.000	0.001
P	6	6.209	7.397	0.001	0.006	300.158	2708.208	0.000	0.005
S*P	6	1.356	6.993	0.000	0.001	440.846	3956.167	0.000	0.003
Error (P)	14	3.637	5.485	0.001	0.006	441.845	3978.322	0.000	0.003
Total	27	3.567	6.708	0.040	0.006	441.473	3973.667	0.000	0.003

NOTE: *, **, * represents significance at 5%, 1% and 0.1 % probability levels, respectively.**

S: Type of seeds, P: Plasma treatments

Table 4.4 Analysis of variance for radicle emergence at 25°C and 15°C in paddy (RNR15048) after cold plasma treatments

Source of variance	d.f	Radicle emergence at 25 °C	Radicle emergence at 15°C
S	6	17.905	547.810***
Error (S)	7	29.714	30.857
Total	13	24.264	269.451

NOTE: *, **, * represents significance at 5%, 1% and 0.1 % probability levels, respectively.**

S: Type of seeds

4.1.2.1 Germination per cent at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on germination per cent at 25°C in paddy were presented in Table 4.5 and the data revealed the presence of significant difference between type of seeds (S) *i.e.*, normal seed and sprouted seed which ranged from 76.0 to 88.0 per cent respectively. Among plasma treatments (P) the germination per cent at 25°C ranged from 78 to 87 per cent. The plasma treatment P3 (20 kV for 15 min) showed highest germination per cent at 25°C (87%) while the plasma treatments P1 (10 kV for 15min), P2 (10 kV for 20 min), P5 (30 kV for 15 min) and P7 (control) were on par with treatment P3. The plasma treatment P4 (20 kV for 20 min) and P6 (30 kV for 20 min) showed significant differences with P3 and the lowest germination per cent at 25°C was recorded in P4 (20 kV for 20 min). The interaction effects between type of seed (S) and cold plasma treatments (P) for germination per cent at 25°C ranged from 69 to 91 per cent. The interaction effects between S1P3 (normal seed treated with 20 kV for 15 min) showed high germination per cent of 91, while the interactions S1P1, S1P2, S1P4, S1P5, S1P6 and S1P7 were on par with S1P3. The interaction effects of sprouted seed S2P1, S2P2, S2P3, S2P4, S2P5, S2P6 and S2P7 showed significant differences with S1P3 and the lowest germination per cent at 25°C was observed in S2P6 (sprouted seed treated with 30 kV plasma for 20 min). The results revealed that normal seed treated with cold plasma (20 kV for 15 min) had recorded more per cent germination at 25°C compared to the control (Plate 4.1,4.2). High per cent difference for germination at 25°C over untreated control was recorded in S1P3 and S2P3 (Fig 4.1). The increase in germination per cent at 25°C in cold plasma treatments may be due to the production RONS which increased germination per cent by weakening the seed coat. The H₂O₂ and NO₂ produced due to plasma treatment might have decreased the ABA levels; limiting dormancy and promoting GA biosynthesis for germination. The results are in accordance with the Rongsangchaicharean *et al.* (2022) who reported increase in germination per cent after plasma treatment in paddy seeds.

4.1.2.2 Germination per cent at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on germination per cent at 15°C in paddy are presented in Table 4.5. The data on germination per cent at 15°C revealed the presence of significant differences between normal and sprouted seed which ranged from 58 to 82 per cent. Normal seed recorded higher germination per cent at 15°C than sprouted seed. Among cold plasma treatments (P) germination per cent

at 15°C ranged from 62 to 78 per cent. Among the treatment (20 kV for 15 min) recorded high germination per cent at 15°C *i.e.*, 78 per cent, while the treatments P1 (10 kV for 15min) and P2 (10 kV for 20min) were on par with P3 and the treatments P4 (20 kV for 20 min), P5 (30 kV for 15 min), P6 (30 kV for 20 min) and P7 (control) showed significant difference with P3 and low germination per cent at 15°C was observed in P7 (control). The interaction effects between type of seed (S) and cold plasma treatments (P) for germination per cent at 15°C ranged from 41 to 87 per cent. The interaction effect of S1P3 (normal seed treated with 20 kV plasma for 15 min) recorded high germination per cent at 15°C *i.e.*, 87 per cent, the interactions S1P1, S1P2 and S1P7 were on par with S1P3 while the interaction effects S1P4, S1P5, S1P6, S2P1, S2P2, S2P3, S2P4, S2P5, S2P6 and S2P7 showed significant differences with S1P3. The low germination per cent at 15°C was observed in S2P7. The results revealed that normal seed treated with cold plasma had recorded more germination per cent at 15°C. High per cent difference for germination at 15°C over untreated control was recorded in S1P3 and S2P3 (Fig 4.1). The per cent germination at 25°C was higher than the per cent germination at 15°C and the lowest germination values were recorded at 15°C due to the imposition of cold stress conditions. Under stress conditions H₂O₂ will activate various defense mechanisms through the redox signaling pathway. The results are in compliance with the Gierczik *et al.* (2020) wherein plasma activated water improved per cent germination compared to untreated control under low temperature conditions in barley. Low germination in seed treated with cold stress may be due to reduction in GA levels by the activation of GA catabolic genes and SLR1a gene encoding the della protein as reported by Tang *et al.*, 2022.

4.1.2.3 Seedling dry weight at 25°C (mg)

The influence of type of seed (S) and cold plasma treatments (P) on seedling dry weight at 25°C in paddy are presented in Table 4.5. The data on seedling dry weight at 25°C revealed the presence of significant differences between normal seed and sprouted seed and the values ranged from 4.03 mg to 5.10 mg. Normal seed treated with plasma showed more seedling dry weight at 25°C than sprouted seed treated with plasma. Among cold plasma treatments (P) seedling dry weight at 25°C ranged from 4.30 mg to 5.20 mg. The plasma treatment P3 (20 kV for 15 min) recorded high seedling dry weight at 25°C *i.e.*, 5.20 mg, the treatments P1 (10kV for 15 min), P2 (10 kV for 20 min), P4 (20 kV 20 for min), P5 (30 kV for 15 min), P6 (30 kV for 20 min) and P7 (control) showed significant difference with P3 and lowest seedling dry weight at 25°C was observed in P4 and P7. The interaction effects for seedling dry weight at 25°C between type of seed

Table. 4.5 Effect of type of seed (S) and cold plasma treatments (P) on germination (%) at 25⁰C and 15⁰C and seedling dry weight at 25⁰C and 15⁰C in paddy variety RNR15048

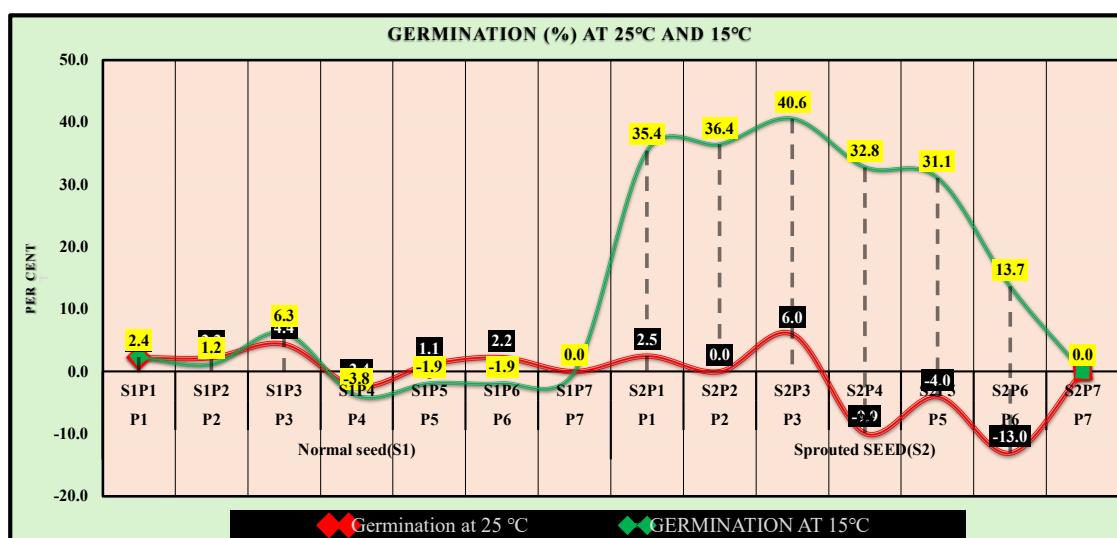
Treatments	Germination (%) at 25 ⁰ C	Germination (%) at 15 ⁰ C	Seedling dry weight (mg) at 25 ⁰ C	Seedling dry weight (mg) at 15 ⁰ C
TYPE OF SEED (S)				
S1: Normal seed	88 (70)	82 (65)	5.10	3.24
S2: Sprouted seed	76 (61)	58 (50)	4.03	2.78
CD (5%)	2.787	2.161	0.279	0.271
SE (m)	0.918	0.712	0.092	0.089
COLD PLASMA TREATMENTS (P)				
P1: 10 kV 15 Min	84 (66)	74 (59)	4.47	3.05
P2: 10 kV 20 Min	83 (66)	74 (59)	4.45	2.50
P3: 20 kV 15 Min	87 (69)	78 (62)	5.20	3.95
P4: 20 kV 20 Min	78 (62)	70 (57)	4.30	2.75
P5: 30 kV 15 Min	82 (65)	70 (57)	4.52	2.45
P6: 30 kV 20 Min	79 (63)	64 (53)	4.62	2.95
P7: Control	82 (65)	62 (52)	4.37	3.42
CD (5%)	5.214	4.043	0.522	0.508
SE(m)	1.719	1.333	0.172	0.167
INTERACTIONS – TYPE OF SEED (S) X COLD PLASMA TREATMENTS (P)				
S1P1	89 (71)	84 (66)	5.15	3.05
S1P2	89 (71)	83 (66)	4.95	2.90
S1P3	91 (72)	87 (69)	5.85	3.70
S1P4	85 (67)	79 (63)	4.80	3.10
S1P5	88 (70)	80 (64)	5.05	3.20
S1P6	89 (71)	80 (64)	5.10	3.30
S1P7	87 (69)	82 (64)	4.80	3.45
S2P1	80 (63)	63 (53)	3.80	3.05
S2P2	78 (62)	64 (53)	3.95	2.10
S2P3	83 (66)	69 (57)	4.55	4.20
S2P4	71 (57)	61 (51)	3.80	2.40
S2P5	75 (60)	59 (50)	4.00	1.70
S2P6	69 (56)	47 (43)	4.15	2.60
S2P7	78 (62)	41 (40)	3.95	3.40
GRAND MEAN	82.17	70.17	4.56	3.01
CD	2.43	1.885	0.243	0.231
SE(m)	7.374	5.717	0.738	0.719
CV (%)	4.18	3.79	7.54	10.14



Plate 4.1: Per cent germination of RNR15048 treated with cold plasma treatment (20 kV 15 min) under normal conditions



Plate 4.2: Per cent germination of control under normal conditions



4.1 Per cent change over control for germination per cent at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10kV for 20 min, P3: 20kV for 15 min, P4: 20kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

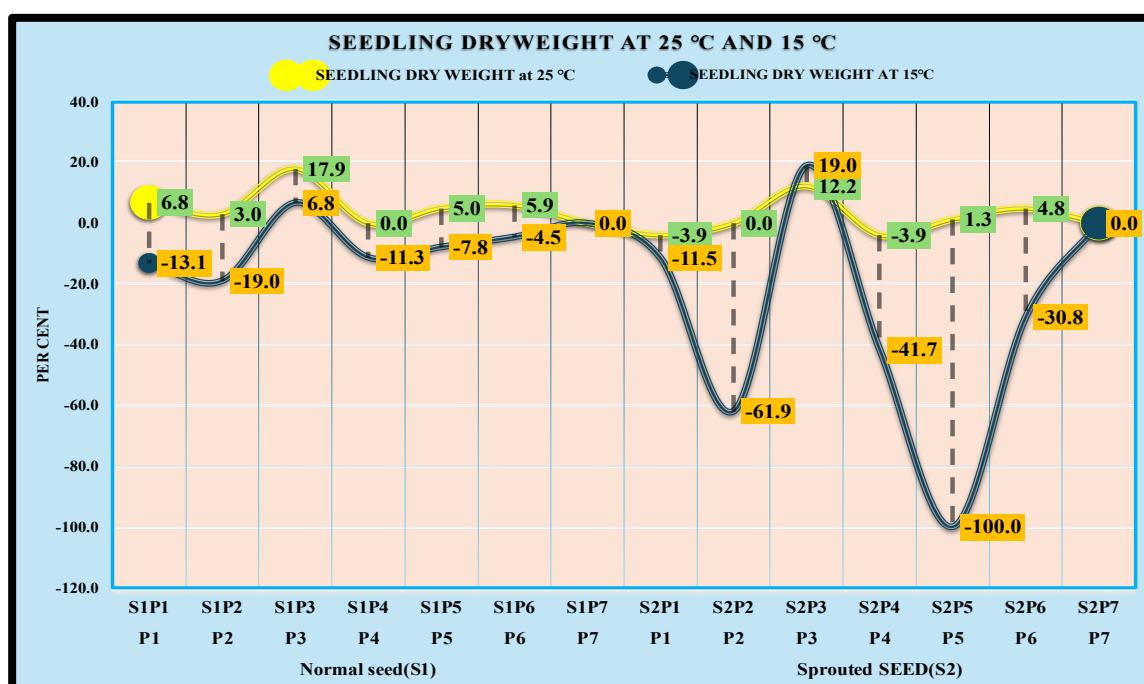


Figure 4.2 Per cent change over control for seedling dry weight at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10kV for 20 min, P3: 20kV for 15 min, P4: 20kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

(S) and cold plasma treatments (P) ranged from 3.80 mg to 5.85 mg. The interaction treatment S1P3 (normal seed treated with 20 kV plasma for 15min) showed high seedling dry weight at 25°C *i.e.*, 5.85 mg while all interaction effects showed significant difference except S1P1 (normal seed treated with 10 kV for 15 min) and S1P6 (normal seed treated with 30 kV for 20 min) which recorded non-significant difference with S1P3. The per cent difference over control was high in normal seed treated with 20 kV plasma for 15 minutes (Fig 4.2). The results revealed that normal seed treated with 20 kV 15 min recorded more seedling dry weights at 25°C, the results are in agreement with Harish *et al.* (2024) where in plasma treated plants showed more seedling dry weight in mustard.

4.1.2.4 Seedling dry weight at 15°C (mg)

The influence of type of seed (S) and cold plasma treatments (P) on seedling dry weight at 15°C in paddy are presented in Table 4.5. The data on seedling dry weight at 15°C revealed the presence of significant differences and it ranged from 2.78 mg to 3.24 mg. The cold plasma treatments (P) for seedling dry weight at 15°C ranged from 2.45 to 3.95 mg. The treatment P3 (20 kV for 15 min) exhibited high seedling dry weight at 15°C *i.e.*, 3.95 mg and the treatments P1 (10 kV for 15 min), P2 (10 kV for 20 min), P4 (20 kV for 20 min), P5 (30 kV for 15 min) and P6 (30 kV for 20 min) and P7 (control) recorded significant differences with P3. The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of significant differences and the seedling dry weight at 15°C ranged from 1.70 mg to 4.20 mg. The interaction effect S2P3 (sprouted seed treated with 20 kV plasma for 15 min) recorded high seedling dry weight at 15°C *i.e.*, 4.2 mg, and it showed significant difference with all other interaction effects except with S1P3 (normal seed treated with 20 kV for 15 min plasma) and low seedling dry weight at 15°C was observed in S2P5. The seedling dry weight at 25°C was higher than the seedling dry weight at 15°C and the low seedling dry weight values were recorded at 15°C due to imposition of cold stress conditions. The per cent difference over control was high in sprouted seed treated with 20 kV plasma for 15 minutes (Fig 4.2).

4.1.2.5 Seedling vigour index- II at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on seedling vigour index-II at 25°C in paddy are presented in Table 4.6. The data on seedling vigour index-II at 25°C revealed the presence of significant differences between normal seed and sprouted seed and it ranged from 308 to 449. Normal seed (S1) recorded high seedling vigour index-II at 25°C *i.e.*, 449. Among cold plasma treatments (P) seedling

vigour index-II at 25°C ranged from 339 to 454. The treatment P3 (20 kV for 15 min) recorded high seedling vigour index-II at 25°C *i.e.*, 454, the treatments P1, P2, P4, P5, P6 and P7 were significantly different with P3. The low seedling vigour index-II at 25°C was observed in P4 (20 kV for 20 min). The interaction effects between types of seed (S) and cold plasma treatments (P) revealed significant differences and the seedling vigour index ranged from 270 mg to 532 mg. The interaction effect of normal seed treated with 20 kV plasma for 15 min (S1P3) showed high seedling vigour index-II at 25°C (532), and the interaction effects S2P1, S2P2, S2P3, S2P4, S2P5, S2P6, S2P7, S1P1, S1P2, S1P4, S1P5, S1P6 and S1P7 with S1P3 recorded significant difference. Low seedling vigour index-II at 25°C was observed in S2P4. The per cent difference over control was high in normal seed treated with 20 kV plasma for 15 minutes (Fig 4.3). The improvement in vigour may be due to modulation of NO levels through NO₂⁻, NO₃⁻ in seeds of cold plasma treatments and ABA levels are reduced while GA levels are increased in plasma treated seeds which result in seed vigour improvement. The above results are in confirmation with Ling *et al.* (2014) who reported the seedling vigour improvement with cold plasma treatments in soybean.

4.1.2.6 Seedling vigour index-II at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on seedling vigour index-II at 15°C in paddy are presented in Table 4.6. The data on seedling vigour index-II at 15°C revealed the presence of significant differences between normal seed and sprouted seed and values ranged from 161 to 267. Among cold plasma treatments (P) seedling vigour index-II at 15°C ranged from 179 to 306. The treatment P3 (20 kV for 15 min) recorded high seedling vigour index-II at 15°C *i.e.*, 306 and the treatments P1, P2, P4, P5, P6 and P7 showed significant different with P3. The interaction effects between types of seed (S) and cold plasma treatments (P) revealed the presence of significant differences which ranged from 101 to 323. The interaction between normal seed and 20 kV plasma treatment for 15 min (S1P3) showed highest seedling vigour index-II at 15°C *i.e.*, 323, the treatments S2P1, S2P2, S2P3, S2P4, S2P5, S2P6 and S2P7, S1P2, S1P4, S1P5, S1P6 and S1P7 recorded significant difference with S1P3. Low seedling vigour index-II at 15°C was observed in S2P5. The seedling vigour index at 25°C was higher than the seedling vigour index at 15°C and the low seedling vigour index values were recorded at 15°C due to imposition of cold stress conditions. The per cent difference over control was high in sprouted seed treated with 20 kV plasma for 15 minutes (Fig 4.3)

4.1.2.7 Per cent seed infection at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on per cent seed infection at 25°C in paddy are presented in Table 4.6 and (Fig 4.4). The per cent seed infection at 25°C for normal seed (S1) and sprouted seed (S2) showed significant difference and seed infection per cent ranged from 2.07 to 7.86 per cent. Low seed infection per cent at 25°C was recorded in normal seed (2.07%). Cold plasma treatments (P) revealed the presence of significant differences for seed infection and the infection percent ranged from 3.00 to 5.75 per cent. The treatment P3 (20 kV for 15 min) showed low per cent seed infection at 25°C (3.00%) and the treatments P1 and P2 are on par with P3. However, the treatments P4, P5, P6 and P7 are significantly different with P3. High per cent seed infection at 25°C was observed in treatments P4 (20 kV for 20 min), P5 (30 kV for 15 min) and P6 (30kV for 20 min). The interaction effects between types of seed (S) and cold plasma treatments (P) revealed the presence of significant differences for per cent seed infection at 25°C and infection per cent ranged from 1.50 to 9.50%. The interaction effect S1P3 (normal seed treated with 20 kV plasma for 15 min), S1P1 (normal seed treated with 10 kV plasma for 15 min) showed low per cent seed infection at 25°C (1.50%) and interaction effects S1P4 and S1P6 were on par with S1P3 and S1P1. All the other interactions showed significant difference with S1P3 and S1P1. High per cent seed infection at 25°C was observed in S2P6 and S2P4. The results revealed that normal seed treated with 20 kV plasma for 15min recorded less per cent seed infection at 25°C and this may be due to increase in the accumulation of H₂O₂ in plasma treated seed which resulted in reduction of disease severity. The results are in concurrence with the Nedyalkova *et al.* (2019) wherein plasma treated seeds recorded less seed infection compared to untreated control in wheat seeds against *Fusarium monacearum*.

4.1.2.8 Per cent seed infection at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on per cent seed infection at 15°C in paddy are presented in Table 4.6 and (Fig 4.4). There is no significant difference between normal seed (S1) and sprouted seed (S2) for per cent seed infection at 15°C and the mean values ranged from 3.86 to 4.29%. Low seed infection at 15°C was recorded in sprouted seed (3.86%). Cold plasma treatments (P) revealed the presence of significant differences for per cent seed infection at 15°C and infection per cent ranged from 2.00 to 5.25%. The treatment P3 (20 kV for 15 min) showed low per cent seed infection at 15°C (2.00%) and the treatment P1 was on par with P3. The treatments P2, P4, P5, P6 and P7 were significantly different with P3. High per cent seed infection at

15°C was recorded in P6. The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of significant differences and the seed infection per cent ranged from 1.50 to 7.00 per cent. The interaction effect S1P3 (normal seed treated with 20 kV plasma for 15 min) showed low per cent seed infection at 15°C (1.50) and the interactions S1P1, S2P1, S2P2, S2P3, S2P6, S1P2, S1P4, S1P5, S1P6, S1P7, S2P4, S2P5 and S2P7 revealed significant difference with S1P3. High per cent seed infection at 15°C was recorded in (S1P6). The per cent seed infection at 25°C was higher than at 15°C and low per cent seed infection at 15°C was recorded which might be due to expose of seed to cold stress conditions might have reduced the survival of disease inoculum at such low temperatures . According to Ma *et al.* (2022) jasmonic acid dependent signaling pathways are critical for improving cold tolerance and to provide defense against fungal infections.

4.1.2.9 Speed of germination at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on speed of germination at 25°C in paddy are presented in Table 4.7. The data on speed of germination at 25°C revealed non-significant differences between normal seed and sprouted seed and the speed of germination ranged from 14.8 to 15.1. Cold plasma treatments (P) revealed significant differences for speed of germination at 25°C and speed of germination ranged from 9.83 to 22.55. The plasma treatment P3 (20 kV for 15 min) recorded high speed of germination at 25°C (22.55) and the treatments P1 (10 kV for 15 min), P2 (10 kV for 20 min), P4 (20 kV for 20 min), P5 (30 kV for 15 min), P6 (30 kV for 20 min) and P7 (control) were significantly different with P3. Low speed of germination at 25°C was recorded in treatment P4. Speed of germination at 25°C in the interaction effects between type of seed (S) and cold plasma treatments (P) ranged from 9.49 to 22.86. The interaction effect S1P3 recorded high speed of germination at 25°C *i.e.*, 22.86, while the interaction effects S2P1, S2P2, S2P4, S2P5, S2P6, S2P7, S1P1, S1P2, S1P4, S1P5, S1P6 and S1P7 recorded significant difference with S1P3 except S2P3 which was on par with S1P3. Low speed of germination at 25°C was recorded in S1P4. The per cent difference over control was highest in normal seed treated with 20 kV plasma for 15 minutes (Fig 4.5). The results represented that normal seed treated with 20 kV plasma for 15 min recorded more speed of germination at 25°C than

Table 4.6 Effect of type of seed (S) and cold plasma treatments (P) on seedling vigour index -II at 25°C and 15°C and per cent seed infection at 25°C and 15°C in paddy variety RNR15048

Treatments	Seedling vigour index - II at 25°C	Seedling vigour index – II at 15°C	Per cent seed infection at 25°C	Per cent seed infection at 15°C
TYPE OF SEED (S)				
S1:Normal seed	449	267	2.07 (8.2)	4.29 (11.9)
S2: Sprouted seed	308	161	7.86 (16.2)	3.86 (11.3)
CD (5%)	26.650	15.970	0.790	NS
SE(m)	8.787	5.266	0.262	0.247
COLD PLASMA TREATMENTS (P)				
P1: 10 kV 15 Min	379	224	4.50 (12.24)	3.00 (9.90)
P2: 10 kV 20 Min	373	188	4.50 (12.24)	3.75 (11.16)
P3: 20 kV 15 Min	454	306	3.00 (9.97)	2.00 (8.13)
P4: 20 kV 20 Min	339	195	5.75 (13.87)	4.75 (12.58)
P5: 30 kV 15 Min	372	179	5.75 (13.87)	5.00 (12.92)
P6: 30 kV 20 Min	369	193	5.75 (13.87)	5.25 (13.24)
P7: Control	362	211	5.50 (13.56)	4.75 (12.58)
CD (5%)	49.862	29.882	1.489	1.404
SE(m)	16.439	9.851	0.491	0.463
INTERACTIONS – TYPE OF SEED (S) X COLD PLASMA TREATMENTS (P)				
S1P1	456	256	1.50 (7.03)	3.00 (9.97)
S1P2	438	240	2.50 (9.09)	4.00 (11.53)
S1P3	532	323	1.50 (7.03)	1.50 (7.03)
S1P4	408	245	2.00 (8.13)	4.00 (11.53)
S1P5	442	257	2.50 (9.09)	5.50 (13.56)
S1P6	451	265	2.00 (8.13)	7.00 (15.34)
S1P7	415	282	2.50 (9.09)	5.00(12.92)
S2P1	303	194	7.50 (15.89)	3.00 (9.97)
S2P2	308	136	6.50 (14.77)	3.50 (10.7)
S2P3	376	290	4.50 (12.24)	2.50(9.09)
S2P4	270	146	9.50 (17.95)	5.50 (13.56)
S2P5	302	101	9.00 (17.45)	4.50 (12.24)
S2P6	287	120	9.50 (17.95)	3.50(10.78)
S2P7	308	139	8.50 (16.95)	4.50 (12.24)
GRAND MEAN	378	214	4.96	4.07
CD (5%)	23.248	13.932	0.694	0.654
SE(m)	70.51	42.26	2.10	1.98
CV (%)	8.68	9.20	9.78	9.73

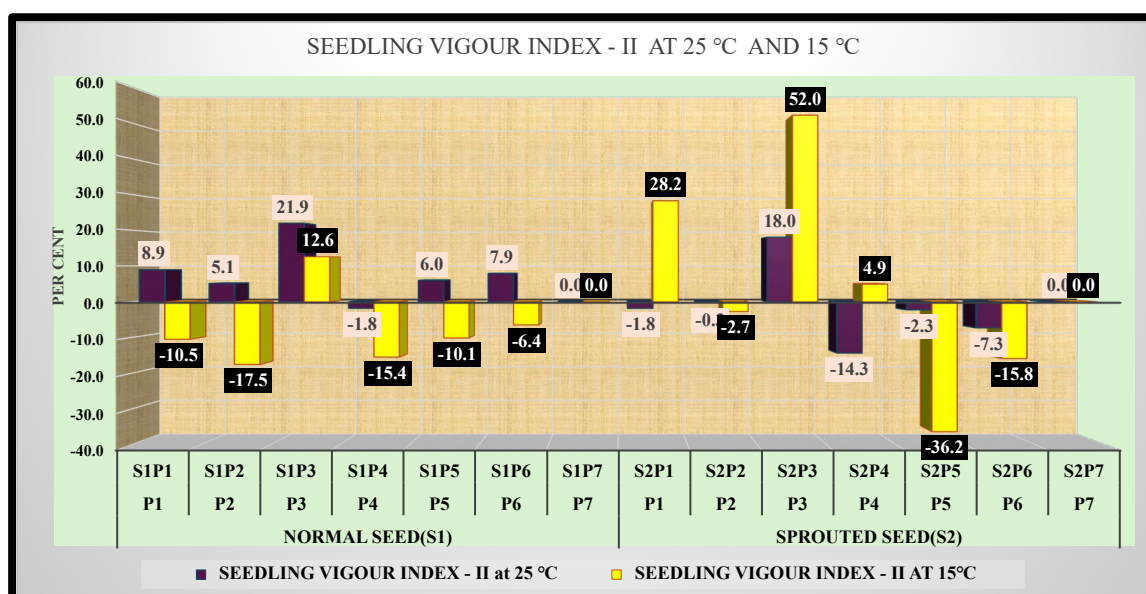


Figure 4.3. Per cent change over control for seedling vigour index -II at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
 P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min
 P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

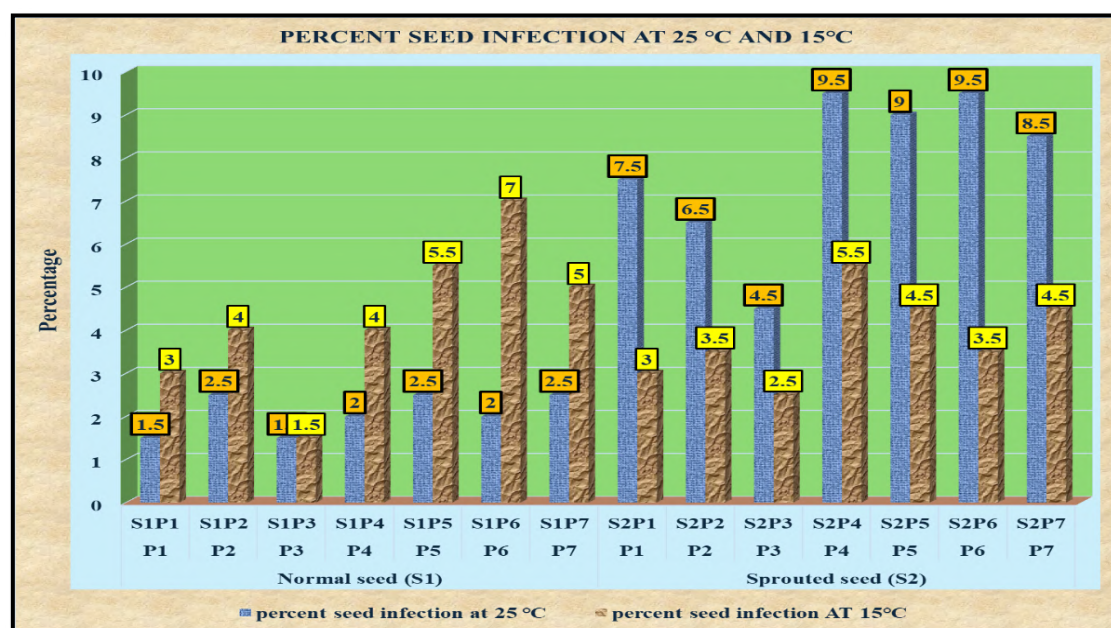


Figure 4.4 Per cent change over control for seed infection at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
 P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min
 P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

untreated control this may be due to H₂O₂ and NO₂ affected ABA levels, limiting dormancy and promoting GA biosynthesis for germination and the speed of germination was increased at 25°C. The above results are in accordance with the Gurgain *et al.* (2021) who reported that speed of germination was faster in plasma treated seed compared to untreated control in carrot.

4.1.2.10 Speed of germination at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on speed of germination at 15°C in paddy are presented in Table 4.7. The data on speed of germination at 15°C revealed the presence of significant differences between normal seed and sprouted seed and the speed of germination ranged from 9.22 to 11.50. Normal seed (S1) exhibited high speed of germination (11.50). The cold plasma treatments (P) revealed significant differences for speed of germination at 15°C and the values ranged from 3.34 to 15.53. The plasma treatment P3 (20 kV for 15 min) showed high speed of germination at 15°C (15.53), and the treatments P1, P2, P4, P5, P6 and P7 were significantly different with P3. Low speed of germination at 15°C was noticed in P5. Speed of germination at 15°C in the interaction effects between type of seed (S) and cold plasma treatments (P) ranged from 1.61 to 16.50. The interaction effect treatments S1P3 recorded high speed of germination at 15°C (16.50) and the interaction effects S2P1, S2P2, S2P4, S2P5, S2P6, S2P7, S1P2, S1P4, S1P5, S1P6 and S1P7 showed significant difference with S1P3 while S1P1 was on par with S1P3. Low speed of germination at 15°C was observed in S2P5. The per cent difference over control was high in sprouted seed treated with 20 kV plasma for 15 minutes as the sprouted seed are advanced in germination process compared to control under cold stress conditions (Fig 4.5)

4.1.2.11 Cold tolerance index (%)

The influence of type of seed (S) and cold plasma treatments (P) on cold tolerance index (%) in paddy are presented in Table 4.7. The data on cold tolerance index (%) revealed the presence of significant differences between normal seed and sprouted seed and per cent cold tolerance index was ranged from 76 to 94 per cent. Cold tolerance index for normal seed (S1) was higher than sprouted seed which is 94 per cent. The cold plasma treatments (P) revealed the presence of significant differences for cold tolerance index and the values ranged from 74 to 90 per cent. The treatment P3 (20 kV for 15 min) and P4 (20 kV for 20 min) showed high per cent cold tolerance index (90%) and the treatments P1, P2 and P5 were on par with P3 and P4, P6 and P7 are significantly

different. Low per cent cold tolerance index (%) was observed in control (P7). The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of significant differences and cold tolerance index ranged from 53 to 97 . The treatments S2P1, S2P2, S2P3, S2P4, S2P5, S2P6 and S2P7 were significantly different and S1P1, S1P2, S1P4, S1P5, S1P6 and S1P7 were on par with S1P3. Low cold tolerance index was observed in S2P7. The result revealed that cold tolerance index was higher in normal seed treated with plasma (20 kV for 15 min) while the per cent difference over control was high in sprouted seed treated with 20 kV plasma for 20 minutes (Fig 4.6).

4.1.2.12 Superoxide dismutase activity (unit/mg protein/min) at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on biochemical parameters *viz.*, superoxide dismutase activity in paddy presented in Table 4.8. The data on superoxide dismutase activity at 25°C revealed the presence of non-significant differences between normal seed and sprouted seed and the values ranged from 7.95 to 7.97 unit/mg protein/min. The cold plasma treatment (P) revealed the presence of non-significant differences for superoxide dismutase activity and it ranged from 5.3 to 9.0 unit/mg protein/min. The treatment P1 (10 kV for 15 min) showed low superoxide dismutase activity at 25°C (5.3 unit/mg protein/min). The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of non-significant differences and values ranged from 4.3 to 9.1 unit/mg protein/min and all the treatments were on par with each other with S2P7. High superoxide dismutase value was observed in sprouted seed untreated control (S2P7). The per cent difference over control for superoxide dismutase activity was negative in all the plasma treatments (Fig 4.7). Sandalio *et al.* (2001) reported that SOD catalyzes the dismutation of superoxide to hydrogen peroxide in maize. Slooten *et al.* (1995) reported that stimulation of SOD activity in response to stresses is possibly attributed to the *de novo* synthesis of the enzymatic protein in transgenic tobacco. Elevated oxidative stress causes increase in production of hydrogen peroxide which may inhibit the activity of Cu Zn SOD which is sensitive to hydrogen peroxide as reported by Sandalio *et al.* (2001) and they assumed that SOD will be the main enzyme which has a dominant role in quenching oxidative stress in maize seeds treated with low thermal plasma.

Table 4.7 Effect of type of seed (S) and cold plasma treatments (P) on speed of germination at 25⁰C and 15⁰C and cold tolerance index (%) at 25⁰C and 15⁰C in paddy variety RNR15048

Treatments	Speed of germination at 25 ⁰ C	Speed of germination at 15 ⁰ C	Cold tolerance index (%)
TYPE OF SEED (S)			
S1: Normal seed	15.10	11.50	94 (75)
S2: Sprouted seed	14.80	9.22	76 (61)
CD (5%)	0.919	0.527	2.970
SE(m)	0.303	0.174	0.980
COLD PLASMA TREATMENTS (P)			
P1: 10 kV 15 Min	15.60	13.85	87 (69)
P2: 10 kV 20 Min	15.33	12.49	88 (70)
P3: 20 kV 15 Min	22.55	15.53	90 (72)
P4: 20 kV 20 Min	9.83	6.38	90 (72)
P5: 30 kV 15 Min	10.38	3.34	86 (68)
P6: 30 kV 20 Min	15.03	9.43	80 (63)
P7: Control	15.98	11.47	74 (59)
CD (5%)	1.721	0.985	5.557
SE(m)	0.567	0.324	1.832
INTERACTIONS – TYPE OF SEED (S) X COLD PLASMA TREATMENTS (P)			
S1P1	16.06	15.11	95 (76)
S1P2	15.40	12.93	94 (75)
S1P3	22.86	16.50	97 (79)
S1P4	9.48	8.06	93 (75)
S1P5	10.33	5.07	92 (74)
S1P6	15.33	10.60	91 (73)
S1P7	16.10	12.21	95 (77)
S2P1	15.14	12.61	80 (63)
S2P2	15.28	12.07	83 (65)
S2P3	22.25	14.58	84 (66)
S2P4	10.17	4.70	86 (68)
S2P5	10.42	1.61	79 (62)
S2P6	14.74	8.27	69 (56)
S2P7	15.86	10.73	53 (53)
GRAND MEAN	14.96	10.35	85
CD (5%)	2.433	1.393	7.859
SE(m)	0.802	0.459	2.590
CV (%)	7.584	6.270	4.320

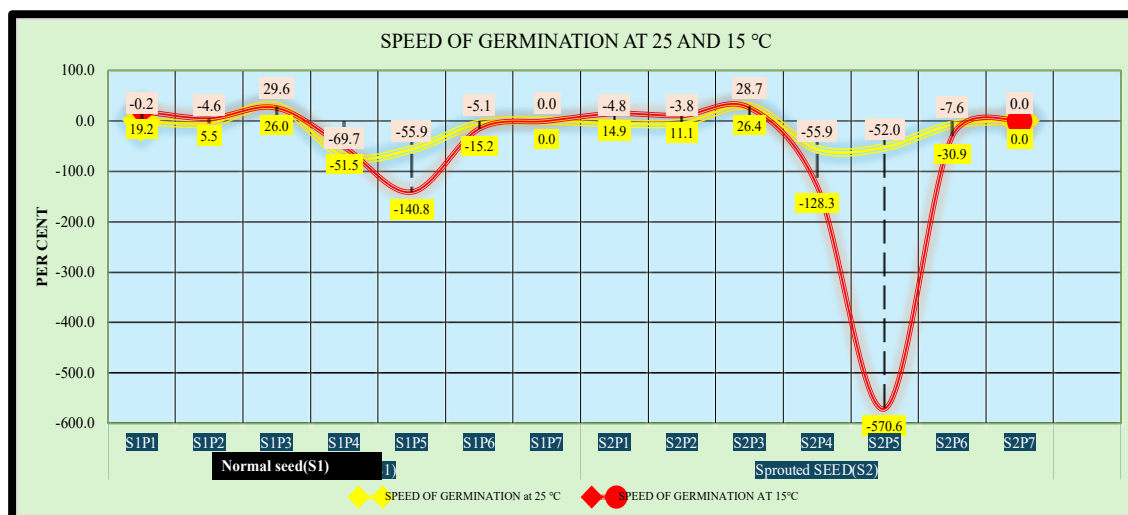


Figure 4.5 Per cent change over control for speed of germination at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min
P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

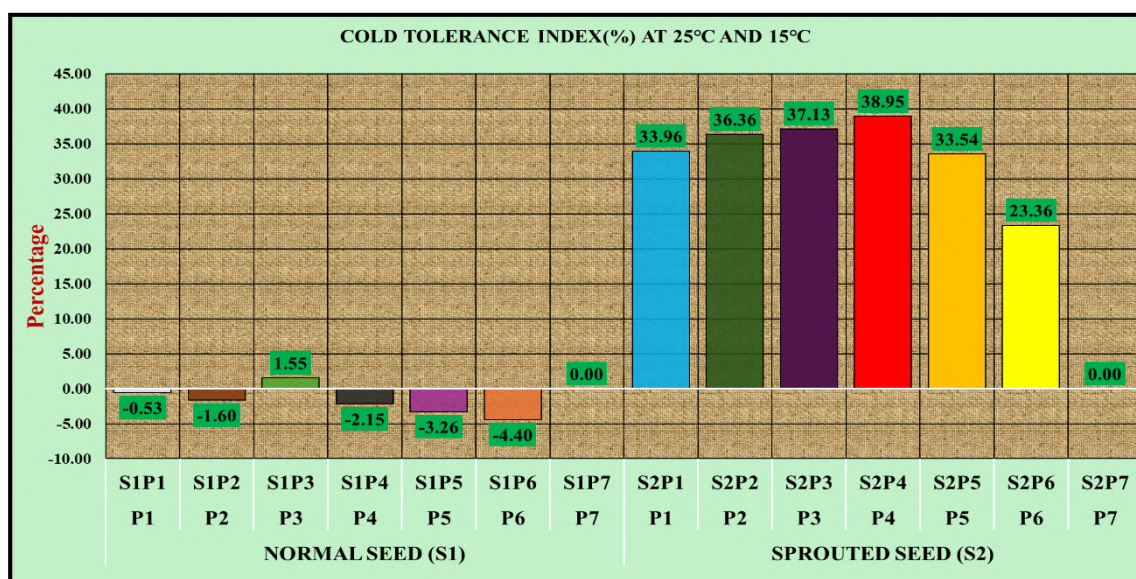


Figure 4.6 Per cent change over control for cold tolerance(%) at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min
P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

4.1.2.13 Superoxide dismutase activity (unit /mg /protein min) at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on biochemical parameters *viz.*, superoxide dismutase activity in paddy is presented in Table 4.8. The data on super oxide dismutase activity at 15°C revealed non-significant differences between normal seed and sprouted seed and superoxide dismutase activity at 15°C values ranged from 19.3 to 20.9 unit/mg protein/min. Normal seed (S1) recorded high superoxide dismutase activity at 15°C than sprouted seed (20.9). Superoxide dismutase activity at 15°C ranged from 18.17 to 21.93 unit/mg protein/min in plasma treatments. The cold plasma treatment P2 (10 kV for 20 min) recorded low superoxide dismutase activity at 15°C (18.17 unit/mg protein/min). All the plasma treatments showed non-significant difference with control. The interaction effect between type of seed (S) and cold plasma treatments (P) revealed non-significant difference and values ranged from 15.37 to 21.97 unit/mg protein/min. The per cent difference over control for superoxide dismutase activity was negative in all the plasma treatments (Fig 4.7). Bian *et al.*, (2024) reported that cold plasma treatments improved the superoxide dismutase activity and our results are in accordance with them as under low temperature of 15°C the superoxide dismutase activity was increased compared to 25°C.

4.1.2.14 Peroxidase activity (units/mg/protein/min) at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on peroxidase activity at 25°C in paddy are presented in Table 4.8. The data on peroxidase activity at 25°C revealed the presence of significant differences between normal seed and sprouted seed and peroxidase activity ranged from 0.029 to 0.051 units/mg/protein/min. Normal seed (S1) recorded high peroxidase activity compared to sprouted seed. Among cold plasma treatments (P) peroxidase activity ranged from 0.027 to 0.060 units/mg/protein/min. The treatment P2 (10 kV for 20 min) recorded high peroxidase activity at 25°C (0.060 units/mg/protein/min) and all the treatments were non-significant with P2. Low peroxidase activity at 25°C was observed in P1 (10 kV for 15 min) *i.e.*, 0.027 units/mg/protein/min. The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of non-significant differences which ranged from 0.014 to 0.069 units/mg/protein/min. Highest peroxidase activity was observed in S1P2 and all interaction effects recorded significant difference with S1P2. Low peroxidase activity was observed in S2P5 *i.e.*, sprouted seed treated with 30 kV plasma for 15min. The per cent difference over control for peroxidase activity was high in sprouted seed treated with 10 kV plasma for 20 minutes (Fig. 4.8). Peroxidase activity

was decreased as the age of the seedlings increased after non-thermal plasma treatments in maize (Henselova *et al.*, 2012) and our results are in accordance with them. Rashid *et al.* (2021) reported decrease in peroxidase activity in paddy may be due to the presence of high concentrations of nitrate and nitrite with the increased applications of plasma activated water.

4.1.2.15 Peroxidase activity (units/mg /protein /min) at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on peroxidase activity at 15°C in paddy are presented in Table 4.8. The data on peroxidase activity at 15°C revealed the presence of significant differences between normal seed and sprouted seed and peroxidase activity ranged from 0.087 to 0.153 units/mg/protein/min. Normal seed (S1) recorded higher peroxidase activity than the sprouted seed (0.153 units/mg/protein/min). Among cold plasma treatments (P) peroxidase activity ranged from 0.07 to 0.18 units/mg/protein/min. The treatment P2 (10 kV for 20 min) showed highest peroxidase activity at 15°C (0.18 units/mg/protein/min) and remaining all treatments were non-significant with respect to treatment P2. Low peroxidase activity at 15°C was observed in P5 (30 kV for 15 min) which is 0.07 units/mg/protein/min. The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of non-significant differences and peroxidase activity ranged from 0.042 to 0.207 units/mg/protein/min. High peroxidase activity was observed in S1P2 (0.207 units/mg/protein/min) and remaining all treatments were on par with S1P2. Low peroxidase activity was observed in S2P5 *i.e.*, sprouted seed treated with 30 kV plasma for 15 min. The per cent difference over control for peroxidase activity was high in S2P2 *i.e.*, sprouted seed treated with 10 kV plasma for 20 minutes (Fig 4.8). Cold plasma treatments improved the activity of antioxidant enzymes such as peroxidase under low temperature conditions and our results are in accordance with Bian *et al.* (2024) who reported in paddy crop wherein under 15°C peroxidase activity was increased compared to 25°C.

4.1.2.16 Catalase activity (n moles of H₂O₂ used /min /g weight of sample) at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on catalase activity at 25°C in paddy are presented in Table 4.9. The data on catalase at 25°C revealed the presence non-significant differences between normal seed and sprouted seed. Where it catalase activity ranged from 117 to 131 n moles of H₂O₂ used /min/g weight of sample. Normal seed (S1) recorded high catalase activity (131 n moles of H₂O₂ used /min/g weight of sample) than sprouted seed. Among cold plasma treatments (P) catalase activity ranged from 111 to 132 n moles of H₂O₂ used /min/g weight of sample. The

Table 4.8 Effect of type of seed (S) and cold plasma treatments (P) on bio chemical parameters viz., superoxide dismutase activity (units/mg) at 25°C and 15°C , peroxidase activity (units/min/mg) at 25°C and 15°C in paddy variety RNR15048

Treatments	Super oxide dismutase activity (units/mg) at 25°C	Superoxide dismutase activity (units/mg) at 15°C	Peroxidase activity (units/min/mg) at 25°C	Peroxidase activity (units/min/mg) at 15°C
TYPE OF SEED (S)				
S1: Normal seed	7.97	20.90	0.051	0.153
S2: Sprouted seed	7.95	19.30	0.029	0.087
CD (5%)	NS	NS	0.021	0.064
SE(m)	0.509	0.692	0.007	0.021
COLD PLASMA TREATMENTS (P)				
P1: 10 kV 15 Min	5.30	18.37	0.027	0.080
P2: 10 kV 20 Min	7.50	18.17	0.060	0.180
P3: 20 kV 15 Min	8.40	20.38	0.052	0.150
P4: 20 kV 20 Min	8.30	20.18	0.031	0.090
P5: 30 kV 15 Min	8.30	20.18	0.025	0.070
P6: 30 kV 20 Min	8.70	21.07	0.038	0.110
P7: CONTROL	9.00	21.93	0.045	0.130
CD (5%)	NS	NS	0.040	0.120
SE(m)	0.950	1.170	0.013	0.130
INTERACTIONS – TYPE OF SEED (S) X COLD PLASMA TREATMENTS (P)				
S1P1	4.30	21.37	0.036	0.107
S1P2	8.30	20.12	0.069	0.207
S1P3	8.10	19.67	0.059	0.178
S1P4	8.70	21.09	0.040	0.122
S1P5	8.70	21.14	0.036	0.108
S1P6	8.50	20.72	0.058	0.173
S1P7	9.00	21.88	0.058	0.174
S2P1	6.30	15.37	0.019	0.059
S2P2	6.70	16.23	0.052	0.156
S2P3	8.70	21.09	0.045	0.135
S2P4	7.90	19.28	0.022	0.065
S2P5	8.00	19.43	0.014	0.042
S2P6	8.80	21.42	0.018	0.055
S2P7	9.10	21.97	0.032	0.096
GRAND MEAN	7.96	20.05	0.040	0.120
CD (5%)	NS	NS	NS	0.169
SE(m)	1.340	1.650	0.018	0.055
CV (%)	8.900	8.600	9.900	8.500

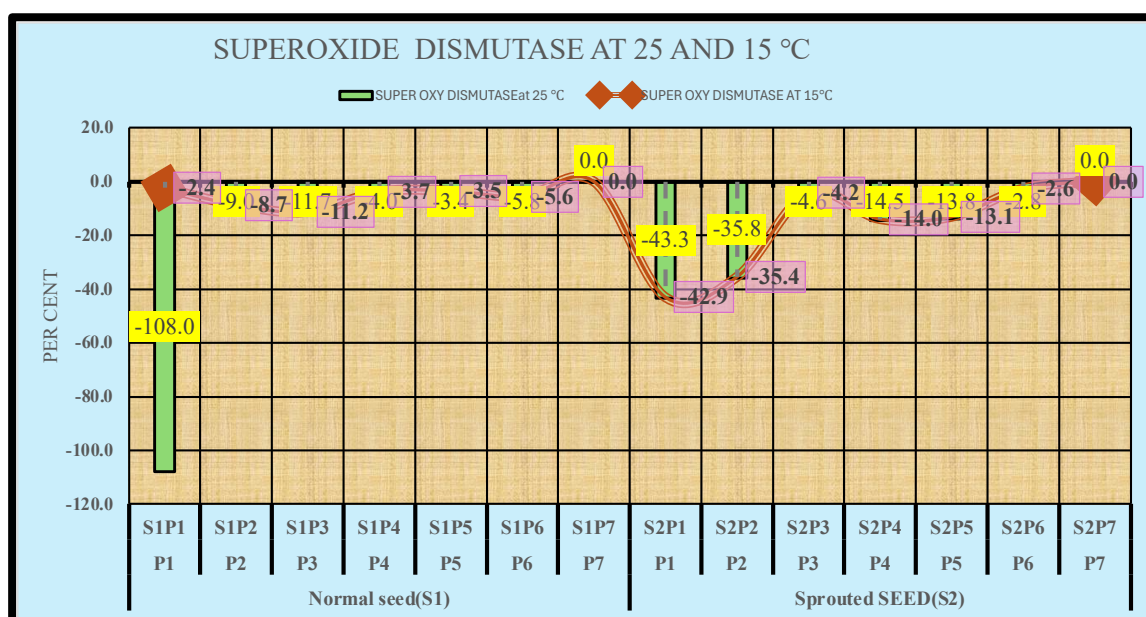


Figure 4.7 Per cent change over control for superoxide dismutase at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

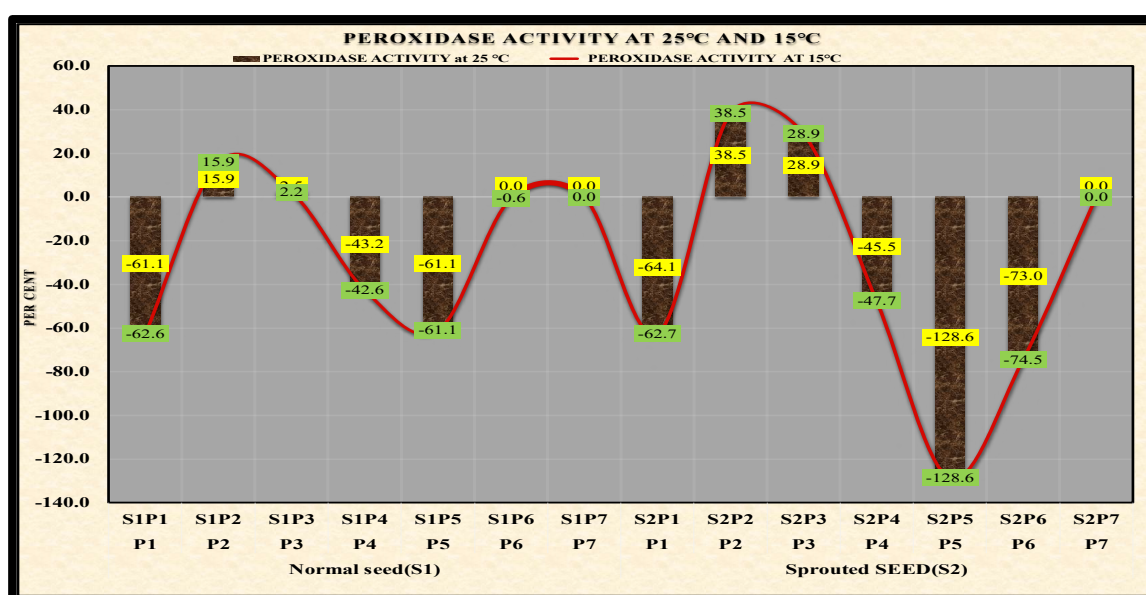


Figure 4.8 Per cent change over control for peroxidase activity (units/min/mg) at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

treatment P6 (30 kV for 20 min) recorded high catalase activity at 25°C (132 n moles of H₂O₂ used /min /g weight of sample), and remaining all treatments are on par with P6. Low catalase activity at 25°C was observed in P2 (10 kV for 20min) which is 111 n moles of H₂O₂ used /min /g weight of sample. The interaction effect readings between type of seed (S) and cold plasma treatments (P) revealed a non-significant differences for catalase activity which ranged from 96 to 144 n moles of H₂O₂ used /min /g weight of sample. High catalase activity at 25°C was observed in S2P5 (144 n moles of H₂O₂ used /min /g weight of sample) and remaining all treatments were on par with S2P5. Low catalase activity was observed in S2P3 (sprouted seed treated with 20 kV plasma for 15 min). The per cent difference over control for catalase activity was high in S2P5 plasma treatments *i.e.*, sprouted seed treated with 30 kV plasma for 15 minutes (Fig 4.9). The antioxidant enzyme CAT activity in 3 day and 6-day old roots significantly decreased after 60 seconds of low thermal plasma treatment *i.e.*, 50% reduction in 3-day and 75% reduction in 6-day old seedlings of maize. In roots grown from seeds treated with LTP for 120 seconds the CAT activity was also reduced but this reduction was not as substantial (Henselova *et al.*, 2012). Rashid *et al.* (2021) reported decrease in catalase activity after plasma treatments in paddy may be due to the presence of high concentrations of nitrate and nitrite and increased applications of plasma activated water and the above results are in accordance with them.⁷

4.1.2.17 Catalase activity (n moles of H₂O₂ used ⁻¹ min⁻¹ g weight of sample) at 15°C

The influence of type of seed (S) and cold plasma treatments (P) on catalase activity at 15°C in paddy are presented in Table 4.9. The data on catalase activity at 15°C revealed a non-significant differences between normal seed and sprouted seed and catalase activity at 15°C ranged from 353 to 394 n moles of H₂O₂ used /min /g weight of sample. Normal seed (S1) recorded high catalase activity (394 n moles of H₂O₂ used /min /g weight of sample). Among cold plasma treatments (P) catalase activity ranged from 333 to 398 n moles of H₂O₂ used /min /g weight of sample. The treatment P6 (30 kV for 20 min) recorded high catalase activity at 15°C (398) and remaining all treatments were on par with P6. Low catalase activity at 15°C was observed in P2 (333). The interaction effects between type of seed (S) and cold plasma treatments (P) revealed a non-significant differences and the catalase activity ranged from 287 to 439 n moles of H₂O₂ used /min /g weight of sample. High catalase activity was observed in S2P5 which is 439 n moles of H₂O₂ used /min /g weight of sample and remaining all treatments were non-significant with S2P5. Low catalase activity was observed in S2P3 *i.e.*, sprouted seed treated with 20 kV plasma for 15 min (287 n moles of H₂O₂ used /min /g weight of

sample). The per cent difference over control for catalase activity was high in S2P5 plasma treatments *i.e.*, sprouted seed treated with 30 kV plasma for 15 minutes (Fig 4.9). Cold plasma treatments improved the activity of antioxidant enzymes such as catalase under low temperature conditions and our results are in agreement with Bian *et al.* (2024) reported in paddy. The catalase activity in the seed kept at 15 °C was increased when compared to plasma treated seeds kept at 25°C.

4.1.2.18 Proline content (µmoles of proline/g of tissue) at 25°C

The influence of type of seed (S) and cold plasma treatments (P) on proline content at 25° C in paddy are presented in Table 4.9. The data on proline content at 25°C revealed the non-significant differences between normal seed and sprouted seed and proline content ranged from 0.020 to 0.023 µmoles of proline/g of tissue. Cold plasma treatments (P) for proline content at 25°C ranged from 0.016 to 0.031 µmoles of proline/g of tissue. The treatment P7 (control) recorded high proline content at 25°C (0.031 µmoles of proline/g of tissue) and low proline content at 25°C was recorded in P2 which is 0.016 µmoles of proline/g of tissue. The interaction effects between type of seed (S) and cold plasma treatments (P) revealed the presence of non-significant differences where the proline content at 25°C ranged from 0.014 to 0.033 µmoles of proline/g of tissue. High proline content was recorded in sprouted seed untreated control (S2P7) *i.e.*, is 0.033 µmoles of proline/g of tissue and remaining all treatments are non-significant with sprouted seed control. Low proline content was observed in sprouted seed treated with 10 kV 20 min (S2P2). The per cent difference over control for proline activity was negative in all the plasma treatments (Fig. 4.10). Abarghuei *et al.* (2021) reported the interaction between radiation time and voltage did not significantly affected the proline content in Basil crop. The proline upregulates the oxidative pentose phosphate pathway and proline can stabilize sub-cellular pathways quench the active oxygen and protects the cell against disadvantageous effects of stress conditions.

4.1.2.19 Proline content (µmoles of proline g⁻¹ of tissue) at 15°C

The influences of type of seed (S) and cold plasma treatments (P) on proline content at 15°C in paddy are presented in Table 4.9. The data on proline content at 15°C revealed the non-significant differences between normal seed and sprouted seed and proline content at 15°C ranged from 0.154 to 0.168 µmoles of proline/g of tissue. Cold plasma treatments (P) revealed the presence of significant differences between treatments and values ranged from 0.121 to 0.238 µmoles of proline/g of tissue.

Table 4.9 Effect of type of seed (S) and cold plasma treatments (P) on bio chemical parameters like catalase (n moles/min/g) at 25°C and 15°C ,proline content (μ moles of proline/g) at 25°C and 15°C in paddy variety RNR15048

Treatments	Catalase (n moles/min/g) At 25°C	Catalase (n moles/min/g) at 15°C	Proline content (μ moles of proline/g) at 25°C	Proline content (μ moles of proline/g) at 15°C
TYPE OF SEED(S)				
S1: Normal seed	131	394	0.023	0.168
S2: Sprouted seed	117	353	0.020	0.154
CD (5%)	NS	NS	NS	NS
SE(m)	5.61	16.85	0.001	0.013
COLD PLASMA TREATMENTS(P)				
P1: 10 kV 15 Min	127	381	0.021	0.164
P2: 10 kV 20 Min	111	333	0.016	0.121
P3: 20 kV 15 Min	119	351	0.021	0.160
P4: 20 kV 20 Min	116	356	0.018	0.144
P5: 30 kV 15 Min	130	392	0.018	0.140
P6: 30 kV 20 Min	132	398	0.020	0.157
P7: Control	131	394	0.031	0.238
CD (5%)	NS	NS	NS	NS
SE(m)	10.51	31.53	0.003	0.025
INTERACTIONS – TYPE OF SEED (S) x COLD PLASMA TREATMENTS (P)				
S1P1	129	389	0.027	0.207
S1P2	120	360	0.017	0.132
S1P3	140	421	0.027	0.206
S1P4	121	363	0.014	0.111
S1P5	121	363	0.020	0.150
S1P6	141	424	0.019	0.143
S1P7	142	433	0.030	0.225
S2P1	124	372	0.016	0.121
S2P2	101	305	0.014	0.111
S2P3	96	287	0.015	0.115
S2P4	115	346	0.023	0.177
S2P5	144	439	0.017	0.130
S2P6	124	372	0.022	0.170
S2P7	118	354	0.033	0.250
GRAND MEAN	124.5	373.5	0.021	0.160
CD	NS	NS	NS	NS
SE(m)	14.8	44.6	0.004	0.035
CV (%)	8.88	7.88	7.28	9.43

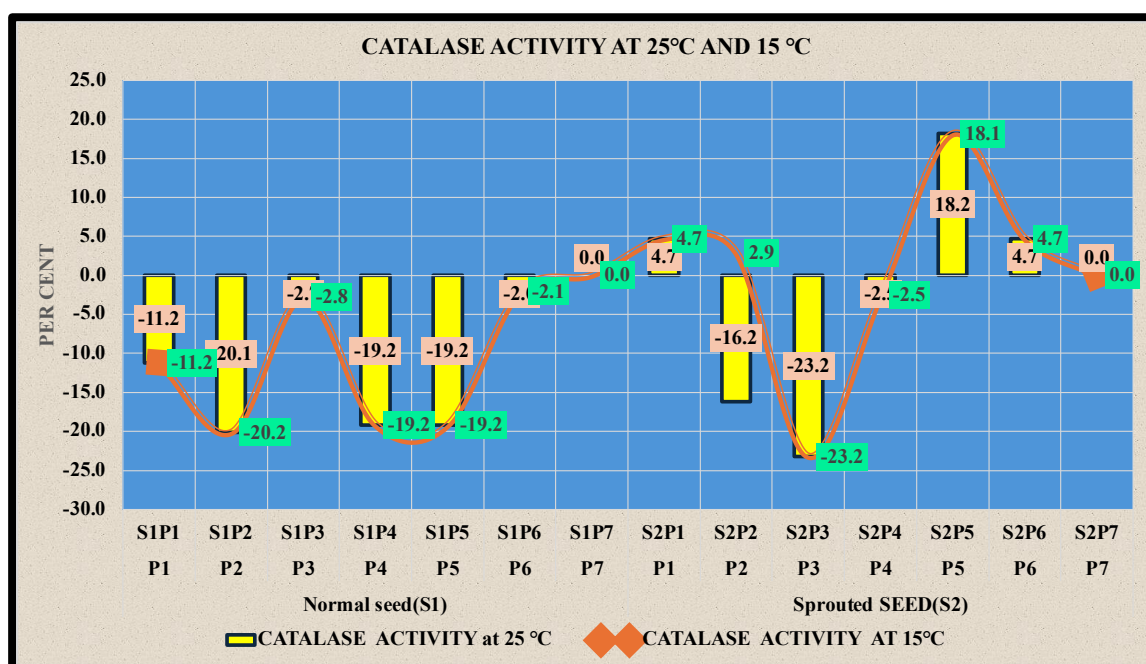


Figure 4.9 Per cent change over control for catalase activity (n moles/min/g) at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

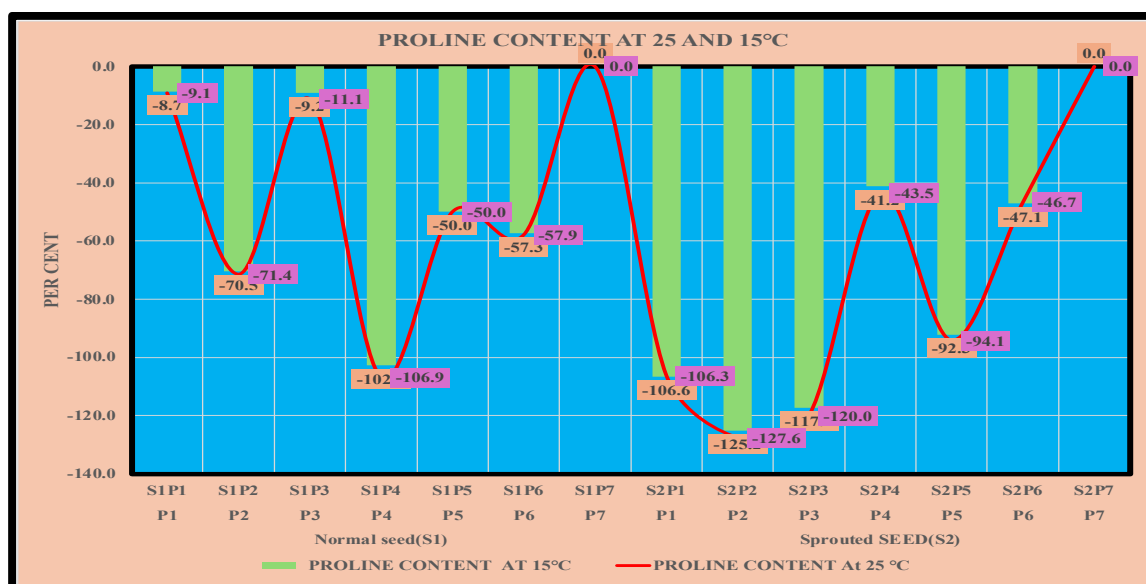


Figure 4.10 Per cent change over control for proline content (μ moles of proline/g) at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

The treatment, P7 control recorded higher proline content at 15°C (0.238 µmoles of proline/g of tissue) and plasma treatments were on par with control. The interaction effect between type of seed (S) and cold plasma treatments (P) revealed the presence of non-significant differences which ranged from 0.111 to 0.250 µmoles of proline/g of tissue. High proline content was observed in sprouted control seed (0.250 µmoles of proline/g of tissue) and all plasma treatments were on par with S2P7. Low proline content was observed in sprouted seed treated with 20 kV plasma for 20 min (S2P2) and normal seed treated with 10 kV plasma for 20 min (S1P4) which is 0.111 µmoles of proline/g of tissue. The per cent difference over control for proline activity was negative in all the plasma treatments (Fig 4.10). Cold plasma treatments improved the activity of proline under low temperature conditions and our results are in agreement with Bian *et al.* (2024) at 15°C proline activity was increased compared to 25°C.

4.1.2.20 Radicle emergence at 25°C (%)

Influence of type of seed (S) and cold plasma treatments (P) on radicle emergence at 25°C after 96 hours in paddy were presented in Table 4.10 and (Fig 4.11). The cold plasma treatments (P) revealed non-significant difference among treatments and radicle emergence per cent ranged from 90 to 98 per cent. The plasma treatment P3 (20 kV for 15 min) recorded high radicle emergence per cent at 25°C *i.e.*, 98 per cent and treatments P1 (10 kV for 15min), P2 (10 kV for 20min), P4 (20 kV for 20 min), P5 (30 kV for 15 min), P6 (30 kV for 20 min), P7 (control) were on par with P3. Low per cent radicle emergence at 25°C was noticed in treatments P1 (10 kV for 15min) and P2 (10 kV for 20min) and P5 (30 kV for 15 min) which is 90 per cent. Chueauan *et al.* (2023) reported that radicle emergence at 48 and 72 hours enhanced the radicle growth after plasma activated water treatment in paddy whereas after 24 hours of treatment there was no significant difference observed in radicle growth and the present results are in accordance with them.

4.1.2.21 Radicle emergence at 15°C (%)

Influence of type of seed (S) and cold plasma treatments (P) on radicle emergence at 15°C after 96 hours in paddy were presented in Table 4.10 and (Fig 4.11). Among cold plasma treatments (P) presence of significant difference was noticed between treatments and the values ranged from 2 to 52% after 96 hrs. The plasma treatment P3 (20 kV for 15 min) showed highest radicle emergence per cent at 15°C *i.e.*, 52 per cent, remaining all treatments P1 (10 kV for 15min), P2 (10 kV for 20 min), P4 (20 kV for 20 min), P5

(30 kV for 15 min), P6 (30 kV for 20 min), and P7 (control) recorded significant difference with P3 (20 kV for 15min). Low radicle emergence at 15°C (96 hrs) was observed in P2 (10 kV for 20 min) *i.e.*, 2 per cent.

Table 4.10 Effect of type of seed (S) and cold plasma treatments (P) on radicle emergence at 25°C and radicle emergence at 15°C in paddy variety RNR15048

S. No	Treatments	Radicle emergence (%) at 25°C	Radicle emergence (%) at 15°C
1	P1: 10 kV for 15 Min	90	16
2	P2: 10 kV for 20 Min	90	2
3	P3: 20 kV for 15 Min	98	52
4	P4: 20 kV for 20 Min	94	10
5	P5: 30 kV for 15 Min	90	12
6	P6: 30 kV for 20 Min	94	8
7	P7: Control	92	10
	GRANDMEAN	93	16
	CD	NS	13
	SE(m)	3.85	3.92
	CV	5.88	5.34

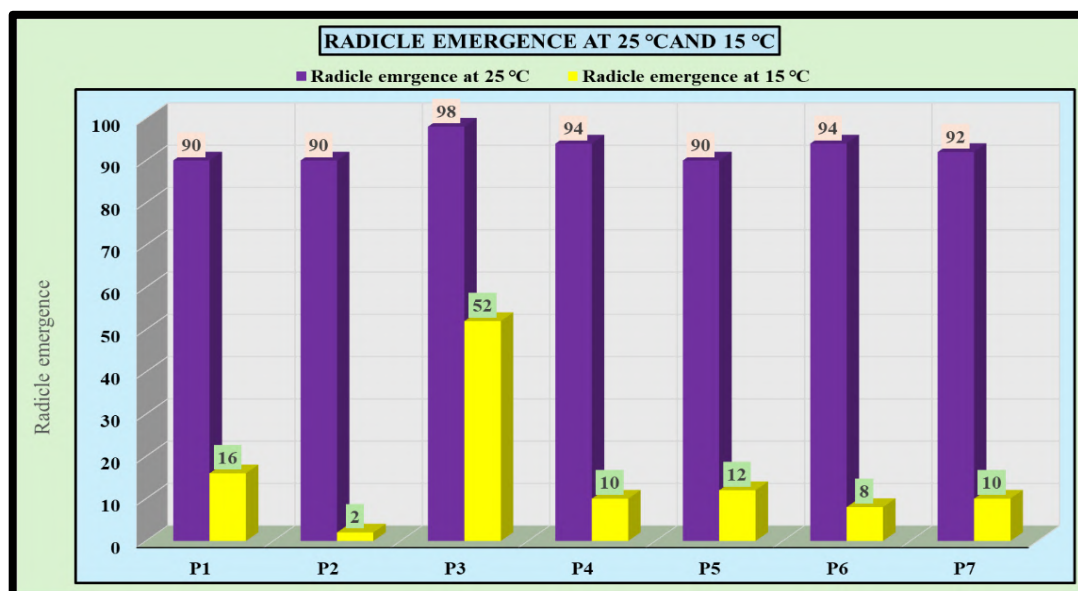


Figure 4.11 Radicle emergence (%) at 25°C and 15°C in normal seed (S1) and sprouted seed (S2) treated with different cold plasma treatments

S1: Normal seed treated with cold plasma, S2: Sprouted seed treated with cold plasma
P1: 10 kV for 15 min, P2: 10 kV for 20 min, P3: 20 kV for 15 min, P4: 20 kV for 20 min, P5: 30 kV for 15 min, P6: 30 kV for 20 min and P7: untreated control

4.2 Field evaluation of effective cold plasma treatment on vegetative growth and yield contributing characters in paddy during *Rabi* 2023-24.

The weather data pertaining to the crop growth period was mentioned in Annexure - I and II. Even though during nursery period the temperatures dropped below 15°C (Plate 4.3). we did not observe any chilling injury after transplanting to main field during vegetative growth stage the temperatures fallen below low temperature. The paddy crop is not subjected to any chilling injury currently in the present study.

4.2.1 Analysis of variance:

Sprouted and normal seed of three varieties of paddy RNR 15048, JGL 24423 and Tellahamsa were subjected to 20 kV cold plasma for 15 min which is identified as the effective plasma treatment based on seed quality parameters studied in experiment 1. Nursery was raised on 16.12.2023 and transplanted to the main field was done on 13.01.2024 when the temperatures are very low so that the paddy crop will subject to natural cold stress during vegetative stage and the observations recorded are mentioned below.

Analysis of variance for varieties (V) and type of seeds (S) treated with cold plasma (20 kV for 15 min) and its effects on different seed quality parameters *viz.*, per cent germination, dry matter for shoot at 15 days (g), dry matter for shoot at 30 days (g), dry matter for shoot at 45 day (g), dry matter for root at 15 days (g), dry matter for root at 30 days (g), dry matter for root at 45 days (g) were recorded and presented in Table 4.11. The results indicated the existence of significant differences for varieties (V) for per cent germination, dry matter for shoot at 15 days (g), dry matter for shoot for at 30 days (g), dry matter for shoot at 45 days (g), dry matter for root at 15 days (g) and dry matter for root at 45 days (g) while dry matter for root at 30 days (g) was non-significant. For different types of seeds (S) per cent germination, dry matter for shoot at 30 days (g), dry matter for shoot at 45 days (g), dry matter for root at 15 days (g) found non-significant while dry matter for root at 30 days (g) and dry matter for root at 45 days (g) and dry matter shoot at 15 days (g) are significant (Table 4.11). The interaction effects between varieties (V) and types of seeds (S) revealed the presence of significance difference between dry matter for shoot at 30 days (g), dry matter for shoot at 45 days (g) and dry matter for root at 30 days (g) and dry matter for root at 45 days (g) while per cent germination, dry matter for shoot at 15 days (g) and dry matter for root at 15 days (g) recorded non-significance difference.



Plate 4.3: Nursery beds naturally affected with severe cold stress during Rabi season, 2023-24.

Table 4.11 Analysis of variance for cold plasma treated paddy seed grown under field conditions on seed physiological parameters viz., per cent germination, dry matter for shoot and root (g) at 15, 30 and 45 days

Source of variance	d.f	Germination %	Dry matter for shoot at 15 days (g)	Dry matter shoot at 30 days (g)	Dry matter for shoot at 45 days (g)	Dry matter for root at 15 days (g)	Dry matter for root at 30 days (g)	Dry matter for root at 45 days (g)
V	2	186.82 ***	0.005 ***	0.85 ***	7.049 ***	0.001 ***	0.024	2.18 ***
S	2	7.82	0.000 *	0.17	0.356	0.000	0.042 *	1.05 **
V*S	4	3.32	0.000	0.23 *	0.595 *	0.000	0.041 *	1.37 ***
Error (V)	16	14.97	0.000	0.06	0.171	0.000	0.010	0.14
Total	26	26.48	0.000	0.15	0.781	0.000	0.018	0.57

NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively.

S: Type of seeds **V:** Varieties

Analysis of variance for varieties (V) and type of seeds (S) treated with cold plasma (20 kV for 15 min) and its effects on chlorophyll content of leaf, days to 50% flowering, days to maturity, number of tillers per hill, number of productive tillers per m² and proline content were studied are presented in Table 4.12. The results indicated the presence of significant difference between varieties (V) and different types of seeds (S) for days to 50% flowering, days to maturity while chlorophyll content of leaf, number of tillers per hill, number of productive tillers per m² and proline content recorded non-significant differences. The interaction effects between varieties (V) and types of seeds (S) revealed the presence of non-significance difference between all parameters. Analysis of variance for varieties (V) and type of seeds (S) treated with cold plasma (20 kV for 15 min) and its effects on plant height (cm), seed yield per hill (g), seed yield (t/ha), 1000 seed weight (g) in field conditions were studied and observations were presented in Table 4.13. The results indicated the presence of significant difference between varieties (V) for all the parameters studied and for different types of seeds (S) seed yield per hill (g), seed yield (t/ha) and 1000 seed weight (g) recorded non-significant difference while plant height (cm) showed significant difference. The interaction effects between varieties (V) and types of seeds (S) revealed the presence of non-significance difference for all parameters.

4.2.2 Field parameters

The results pertaining to the seed yield contributing parameters such as per cent germination, dry matter for shoot at 15 days (g), dry matter for shoot at 30 days (g), dry matter for shoot at 45 days (g), dry matter for root at 15 days (g), dry matter for root at 30 days (g), dry matter for root at 45 days (g), chlorophyll content of leaf, days to 50% flowering, days to maturity, number of tillers per hill, number of productive tillers per m², proline content of leaf, plant height (cm), seed yield per hill (g), seed yield (t/ha) and 1000 seed weight (g) were presented below. The crop was not affected with chilling injury during vegetative stage even though the temperatures fall below 15°C. The per cent incidence of blast was also not observed in paddy during crop growth period. So, the data pertaining to these two observations are not given here.

4.2.2.1 Per cent germination

Influence of type of seed (S) and varieties (V) on per cent germination was presented in Table 4.14. The data on per cent germination revealed the presence of non-significant differences between RNR 15048 (V1) and JGL 24423 (V2) while Tellahamsa

(V3) showed significant difference with RNR 15048 and JGL 24423 and mean values ranged from 85 per cent to 93 per cent. Tellahamsa (V3) recorded high germination per cent than RNR 15048 and JGL 24423. Among types of seeds (S) presence of non-significant differences was observed and per cent germination ranged from 86% to 88%. per cent germination in the interaction effects between varieties (V) and type of seed (S) ranged from 83% to 93%. Tellahamsa untreated control (S3V3) and Tellahamsa sprouted seed treated with cold plasma (S2V3) recorded high germination per cent of 93. The interaction effects S1V1, S2V1, S3V1, S1V2, S2V2 and S3V2 were significantly different with S2V3 and low germination per cent was recorded in S3V1 (RNR 15048 untreated control) and S3V2 (JGL 24423 untreated control). The results revealed that Tellahamsa sprouted seed treated with cold plasma and Tellahamsa untreated control were having good germination per cent. The per cent difference over control for per cent germination was high in RNR 15048 sprouted seed treated with plasma and JGL 24423 normal seed treated with plasma (Fig 4.12). The results are in accordance with Ivankov *et al.* (2021) who reported that plasma treated plants under field conditions exhibited more germination % than control seed in buck wheat. The plasma treatment results in chemical modifications in seed coat leading to increased surface wettability and rapid change in the balance of phytohormones and DNA methylation in seeds resulted in changes of protein expression and enzymatic activities underlying of germination mechanism which may be one of the causes for increase in germination % in plasma treatments.

4.2.2.2 Dry matter for shoot at 15 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for shoot at 15 days (g) was presented in Table 4.14. The data on dry matter for shoot at 15 days (g) revealed the presence of significant differences between three varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 0.040 to 0.087 g. Tellahamsa (V3) recorded more dry matter for shoot at 15 days. Among different types of seeds (S) a significant difference was recorded between normal seed (S1) and sprouted seed (S2) and mean values ranged from 0.056 to 0.070 g. Normal seed treated with cold plasma recorded more dry matter for shoot at 15 days *i.e.*, 0.070 g. Dry matter for shoot at 15 days in the interaction effects between varieties (V) and type of seed (S) ranged from 0.032 to 0.092 g. Tellahamsa normal seed treated with cold plasma (S1V3) recorded high dry matter for shoot at 15 days (0.092 g), and the treatments S1V1, S2V1, S3V1, S2V2 and S3V2 were significantly different with S1V3 while S1V2, S2V3 and S3V3

Table 4.12 Analysis of variance for cold plasma treated paddy seed grown under field conditions on parameters viz., chlorophyll content of leaf, days to 50% flowering, days to maturity, number of tillers/hill, number of productive tillers per m² and proline content (μmoles/g)

Source of variance	d.f	Chlorophyll content of leaf	Days to 50% flowering	Days to maturity	Number of tillers/ hill	Number of productive tillers per m²	Proline content(μmoles/g)
V	2	87.40	30.71**	30.70 **	35.11	2588.44	0.002
S	2	86.61	41.37 **	41.37 **	20.11	1004.11	0.001
S*V	4	79.1511	5.42	5.43	15.72	2294.22	0.000
Error (V)	16	103.25	4.80	4.81	20.44	5505.53	0.001
Total	26	96.32	15.13	15.12	28.11	4081.21	0.001

NOTE: *, **, * represents significance at 5%, 1% and 0.1 % probability levels, respectively.**

S: Type of seeds V: Varieties

Table 4.13 Analysis of variance for cold plasma treated paddy seed grown under field conditions on yield and yield attributing characters viz., like plant height (cm), seed yield per hill (g), seed yield (t/ha) and 1000 seed weight (g)

Source of variance	d.f	Plant height (cm)	Seed yield per hill (g)	Seed yield (t/ha)	1000 seed weight (g)
V	2	402.49 ***	962.75 ***	8.990**	348.20 ***
S	2	100.05 **	108.45	1.461	0.63
S*V	4	3.308	62.40	2.551	2.72
Error (V)	16	13.686	77.794	1.336	0.900
Total	26	48.275	140.314	2.038	27.892

NOTE: *, **, * represents significance at 5%, 1% and 0.1 % probability levels, respectively.**

S: Type of seeds V: Varieties

Table 4.14 Effect of type of seed (S) and varieties (V) on field parameters viz., germination per cent, dry matter for shoot (g) at 15, 30 and 45 days in paddy

Treatments	Germination %	Dry matter for shoot at 15 days (g)	Dry matter for shoot at 30 days (g)	Dry matter for shoot at 45 days (g)
VARIETIES (V)				
V1: RNR15048	85 (67)	0.040	0.345	0.570
V2: JGL24423	85(67)	0.063	0.494	1.588
V3:Tellahamsa	93(74)	0.087	0.935	2.333
CD (5%)	3.866	0.008	0.240	0.413
SE(m)	1.289	0.002	0.080	0.137
TYPE OF SEED (S)				
S1: Normal seed	88(70)	0.070	0.739	1.662
S2: Sprouted seed	88(70)	0.056	0.468	1.551
S3: Untreated control	86(68)	0.064	0.565	1.276
CD (5%)	NS	0.008	NS	NS
SE(m)	1.289	0.002	0.080	0.137
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)				
S1V1	85(67)	0.047	0.319	0.343
S2V1	86(68)	0.032	0.316	0.512
S3V1	83(67)	0.041	0.398	0.853
S1V2	86(66)	0.070	0.458	1.734
S2V2	85(73)	0.049	0.533	1.883
S3V2	83(65)	0.069	0.490	1.145
S1V3	92(73)	0.092	1.439	2.909
S2V3	93(75)	0.087	0.557	2.259
S3V3	93(75)	0.082	0.807	1.829
GRAND MEAN	87	0.063	0.591	1.496
CD	6.696	0.015	0.415	0.715
SE(m)	2.233	0.005	0.138	0.238
CV (%)	4.42	13.77	14.65	12.62

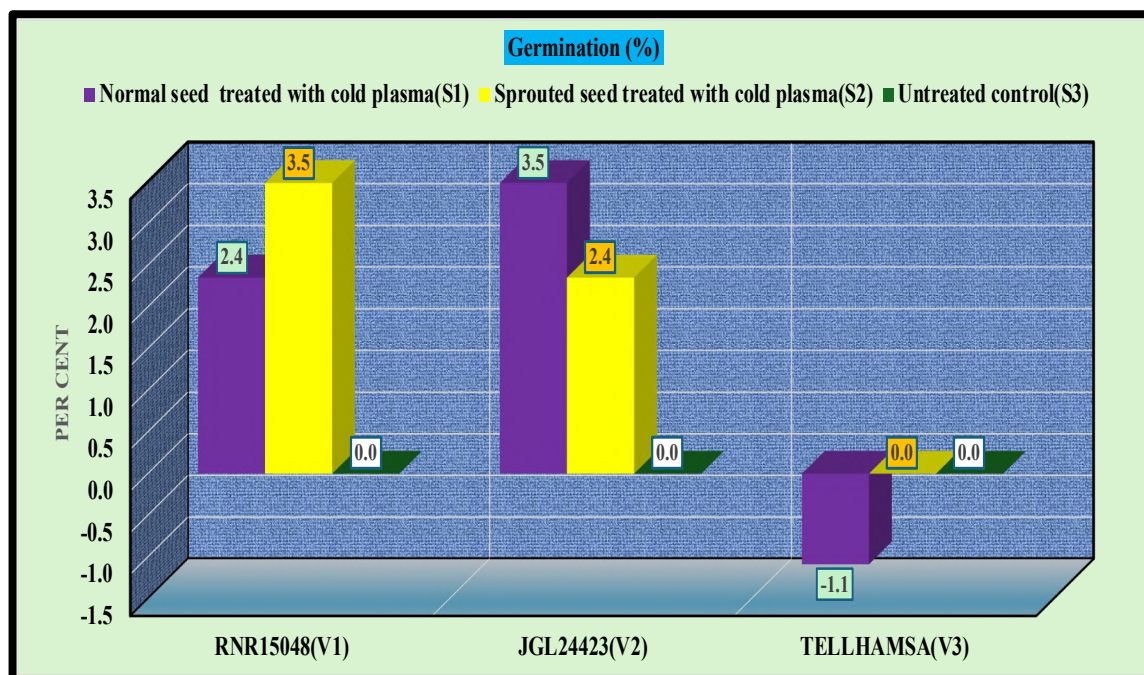


Figure 4.12 Per cent change over untreated control for germination (%) in three paddy varieties treated with cold plasma (20 kV for 15 min)

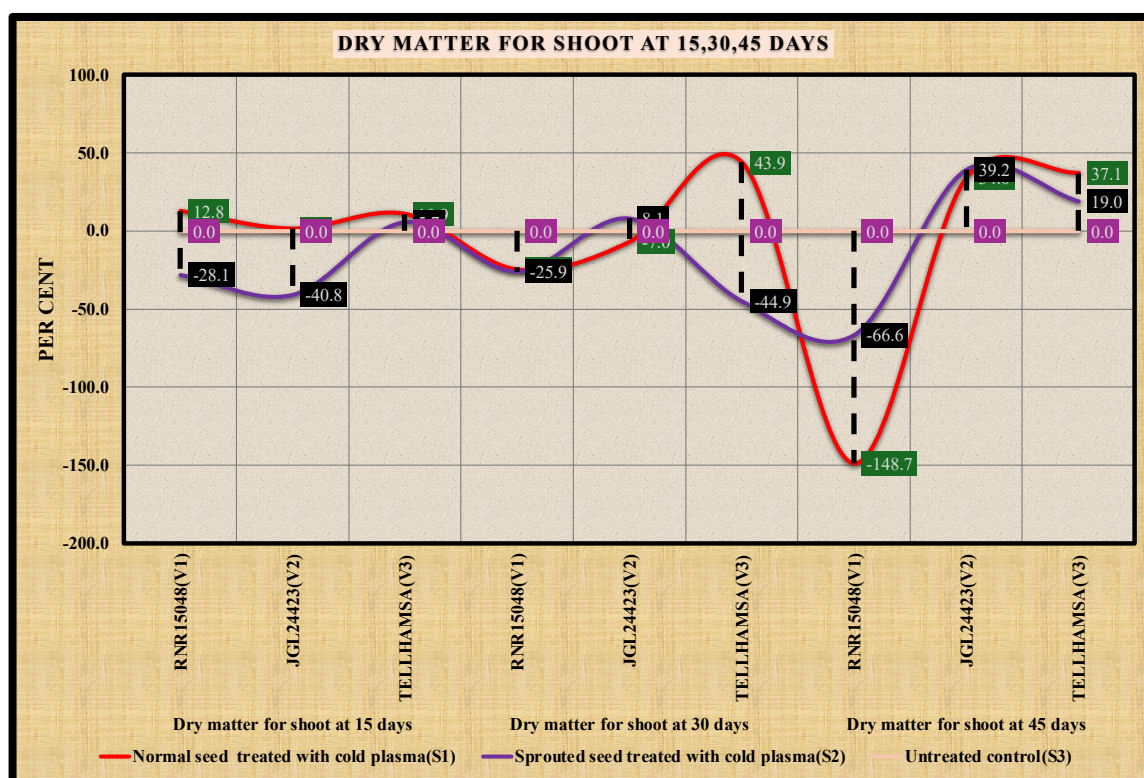


Figure 4.13 Per cent change over untreated control for dry matter for shoot at 15, 30 and 45 days (g) in three paddy varieties treated with cold plasma (20 kV for 15 min)

are on par with S1V3 and low dry matter for shoot at 15 days (g) was recorded in S2V1 (RNR 15048 sprouted seed treated with cold plasma). The results revealed that Tellahamsa normal seed treated with cold plasma recorded more dry matter for shoot at 15 days. The high per cent difference over control for dry matter for shoot at 15 days was recorded highest in RNR 15048 normal seed treated with cold plasma (Fig 4.13). The above results are in accordance with the Ling *et al.* (2016) who reported that dry weight of shoot treated with cold plasma in pea was more than the dry weight of shoot in untreated control.

4.2.2.3 Dry matter for shoot at 30 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for shoot at 30 days (g) was presented in Table 4.14. The data on dry matter for shoot at 30 days (g) revealed the presence of significant differences between three different varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 0.345 to 0.935 g. Tellahamsa (V3) recorded more dry matter for shoot at 30 days. Among types of seeds (S) significant difference was noticed between normal seed (S1) and sprouted seed (S2) and mean values ranged from 0.468 to 0.739 g. Normal seed treated with cold plasma showed better results than sprouted seed treated with cold plasma. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences and mean values ranged from 0.316 to 1.439 g. Tellahamsa normal seed treated with cold plasma (S1V3) recorded high dry matter for shoot at 30 days (1.439 g) and all interactions were significantly different and low dry matter for shoot at 30 days (g) was recorded in S2V1 (RNR 15048 sprouted seed treated with cold plasma). The high per cent difference over control for dry matter for shoot at 30 days was recorded in Tellahamsa normal seed treated with cold plasma (Fig 4.13).

4.2.2.4 Dry matter for shoot at 45 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for shoot at 45 days (g) was presented in Table 4.14. The data on dry matter for shoot at 45 days (g) revealed the presence of significant differences between three varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) where, the mean values ranged from 0.570 to 2.333 g. Among types of seeds (S) non-significant difference was noticed between normal seed treated with cold plasma (S1), sprouted seed treated with cold plasma (S2) and untreated control (S3) and the mean values ranged from 1.276 to 1.662 g. Normal seed treated with cold plasma recorded high dry matter than sprouted seed treated with cold plasma. The interaction effect between varieties (V) and type of seed (S) revealed significant

differences and mean values ranged from 0.343 to 2.909. Tellahamsa normal seed treated with cold plasma (S1V3) recorded high dry matter for shoot at 45 days (2.909g) and treatments S1V1, S2V1, S3V1, S1V2, S2V2, S3V2 and S3V3 were significantly different with S1V3 while S2V3 (Tellahamsa sprouted seed treated with cold plasma) was on par with S1V3. low dry matter for shoot at 45 days (g) was recorded in S1V1 (RNR 15048 normal seed treated with cold plasma). The high per cent difference over control for dry matter for shoot at 45 days was recorded in JGL 24423 sprouted seed treated with cold plasma (Fig 4.13).

4.2.2.5 Dry matter for root at 15 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for root at 15 days (g) was presented in Table 4.15. The data on dry matter for root at 15 days (g) revealed significant differences between three varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) in where mean values ranged from 0.013 to 0.030g. Among types of seeds (S) the presence of non-significant differences was noticed between normal seed (S1) and sprouted seed (S2) and untreated control (S3) and mean values ranged from 0.22 to 0.24. Dry matter for root at 15 days in the interaction effects between varieties (V) and type of seed (S) ranged from 0.013 to 0.031g. Tellahamsa normal seed treated with cold plasma (S1V3) and Tellahamsa untreated control (S3V3) recorded high dry matter for root at 15 days (0.031g) and interactions S1V1, S2V1, S3V1 and S2V2 were significantly different with S1V3 and S3V3 whereas, S1V2, S3V2 and S2V3 are on par with S1V3 and S3V3. Low dry matter for root at 15 days (g) was recorded in S2V1 (RNR 15048 sprouted seed treated with cold plasma). The results revealed that Tellahamsa normal seed treated with cold plasma and Tellahamsa untreated control recorded more dry matter for root at 15 days (g). The high per cent difference over control for dry matter for root at 15 days was recorded in JGL 24423 normal seed treated with cold plasma (Fig 4.14). Abeysingha *et al.* (2024) reported that cold plasma treatments elevated the cytokinin levels in soybean roots promoting root dry matter and the present results are in accordance with them. Cold plasma treatments will influence root growth and development, including carbohydrate availability, hormone signaling and nutrient uptake during embryogenesis which is regulated by auxin flow and cytokinin-induced differentiation.

4.2.2.6 Dry matter for root at 30 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for root at 30 days (g) was presented in Table 4.15. The data on dry matter for root at 30 days (g) revealed

Table 4.15 Effect of type of seed (S) and varieties (V) on field parameters viz., dry matter for root (g) at 15, 30 and 45 days in paddy

Treatments	Dry matter for root at 15 days (g)	Dry matter for root at 30 days (g)	Dry matter for root at 45 days (g)
VARIETIES (V)			
V1: RNR15048	0.013	0.257	0.360
V2: JGL24423	0.007	0.232	0.921
V3: Tellahamsa	0.030	0.331	1.343
CD (5%)	0.004	0.101	0.378
SE(m)	0.001	0.033	0.126
TYPE OF SEED (S)			
S1: Normal seed	0.024	0.316	1.148
S2: Sprouted seed	0.022	0.310	0.984
S3: Untreated control	0.024	0.194	0.492
CD (5%)	NS	0.101	0.3783
SE(m)	0.001	0.034	0.126
Interactions – TYPE OF SEED (S) x VARIETIES (V)			
S1V1	0.014	0.352	0.172
S2V1	0.013	0.190	0.376
S3V1	0.014	0.229	0.532
S1V2	0.028	0.232	0.837
S2V1	0.022	0.242	1.361
S3V2	0.026	0.222	0.564
S1V3	0.031	0.364	2.434
S2V3	0.030	0.497	1.215
S3V3	0.031	0.132	0.379
GRAND MEAN	0.024	0.274	0.87
CD	0.006	0.175	0.655
SE(m)	0.002	0.058	0.218
CV (%)	16.66	15.06	13.27

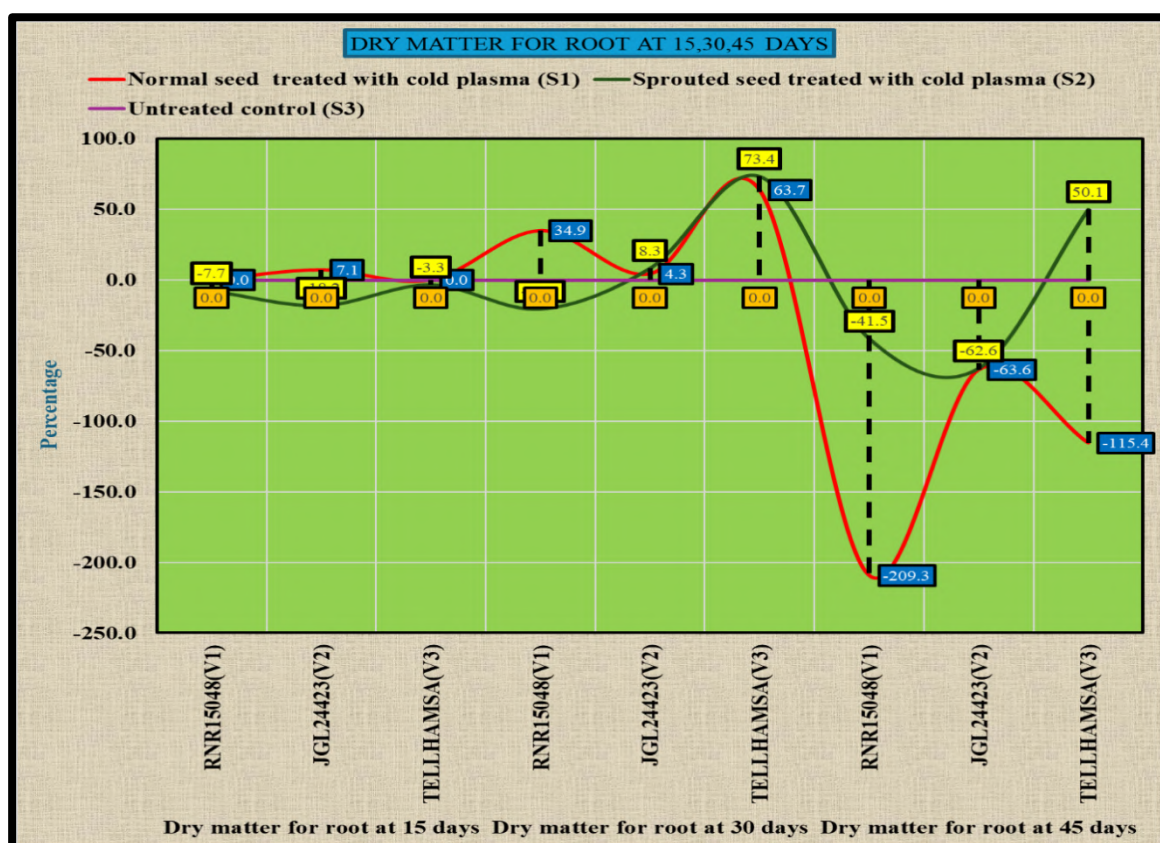


Figure 4.14 Per cent change over untreated control for dry matter for root at 15, 30 and 45 days (g) in three paddy varieties treated with cold plasma (20 kV for 15 min)

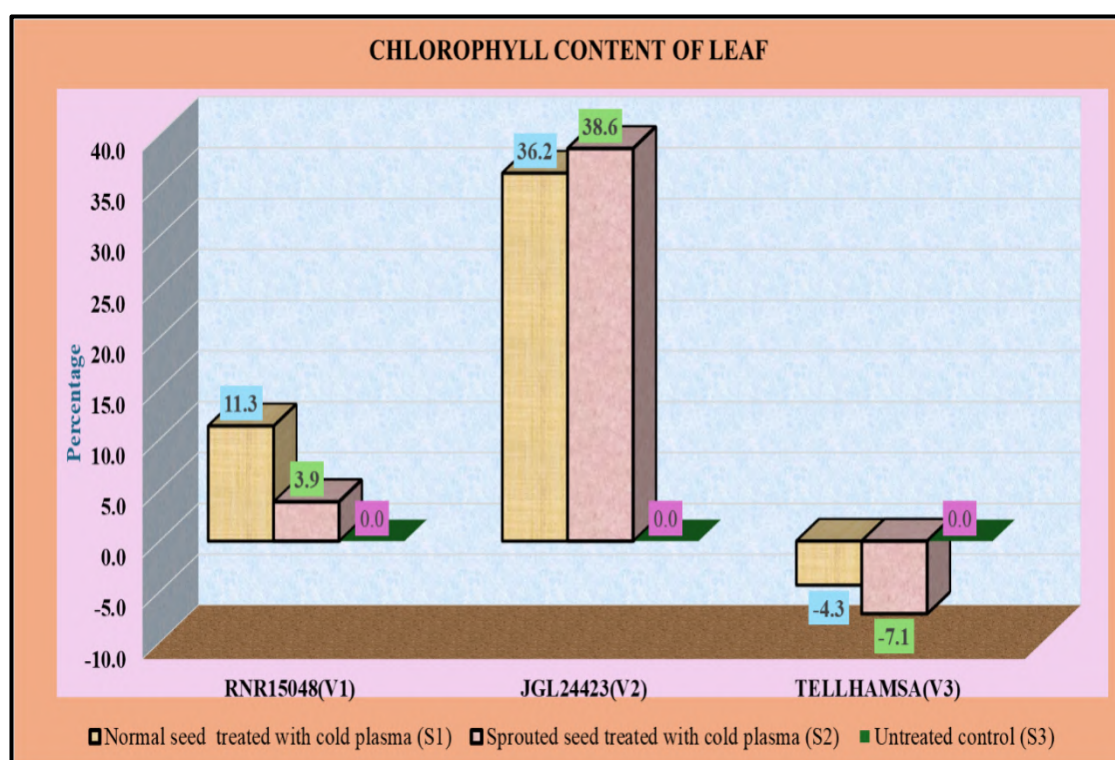


Figure 4.15 Per cent change over untreated control for chlorophyll content of leaf in three paddy varieties treated with cold plasma (20 kV for 15 min)

non-significant differences between three varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 0.232 to 0.331 g. Tellahamsa (V3) recorded more dry matter for root at 30 days. Among types of seeds (S) significant differences were noticed between normal seed (S1) and untreated control (S3). The mean values ranged from 0.194 to 0.316 g. Normal seed treated with cold plasma recorded better result than untreated control. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences and mean values ranged from 0.132 to 0.497 g. Tellahamsa sprouted seed treated with cold plasma (S2V3) showed high dry matter for root at 30 days (0.497 g), interactions S2V1, S3V1, S1V2, S2V2, S3V2 and S3V3 were significantly different with respect to S2V3 while S1V1 and S1V3 are on par with S2V3 and low dry matter for root at 30 days (g) was recorded in S3V3 (Tellahamsa untreated control). The high per cent difference over control for dry matter for root at 30 days was recorded in Tellahamsa sprouted seed treated with cold plasma (Fig 4.14).

4.2.2.7 Dry matter for root at 45 days (g)

Influence of type of seed (S) and varieties (V) on dry matter for root at 45 days (g) was presented in Table 4.15. The data on dry matter for root at 45 days (g) revealed significant differences between three different varieties RNR15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 0.360 to 1.343 g. Tellahamsa (V3) recorded more dry matter for root at 45 days. Among types of seeds (S) significant differences were observed between normal seed (S1) and untreated control (S3) and mean values ranged from 0.492 to 1.148 g. The interaction effects between varieties (V) and type of seeds (S) revealed the presence of significant differences and mean values ranged from 0.172 to 2.434 g. Tellahamsa normal seed treated with cold plasma (S1V3) recorded high dry matter for root at 45 days (2.434 g) and interaction effects S1V1, S2V1, S3V1, S1V2, S2V2, S3V2, S2V3 and S3V3 were significantly different with S1V3 and low dry matter for root at 45 days (g) was recorded in S1V1 (RNR15048 normal seed treated with cold plasma). The high per cent difference over control for dry matter for root at 45 days was recorded in Tellahamsa sprouted seed treated with cold plasma (Fig 4.14).

4.2.2.8 Chlorophyll content of leaf

Influence of type of seed (S) and varieties (V) on chlorophyll content of leaf was presented in Table 4.16. The data on chlorophyll content of leaf revealed the presence of

non-significant difference between three varieties RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 34.60 to 40.80. The treatment RNR 15048 (V1) has recorded more chlorophyll content of leaf and less in JGL 24423 (V2). Among types of seeds (S) non-significant difference was observed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values ranged from 34.14 to 39.92. The interaction effects between varieties (V) and type of seed (S) revealed the presence of non-significant differences and values ranged from 24.72 to 43.59. RNR 15048 normal seed treated with plasma (S1V1) recorded chlorophyll content of leaf (43.59) and low chlorophyll content of leaf was recorded in S3V2 (JGL 24423 untreated control seed). The per cent difference over control for chlorophyll content in leaf was recorded in JGL 24423 sprouted seed treated with cold plasma (Fig 4.15). The present results are in agreement with them but Henselova *et al.* (2012) reported that plasma effects increased chlorophyll content in maize and tomato under non-stress conditions but the present results are not in agreement with them.

4.2.2.9 Days to 50% flowering

Influence of type of seed (S) and varieties (V) on days to 50% flowering was presented in Table 4.16 (plate 4.4) and (Fig. 4.16). The data on days to 50% flowering revealed significant difference between RNR15048 (V1) and Tellahamsa (V3) while JGL24423 (V2) was on par with RNR 15048 (V1) and mean values ranged from 93.60 to 97.20. The treatment RNR 15048 (V1) was early to days to 50% flowering while JGL24423 (V2) was late to flowering. Among types of seeds (S) significant difference was noticed between sprouted seed (S2) and untreated control (S3) while normal seed (S1) was on par with sprouted seed (S2) and mean values ranged from 93.56 to 97.67. Sprouted seed has taken less number of days to 50% flowering compared to normal seed treated with plasma and untreated control. Days to 50% flowering in the interaction effects between varieties (V) and type of seed (S) ranged from 90.67 to 100.33. RNR15048 sprouted seed treated with cold plasma (S2V1) recorded less number of days to 50% flowering (90.67) and S3V1, S1V2, S2V2, S3V2, S1V3 and S3V3 were recorded significant difference with S2V1 while S1V1 and S2V3 are on par with S2V1 and more number of days to 50% flowering was recorded in S3V2 (JGL24423 untreated control). The results revealed among varieties RNR15048 sprouted seed treated with cold plasma was early to days to 50 % flowering. It may be due to completion of pre-germination metabolic activities making the seeds ready for radicle protrusion and plasma treated seed germinated earlier as compared to untreated control seeds. The results are in

Table 4.16 Effect of type of seed (S) and varieties (V) on field parameters viz., chlorophyll content of leaf, days to 50% flowering and days to maturity in paddy

Treatments	Chlorophyll content of leaf	Days to 50% flowering	Days to maturity
VARIETIES(V)			
V1: RNR15048	40.80	93.60	121.60
V2: JGL24423	34.60	97.20	125.20
V3: Tellahamsa	37.60	95.00	123.00
CD (5%)	NS	2.189	2.189
SE(m)	3.386	0.730	0.730
TYPE OF SEED(S)			
S1: Normal seed	39.92	94.56	122.56
S2: Sprouted seed	38.98	93.56	121.56
S3: Untreated control	34.14	97.67	125.67
CD (5%)	NS	2.189	2.189
SE(m)	3.386	0.730	0.730
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)			
S1V1	43.59	93.33	121.33
S2V1	40.21	90.67	118.67
S3V1	38.65	96.67	124.67
S1V2	38.75	95.33	123.33
S2V2	40.28	96.00	124.00
S3V2	24.72	100.33	128.33
S1V3	37.43	95.00	123.00
S2V3	36.45	94.00	122.00
S3V3	39.05	96.00	124.00
GRAND MEAN	37.68	95.25	123.25
CD	NS	3.792	3.792
SE(m)	5.866	1.265	1.265
CV(%)	16.96	2.30	1.77



Plate 4.4 : Three paddy varieties both normal and sprouted seed treated with 20kV plasma for 15 minutes along with untreated control at days to 50 % flowering stage in main field during *Rabi*, 2023-24

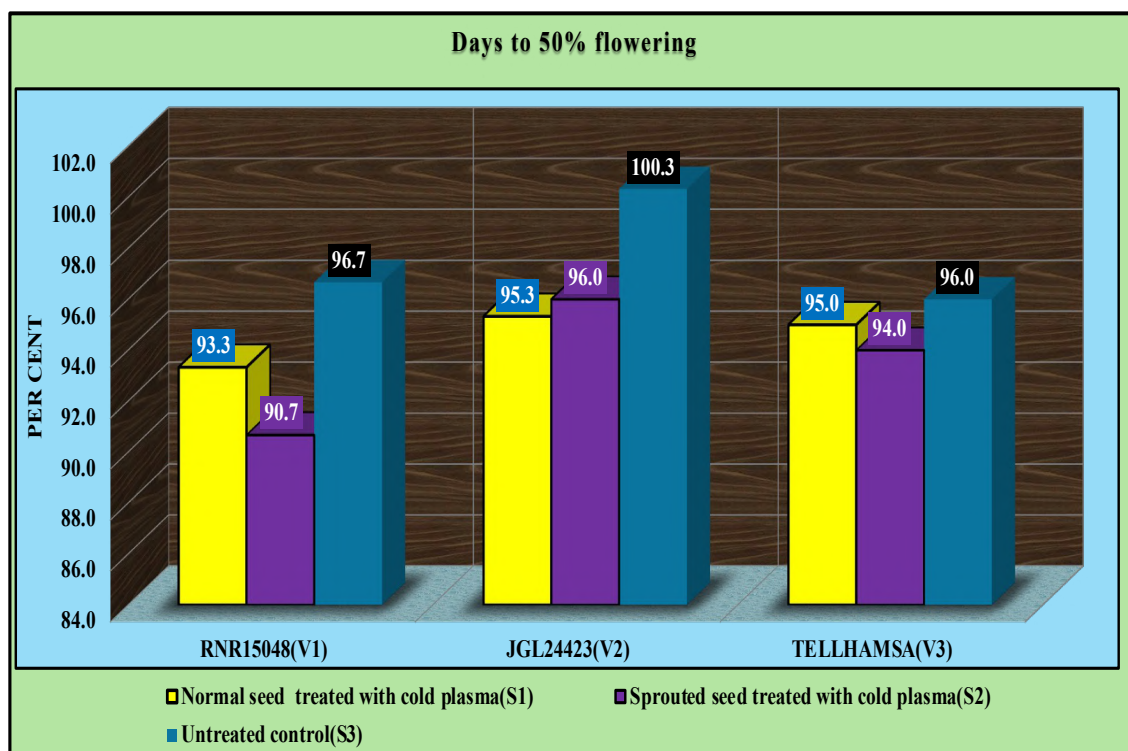


Figure 4.16 Comparison for days to 50 % flowering in three paddy varieties treated with cold plasma (20 kV for 15 min) along with untreated control

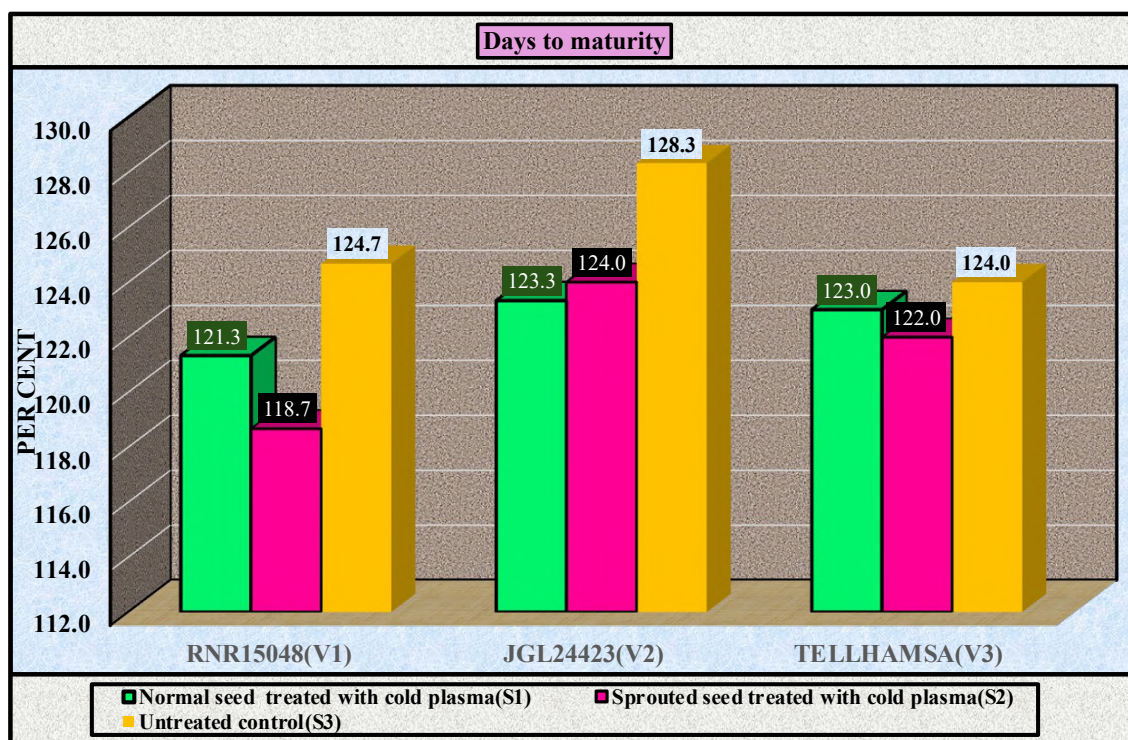


Figure 4.17 Comparison for days to maturity in three paddy varieties treated with cold plasma (20 kV for 15 min) along with untreated control

accordance with the Kumar *et al.* (2017) as they reported early days to 50%

flowering in plasma treated okra plants compared to untreated control.

4.2.2.10 Days to maturity

Influence of type of seed (S) and varieties (V) on days to maturity was presented in Table 4.16 and (Fig 4.17) The data on days to maturity revealed the presence of significant difference between RNR 15048 (V1) and JGL 24423 (V2) while Tellahamsa (V3) was on par with RNR 15048 (V1) and mean values ranged from 121.60 to 125.20 days. RNR 15048 (V1) was early to days to maturity and JGL 24423 (V2) was late to days to maturity. Among types of seeds (S) significant difference was noticed between sprouted seed (S2) and untreated control (S3) while normal seed (S1) was on par with sprouted seed and the mean values ranged from 121.56 to 125.67. Sprouted seed has taken less number of days to maturity when compared to normal seed treated with plasma and untreated control. Days to maturity in the interaction effects between varieties (V) and type of seed (S) ranged from 118.67 to 128.33. RNR 15048 sprouted seed treated with plasma (S2V1) was early to days to maturity of 118.67, and interaction effects S3V1, S1V2, S2V2, S3V2, S1V3 and S3V3 recorded significant difference with S2V1 while S1V1 and S2V3 were on par with S2V1 and more number of days to maturity was taken by JGL 24423 untreated control (S3V2). The results revealed RNR 15048 sprouted seed treated with cold plasma taken less number of days to maturity compared with control. The above results are in accordance with the Nalwa *et al.* (2018) who reported that plasma treated seeds attain early maturity than untreated control seed in chilli (*Capsicum annum longum* L.). The early maturity of the crop raised from plasma treated seed may be due to the fact that the plasma treated seeds took fewer days to emergence and to flower so that ultimately matured earlier than untreated seed.

4.2.2.11 Number of tillers per hill

Influence of type of seed (S) and varieties (v) on number of tillers per hill was presented in Table 4.17. The data on number of tillers per hill revealed the presence of non-significant difference between RNR 15048 (V1) and JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 18.22 to 22.00. Tellahamsa (V3) recorded high number of tillers per hill while JGL 24423 (V2) recorded low number of tillers per hill. Among different types of seeds (S) non-significant difference was observed between sprouted seed (S2), untreated control (S3) and normal seed (S1) and mean values ranged from 19.22 to 22.11. Normal seed recorded high number of tillers per hill compared to

sprouted seed and untreated control. The interaction effects between varieties (V) and type of seed (S) revealed non-significant differences and mean values ranged from 17.30 to 25.33. RNR 15048 normal seed (S1V1) showed high number of tillers per hill of 25.33 and low number of tillers per hill was recorded in S1V2 (JGL 24423 normal seed treated with cold plasma). The high per cent difference over control for number of tillers per hills was recorded in RNR 15048 normal seed treated with cold plasma (Fig 4.18).

4.2.2.12 Number of productive tillers per m²

Influence of type of seed (S) and varieties (V) on number of productive tillers per m² were presented in Table 4.17. The data on number of productive tillers per m² revealed non-significant difference between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values for number of productive tillers per m² were ranged from 384 to 414. Tellahamsa (V3) recorded high number of productive tillers per m² and low in JGL 24423 (V2). Among data on different types of seeds (S) revealed the presence of non-significant difference between sprouted seed (S2) and untreated control (S3) and mean values ranged from 384 to 405. Sprouted seed showed higher number of productive tillers per m² compared to normal seed and untreated control. The interaction effects between varieties (V) and type of seed (S) revealed the presence of non-significant differences and mean values ranged from 361 to 447. Tellahamsa sprouted seed treated with cold plasma (S2V3) showed high number of productive tillers per m² (447) and interaction effects S1V1, S2V1, S3V1, S1V2, S2V2, S3V2, S1V3 and S3V3 were non-significant with S2V3. However low number of productive tillers per m² was recorded in S3V2 (JGL 24423 untreated control seed). The high per cent difference over control for number of productive tillers/m² was recorded high in JGL 24423 sprouted seed treated with cold plasma (Fig 4.19).

4.2.2.13 Proline content of leaf (µmoles of proline/g of tissue)

Influence of type of seed (S) and varieties (V) on proline content of leaf are presented in Table 4.17. The data on proline content of leaf revealed significant difference between RNR 15048 (V1) and Tellahamsa (V3) and mean values ranged from 0.022 to 0.049 µmoles of proline/g of tissue. RNR 15048 (V1) recorded high proline content while, Tellahamsa recorded low proline content (V3). Among types of seeds (S) significant difference was observed between sprouted seed (S2) and untreated control (S3) and mean values ranged from 0.020 to 0.045 µmoles of proline/g of tissue. Untreated control showed high proline content than normal seed and sprouted seed treated with plasma. The interaction effects between varieties (V) and type of seed (S) revealed the

Table 4.17 Effect of type of seed (S) and varieties (V) on field parameters viz., number of tillers/hill, number of productive tillers per m², proline content (µ moles/g) and plant height (cm) in paddy

Treatments	Number of tillers/ hill	Number of productive tillers per m ²	Proline content (µmoles/g)	Plant height (cm)
VARIETIES (V)				
V1: RNR15048	21.11	385	0.049	86.80
V2: JGL24423	18.22	384	0.026	83.90
V3:Tellahamsa	22.00	414	0.022	96.70
CD (5%)	NS	NS	NS	3.696
SE(m)	1.500	24.730	0.008	1.233
TYPE OF SEED (S)				
S1: Normal seed	22.11	384	0.037	92.08
S2: Sprouted seed	20.00	405	0.020	89.80
S3: Untreated control	19.22	394	0.045	85.51
CD (5%)	NS	NS	NS	3.696
SE(m)	1.507	24.730	0.008	1.233
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)				
S1V1	25.3	379	0.061	90.27
S2V1	20.0	372	0.021	88.10
S3V1	18.0	405	0.067	81.93
S1V2	17.3	394	0.027	86.30
S2V2	19.0	396	0.020	84.00
S3V2	18.3	361	0.032	81.53
S1V3	23.6	379	0.020	99.67
S2V3	21.0	447	0.018	97.30
S3V3	21.3	415	0.027	93.07
GRAND MEAN	20.44	394.33	0.030	89.12
CD	NS	NS	NS	6.403
SE(m)	7.820	42.830	0.014	2.135
CV (%)	12.12	10.82	15.82	4.15

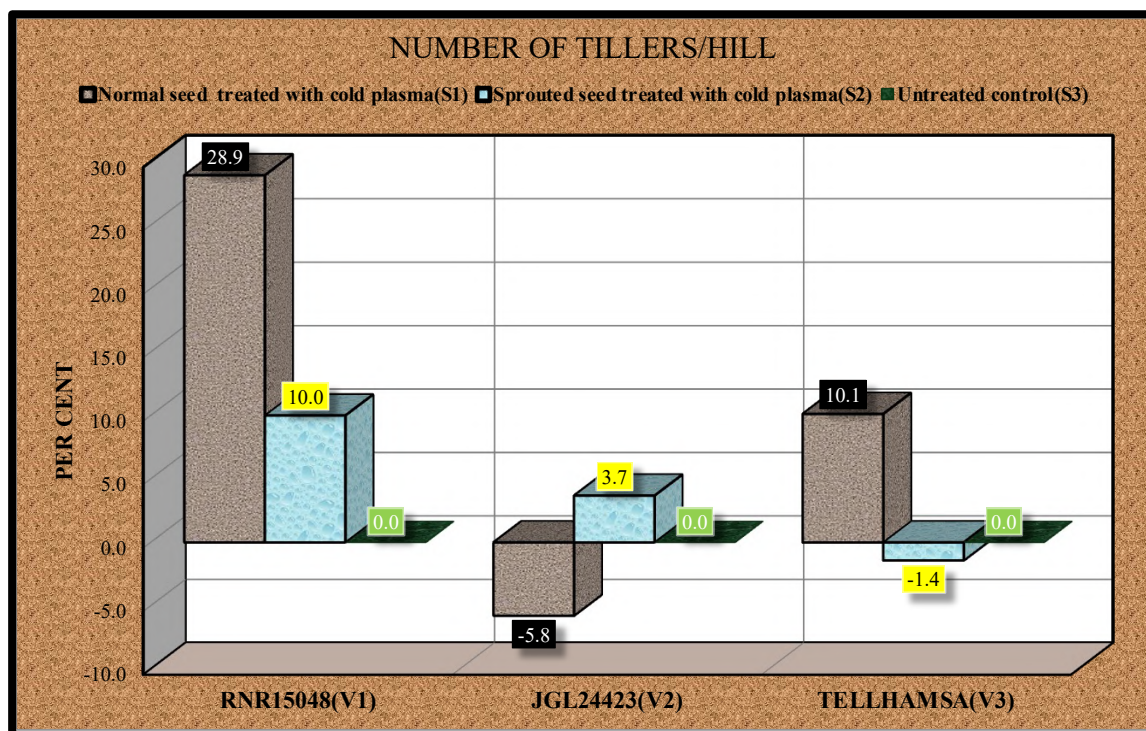


Figure 4.18 Per cent change over untreated control for Number of tillers per hill in three paddy varieties treated with cold plasma (20 kV for 15 min)

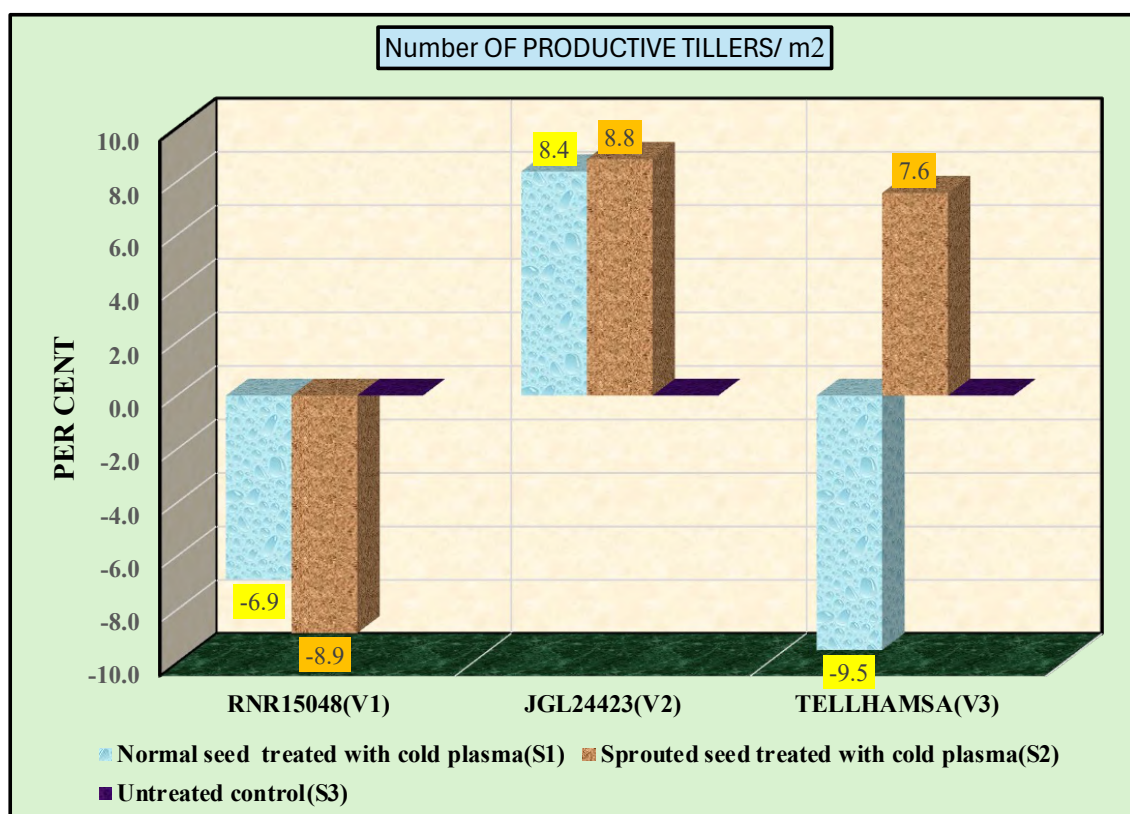


Figure 4.19 Per cent change over untreated control for in three paddy varieties treated with cold plasma (20 kV for 15 min)

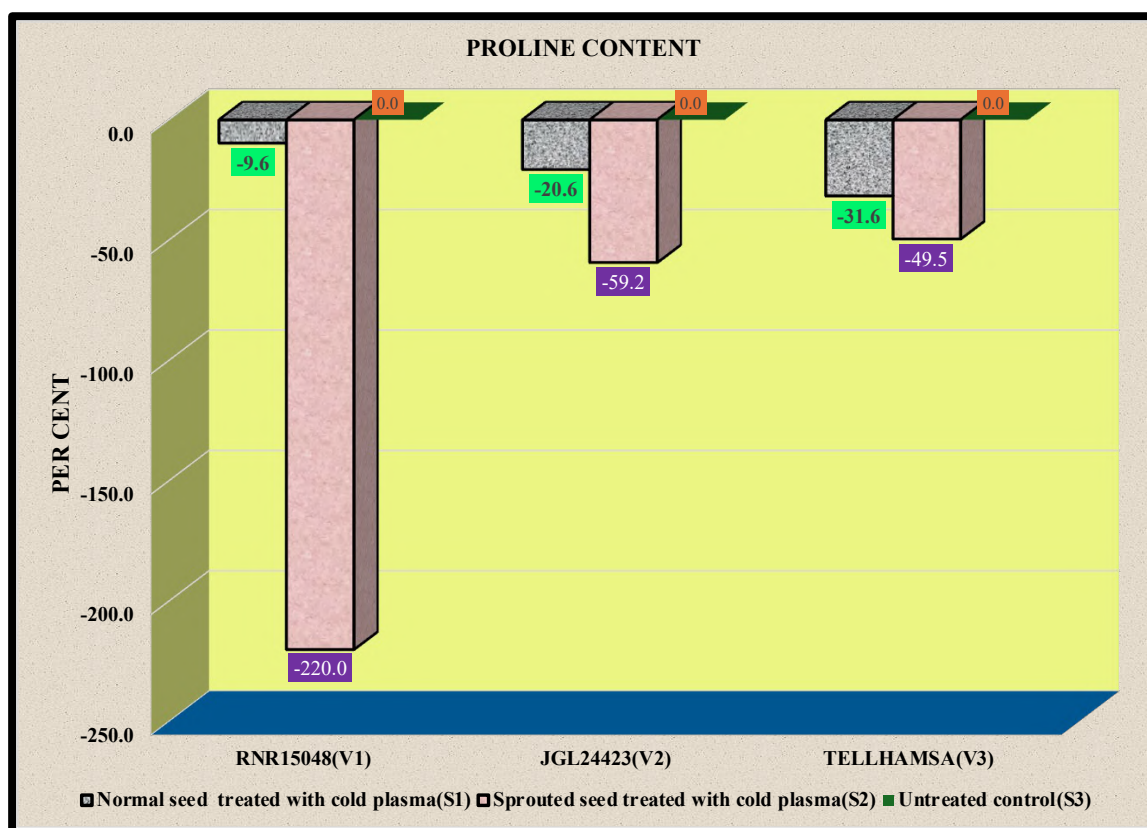


Figure 4.20 Per cent change over untreated control for proline content (μ moles of proline/g) in three paddy varieties treated with cold plasma (20 kV for 15 min)

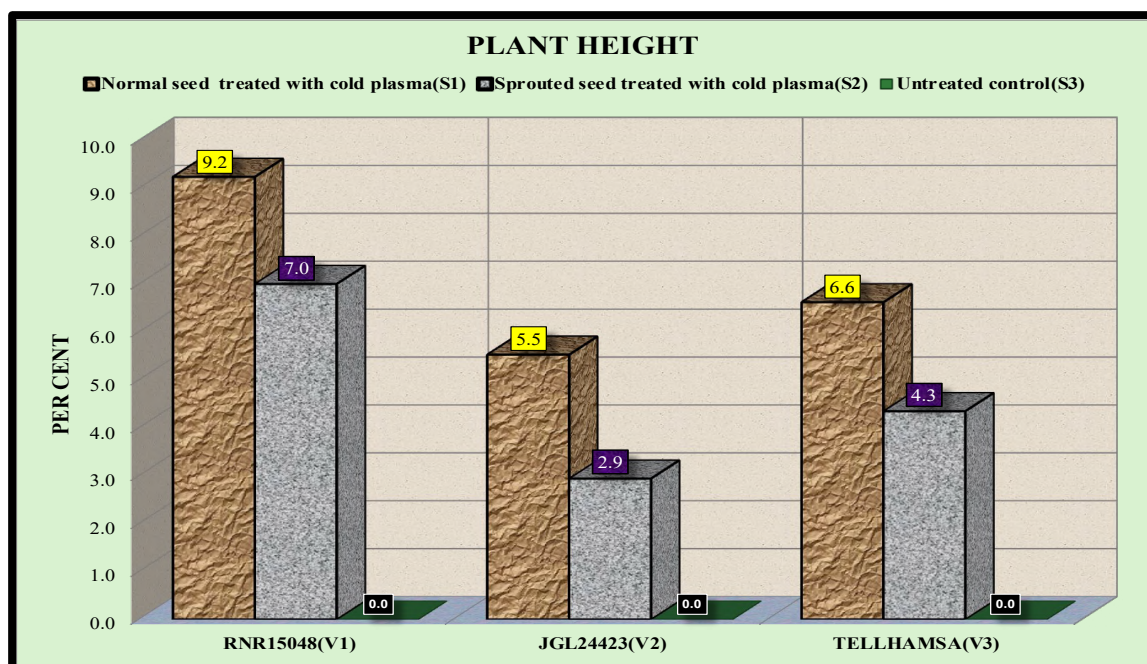


Figure 4.21 Per cent change over untreated control for plant height (cm) in three paddy varieties treated with cold plasma (20 kV for 15 min)

presence of significant differences and mean values ranged from 0.018 to 0.067 μ moles of proline/g of tissue. RNR 15048 untreated control (S3V1) recorded highest proline content of 0.067 μ moles of proline/g of tissue and treatments S2V1, S2V2, S1V3 and S2V3 were significantly different with S3V1 while treatments S1V1, S1V2, S3V2 and S3V3 were on par with S3V1 and low proline content was recorded in S2V3 (Tellahamsa sprouted seed treated with cold plasma). The per cent difference over control for proline content in leaf was negative in all the cold plasma treatments (Fig. 4.20). Abarghuei *et al.* (2021) reported the interaction between radiation time and voltage did not significantly affect the proline content in Basil crop and the results are in accordance with them. The proline upregulates the oxidative pentose phosphate pathway and it stabilizes sub-cellular pathways quench the active oxygen and protects the cell stress conditions.

4.2.2.14 Plant height (cm)

Influence of type of seed (S) and varieties (V) on plant height (cm) was presented in Table 4.17. The data on plant height (cm) revealed significant difference between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 83.90 to 96.70 cm. Tellahamsa (V3) recorded more plant height (cm) and JGL 24423 (V2) recorded less plant height. Among types of seeds (S) significant difference was noticed between normal seed (S1) and untreated control (S3) while sprouted seed (S2) was on par with normal seed (S1) and the mean values ranged from 85.50 to 92.08 cm. Normal seed recorded more plant height (cm) than sprouted seed and untreated control. Plant height in the interaction effects between varieties (V) and type of seed (S) ranged from 81.53 to 99.67 cm. Tellahamsa normal seed treated with cold plasma (S1V3) recorded more plant height (99.67 cm) and interactions S1V1, S2V1, S3V1, S1V2, S2V2 and S3V2 recorded significant difference with S1V3 while S2V3, S3V3 were on par with S1V3 and less plant height was recorded in (S3V2) JGL 24423 untreated control. The high per cent difference over control for plant height was recorded in RNR 15048 normal seed treated with cold plasma (Fig 4.21). Nalwa *et al.*, 2018 reported that plant height after 30 days of transplanting significantly differed due to plasma treatments. After 6 min of plasma treatment more plant height was recorded when compared with control in chilli (*Capiscicum annum longum* L.) and the results are in agreement with them. Increased plant height may be due to early emergence and rapid cell division in meristematic divisions, increase in cell elongation due to multiplication of various parts of the plant tissue, auxin metabolism, cell wall plasticity and permeability of cell

membrane and increase in photosynthates (Sadavarthe and Gupta,1963).

4.2.2.15 Seed yield per hill (g)

Influence of type of seed (S) and varieties (V) on seed yield per hill (g) were presented in Table 4.18. The data on seed yield per hill (g) revealed significant difference between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values for seed yield per hill ranged from 29.47 to 49.57 g. The treatment JGL 24423 (V2) recorded high seed yield per hill and low was recorded in RNR 15048 (V1). Among types of seeds (S) non- significant difference was noticed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values ranged from 35.79 g to 42.10 g. Sprouted seed recorded high seed yield per hill (g) compared to normal seed and untreated control. The interaction effects between varieties (V) and type of seed (S) revealed a non-significant differences and mean values ranged from 27.99 g to 58.85g. JGL 24423 with sprouted seed (S2V2) recorded high seed yield per hill (58.85 g) and interactions S1V1, S2V1, S3V1, S1V3, S2V3 and S3V3 recorded significant difference with respect to S2V2 while S1V2 and S3V2 were on par with S2V2 and low seed yield per hill was recorded in S3V1 (RNR 15048 untreated control). The results revealed JGL 24423 sprouted seed recorded high seed yield per hill. The high per cent difference over control for seed yield per hill was recorded in JGL 24423 sprouted seed treated with cold plasma (Fig. 4.22). Hazhizume *et al.* (2021) reported that irradiation of plasma treatments in rice seedlings during vegetative growth increased seed yield per hill and the present results are in accordance with them as the plant hormone cytokinin is important for panicle differentiation and grain yield especially in paddy. Plasma treatments will improve cytokoinin as it promotes the cell division in shoot apical meristem by up regulating the cytokinin pathway.

4.2.2.16 Seed yield (t/ha)

Influence of type of seed (S) and varieties (V) on seed yield (t/ha) was presented in Table 4.18. The data on seed yield (t/ha) revealed significant difference between RNR 15048 (V1), JGL 24423 and Tellahamsa (V3) and mean values for seed yield (t/ha) ranged from 9.84 to 11.80 t/ha. Tellahamsa (V3) recorded high seed yield (t/ha) and RNR15048 (V1) recorded low seed yield. Among types of seeds (S) non-significant difference was observed between normal seed (S1), untreated control (S3) and sprouted seed(S2) and mean values ranged from 10.51 to 11.32 t/ha. Normal seed recorded more seed yield (t/ha) compared to sprouted seed and untreated control. seed yield in the

Table 4.18 Effect of type of seed (S) and varieties (V) on field parameters viz., seed yield per hill (g), seed yield (t/ha) and 1000 seed weight (g) in paddy

Treatments	Seed yield per hill (g)	Seed yield (t/ha)	1000 seed weight (g)
VARIETIES (V)			
V1: RNR15048	29.47	9.84	11.80
V2: JGL24423	49.57	11.14	22.45
V3: Tellahamsa	35.29	11.80	22.70
CD (5%)	8.814	NS	0.948
SE(m)	2.940	0.385	0.316
TYPE OF SEED (S)			
S1: Normal seed	35.79	11.32	18.95
S2: Sprouted seed	42.10	10.51	19.26
S3: Untreated control	36.43	10.94	18.73
CD (5%)	NS	NS	NS
SE(m)	2.940	0.385	0.316
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)			
S1V1	28.55	9.68	11.52
S2V1	31.86	9.63	11.38
S3V1	27.99	9.01	12.49
S1V2	46.31	11.37	23.24
S2V2	58.85	10.13	22.67
S3V2	43.55	10.04	21.41
S1V3	32.51	12.94	22.10
S2V3	35.59	11.74	23.72
S3V3	37.75	10.70	22.27
GRAND MEAN	38.11	10.92	18.98
CD	15.266	2.000	1.642
SE(m)	5.092	0.667	0.547
CV (%)	13.14	6.27	4.99

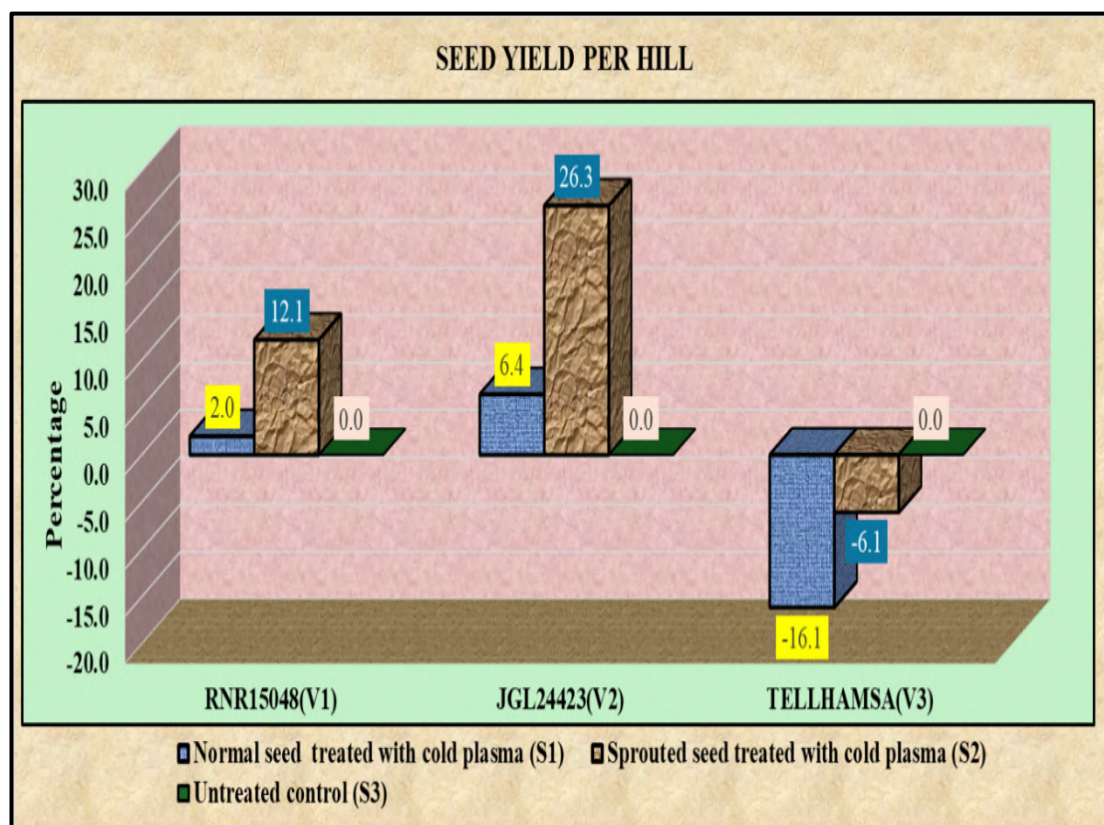


Figure 4.22 Per cent change over untreated control for seed yield per hill (g) in three paddy varieties treated with cold plasma (20 kV for 15 min)

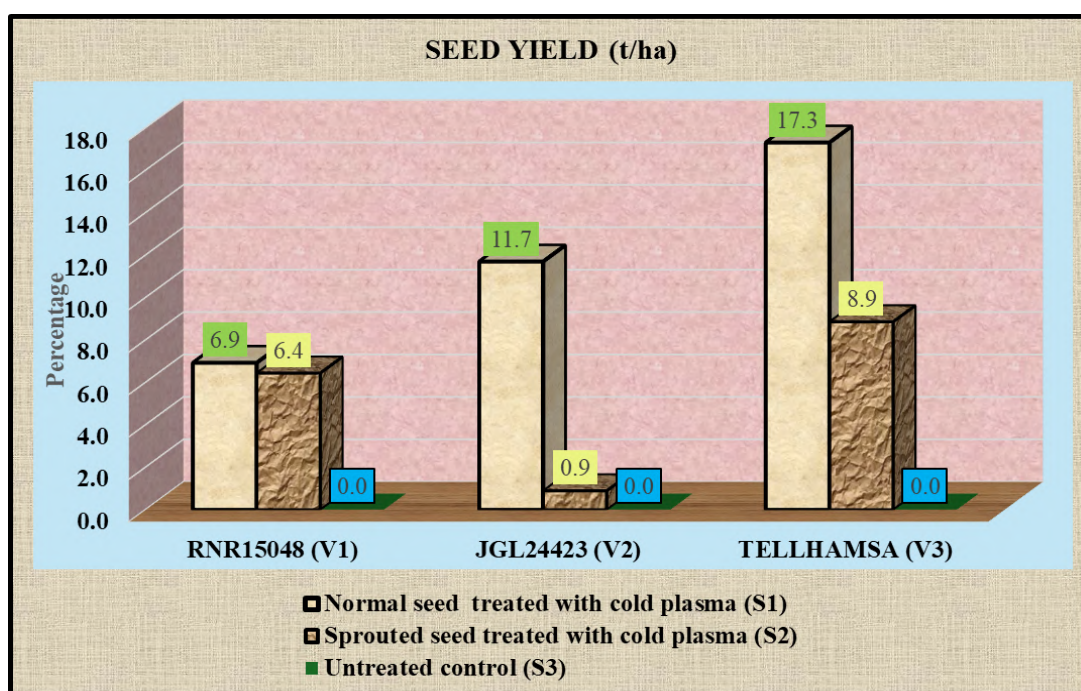


Figure 4.23 Per cent change over untreated control for seed yield (t/ha) in three paddy varieties treated with cold plasma (20 kV for 15 min)

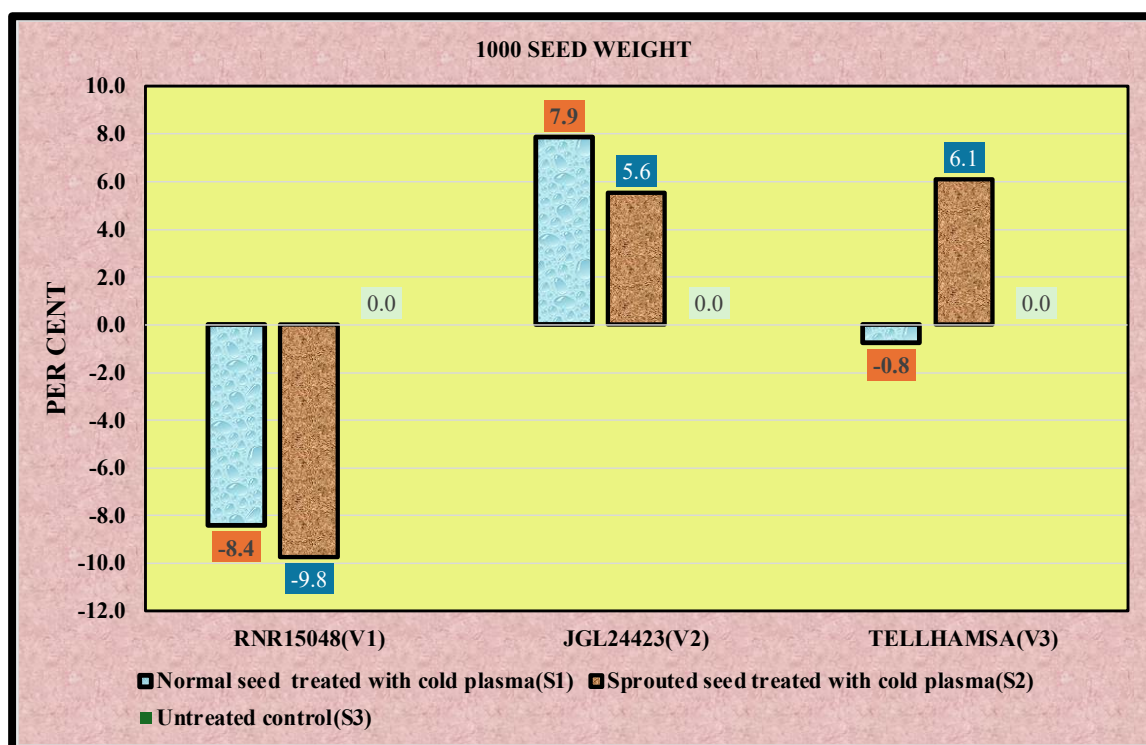


Figure 4.24 Per cent change over untreated control for 1000 seed weight (g) in three paddy varieties treated with cold plasma (20 kV for 15 min)

interaction effects between varieties (V) and type of seed (S) ranged from 9.01 to 12.94 t/ha. Tellahamsa normal seed treated with cold plasma (S1V3) recorded high seed yield (12.94 t/ha) and interactions S1V2, S2V3 and S3V3 were on par with S1V3 while S1V1, S2V1, S3V1, S2V2 and S3V2 were significant with S1V3 and less seed yield (t/ha) was recorded in S3V1 (RNR15048 untreated control). The high per cent difference over control for seed yield (t/ha) was recorded in Tellahamsa normal seed treated with cold plasma (Fig. 4.23). Hazhizume *et al.* (2021) reported irradiation of plasma treatments in rice seedlings during vegetative growth increased seed yield per hectare and the results are in accordance with them. Rashid *et al.* (2022) reported increase in 16% seed yield in plasma treated in paddy and the above results are in accordance with them as the per cent improvement in seed yield was 17 per cent in the present study.

4.2.2.17 1000 seed weight (g)

Influence of type of seed (S) and varieties (V) on 1000 seed weight (g) was presented in Table 4.18. The data on 1000 seed weight (g) revealed the presence of significant difference between RNR 15048 (V1) and Tellahamsa (V3) and mean values ranged from 11.80 to 22.70 g. Among types of seeds (S) non-significant difference was noticed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values ranged from 18.73 to 19.26 g. The 1000 seed weight in the interaction effects between varieties (V) and type of seed (S) ranged from 11.38 to 23.72 g. Tellahamsa sprouted seed (S2V3) showed high 1000 seed weight (23.72 g) and interactions S1V2, S2V2, S1V3 and S3V3 were non-significant with S2V3 and treatments S1V1 S2V1, S3V1 and S3V2 were significant with S2V3 and low 1000 seed weight (g) was recorded in S2V1 (RNR 15048 sprouted seed). The high per cent difference over control for 1000 seed weight (g) was recorded in JGL 24423 normal seed treated with cold plasma (Fig 4.24). Ling *et al.* (2018) reported in oil seed and rape that the yield component 1000 seed weight was increased by cold plasma treatments and the present results are in concurrence with them.

4.3 EVALUATION OF SEED QUALITY PARAMETERS OF PADDY SEED HARVESTED FROM COLD PLASMA TREATMENTS

4.3.1 Analysis of variance:

Both sprouted and normal seed of three varieties RNR 15048, JGL 24423 and Tellahamsa treated with 20 kV plasma for 15 min was used to raise the crop during *Rabi*, 2023-24. The seed quality parameters of harvested paddy seed recorded were mentioned below. Analysis of variance for germination percentage (%), radicle emergence (%), seedling dry weight (mg), seedling vigour index-II, speed of germination, seed moisture content (%) and electrical conductivity of seed leachate ($\mu\text{S}/\text{cm}/\text{g}$) were studied and presented in Table 4.19. The results indicated the existence of significant differences between varieties (V) for radicle emergence (%), seedling dry weight (mg), seedling vigour index-II, speed of germination, seed moisture content (%) and electrical conductivity of seed leachate ($\mu\text{S}/\text{cm}/\text{g}$). For types of seeds (S) germination percentage (%), seedling dry weight (mg), seedling vigour index-II, seed moisture content (%) and electrical conductivity of seed leachate ($\mu\text{S}/\text{cm}/\text{g}$) were found non-significant while radicle emergence (%), speed of germination were found significant. The interaction effects between varieties (V) and types of seeds (S) revealed significance difference for speed of germination and electrical conductivity of seed leachate ($\mu\text{S}/\text{cm}/\text{g}$) and non-significant difference were noticed for germination (%), radicle emergence (%), seedling dry weight (mg), seedling vigour index-II and seed moisture (%) content.

4.3.2 Seed quality parameters of harvested produce obtained from cold plasma treated rice varieties grown during *Rabi*, 2023-24

4.3.2.1 Germination (%)

Influence of type of seed (S) and varieties (V) on germination per cent were presented in Table 4.20. The data on germination per cent revealed the presence of non-significant differences between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3). Among types of seeds (S) non-significant difference between normal seed (S1) and untreated control (S3) were noticed while sprouted seed (S2) was on par with normal seed (S1) and germination per cent ranged from 97 to 99 per cent. The interaction effect between varieties (V) and type of seed (S) revealed the presence of non-significant differences and germination per cent ranged from 96 to 99 percent. The per cent difference over control for germination per cent was high in JGL 24423 sprouted seed treated with cold plasma (Fig 4.25) (Plate 4,5) (Plate 4.6). The results revealed that among interaction effects non-significant difference was noticed. The results were in

Table 4.19 Analysis of variance in seed quality parameters viz., germination percentage (%), radicle emergence (%), seedling dry weight (g), seedling vigour index-II, speed of germination, seed moisture content (%) and electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) in paddy seed harvested from cold plasma treatments

Source of variance	D.F	Germination percentage (%)	Radicle emergence (%)	Seedling dry weight (g)	Seedling vigour index II	Speed of germination	Seed moisture content (%)	Electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$)
V	2	1.778	192.000**	81.231***	2770273.900***	1.790**	15.887*	15236.580***
S	2	3.444	112.000*	0.158	1449.073	2.519***	4.664	184.783
V*S	4	5.722	21.333	0.815	12072.550	1.868***	8.458	1261.215*
Error (V)	18	2.370	21.926	0.375	3110.426	0.213	3.822	293.307
Total	26	2.923	41.846	6.646	63374.000	0.767	5.528	1583.766

NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively.

S: Type of seeds **V:** Varieties

Table 4.20 Effect of type of seed (S) and varieties (V) on seed quality parameters viz., germination percentage (%), radicle emergence (%), seedling dry weight (mg) and seedling vigour index-II in paddy seed harvested from cold plasma treatments.

Treatments	Germination percentage (%)	Radicle emergence (%)	Seedling dry weight (mg)	Seedling vigour index- II
VARIETIES (V)				
V1: RNR15048	98 (84)	96 (78)	4.23	417
V2: JGL24423	98 (81)	96 (78)	8.90	869
V3: Tellahamsa	98 (81)	88 (70)	9.84	965
CD (5%)	NS	4.63	0.60	55.23
SE(m)	0.51	1.56	0.20	18.59
TYPE OF SEED (S)				
S1: Normal seed	99 (84)	95 (77)	7.60	750
S2: Sprouted seed	98 (81)	96 (78)	7.81	763
S3: Untreated control	97 (80)	89 (71)	7.56	737
CD (5%)	NS	4.63	0.60	55.
SE(m)	0.51	1.56	0.20	19
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)				
S1V1	97 (80)	100 (90)	4.10	404
S2V1	99 (84)	99 (83)	4.60	454
S3V1	98 (81)	89 (71)	4.00	392
S1V2	98 (81)	97 (81)	9.00	885
S2V2	99 (84)	97 (81)	9.30	924
S3V2	96 (78)	93 (75)	8.30	797
S1V3	99 (84)	86 (69)	9.70	960
S2V3	96 (78)	92 (74)	9.40	910
S3V3	99 (84)	85 (67)	10.30	1023
GRAND MEAN	98.00	93.34	7.65	749.93
CD	NS	8.03	1.05	95.66
SE(m)	0.88	2.70	0.35	32.19
CV(%)	1.57	5.01	7.99	7.43

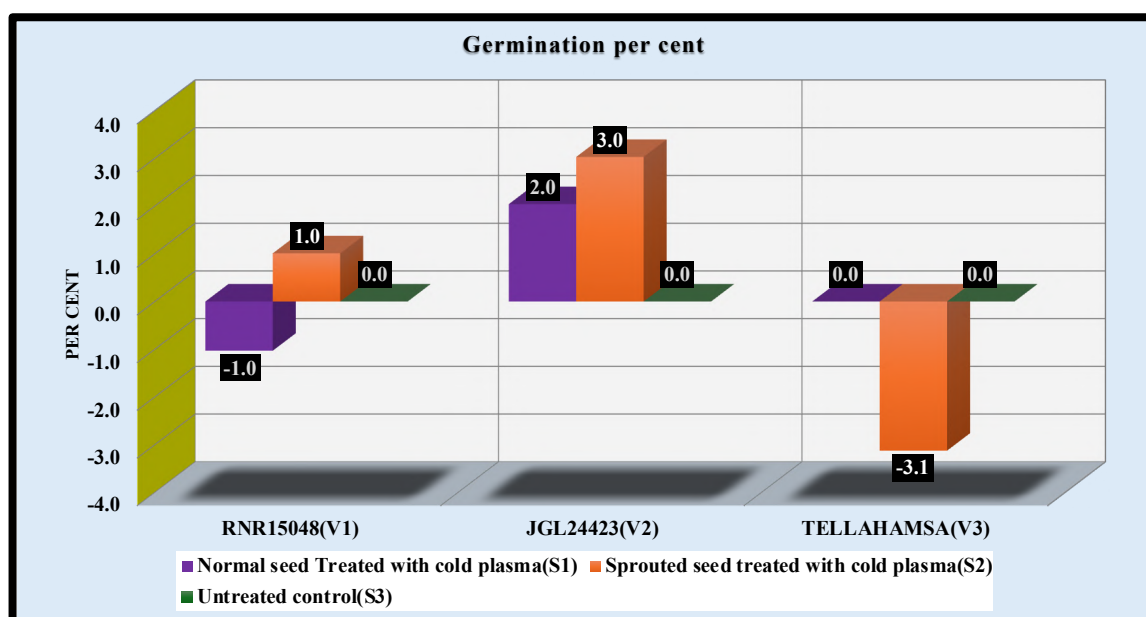


Figure 4.25 Per cent change over untreated control for germination per cent in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

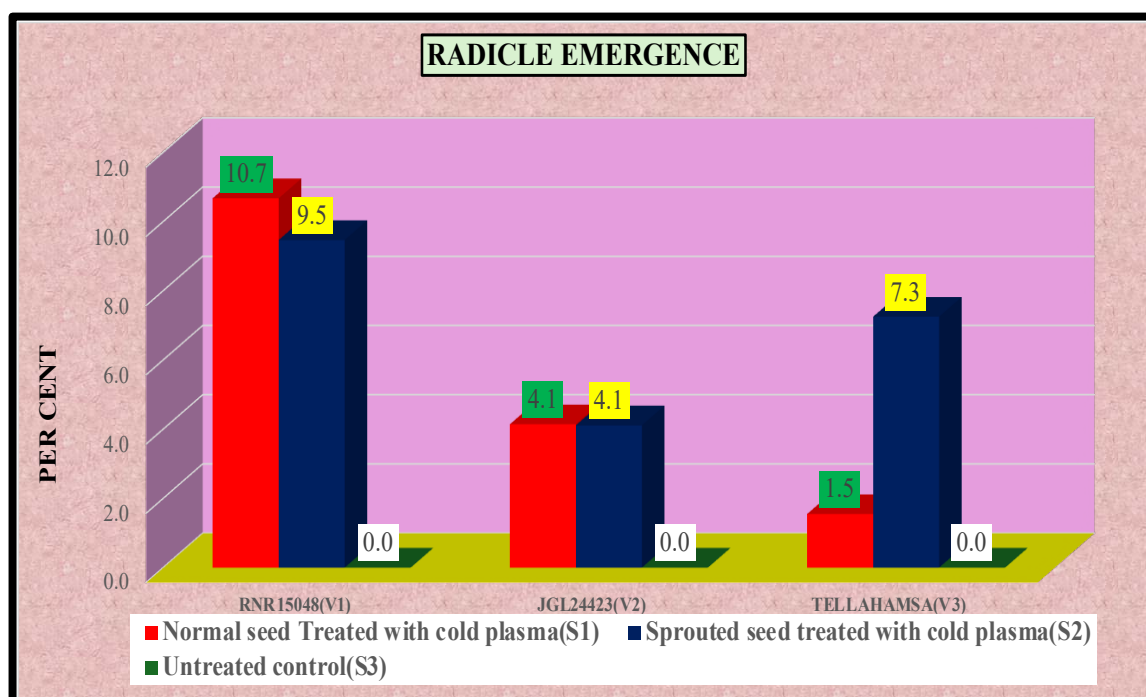


Figure 4.26 Per cent change over untreated control for radicle emergence per cent in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)



**Plate 4.5: Per cent germination in post harvest seed of paddy variety JGL24423
normal seed treated with cold plasma**



**Plate 4.6: Per cent germination in post harvest seed of paddy variety JGL24423
control**

contradictory with Volkov *et al.* (2020) and Billah *et al.* (2021) wherein they reported that non-thermal plasma treatment improved the seed germination per cent.

4.3.2.2 Radicle emergence (%)

Influence of type of seed (S) and varieties (V) on radicle emergence (%) were presented in Table 4.20. The data on radicle emergence revealed the presence of significant differences between RNR 15048 (V1) and JGL 24423 (V2) and mean values ranged from 88% to 96 per cent. Among types of seeds (S) presence of non-significant differences was noticed between sprouted seed (S2), normal seed (S1) and radicle emergence (%), which ranged from 89 to 96 per cent. Radicle emergence (%) in the interaction effects between varieties (V) and type of seed (S) ranged from 85 to 100 per cent. RNR 15048 normal seed treated with plasma (S1V1) showed high radicle emergence (%) (100) and the treatments S1V2, S2V1, S2V2, S2V3 and S3V2 were on par with S1V1 while S1V3, S3V1 and S3V3 showed significant differences with S1V1 and low radicle emergence per cent was recorded in treatments S3V3. The results revealed RNR 15048 normal seed recorded faster radicle emergence than untreated control. The high per cent difference over control for radicle emergence was recorded in RNR 15048 normal seed treated with cold plasma (Fig 4.26). The results are in accordance with the Chueauan *et al.* (2023) as they reported that increased radicle emergence at 48 h, 72 h and 96 h of germination in paddy as the seeds absorbed Plasma activated water containing N_2 species more rapidly and has a clear effect on stimulating radicle emergence. The plasma activated water permeates into the seed, making embryo to signal GA_3 to stimulate the cells in the aleurone layer and synthesize hydrolytic enzymes that leads to radicle emergence.

4.3.2.3 Seedling dry weight (mg)

Influence of type of seed (S) and varieties (V) on seedling dry weight (mg) was presented in Table 4.20. The data on seedling dry weight (mg) revealed the presence of significant differences between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values for seedling dry weight (mg) ranged from 4.23 mg to 9.84 mg. Highest seedling dry weight was recorded in Tellahamsa. Among types of seeds (S) non-significant difference was found between normal seed (S1), sprouted seed (S2) and untreated control (S3) and mean values for seedling dry weight ranged from 7.56 mg to 7.81 mg. Seedling dry weight in the interactions between varieties (V) and type of seed (S) ranged from 4.00mg to 10.30mg. Tellahamsa control (S3V3) recorded high seedling

dry weight of 10.30 mg and treatments S1V1, S2V1, S3V1 and S3V2 significantly different with S3V3 and low seedling dry weight was recorded in S3V1. The high per cent difference over control for seedling dry weight was recorded in RNR 15048 sprouted seed treated with cold plasma (Fig 4.27).

4.3.2.4 Seedling vigour index-II

Influence of type of seed (S) and varieties (V) on seedling vigour index- II was presented in Table 4.20. The seedling vigour index- II revealed significant differences between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 417 to 965. Tellahamsa (V3) recorded better seedling vigour index-II. Among types of seeds (S) non-significant differences were observed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values for seedling vigour index-II ranged from 737 to 763. Seedling vigour index-II in the interaction effects between varieties (V) and type of seed (S) ranged from 392 to 1023. S3V3 (Tellahamsa control) showed high seedling vigour index-II of 1023 and all interactions effects recorded significant difference with S3V3 except S1V3. The per cent difference over control for seedling vigour index - II was high in JGL 24423 sprouted seed treated with cold plasma (Fig 4.28).

4.3.2.5 Speed of germination

Influence of type of seed (S) and varieties (V) on speed of germination was presented in Table 4.21. The data on speed of germination revealed the presence of significant differences between RNR 15048 (V1) and JGL 24423 (V2) and mean values ranged from 14.17 to 15.05. The treatment JGL 24423 (V2) recorded high speed of germination followed by RNR 15048 (V1) and Tellahamsa (V3). Among types of seeds (S) significant differences were noticed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values ranged from 14.31 to 15.28. The interaction effects between varieties (V) and type of seed (S) revealed significant differences and speed of germination values ranged from 13.27 to 15.95. The treatment S1V2 (JGL 24423 normal seed treated with cold plasma) recorded high speed of germination of 15.95 and treatments S1V1, S2V1, S3V1, S2V2, S3V2 and S3V3 recorded significant difference with S1V2. The high per cent difference over control for speed of germination was recorded in Tellahamsa sprouted seed treated with cold plasma (Fig 4.29). The results revealed JGL 24423 normal seed recorded high speed of germination compared to control. This may be due to changes in wetting properties of seed and seed coat, due to oxidation of seed surface which leads to faster germination (Bormashenko *et al.*,

2012). The results are in accordance with the Sherawat *et al.* (2017) wherein, they reported that high speed of germination in the seeds treated with cold plasma compared to untreated control in okra.

4.3.2.6 Seed Moisture content (%)

Influence of type of seed (S) and varieties (V) on seed moisture content (%) was presented in Table 4.21 and (Fig 4.30). The data on seed moisture content (%) revealed presence of significant differences between Tellahamsa and JGL 24423 (V2) and mean values ranged from 9.46 to 12.09. JGL 24423 (V2) had recorded low seed moisture content. Among types of seeds (S) non-significant difference was noticed and mean values ranged from 9.90 to 11.33 per cent. It was observed that the sprouted seed recorded low moisture content than normal seed treated with cold plasma and untreated control. Seed moisture content in the interaction effects between varieties (V) and type of seed (S) ranged from 6.55 to 12.41%. However, in the present study S2V2 (JGL 24423 sprouted seed) recorded low seed moisture content (6.55%). The results are accordance with the Staric *et al.* (2022) reported in wheat. Los *et al.* (2019) reported that wheat seeds treated with cold plasma showed lower seed moisture content than untreated control.

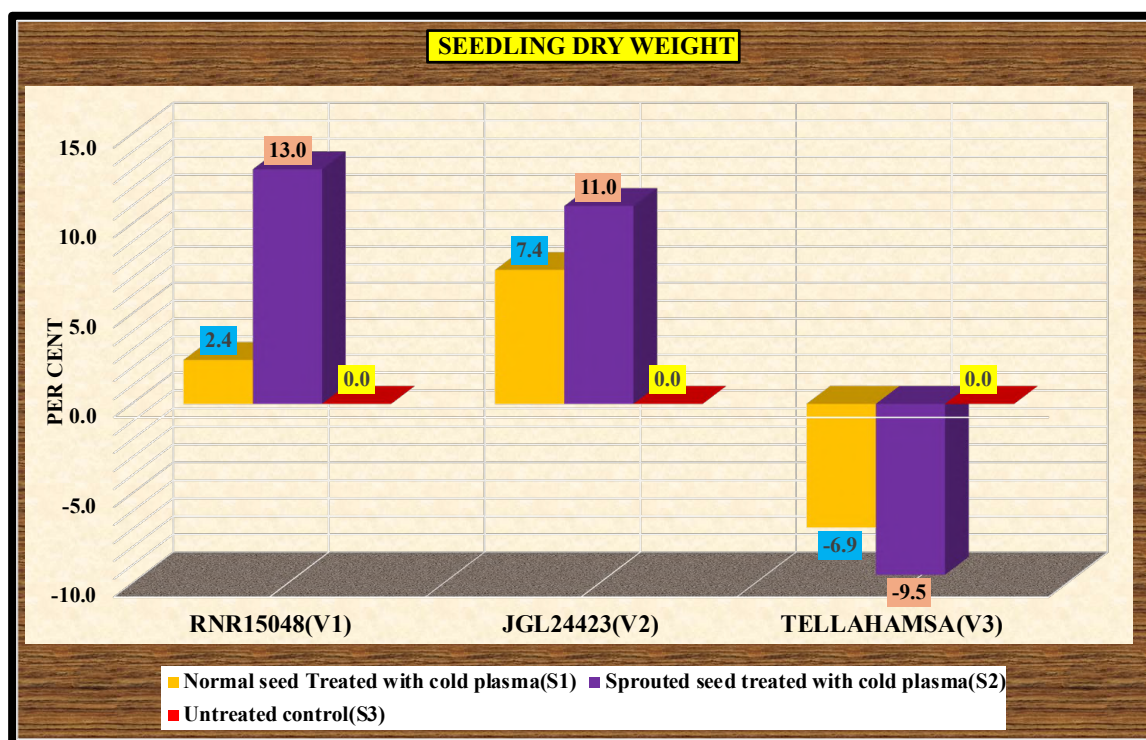


Figure 4.27 Per cent change over untreated control for seedling dry weight (g) in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

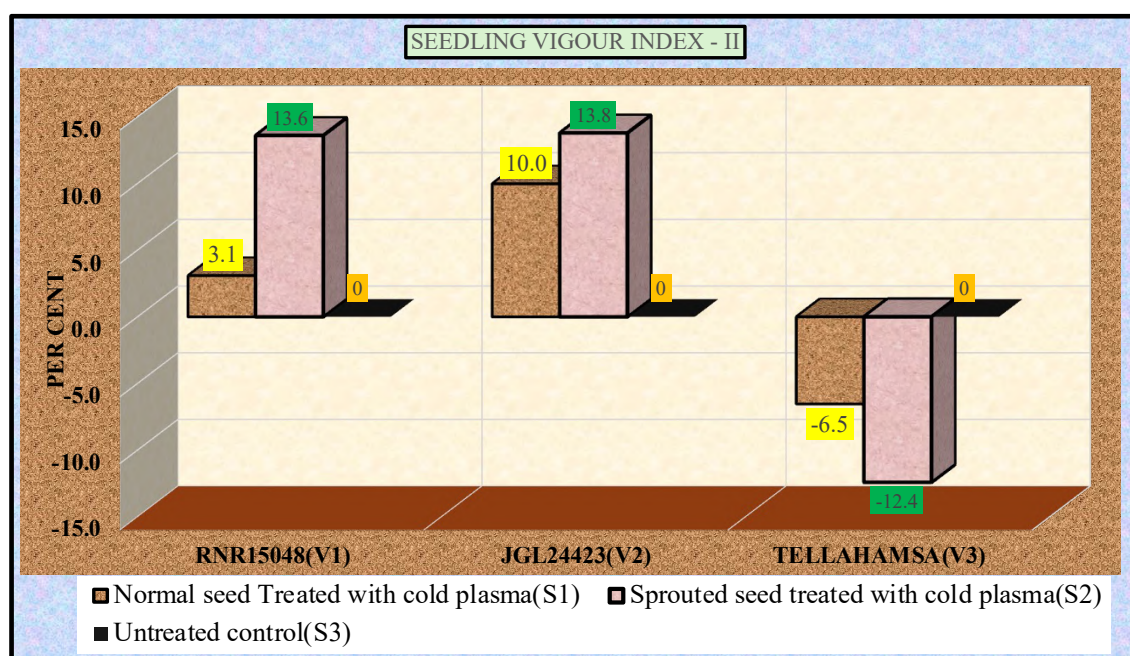


Figure 4.28 Per cent change over untreated control for seedling vigour index-II in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

Table 4.21 Effect of type of seed (S) and varieties (V) on seed quality parameters viz., speed of germination, seed moisture % and electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) in paddy seed harvested from cold plasma treatments

Treatments	Speed of germination	Seed moisture %	Electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$)
VARIETIES (V)			
V1: RNR15048	14.17	10.43 (18.84)	177.40
V2: JGL24423	15.05	9.46 (17.91)	96.40
V3: Tellahamsa	14.72	12.09 (20.34)	124.30
CD (5%)	0.45	1.93	16.97
SE(m)	0.21	0.65	5.71
TYPE OF SEED (S)			
S1: Normal seed	15.28	10.75 (19.13)	127.57
S2: Sprouted seed	14.38	9.90 (18.33)	134.43
S3: Untreated control	14.31	11.33 (19.66)	136.13
CD (5%)	0.45	NS	NS
SE(m)	0.15	0.651	5.71
INTERACTIONS – TYPE OF SEED (S) X VARIETIES (V)			
S1V1	14.74	9.94 (18.37)	182.02
S2V1	13.27	10.74 (19.13)	153.50
S3V1	14.50	10.62 (19.01)	196.72
S1V2	15.95	10.17 (18.59)	87.07
S2V2	14.48	6.55 (14.82)	117.21
S3V2	14.72	11.67 (19.97)	84.98
S1V3	15.08	12.14 (20.39)	113.62
S2V3	15.41	12.41 (20.62)	132.58
S3V3	13.68	11.72 (20.01)	126.71
GRAND MEAN	14.64	10.66	132.71
CD (5%)	0.79	3.35	29.40
SE(m)	0.26	1.12	9.89
CV(%)	3.15	8.34	8.92

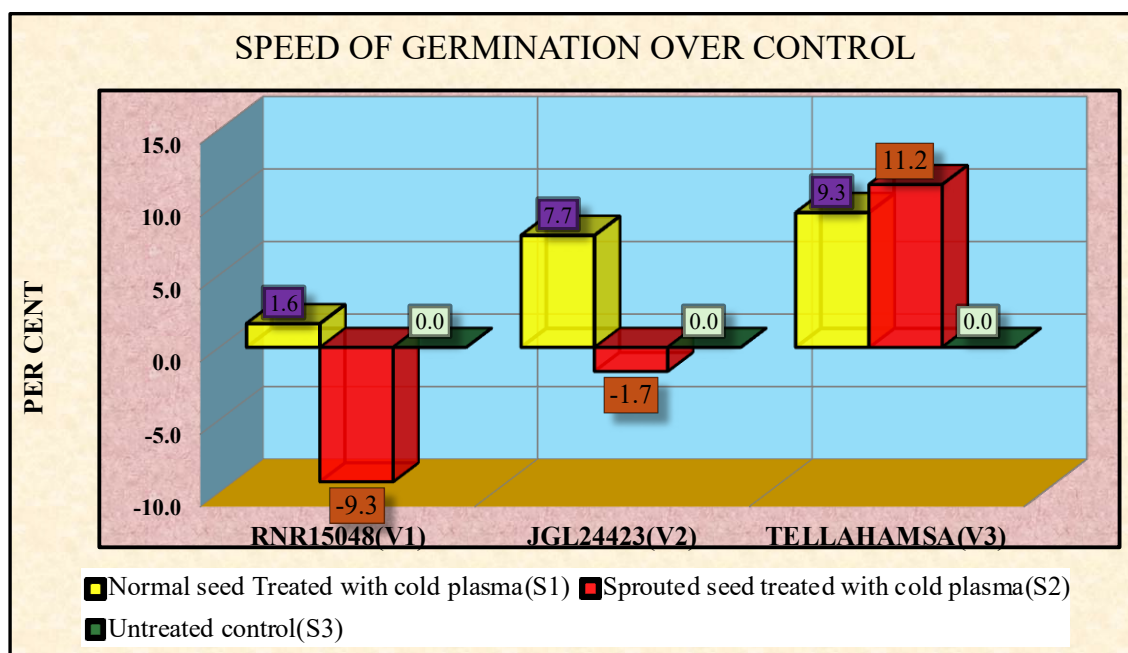


Figure 4.29 Per cent change over untreated control for speed of germination in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

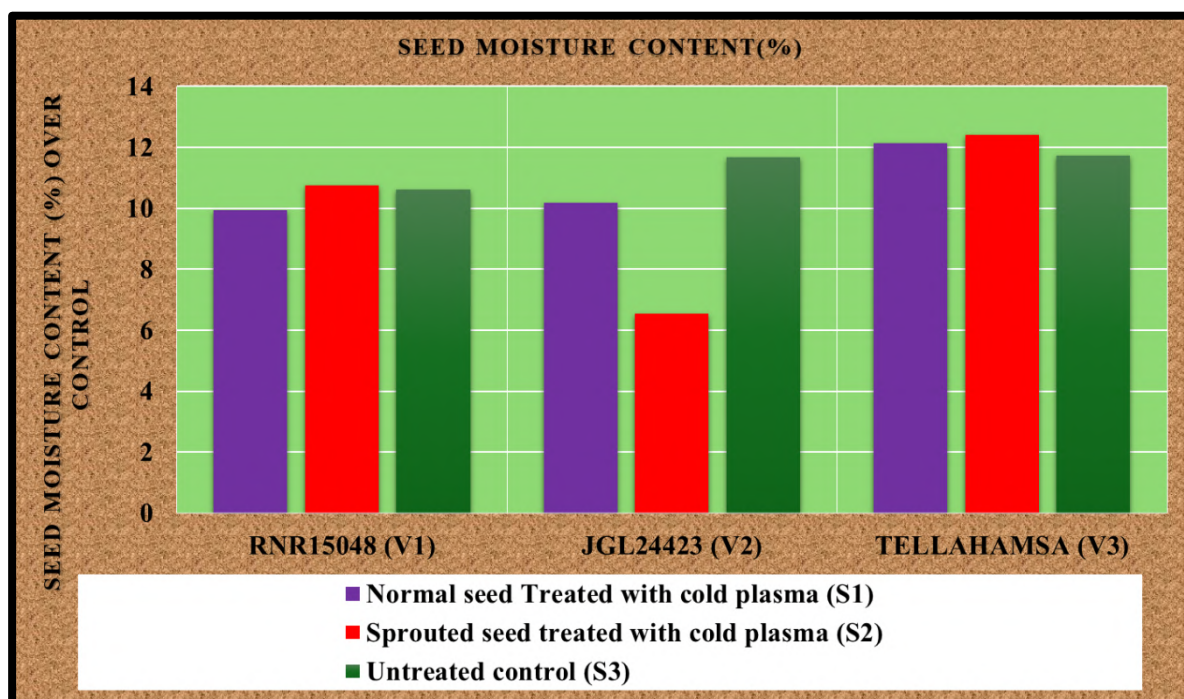


Figure 4.30 Comparison for seed moisture content in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

4.3.2.7. Electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$)

Influence of type of seed (S) and varieties (V) on electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) was presented in Table 4.21 and (Fig 4.31). The data on electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) revealed significant differences between RNR 15048 (V1), JGL 24423 (V2) and Tellahamsa (V3) and mean values ranged from 96.40 to 177.40 $\mu\text{S}/\text{cm}/\text{g}$. JGL 24423 (V2) recorded low electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) compared to RNR 15048 (V1) and Tellahamsa (V3). Among types of seeds (S) the presence of non-significant difference was noticed between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values ranged from 127.57 to 136.13. Normal seed recorded low electrical conductivity of seed leachates ($\mu\text{S}/\text{cm}/\text{g}$) than sprouted seed and untreated control. The interaction effect between varieties (V) and type of seed (S) revealed significant differences and electrical conductivity ranged from 84.98 to 196.72 $\mu\text{S}/\text{cm}/\text{g}$. S3V2 (JGL 24423 control seed) showed low electrical conductivity of 84.98 $\mu\text{S}/\text{cm}/\text{g}$ and treatments S1V1, S2V1, S3V1, S2V2, S2V3 and S3V3 recorded significant difference with S3V2 and high electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$) was recorded in S3V1. The results revealed that JGL 24423 control seed showed low electrical conductivity of seed leachates. High electrical conductivity in control group against the plasma treated seed confirmed that there was less release of seed leachates when treated with cold plasma. The results are in accordance with Sayahi *et al.* (2024) reported that plasma treated seeds recorded less electrical conductivity of leachates compared to control in soybean.

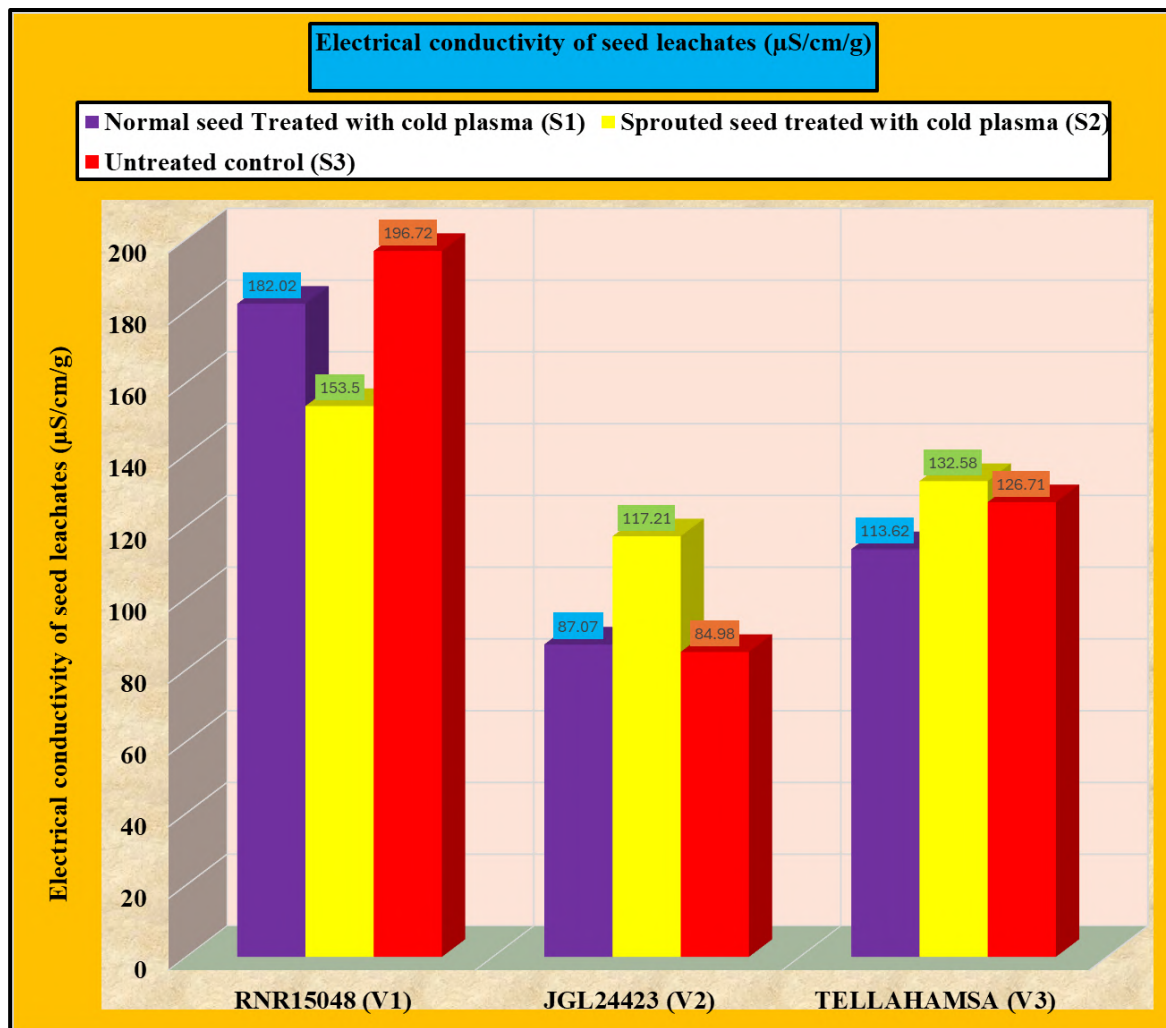


Figure 4.31 Comparison for electrical conductivity of seed leachates in the harvested produce obtained from three paddy varieties treated with cold plasma (20 kV for 15 min)

CHAPTER V

SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSIONS

Present investigation entitled “Studies on cold plasma treatments for vegetative cold tolerance in *Rabi* rice (*Oryza sativa* L.)” was aimed at improving cold tolerance in rice during *Rabi* season especially at vegetative stage. The experiment on standardization of cold plasma treatments using RNR 15048 was carried out under laboratory conditions and the effective plasma treatment was identified based on seed quality parameters and it was imposed on three rice varieties, RNR 15048, JGL 24423 and Tellahamsa and field studies were conducted during *Rabi*, 2023-24 at seed production area of Seed Research and Technology Centre. The seed quality parameters, yield contributing characters and post-harvest seed quality parameters of three rice varieties were recorded. The summary and conclusion pertaining to the study is given below.

In standardisation experiment among six different cold plasma treatments imposed on RNR 15048 using both normal and sprouted seed the normal seed performed better than the sprouted seed for majority of the seed quality parameters. Among the cold plasma treatments the seed treated with 20 kV plasma for 15 min (P3) has recorded best seed quality parameters. The high seed germination per cent at 25°C and 15°C *i.e.*, 91% and 87% respectively, seedling dry weight at 25°C and 15°C was 5.85 mg and 3.70 mg, respectively and seedling vigour index-II at 25°C and 15°C was 532 and 323, respectively, in normal seed treated with 20 kV plasma for 15 min (S1P3).

High per cent difference over untreated control for germination at 25°C and 15°C was recorded in S1P3 (normal seed treated with 20 kV plasma for 15 min) (6.3) and S2P3: sprouted seed treated with 20 kV plasma for 15 min (40.6), respectively. The per cent difference over control was more in seeds kept for germination at 15°C. High per cent difference over untreated control for seedling dry weight at 25°C and 15°C was recorded in S1P3: normal seed treated with 20 kV plasma for 15 min *i.e.*, 17.9 and in S2P3: Sprouted seed with 20 kV plasma for 15 min *i.e.*, 19.0.

High per cent difference over untreated control for seedling vigour index-II at 25°C and 15°C was recorded in S1P3: normal seed treated with 20 kV plasma for 15 min (21.9) and S2P3: sprouted seed treated with 20 kV plasma for 15 min (52), respectively. The per cent difference over control for seedling vigour index-II was more in seeds kept for germination at 15°C. High per cent difference over untreated control for speed of germination at 25°C and 15°C was recorded in S1P3: normal seed treated with 20 kV

plasma for 15 min (29.6) and S2P3: sprouted seed treated with 20 kV plasma for 15 min (28.7), respectively.

While low seed germination per cent at 25°C and 15°C was observed in S2P6: sprouted seed treated with 30 kV plasma for 20 minutes (69) and S2P7: sprouted seed untreated control (41), respectively. Low seedling dry weight at 25°C and 15°C was recorded in S2P1: sprouted seed treated with 10 kV plasma for 15 min (3.80 mg) and S2P5: sprouted seed treated with 30 kV plasma for 15 min (1.70 mg), respectively. Low seedling vigour index-II at 25°C and at 15°C was recorded in S2P4: sprouted seed treated with 20 kV plasma for 20 min (270) and S2P5; sprouted seed treated with 30 kV plasma for 15 min (101), respectively.

The low seed infection per cent at 25°C (1.5%) and 15°C (1.5%), high speed of germination at 25°C (22.86) and 15°C (16.49) and high cold tolerance index (97%) was recorded in normal seed treated with 20 kV plasma for 15 min (S1P3).

High seed infection per cent at 25°C and at 15°C was observed in S2P6 & S2P4 (9.50), and S1P6 (7.00), respectively, low speed of germination at 25°C and at 15°C was recorded in S1P4 (9.48) and S2P5 (1.605), respectively, low cold tolerance index (%) was observed in S2P7: sprouted seed untreated control (53), low peroxidase activity at 15°C was observed in S2P5: sprouted seed treated with 30 kV plasma for 15 minutes (0.042).

Among biochemical parameters non-significant difference was observed between cold plasma treatments for superoxide dismutase activity at 25°C and 15°C, peroxidase activity at 25°C, catalase activity at 25°C and 15°C and proline content at 25°C and 15°C. High peroxidase activity at 15°C (0.207) was recorded in normal seed treated with 10 kV plasma for 20 min (S1P2). Radicle emergence % at 25°C recorded non-significant differences while radicle emergence at 15°C recorded significant differences. High radicle emergence % was recorded in S1P3 (normal seed treated with 20 kV plasma for 15 min). High % differences over untreated control for radicle emergence was recorded in seed kept at 15°C.

The best cold plasma treatment *i.e.*, 20 kV for 15 min identified based on seed quality parameters, was imposed on three rice varieties RNR 15048, JGL 24423 and Tellahamsa and field experiment was laid out to study the effects of cold plasma treatments on cold tolerance at vegetative stage and on yield contributing characters. The high germination per cent was recorded in S2V3: Tellahamsa sprouted seed treated with plasma (93%) and S3V3: Tellahamsa untreated control (93%). High dry matter for shoot

at 15 (0.092 g), 30 (1.439 g) and 45 (2.909 g) days was recorded in S1V3: Tellahamsa normal seed treated with plasma. Over all high dry matter for shoot at 15, 30 and 45 days was recorded in Tellahamsa normal seed treated with plasma. High dry matter for root at 15 (0.031g) and 45 (2.434 g) days was recorded in S1V3: Tellahamsa normal seed treated with plasma and high dry matter for root at 30 days was recorded in S2V3: Tellahamsa sprouted seed treated with plasma (0.497g). Over all high dry matter for root at 15, 30 and 45 days were recorded in Tellahamsa normal & sprouted seed treated with plasma. As Tellahamsa is naturally tolerant to cold, cold plasma treatment further improved its tolerance further so it has recorded high shoot & root dry matter at 15, 30 and 45 days. Non-significant differences were observed for chlorophyll content of leaf among varieties. RNR 15048 sprouted seed treated with cold plasma (S2V1) was early to days to 50% flowering (90.67) and early to days to maturity (118.67). The parameters numbers of tillers/hill and number of productive tillers per m² recorded non-significant differences between varieties.

RNR15048 untreated control (S3V1) recorded high proline content (0.067), Tellahamsa normal seed treated with cold plasma (S1V3) recorded high plant height (99.67cm). JGL24423 sprouted seed treated with cold plasma (S2V2) recorded high seed yield per hill (58.85 g), Tellahamsa normal seed treated with cold plasma (S1V3) recorded more seed yield (12.94 t/ha), Tellahamsa sprouted seed treated with cold plasma recorded high 1000 seed weight (23.72 g).

Low per cent germination was recorded in S3V1: RNR 15048 untreated control (83) and S3V2: JGL 24423 untreated control (83), low dry matter for shoot at 15 (0.032 g) and 30 (0.316 g) days was as recorded in RNR 15048 sprouted seed treated with plasma and for 45 days it was recorded in S1V1: RNR 15048 normal seed treated with plasma (0.343 g). Over all low dry matter for shoot at 15, 30 and 45 days as recorded in RNR 15048 variety. Low dry matter for root at 15 days (0.013 g) and 30 days (0.190 g), was recorded in S2V1: RNR 15048 sprouted seed treated with plasma and low dry matter for root at 45 days was recorded in S1V1: RNR 15048 normal seed treated with plasma (0.172 g). Over all low dry matter for root at 15, 30 and 45 days was recorded in RNR 15048 variety.

Low chlorophyll content was recorded in S3V2: JGL 24423 untreated control (24.72), and it was also late to days to 50% flowering (100.33) and late to days to maturity (128.33). Among varieties RNR 15048 was early to flowering and maturity while JGL 24423 was late to flowering and maturity. Low proline content was observed in S2V3:

Tellahamsa sprouted seed treated with plasma (0.018). Low plant height was recorded in the S3V2: JGL 24423 untreated control (81.53). Low seed yield per hill (27.99 g) and seed yield (9.01 t/ha) was recorded in S3V1: RNR 15048 untreated control while low 1000 seed weight was observed in S2V1: RNR 15048 sprouted seed treated with plasma (11.38 g). Overall low seed yield was recorded in RNR 15048 among varieties. The plasma treatment increased plant height and reduced number of days to flowering and maturity when compared to untreated control. The plasma treatments also improved seed yield compared to control.

High per cent difference over untreated control for germination% (3.5) was recorded in RNR 15048 sprouted seed treated with plasma (S2V1) and JGL 24423 normal seed treated with plasma (S1V2). High per cent difference over untreated control for dry matter at 15 days, 30 days and 45 days was recorded in S1V1: RNR 15048 normal seed treated with plasma (12.8), S1V3: Tellahamsa normal seed treated with plasma (43.9) and S2V2: JGL 24423 sprouted seed (39.5), respectively. High per cent difference over untreated control for chlorophyll content of leaf, number of tillers/hill, productive tillers/m², plant height, seed yield per hill, seed yield (t/ha) and 1000 seed weight was recorded in S2V3: Tellahamsa sprouted seed treated with plasma (38.6), S1V1: RNR 15048 normal seed treated with plasma (28.9), S2V2: JGL 24423 sprouted seed treated with plasma (8.8) and S1V1: RNR 15048 normal seed treated with plasma (9.2) S2V2: JGL 24423 sprouted seed treated with plasma (26.3) S1V3: Tellahamsa normal seed treated with plasma (17.3) and S1V2: JGL 24423 normal seed treated with plasma (7.9), respectively.

Paddy seed harvested from three rice varieties RNR 15048, JGL 24423 and Tellahamsa treated with cold plasma (20kV for 15 min) was used to study the seed quality parameters. For germination per cent non-significant difference was observed among varieties while high radicle emergence per cent was recorded in S1V1: RNR15048 normal seed treated with cold plasma (100). High seedling dry weight (10.3 mg) and seedling vigour index-II (1023) was recorded in S3V3: Tellahamsa untreated control while, high speed of germination was observed in S1V2: JGL 24423 normal seed treated with plasma (15.95). Low seed moisture content was recorded in S2V2: JGL 24423 sprouted seed treated with plasma (6.55) and low electrical conductivity of seed leachates was observed in S3V2: JGL 24423 untreated control (84.98).

High per cent difference over untreated control for germination per cent, radicle emergence, seedling dry weight, seedling vigour index- II was recorded in S2V2: JGL

24423 sprouted seed treated with plasma (3.0), S1V1: RNR15048 normal seed treated with plasma (10), S2V1: RNR15048 sprouted seed treated with plasma (13), S2V2: JGL 24423 sprouted seed treated with plasma (13.8), respectively.

Based on the above summary, we conclude that:

High germination per cent, seedling dry weight (mg), seedling vigour index-II and speed of germination both at 25°C and 15°C was recorded in normal seed treated with 20 kV plasma for 15 min. The per cent difference over control for seedling vigour index-II was more in seeds kept at 15°C.

Low seed infection per cent at 25°C was recorded in normal seed treated with 20 kV plasma for 15 min whereas, sprouted seed treated with 20 kV plasma for 15 min recorded low seed infection % at 15°C. High per cent difference over control for cold tolerance index (%) was recorded in sprouted seed compared to normal seed.

Non-significant differences were recorded for most of the biochemical parameters studied. Radicle emergence per cent after 96 hrs was almost 100 % in all plasma treatments kept at 25°C while it is delayed in the plasma treatments kept at 15°C.

Among varieties Tellahamsa treated with cold plasma recorded higher seedling dry matter for shoot and root at 15, 30 and 45 days under field conditions. Non-significant difference was recorded for chlorophyll content between varieties.

The RNR 15048 variety treated with cold plasma was early to days to 50% flowering and days to maturity whereas, for number of tillers/hill and number of productive tillers/m² non-significant difference was noticed between varieties.

High proline content was recorded in RNR15048 treated with cold plasma and high plant height was observed in Tellahamsa treated with cold plasma.

JGL 24423 treated with cold plasma recorded high seed yield/hill whereas, for seed yield (t/ha) non-significant difference was recorded among varieties but for 1000 seed weight significant difference were observed among varieties.

For seed quality parameters of harvested produce of plasma treated paddy non-significant difference was recorded for germination per cent while for per cent radicle emergence after 96 hrs, RNR 15048 and JGL 24423 treated with cold plasma recorded good radicle emergence.

Tellahamsa treated with cold plasma recorded high seedling dry weight and seedling vigour index-II while JGL 24423 recorded high speed of germination, low moisture% and low electrical conductivity of seed leachates.

Future line of work:

- The cold plasma effects on plant growth, development and sustainability must be verified by abundant experimental data.
- The cold plasma effects on molecular pathways need to be studied extensively. The cold plasma effects on yield contributing pathways and plasma effects on genes responsible for yield contributing characters need to be explored.
- The underlying mechanisms of plasma effects are relatively unexplored compared to phenotypic effects and more information about the modes of plasma action on plant production and sustainability is necessary to optimize plasma systems and its applications.

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LITERATURE CITED

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APPENDIX – I

**Monthly mean meteorological data recorded during the crop growth period of paddy during *Rabi*,
2023 - 24**

STANDARD MONTH WISE METEOROLOGICAL DATA DURING CROP GROWTH PERIOD 2023-24									
RECORDED AT ACRC, ARI, RAJENDRANAGAR									
Standard month	T Max(°C)	T Min(°C)	RH I(%)	RH II(%)	Rainfall (mm)	Rainydays	Sunshine (hrs)	Wind speed (km/hr)	Evaporation (mm)
December 2023	29.8	16.9	85	42.9	0.1	0	6.2	3.6	3.5
January 2024	29.9	16.0	87	37	0.0	0	6.9	3.6	3.6
February 2024	33.3	17.5	83	29	0.0	0	8.7	4.1	5.2
March 2024	36.3	21.4	73	30	0.1	0	7.2	4.3	6.5
April 2024	39.0	23.8	69	32	0.1	0	7.7	4.3	4.6
Average	33.66	19.12	79.4	34.18	0.06	0	7.34	3.98	4.68

APPENDIX – II

Weekly mean meteorological data recorded during the crop growth period of paddy during *Rabi*, 2023 - 24

STANDARD WEEKLY WISE METEOROLOGICAL DATA DURING CROP GROWTH PERIOD 2023-24										
RECORDED AT ACRC, ARI, RAJENDRANAGAR										
Standard week no	Date	T Max (°C)	T Min (°C)	RH I (%)	RH II (%)	Rainfall (mm)	Rainy days	Sunshine (hrs)	Wind speed (km/hr)	Evaporation (mm)
50	10th Dec – 16th Dec	31.5	16.2	90	37	0.0	0	7.1	2.1	3.6
51	17th Dec- 23rd Dec	31.2	16.6	85	35	0.0	0	7.2	3.5	4.0
52	24th Dec-31st Dec	29.6	15.8	85	40	0.0	0	7.1	3.7	3.8
1	01st ^t - 07th Jan	28.7	15.4	90	38	0.0	0	7.6	3.9	3.1
2	08th - 14th Jan	29.4	16.6	88	36	0.0	0	5.2	3.7	3.0
3	15th -2st ^t June	29.9	16.0	87	37	0.0	0	6.9	3.6	3.5
4	22nd Jan - 28th Jan	30.6	17.2	84	39	0.0	0	6.7	3.9	4.1

5	29th Jan - 04th feb	30.8	15.6	87	32	0.0	0	8.1	3.4	4.3
6	05th Feb -11 Feb	33.9	18.6	84	35	0.0	0	8.2	3.8	4.9
7	12th Feb - 18th Feb	33.1	15.3	83	21	0.0	0	9.3	4.4	5.5
8	19th Feb -25th Feb	34.3	17.7	81	28	0.0	0	8.8	3.6	5.5
9	26th Feb - 04th Mar	34.0	19.4	74	29	0.0	0	8.9	4.7	5.8
10	05th Mar - 11th Mar	36.2	21.1	76	28	0.0	0	8.1	4.3	6.4
11	12th Mar – 18th Mar	36.1	22.5	77	33	0.0	0	5.4	4.9	6.0
12	19th Mar – 25th Mar	35.4	21.6	74	32	0.2	0	6.7	4.1	6.1
13	26 Mar – 01 Apr	38.6	21.3	65	32	0.0	0	7.6	3.7	7.9
14	02 Apr – 08 Apr	39.9	24.1	66	32	0.0	0	7.9	3.9	8.4
15	09 Apr – 15 Apr	36.2	23.5	80	40	0.0	0	6.7	6.3	6.7
16	16 Apr – 22 Apr	39.3	24.9	71	31	0.4	0	6.3	3.6	7.1
17	23 Apr – 29 Apr	40.4	23.0	61	26	0.0	0	9.7	3.5	8.0