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# STUDIES ON SEED QUALITY PARAMETERS IN HARVESTED SEED OF COLD PLASMA TREATED RICE (ORYZA SATIVA L) GROWN DURING RABI SEASON

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Development of climate resilient varieties is critical as cold stress is a polygenic trait, identifying the germplasm for this trait is highly challenging. Cold plasma treatment to rice seeds act as a "mild stressor" that could induce signalling pathways and strengthen the rice plants to combat cold stress, thus eliciting a response from the plant immune system to protect itself from stress. Both normal seed and sprouted seed of three varieties of rice viz., RNR15048, JGL24423 and Tellahamsa were subjected to 20 kV cold plasma for 15 minutes using dielectric barrier discharge method. The nursery was raised during Rabi 2023-24 when the temperatures dropped below 15°C. The harvested produce seed quality parameters viz., seed germination rate (%), radicle emergence (%), seedling dry weight (g), seedling vigour index- II, germination index, seed moisture content (%) and electrical conductivity of seed leachate (µS/cm/g) were recorded. The results revealed that, cold plasma treatment showed nonsignificant difference for germination rate for varieties; types of seeds and their interaction effects. In radicle emergence test after 96 hrs RNR 15048 showed 8.4% increase over Tellahamsa, among types of seed sprouted seed had showed 6% increase over untreated control and in interaction effects RNR15048 ABSTRACT normal seed treated with plasma revealed 15% increase over Tellahamsa control seed. For seedling dry weight (g) Tellahamsa had showed 58% increase over RNR15048, while types of seeds were on par with each other and interactions effects revealed that Tellahamsa control had recorded 62% increase over RNR15048 normal seed treated with plasma. Tellahamsa recorded 61.6% higher seedling vigour index-II compared to RNR15048 while interaction effects showed Tellahamsa control recorded 61% higher seedling vigour index-II compared to Tellahamsa normal seed treated with plasma. JGL24423 had showed 5.8% increase over RNR15048 for germination index while interaction effects revealed that JGL24423 normal seed treated with plasma recorded 16.80% increase over Tellahamsa control. Low moisture content was recorded in JGL24423 sprouted seed treated with plasma and high in Tellahamsa sprouted seed. Low electrical conductivity of seed leachate was recorded in JGL24423 control seed while high electrical conductivity of seed leachate was recorded in RNR15048 normal seed treated with cold plasma.

*Keywords*: Cold plasma, paddy seed quality, Germination rate, germination index, seedling vigour index-II, seed moisture content.

# Introduction

Rice is one of the world's most important food crops; it feeds more than half of the world's population. Rice cultivation provides employment and livelihoods for millions of people; it can be grown in a variety of environments, from mountains to coastal areas to plains. 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 5.51 lakh ha and Assam 1.84 lakh ha (Agricultural Market Intelligence Centre, PJTSAU Paddy Outlook-April 2023).

Abiotic stresses are the major problem in agriculture and several abiotic stresses such as drought, heat, cold, salinity and heavy metal toxicity often affect the plant growth resulting in poor crop stand and reduced agricultural productivity (Soltani *et al.*, 2006; Jabbari *et al.*, 2013). Abiotic stresses cause osmotic stress in cells, retard cell development, reduce photosynthetic activity, induce seed dormancy and delay reproduction in plants and eventually show a negative impact on yield. Similarly, cold stresses in plants affect tissue water content, membrane fluidity and chlorophyll content (Zhang *et al.*, 2012).

Cold stress is classified as chilling  $(0-15^{\circ}C)$  and freezing (<0°C) stress and is a major environmental factor limiting the growth, productivity and geographical distribution of crops (Zhu *et al.*, 2007). Cold temperatures of 0-15°C can reduce the crop survival rate, inhibit photosynthetic activity, retard growth and block the synthesis of proteins, lipids and carbohydrates (Setter and Greenway 1988; Aghaee *et al.*, 2011; Liu *et al.*, 2013).

Rice is more sensitive to low temperatures because they can inhibit seed germination (Morsy *et al.*, 2006; Baruah *et al.*, 2009) and also retard seedling growth, resulting in leaf curving, shoot shortening and few tillers (Dashtmian *et al.*, 2014). In addition, low temperatures may cause the accumulation of reactive oxygen species (ROS), such as superoxide anion, singlet oxygen and hydrogen peroxide ( $H_2O_2$ ), which leads to lipid peroxidation, electrolyte leakage and membrane damage (Kuk *et al.*, 2003; Hung *et al.*, 2008; Bhattacharjee, 2013).

Since, abiotic stress is a polygenic trait, identifying the germplasm for this trait is highly challenging. Practical use of cold stress tolerant varieties has their own limitations similar to disease resistant varieties (Hsu *et al.*, 2003; Huang *et al.*, 2013; Yang *et al.*, 2014; Godoy *et al.*, 2021).

Non-thermal plasma treatment on seeds is anticipated to act as a "mild stressor" that could induce signalling pathways and strengthen the plant to combat abiotic stress factors. Huge research was conducted to assess the role of non-thermal plasma treatment in the alleviation of abiotic stresses and demonstrated multiple positive effects of plasma treatment over the conventional technologies (Wu *et al.*, 2007; Ling *et al.*, 2015; Guo *et al.*, 2017; Iranbakhsh *et al.*, 2017; de Groot *et al.*, 2018; Kabir *et al.*, 2019). The plasma composition depends on the operating parameters such as voltage, frequency, humidity and flow rate and gas mixture. Gases such as argon, oxygen, nitrogen, helium and or/air can be ionized by electric fields to form electrons ions, UV, thermal radiation and reactive species. In the present study we used non thermal dielectric barrier discharge plasma treatments which are safe and sustainable approaches for seed treatments.

# **Materials and Methods**

The cold plasma (20 kV for 15 min) treated paddy seeds of three varieties *viz.*, RNR15048 (V1), JGL24423 (V2) and Tellahamsa (V3) both normal seed and sprouted along with untreated control were sown in the nursery during *Rabi*, 2023 when the temperatures fall below  $15^{\circ}$ C during the month of December and transplanted to the main field in January, 2024 and all the agronomic practices were carried and harvested the paddy seed. The harvested paddy seed was taken and studied the seed quality parameters in order to evaluate the effects of cold plasma on the harvested produce.

**Location:** The cold plasma treatments were carried out at MPMI-QC lab, and the crop was grown at the seed production farm of Seed Research and Technology Centre and seed quality parameters were recorded at Seed Testing Laboratory (STL) of Department of Seed Science and Technology, PJTSAU, Rajendranagar, Hyderabad.

**Statistical analysis:** The mean data generated through evaluation of above parameters was subjected to two Factorial Completely Randomized Design (FCRD), Statistical analysis was done using INDOSTAT software and analysis of means and variances were carried out by following the method developed by Gomez and Gomez. 1984.

# Methods

Factor 1Varieties (V)RNR15048 (V1)Factor 1Varieties (V)JGL24423 (V2)Tellahamsa (V3)Tellahamsa (V3)Factor 2Types of seeds<br/>(S)Normal seed(S1)Sprouted seed(S2)Untreated control (S3)

**Treatments:** The treatment details are mentioned below:

# Germination rate (%)

The germination test was conducted as per ISTA, 2019. Hundred seeds each in four replications were taken from each treatment and placed on germination

paper. The paper towel then placed in seed germination chamber, maintained with temperature of  $25 \pm 0.5^{\circ}$ C and  $95 \pm 2\%$  relative humidity. On the  $14^{\text{th}}$  day, the normal seedlings (seedlings with normal shoot and root growth and which did not having secondary infections) were calculated and the mean was expressed as percentage.

Germination rate (%) = 
$$\frac{\text{Number of normal seedlings}}{\text{total number of seeds placed}} \times 100$$

#### Radicle emergence (%)

The radicle emergence was calculated as per the method suggested by the (Chuea-uan *et al.*, 2024). After placing the seed in petriplates the length of the radicle emergence that exceeds 2 mm was measured and recorded. The percentage radicle emergence was calculated as follows:

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

# Seedling dry weight (mg)

Ten normal seedlings were picked randomly which were used for calculating germination percentage and were put in the butter paper bags and kept in hot air oven at  $100 \pm 1^{\circ}$ C for 24hrs. Later they were removed and allowed to cool in desiccators for 30 minutes before weighing in an electronic balance. The mean dry weight of the seedlings were recorded and expressed in milligrams (ISTA, 2019).

#### Seedling vigour index-II

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

Seedling vigour index-II = Germination (%) X Seedling dry weight (mg)

#### Germination index

Germination index was estimated by using ISTA recommended method. In this method three replications of 100 seeds were placed in sand. The number of seedlings (2cm radical length) germinated at each day up to 14 days after setting the test were recorded regularly. Germination index was calculated as

Germination index =

$$\sum \left(\frac{N1}{d1} + \frac{N2 - N1}{d2} \dots \dots \dots \dots \frac{Nn - Nn - 1}{dn}\right)$$

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

d = serial number of days

#### Seed Moisture content (%)

The moisture content was determined by the hot air oven method as per ISTA (2019) 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 the hot air oven and dried at  $130^{\circ}$ C for a period of 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.

Moisture per cent (%) = 
$$\frac{W2 - W3}{W2 - W1} \times 100$$

- W1 Empty container weight along with lid (gm)
- W2 Weight of the grounded seed sample before drying (gm) kept in container along with the lid
- W3 Weight of the grounded seed sample after drying (gm) kept in container along with the lid

# Electrical conductivity of seed leachates (µS/cm/g)

Electrical conductivity test was performed as per ISTA (2019) 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 20<sup>°</sup>C temperature for 1 day. After 1 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 gram of seed. The conductance was calculated using the formula given below

 $\frac{\text{Electrical conductivity}}{(\mu S \text{ cm}^{-1}\text{g}^{-1})} = \frac{\text{Conductivity reading - Control reading}}{\text{Weight of replicate (g)}}$ 

#### **Results and Discussion**

Both normal and sprouted seed of three rice varieties RNR15048, JGL24423 and Tellahamsa treated with non-thermal dielectric barrier discharge plasma (20kV for 15min) and with untreated control. The seeds were harvested from the plasma treated paddy and un treated control and seed quality parameters of the harvested rice seed are estimated. Analysis of variance for cold plasma treatments on germination rate (%), radicle emergence (%), seedling

dry weight (mg), seedling vigour index-II, germination seed moisture content (%), index, electrical conductivity of seed leachate (µS/cm/g) of seed harvested from cold plasma treated rice varieties grown under field conditions were studied and presented in Table. 1. The results indicated the existence of significant differences between varieties (V) for radicle emergence (%), seedling dry weight (mg), seedling vigour index-II, germination index, seed moisture content (%), electrical conductivity of seed leachate  $(\mu S/cm/g)$ . For types of seeds (S) germination rate (%), seedling dry weight (mg), seedling vigour index-II, seed moisture content (%) and electrical conductivity of seed leachate (µS/cm/g) recorded non-significant differences while radicle emergence (%), germination index had observed significant difference. The interaction effects between varieties (V) and types of seeds (S) revealed the presence of significant seedling differences between vigour index-II, germination index and electrical conductivity of seed leachate (µS/cm/g) and germination rate, radicle emergence (%), seedling dry weight (mg) and seed moisture % recorded non-significant difference (Table. 1).

#### Germination rate

Influence of type of seed (S) and varieties (V) on germination per cent were presented in Table. 2 and Fig. 1. The data on germination rate revealed the presence of non significant differences between RNR15048 (V1), JGL24423 (V2) and Tellahamsa (V3). Among types of seeds (S) significant differences between normal seed (S1) and untreated control were observed (S3) while sprouted seed (S2) was on par with normal seed (S1) and germination rate ranged from 97 to 99 percent. The interaction effect between varieties (V) and type of seed (S) revealed the presence of significant differences and germination rate ranged from 96 to 99. The interaction effects S2V1, S2V2, S1V3 and S3V3 showed highest germination rate of 99 and the treatments S1V1, S3V1and S1V2 were nonsignificant with the above four treatments while S2V3 and S3V2 were significant with S2V1, S2V2, S1V3 and S3V3 and lowest germination rate was recorded in S2V3 and S3V2. The results obtained are in agreement with Bian et al. (2024) and Billah et al. (2021) who reported that non thermal plasma treatment under low temperature stress showed increase in seed germination rate when compared to control seeds.

# **Radicle emergence** (%)

Influence of type of seed (S) and varieties (V) on radicle emergence (%) after 96 hrs was presented in Table. 2 and Fig. 2. The data on radicle emergence (%)

after 96 hrs revealed the presence of significant differences for RNR15048 (V1) and JGL24423 (V2) with Tellahamsa (V3) and mean values ranged from 88 to 96%. Among the data on different types of seeds (S) revealed the presence of significant differences between normal seed (S1) and untreated control (S3) while sprouted seed (S2) was on par with normal seed (S1) and radicle emergence % ranged from 89 to 96. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences and radicle emergence % ranged from 85 to 100 %. RNR15048 normal seed treated with plasma (S1V1) showed highest radicle emergence (%) after 96 hrs and the treatments S2V1, S1V2, S2V2, S3V2 and S2V3 were on par with S1V1 while S3V1 and S3V3 recorded significant differences with S1V1 and lowest radicle emergence per cent was recorded in treatments S3V3 (Table. 2). The results revealed that RNR15048 sprouted seed recorded faster radicle emergence than the control seed or untreated control. The results are in accordance with the Chuea-uan et al. (2024) as they reported that increased radicle emergence at 48, 72 h and 96 h of germination as the seeds absorbed plasma activated water containing N2 species more rapidly and has a clear effect on stimulating radicle emergence. The plasma activated water permeates into the seed, causing the embryo to signal GA3 to stimulate the cells in the aleuronic layer to synthesise hydrolytic enzymes that leads to radicle emergence.

## Seedling dry weight (mg)

Influence of type of seeds (S) and varieties (V) on seedling dry weight (mg) were presented in the Table. 2 and Fig. 3. The data on seedling dry weight (mg) revealed the presence of significant differences between RNR15048 (V1), JGL24423 (V2) and Tellahamsa (V3) and mean values of seedling dry weight (mg) ranged from 4.23 to 9.84. Among the data on different types of seeds (S) revealed the presence of non significant differences between normal seed (S1) and untreated control (S3) and mean values for seedling dry weight ranged from 7.56 to 7.81 mg. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences for seedling dry weight which are ranged from 4.0 to 10.3. Tellahamsa control (S3V3) showed highest seedling dry weight (10.3 mg) and treatments S1V1, S2V1, S3V3, S2V1 and S3V2 were significantly different while S1V2, S2V2, S1V3 and S2V3 were on par with S3V3 and lowest seedling dry weight was recorded in S3V1.

#### Seedling vigour index- II

Influence of type of seed (S) and varieties (V) on seedling vigour index-II was presented in Table. 2 and Fig. 4. The seedling vigour index-II data revealed the presence of significant differences between RNR15048 (V1), JGL24423 (V2) and Tellahamsa (V3) where mean values ranged from 417 to 965. Tellahamsa (V3) recorded higher seedling vigour index-II than RNR15048 (V1) and JGL24423 (V2). The data on types of seeds (S) revealed the presence of nonsignificant differences between normal seed (S1), untreated control (S3) and sprouted seed (S2) and mean values for seedling vigour index-II ranged from 737 to 762. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences and values for seedling vigour index-II ranged from 392 to 1023. S3V3 (Tellahamsa control) showed highest seedling vigour index-II (1023) and interaction effects S1V1, S2V1, S3V1, S1V2, S2V,S3V2 and S2V3 recorded significant difference while S2V3 was on par with S3V3 and lowest seedling vigour index-II was recorded in S3V1.

# Germination index

Influence of type of seed (S) and varieties (V) on germination index was presented in the Table. 3 and Fig. 5. The data on germination index revealed the presence of significant differences between RNR15048 (V1) and JGL24423 (V2) and mean values ranged from 14.17 to 15.05. JGL24423 (V2) has recorded higher germination index than RNR15048 (V1) and Tellahamsa (V3). In types of seeds (S) significant differences for germination index was 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) for germination index revealed the presence of significant differences and values ranged from 13.27 to 15.95. RNR15048 sprouted seed (S2V1) recorded highest germination index (15.95) and interaction effects S1V1, S1V2, S1V3, S2V2, S3V2 and S3V3 were recorded significant difference with S1V2 while S1V3 and S2V3 were on par with S1V2 and lowest germination index was recorded in S2V1. The results are in accordance with the results of sehrawat et al. (2017) and Bormashenko et al. (2012) wherein higher speed of germination was observed in the seeds treated with cold plasma when compared to untreated control and this may be due to the changes of the wetting properties of seed and seed coat, due to oxidation of their surface which leads to faster germination. The results revealed that JGL24423 normal seed and RNR15048 sprouted seed recorded higher germination index than control.

#### Seed Moisture content (%)

Influence of type of seed (S) and varieties (V) on seed moisture content (%) were presented in the Table. 3 and Fig. 6. The data on seed moisture content (%) revealed the presence of significant differences between RNR15048 (V1) and JGL24423 (V2) and mean values ranged from 9.46 to 12.09. JGL24423 (V2) recorded lowest seed moisture content. Among types of seeds (S) revealed the presence of non significant difference and mean values ranged from 9.90 to 11.33. Sprouted seed had noticed lowest moisture content than normal seed and untreated control. The interaction effects between varieties (V) and type of seed (S) revealed the presence of significant differences and moisture content ranged from 6.55 to 12.41. JGL24423 sprouted seed (S2V2) showed lowest seed moisture content (6.55) and all the treatments S1V1, S2V1, S3V1, S1V2, S3V2, S1V3 S2V3 and S3V3 were recorded significant difference with S2V2 and highest seed moisture content was observed in S3V3. The results are in accordance with the Staric et al. (2022) who reported that seeds treated with cold plasma showed lower seed moisture content than the control.

# Electrical conductivity of seed leachates (µS/cm/g)

Influence of type of seeds (S) and varieties (V) on electrical conductivity of seed leachates (µS/cm/g) was presented in the Table. 3. and Fig. 6. The data on electrical conductivity of seed leachates (µS/cm/g) revealed the presence of significant differences between RNR15048 (V1) and JGL24423 (V2) and Tellahamsa (V3) and mean values ranged from 96.40 to 177.40. JGL24423 (V2) had recorded low electrical conductivity of seed leachates (µS/cm/g) than RNR15048 (V1) and Tellahamsa (V3). Among types of seeds (S) non-significant difference between normal seed (S1), untreated control (S3) and sprouted seed (S2) were noticed and mean values ranged from 127.57 to 136.13. The interaction effect between varieties (V) and type of seed (S) revealed the presence of significant differences and electrical conductivity values ranged from 84.98 to 182.02. JGL24423 untreated control (S3V2) recorded lowest electrical conductivity of 84.98 and interaction effects S1V1, S2V1, S3V1, S2V2, S2V3, S3V3 were recorded significant difference with S3V2 and highest electrical conductivity (µS/cm/g) was recorded in S1V1 (Table. 3 and Fig. 6). The results revealed that JGL24423 normal seed and JGL24423 untreated control seed showed lowest electrical conductivity of seed leachates compared to control. The results are in agreement with the Sayahi et al. (2024) who reported that plasma treated seeds showed less electrical conductivity of leachates compared to control. Higher amount of electrical conductivity in the control group against the plasma treated seed confirmed low degradation of cold plasma treated seeds.

# Conclusion

The results revealed that, cold plasma treatment showed non-significant difference for germination rate for varieties; types of seeds and their interaction effects. In radicle emergence test RNR 15048 showed 8.4% increase over Tellahamsa, among types of seed sprouted seed had showed 6% increase over untreated control and in interaction effects RNR15048 plasma treated normal seed revealed 15% increase over Tellahamsa control seed. For seedling dry weight (mg) Tellahamsa had showed 58% increase over RNR15048, while types of seeds were on par each other and interactions effects revealed that Tellahamsa control had recorded 62% increase over RNR15048 plasma treated normal seed. Tellahamsa recorded 56% higher seedling vigour index-II compared to RNR15048 interaction while effects showed Tellahamsa control recorded 61% higher seedling vigour index- II compared to Tellahamsa normal seed treated with plasma. JGL24423 had showed 5.8% increase over RNR15048 for germination index while interaction effects showed that JGL24423 normal seed treated with plasma recorded 14.23% increase over Tellahamsa control. Less moisture content was recorded in JGL24423 sprouted seed and highest in Tellahamsa sprouted seed. Lowest electrical conductivity of seed leachate had recorded in JGL24423 control seed while highest was recorded in RNR15048 normal seed. Thus, cold plasma treatments can be used to improve seed quality parameters and protect paddy seeds from cold stress.

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# **Authors' Contributions**

Mr. P.S. has executed the entire work as part of his PG research programme. Dr. YB is the chairperson who formulated and helped in execution of the work, PJMR, MM are the advisory committee helped the student in doing his research work and KLP helped in doing the cold plasma treatments as part of advisory committee.

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