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Standardization of Cold Plasma Treatments for Seed Quality and Crop Productivity: Lab to Field Validation

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ABSTRACT: 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.we have used both normal and sprouted seed of rice variety RNR15048 for standardization of cold plasma 20 Kv 15 min have recorded best treatment in seed physiological parameters and showed non significant difference for biochemical parameters.Three varities RNR15048,Tellahamsa and JGL24423 were used in the field studies by using the randomized block design to study effect of cold plasma treatment.Tellahamsa dry seed with plasma recorded best treatment for dry matter for

shoot and root at 15,30 and 45 days,plant height and seed yield. Non-significant differences were observed for chlorophyll content of leaf numbers of tillers/hill and number of productive tillers per m^2 among varieties. RNR 15048 sprouted seed treated with cold plasma was early to days to 50% flowering and early to days to maturity. Tellahamsa sprouted seed treated with cold plasma recorded high 1000 seed weight.cold plasma treatment have improved seed physiological parameters and yield parameters and in rabi rice yield production .

Introduction

Rice is a vital crop that sustains more than half of the global population, serving as a primary source of nutrition in Asia. It is an essential source of carbohydrates for energy and minerals like iron, magnesium, phosphorus, and zinc, as well as vitamins B1, B2, B3, B6, and B9. However, rice production is plagued by challenges that affect global food security. Global rice production declined to an estimated 503.0 million tons in 2022-23. In India, rice cultivation area during the *Rabi* season of 2022-23 witnessed a significant increase of 37.89% at 55.02 lakh hectares, as compared to 39.90 lakh hectares in 2021-22 [1]. Nonetheless, abiotic stresses, including heat, cold, salinity, drought, and toxicities arising from heavy metals, hinder rice productivity. These stresses affect around 96.5% of the world's arable land [2]. Of these, cold stress is perhaps the most significant problem for rice, a crop that occurs naturally in tropical and subtropical environments, placing it extremely vulnerable to low temperatures compared with other crops such as wheat and barley.

Cold stress, especially during the vegetative phase, affecting it badly, along with poor germination, stunted seedling growth, yellowing of leaves, withering, and lesser tillers. Moreover, reproductive development suffers as well, along with higher sterility of flowers and pollens, ultimately reducing seed yield. This vulnerability is quite pronounced particularly during *Rabi* seasons when the temperatures drop drastically. Recent advancements in agricultural technology have identified cold plasma as a potential tool that can bring relief through abiotic stresses, specifically cold stress, that might be otherwise prevalent in rice culture. Cold plasma is also known as the fourth state of matter that is described as composed of excited atoms, ions, and molecules along with reactive oxygen species (ROS) and reactive nitrogen species (RNS). These species, coupled with free radicals, atoms,

and molecules in different energy states, yield a highly reactive environment with a lot of applications. Cold plasma works at temperatures approaching ambient near 60°C, thus making the technology suitable for agriculture [3,4].

Cold plasma produces reactive species, which penetrate microbes, oxidize cytoplasmic membranes, and disrupt cellular systems to effectively inactivate pathogens [5]. In various crops, this antimicrobial activity has also been proven helpful in diminishing losses due to diseases, accounting for 20-30% of the annual losses [6]. Studies showed that seed germination, water absorption, and total seed vigor improved by several folds following cold plasma treatment under chilling conditions [7]. Plasma-activated water has also been used for plant disease management [8,9]. Its role in inducing defense responses is less explored under cold stress conditions in agricultural research.

In rice, cold stress during germination inhibits metabolic activity, that reduces seedling growth and causes other defects such as curling of leaves, shortening of the shoot, and poor tillering [10]. At low temperatures, root tissues suffer which hampers water and nutrient uptake and thus stunts growth in general [11]. Cold plasma treatment has shown promising effects in the reduction of the abovementioned effects. For example, treatment at 15 kV for 30 seconds could enhance rice germination and seedling growth under cold stress by increasing antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) [12]. Cold plasma application does not only limit to the mitigation of stress but also improve other growth parameters in rice. Scientific studies have shown that plasma treatments increase panicle length, stem length, seedling height, panicle weight, and harvest index [13]. Also improve seed quality through increased permeability of the seed coat without altering the moisture content which favors germination and seedling vigor [14].

Given the potential of cold plasma technology in addressing cold stress and enhancing rice productivity, this study aimed to develop standardized protocols for cold plasma treatments to improve seed quality under in vitro conditions and evaluated their effectiveness in the field during the Rabi season. This research addresses critical stages of rice growth and parameters of yield to set

up the possibility of cold plasma as a potentially available means of mitigating abiotic stresses on rice production with global climatic changes.

Results

RNR15048 seed physiological parameters under both normal and cold stress conditions when treated with and without plasma treatment. Analyis of variance of seed physiological parameters germination(%),seedling dry weight (mg), seedling vigour index-II, per cent seed infection, speed of germination were presented in Table 1& 2. mean comparision results of rice seed of variety RNR15048 physiological parameters seed germination(%),seedling dry weight (mg), seedling vigour index-II, per cent seed infection, speed of germination were presented in the Table3. The results indicate that cold plasma treatment, especially at 20 kV for 15 minutes (P3),enhanced germination % and seedling dry weight, seedling vigour index-II, germination index, seed infection % in rice seeds of variety RNR15048. results at germination at normal and cold stress conditions was enhanced under plasma treatment compared to control or untreated seed under normal conditions dry seeds responded better to plasma treatment compared to sprouted seeds, and germination was particularly improved at normal conditions, with some benefits still observed at under cold stress. there is no significant difference between interaction effects S*P(Table1) mean interaction effects between Germination at normal conditions exhibited a higher germination rate 91% of S1P3 compared to sprouted seed treated with 20 Kv 20 min S2P6, which had a rate of 69%. Cold plasma treatment, particularly S1P3 20 kV for 15 minutes with dry seed, significantly improved germination compared to the sprouted seed with untreated control P7 which is 78%. plasma treatment enhanced 14.2% more germination than control under normal conditions (Table 3). there is a significant difference present between interaction effects (Table1) mean interaction effect results indicated that germination at cold stress conditions. Plasma treated seeds under cold conditions significant enhancement than control. plasma treatment S1P3 which is 20 Kv 15 min treated with cold plasma 87% while low was observed in S2P7 sprouted seed under cold stress with out plasma treatment which is 41%.plasma treatment had enhanced 52.8% more germination compared cold stress with out plasma(Table3)(Fig.1). there is no significant difference between interaction effects

(Table 1).mean interaction effect results indicated that seedling dry weight at normal conditions with out cold stress had significantly enhanced than the control.S1P3 20Kv for 15 min with normal seed treated with cold plasma have shown more seedling dry weight of 5.85mg which is 32.47%more than the S2P7 sprouted seed untreated with cold plasma (Table 3). and there is a significant difference present between interaction effects (Table 1).mean interaction effect results of seedling dry weight at cold stress S2P3 sprouted seeds treated with 20 kV plasma for 15 minutes produced the highest seedling dry weight at cold stress conditions, measuring 4.2 mg when compared to S2P7 sprouted seed with cold stress with out plasma treatment which is 3.40mg. plasma treatment with cold stress have enhanced more significance thn the cold stress with out plasma treatment i.e., 19.04% more than the cold stress with out cold plasma treatment (Table 3). Seedling dry weight was consistently higher at normal temperature compared to cold stress conditions, reflecting the negative impact of cold stress on growth at lower temperature(Fig.2). there is presence of non significance difference between interaction effects (Table 1) and mean comparision effects of the results of seedling vigour index-II indicates at normal temperature conditions indicates that the combination of normal seeds treated with 20 kV plasma for 15 minutes S1P3 achieved the highest seedling vigour index-II at normal temperature 532 mg showing significant improvement over control without cold plasma treatment the lowest value was recorded in S2P4 sprouted seeds treated with 20 kV plasma for 20 minutes. seedling vigour index- II was significantly improved when compared with cold plasma treatment when compared sprouted seed control which is 42.1% more than the control (Table3) and there is presence of significant difference between interaction effects. The result indicated that at seedling vigour index - II at cold stress conditions the normal seeds treated with 20 kV plasma for 15 minutes S1P3 recorded the highest value 323, significantly out performing all other interactions. The lowest value was observed in S2P5 sprouted seeds treated with plasma 101.plasma treatment under cold stress conditions have shown more significant improvement than the cold stress seed without plasma treatment that is 56.96 % greater than the control (Table3). Seedling vigour index-II at normal temperature was notably higher than at cold stress conditions, emphasizing the adverse effects of cold stress (Table 3). there is presence of significance differnce between interaction effects (Table 1) and mean interaction effects of the results of seed infection % indicated that S1P3 20Kv 15 min treated with normal seed recorded lowest seed

infection % at normal conditions of 1.50 and highest was recorded in S2P5. plasma treated seed have recorded significant improvement over control without plasma treatment which is 82.35 % respectively (Table 3)(fig.2). analysis of variance indicated that presence of significant difference between interaction effects (Table 1) and mean interaction effects between the results of seed infection % at cold stress indicated that S1P3 normal seeds treated with 20 kV plasma for 15 minutes recorded the lowest infection rate 1.50 %. High infection rates were observed in S1P6 normal seeds treated with 30 kV plasma for 20 minutes.plasma treated seed have recorded more significant improvement over the control with out plasma treatment under cold stress which is 70% respectivley (Table 3). Cold plasma treatment effectively reduced seed infection rates at cold stress compared with normal temperature conditions which is contradictory to other parameters (Fig.3). there is no signficaant difference among interaction effects and mean comparision results indicated that germination index at normal temperature have recorded that S1P3 have shown that achieved the highest germination index was recorded in 22.86 and lowest germination index was recorded in S1P4 normal seeds treated with 20 kV plasma for 20 minutes.plasma treated seed have shown more significant improvement over control conditions under normal temperature conditions which is 30.35 % respectively (Table 3). There is presence of non significant difference among interaction effects and mean comparision results of germination index at cold stress conditions was recorded that S1P3 normal seed treated with 20Kv 15 min has highest germination index of 16.50 and lowest germination index was observed in S2P5 sprouted seeds treated with 30 kV plasma for 15 minutes. Plasma treated seed have shown more significant improvement over control at normal temperature conditions which is 34.96 % respectively(Table 3)(Fig.4).

RNR15048 variety of rice seed antioxidant parameters under both normal and cold stress conditions with and without plasma treatment. mean comparison results of rice seed of variety RNR15048 antioxidant parameters such as Superoxide dismutase (SOD) ,Peroxidase (POD) , Catalase (CAT) were presented in Table 5.there is presence of non significant between interactions (Table 4) of SOD at normal conditions and mean comparison results indicated that there is non significant improvement compared with control and high super oxide dismutase was observed in S2P7 sprouted seed treated with control. The highest SOD activity was found in sprouted seed untreated control S2P7 which is 9.1 and lowest was followed in S1P1 dry seed with 10Kv 15 min

4.3. there is presence of no significant difference between interaction effects between SOD at cold stress conditions(Table 4) and super oxide dismutase activity at cold stress was recorded non significant improvement plasma treatment and control without plasma treatment (Table 5)(Fig.6). SOD activity was higher at cold stress than at normal temperature, indicating its role in countering oxidative stress during cold exposure. there is presence of non-significant difference between interaction effects (Table 4) and mean interaction effects of the results revealed that peroxidase activity at normal temperature conditions non-significant improvement compared with control. The highest peroxidase activity was recorded in dry seeds treated with 10 kV plasma for 20 minutes S1P2, while the lowest activity was observed in sprouted seeds treated with 30 kV plasma for 15 minutes S2P5 and peroxidase activity at cold stress conditions have revealed that non significant improvement was observed among treatments. The highest activity was recorded in S1P2 dry seed treated with 10 Kv 20 min 0.207 units/mg/protein/min, while the lowest was in S2P5 sprouted seed treated with 30 kV for 15 minutes (Table 5).there is no significant difference between tretments of peroxidase activity at cold stress conditions.Peroxidase activity was at cold stress conditions higher than the normal temperature conditions (Table 5)(Fig.5). there is no significant difference between interaction treatments of catalase activity at normal temperature conditions (Table 4) and mean interaction results of catalase activity at normal temperature have recorded non significant improvement over other, the highest catalase activity was noted in sprouted seeds treated with 30 kV for 15 minutes S2P5, 144 and the lowest activity was observed in S2P3 sprouted seed treated with 20 kV for 15 min. results of catalase activity at cold stress conditions were revealed non-significant improvement among other treatments , though S2P5 sprouted seeds with 30 kV plasma for 15 minutes recorded the highest catalase activity 439 and the lowest activity was observed in S2P3 20 kV for 15 min with 287 (Table 5)(Fig.5) .

RNR15048 seed proline activity under both normal and cold stress conditions with and with out plasma treatment. mean comparision results of RNR15048 variety proline content were presented in the Table 5. there is no presence of significant difference between interaction effects of proline content at normal temperature (Table 4). proline content at normal temperature have revealed that non-significant improvement when compared with control. Highest proline content was

recorded in untreated sprouted seed treated with control S2P7, 0.033 µmoles, while the lowest was in sprouted seeds treated with 10 kV for 20 min S2P2 (Table 5). there is prsence of non significance difference between interaction effects of proline at cold stress conditions (Table 4) and mean comparision effects result of proline content at cold stress conditions have revealed that non significant improvement compared with control without cold plasma treatment under cold stress conditions (Table 5)(Fig.6).

RNR15048 variety radicle emergence under both normal and cold stress conditions with and without plasma treatment. there is no signficant difference present between types of seeds of radicle emergence at normal temperature conditions (Table 6) results indicated that radicle emergence at normal temperature conditions indicated non significant improvement over control (Table 7).there is presence of significant difference between types of seeds of radicle emrgence at cold stress conditions (Table6).mean comparision effects of radicle emergence at cold stress conditions indicated significant improvement over control (Table7)(Fig.7) .

Dry matter for shoot at 15 days : there was presence of non significance difference betweeen types of seeds and varieties for rice under field Rabi conditions (Table 8).mean comparision effects of dry matter at 15 days after nursery sowing indicated that Tellahamsa normal seed treated with cold plasma S1V3 recorded the highest dry matter value 0.092 g.lowest was recorded in recorded in was observed in S2V1 RNR 15048 sprouted seed treated with cold plasma that is 0.032 g.plasma treatment indicated that there is significant difference between compared to control which is 55.43 % respectively (Fig.8).

Dry matter for shoot at 30 days : there was presence of significance difference between interaction effect between types of seeds and varieties (Table 8). mean comparision effects of dry matter at 30 days after nursery sowing indicated that Tellahamsa normal seed treated with cold plasma S1V3 yielded the highest dry matter value 1.439 g. S2V1 RNR 15048 sprouted seed treated with cold plasma recorded the lowest value 0.316 g. .plasma treatment indicated that there is significant difference between compared to control which is 72.34% respectively(FIG.8).

Dry matter for shoot at 45 days : there was presence of significance difference between interaction effect between types of seeds and varieties (Table 8). mean comparision effects of dry matter at 45days after nursery sowing indicated that Tellahamsa normal seed treated with cold plasma S1V3 recorded the highest dry matter value 2.909 g. lowest dry matter value 0.343 g was observed in S1V1 RNR 15048 normal seed treated with cold plasma.plasma treatment was significant improvement over control which is 68.76 %(Fig.8).

Dry matter for root at 15 days : there was presence of non significance difference betweeen types of seeds and varieties for rice under field Rabi conditions (Table 8).mean comparision effects of dry matter at 15 days after nursery sowing indicated that highest values were recorded in both Tellahamsa normal seed treated with cold plasma S1V3 and Tellahamsa untreated control S3V3 that is 0.031 g. lowest dry matter value was observed in S2V1 RNR 15048 sprouted seed treated with cold plasma 0.013 g.plasma treatment have indicated more significant improvement over control which is 54.83% respectively(Fig.8).

Dry matter for root at 30 days : there was presence of significance difference between interaction effect between types of seeds and varieties (Table 8). mean comparision effects of dry matter at 30 days after nursery sowing indicated that Tellahamsa sprouted seed treated with cold plasma S2V3 showed the highest dry matter value 0.497 g. The lowest root dry matter value 0.132 g was observed in S3V3 Tellahamsa untreated control.plasma treatment have indicated significant improvement over control which is 55.33% repectively(Fig.8).

Dry matter for root at 45 days : there was presence of significance difference between interaction effect between types of seeds and varieties (Table 8).). mean comparision effects of dry matter at 30 days after nursery sowing indicated that highest dry matter value (2.434 g) was recorded for Tellahamsa normal seed treated with cold plasma (S1V3) and lowest was recorded for S1V1 RNR 15048 normal seed treated with cold plasma was recorded in 0.172 g.plasma treatment significantly improvement over control which is 78.14% respectively (Fig.8).

Chlorophyll content of leaf : There was no significant difference between interaction effects (Table 10).mean comparision effects of chlorophyll content of leaf indicated that plasma treatment non

significantly over other treatments. S1V1 RNR 15048 normal seed treated with cold plasma exhibited the highest chlorophyll content 43.59, while JGL 24423 untreated control showed the lowest 24.72 (Table 11) (Fig.9).

Days to 50% flowering : there was no significant difference between interaction effects (Table 10). mean comparision effects of days to 50% flowering indicated that plasma treatment significantly over other treatments. RNR 15048 sprouted seeds treated with cold plasma S2V1, which recorded the earliest flowering at 90.67 days. In contrast, untreated control seeds of JGL 24423 S3V2 took the longest time 100.33 days which is 9.6 % respectivley (Table 11)(Fig.10).

Days to maturity : there was no significant difference between interaction effects (Table 10). mean comparision effects of days to maturity indicated that plasma treatment have indicated more significant over other treatments. RNR 15048 sprouted seeds treated with cold plasma (S2V1) matured earliest at 118.67 days and highest days to maturity was 128.28 which is S3V2 JGL24423 untreated control which is 7.49 % respectively (Table 11) (FIG.10).

Number of tillers/ hill : there was no significant difference between interaction effects (Table 10).mean comparision effects of number of tillers/hill indicated that plasma treatment non significantly improved over control or other treatments. RNR 15048 normal seeds S1V1 had the highest number of tillers per hill which is 25.33, while JGL 24423 normal seeds S1V2 recorded the lowest number of tillers per hill which is 17.3 (Table 11) (Fig.11).

Number of productive tillers per m^2 : there was no significant difference between interaction effects (Table 10). mean comparision effects of number of tillers/hill indicated that plasma treatment non significantly improved over control or other treatments. Tellahamsa sprouted seeds treated with cold plasma S2V3 producing the most number of productive tillers/m^2 which is 447, while JGL 24423 untreated control seeds S3V2 had the lowest number of productive tillers/m^2 which is 361 (Table 11) (Fig.11).

Proline content : there was no significant difference between interaction effects (Table 10). mean comparision effects of number of tillers/hill indicated that plasma treatment non significantly improved

over control or other treatments. results indicated that RNR 15048 untreated control S3V1 had the highest proline content 0.067 µmoles/g, while the lowest was in Tellahamsa sprouted seeds treated with plasma S2V3 (Table 11) (Fig.12).

Plant height : there was no significant difference between interaction effects (Table 12).mean comparision effects of plasma treatment have indicated significant improvement over control. The highest plant height was observed in Tellahamsa normal seed treated with cold plasma S1V3, reaching 99.67 cm, while untreated control seeds from JGL 24423 S3V2 had the lowest plant height which is 18.2 % respectively (Table 13)(Fig.13).

Seed yield per hill : there is no significant difference between interaction efects of analysis of variance (Table 12).mean comparision effects of plasma treatment have indicated significant improvement over control. highest seed yield per hill was observed in JGL 24423 with sprouted seed treated with cold plasma S2V2, recording 58.85 g, while the lowest was found in untreated control RNR 15048 S3V1which is 52.34 % respectively (Table 13)(Fig.14).

Seed yield :there is no significant difference between interaction effects (Table 12). mean comparision effects of plasma treatment have indicated significant improvement over control. Tellahamsa normal seed treated with cold plasma S1V3 had the highest yield 12.94 t/ha and lower yield was recorded in S1V1, S2V1 recorded 9.01 t/ha.which is 30.37 % respectively (Table 13)(Fig.14).

1000 seed weight : there is no significant difference between interaction effects (Table 12). mean comparision effects of plasma treatment have indicated significant improvement over control. Tellahamsa sprouted seed S2V3 exhibited the highest 1000 seed weight 23.72 g, while RNR 15048 sprouted seed S2V1 recorded the lowest 11.38 g which is 48.40 % respectively(Fig.14).

Discussion:

Relationship of cold plasma treatment with physiological and biochemical parameters of rice seed under labaraotory and field conditions. Telangana, as the leading rice-cultivating state in India, faces significant crop losses during winter due to cold stress . In the present study we have

investigated effects of cold plasma treatment on both seed quality parameters and biochemical parameters at both normal and cold stress conditions and field parameters under cold stress conditions to overcome this problem. we reported that seeds exposure to cold plasma treatment was significantly improved the seed physiological parameters like germination % and seedling dry weight, seedling vigour index-II, germination index, seed infection %.increase in germinartion at 25°C due tocold plasma treatments can be attributed to the production of reactive oxygen and nitrogen species (RONS). These species likely weakened the seed coat, facilitating water uptake and germination. Additionally, the H_2O_2 and NO_2 generated through plasma treatments may have reduced abscisic acid (ABA) levels, thereby limiting seed dormancy. Concurrently, the plasma treatment appears to have promoted the biosynthesis of gibberellic acid (GA), further enhancing germination [15],reported that improved germination rates in plasma-treated paddy seeds. germination at normal temperature is greater than under cold stress conditions may be due to under stress conditions H2O2 will activate various defense mechanisms through the redox signaling pathway .plasma treatment enhanced the germination under cold stress . [16] reported that plasma-activated water significantly enhanced germination rates in barley under low-temperature stress. [17] reported that reduced germination in seeds exposed to cold stress is likely due to the downregulation of gibberellic acid (GA) levels. this suppression is attributed to the activation of GA catabolic genes and the SLR1a gene, which encodes the DELLA protein.plasma treatment improved seedling dry weight at both normal and cold stress conditions . [18] reported that increase in seedling dry weight in mustard plants after plasma treatment. seedling dry weight at cold stress was improved by plasma treatment but reduced compared to normal temperature conditions may be due to lower temperatures, enzyme activity slows down, affecting various metabolic processes like protein synthesis, carbohydrate metabolism, and other cellular activities crucial for growth and enzyme efficiency limits the energy available for cellular functions, which in turn affects seedling development and dry weight.plasma treatment have improved seedling vigour index - II under both normal and cold stress conditions . seedling vigour index-II with plasma treatment 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. [19] reported that

improvement in seed vigour by plasma treatment was in soyabean. seedling vigour was improved in normal temperature compared to cold stress this may be due to accumulation of reactive oxygen species (ROS), which causes oxidative damage to cell membranes, disrupting normal metabolic activities .cold plasma treatment improved germination index at both normal temperature and cold stress conditions .Germiantion index at normal temperature was improved by plasma treatment this may be due to H2O2 and NO2 decreases ABA levels, limiting dormancy and promoting GA biosynthesis for germination and promoting germination index at normal conditions and germination index at cold stress conditions when compared to normal conditions. [20] reported that Enzymes involved in the breakdown of stored reserves, such as starch and proteins, function less effectively under cold stress, delaying the energy release required for the growth of the emerging seedling in rice . [21] reported that which makes membranes more rigid and less permeable. This disrupts water uptake, which is crucial for seed imbibition and the initiation of germination in maize seedlings.plasma treatment also reduced seed infection% more at cold stress conditions compared to normal temperature conditions might be due to expose of seed to cold stress conditions might have reduced the survival of disease inoculum at such low temperatures. [22] reported that jasmonic acid dependent signaling pathways are critical for improving cold tolerance and to provide defense against fungal infections.plasma treatment also reduced seed infection % at normal temperature conditions this may be due to increase in the accumulation of H_2O_2 in plasma treated seed which resulted in reduction of disease severity. the [23] reported that wherein plasma treated seeds recorded less seed infection compared to untreated control in wheat seeds against *Fusarium monacearum.*

Superoxide dismutase (SOD) is a key antioxidant enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide, the results revealed that plasma treatment did not improved over control. [24] reported that SOD activity can fluctuate depending on the balance between reactive oxygen species (ROS) generation and the synthesis or stability of the enzyme. Elevated stress levels might overwhelm the antioxidant system, leading to decreased SOD activity due to enzyme inactivation or degradation [25] reported that Cold plasma treatments, while stimulating oxidative processes, might reduce SOD activity because of elevated oxidative stress.

Excess hydrogen peroxide generated under such conditions can inhibit Cu/Zn-SOD, which is particularly sensitive to oxidative modifications in maize seedlings.plasma treatment did not significantly improved over control but compared to normal temperature conditions it was improved this may be due to Cold plasma treatments generate reactive oxygen species (ROS) and other radicals while ROS generally activate antioxidant defenses like SOD, in certain conditions, prolonged or excessive ROS production can overwhelm the plant's antioxidant systems, leading to oxidative stress and reduced SOD activity., the plasma treatment at 15ºC may have induced an imbalance between ROS production and the plant's ability to neutralize these molecules, resulting in decreased SOD activity. [12] reported that cold plasma treatments improved the superoxide dismutase activity at lower temperature conditions compared to normal temperature conditions.peroxidase activity at normal temperature revealed non significant improvement over control . [26] reported that Peroxidase activity was decreased as the age of the seedlings increased after non-thermal plasma treatments in maize. [27] reported that 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. peroxidase activity at cold stress conditions also revealed that non significant improvement over control.[12], reported that at lower temperatures, plasma treatment may trigger increased antioxidant enzyme activities, including peroxidase. This could be a response to oxidative stress or a mechanism to protect the seeds from environmental damage.catalase activity at normal temperature plasma treatment significantly not improved over control . [26] reported that 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. [27] reported that 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. plasma treatment activity at catalase activity at cold stress conditions was non significantly improved with control. [28] reported that cold plasma treatments is due to the production of reactive oxygen and nitrogen species (RONS) like hydrogen peroxide and nitric oxide. These act as signaling molecules, boosting the expression of antioxidant

enzymes, including catalase in wheat. 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. plasma treatment of proline content at normal temperature significantly not improved over control. [29] reported the interaction between radiation time and voltage did not significantly affected the proline content in Basil crop.plasma treatment of proline content atcold stress conditions significantly not improved over control .[30] reported that Cold plasma treatments may generate ROS, triggering stress signaling pathways that enhance proline biosynthesis to mitigate cellular stress.

Plasma treatment over radicle emergence at normal temperature indicated non significant improvement over control. [31] 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. plasma treatment over radicle emergence at cold stress indicated significant improvement over control. [32] reported that Plasma treatments alter seed coat permeability, improving water and oxygen diffusion, critical for radicle emergence under low temperatures.

the present study was taken up for study the effects of cold plasma treatments against cold tolerance in paddy during *Rabi*, plasma treatment improved dry matter at 15,30,45 days after treating with cold plasma this may be due to cold plasma treatment is due to improved water uptake, hormonal stimulation, and nutrient acquisition facilitated by the reactive species. [33] reported that cold plasma's reactive species improve seed germination, plant vitality, and physiological processes. The treatment is known to activate auxin and cytokinin signaling pathways, which are crucial for root and shoot development. [34] who reported that dry weight of shoot treated with cold plasma in pea was more than the dry weight of shoot in untreated control.plasma treatment improved dry matter at 15,30,45 days after treating with cold plasma this may be due to cold plasma enhances cytokinin levels, leading to improved root biomass and development. This process is thought to involve upregulation of genes like GmEXP1, which are associated with cell wall expansion and root elongation. [35] 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.

Plasma treatment was indicated that non significant improvement over control. [21] showed that cold plasma treatment at 60 kV reduced chlorophyll breakdown by regulating enzymes like chlorophyllase and pheophorbide a mono-oxygenase, which play a key role in chlorophyll catabolism.plasma treatment indicated significant improvement over other treatments this may be due to Cold plasma generates reactive oxygen and nitrogen species (RONS), which enhance the metabolic activity within seeds. These reactive species help break seed dormancy by weakening the seed coat and improving water uptake, making nutrients readily accessible for metabolic processes. Additionally, cold plasma accelerates pre-germination activities, such as enzyme activation and cellular energy production, which are critical for early plant development and flowering. [36] as they reported early days to 50% flowering in plasma treated okra plants compared to untreated control this may be due to plasma treatment also helps break seed dormancy, improves enzymatic activity, and accelerates the biosynthesis of growth hormones like gibberellins, which are essential for faster flowering and fruiting, ultimately shortening the crop maturity duration. [37] who reported that plasma treated seeds attain early maturity than untreated control seed in chilli (*Capsicum annum longum* L.) plasma treatment does not significantly improved over control in number of tillers per hill this may be due to the generation of reactive species by plasma may not always effectively influence parameters like tiller production due to the complex interplay of growth dynamics and genetic potential.Plasma treatment does not significantly improved over control or other treatments in number of productive tillers per hill [38] reported that complexity aligns with findings in plant response studies under multi-environment trials, where genetic and environmental contributions to traits like yield and tillering are observed to be highly variable . [39] while cold plasma improved certain physiological parameters in rice, its effect on traits like tillering showed variability depending on the genotype and specific environmental conditions.

Plasma treatment have recorded significant improement over control. 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 [40]. [41] highlighted that cold plasma treatments activate molecular mechanisms that enhance cell elongation, division, and metabolic activity, leading to improved growth and the reactive species generated during plasma exposure modify seed coat properties, increase water uptake, and promote nutrient availability, directly enhancing plant height.plasma treatment reported significant improvement over control.[13] observed that plasma treatments increased cytokinin activity in rice, which is crucial for cell division in the shoot apical meristem, leading to better panicle differentiation and higher grain yield. Cytokinins are essential in regulating shoot growth and development, contributing to increased seed yield per hill.plasma treatments recorded significant improvement over control. [42] reported a 16% increase in seed yield in plasma-treated paddy, which aligns with the improvements observed in my study and these results highlight how plasma technology can optimize agricultural productivity, especially under challenging environmental conditions.plasma tretments indicated more significant improvement over control. [43] demonstrated that cold plasma treatment significantly increased 1000 seed weight in oilseed rape (Brassica napus). This improvement is attributed to plasma's impact on physiological processes, such as increased nitrogen levels and the activation of growth-related genes. The plasma treatment also enhances the production of reactive oxygen species (ROS), which promote growth by influencing cell division and expansion.

Materials and Methods

The experiment was conducted during the Kharif and Rabi season of 2023-24 to standardize and evaluate the effects of cold plasma treatments on rice (Oryza sativa L.). The study comprised two phases: the standardization of cold plasma treatment protocols in a controlled laboratory setting and the subsequent field evaluation of the most effective treatment.

Laboratory Standardization of Cold Plasma Treatments. Standardization work was carried out at the Quality Control Lab, Seed Testing Laboratory, and Central Instrumentation Cell (CIC), Professor Jayashankar Telangana Agricultural University (PJTAU), Rajendranagar, Hyderabad. The rice variety RNR 15048 was used for this phase. Cold plasma treatments were conducted using a plasma generator available in the Quality Control Lab at Kharif Season. Three voltage levels (10 kV, 20 kV,

and 30 kV) and two exposure durations (15 minutes and 20 minutes) were tested for both normal and sprouted seeds were subjected to these treatments. The effectiveness of cold plasma treatments was assessed using a combination of Physiological Parameters: Germination percentage, seedling dry weight (mg), seedling vigor index-II, cold tolerance index (%), Seed Infection (%), Speed of Germination, and Radicle Emergence (%). And Biochemical Parameters: Superoxide dismutase (SOD) activity (units/mg of protein), catalase activity (nmoles of H_2O_2 used/min/g of sample), peroxidase activity (units/min/mg of protein), and proline content (µmoles of proline/g).

Field evaluation of the standardized cold plasma treatment (20 kV for 15 minutes) was conducted at the Seed Production Farm at Rabi Season, Seed Research and Technology Centre, PJTAU, Rajendranagar, Hyderabad. Three rice varieties—RNR 15048, JGL 24423, and Tellahamsa were used. Observation Recorded. Growth Parameters: Dry weight of roots and shoots at 15, 30, and 45 days after transplanting, Plant height (cm), Number of tillers per hill and per square meter. Stress Indicators: Chilling injury (%), Per cent incidence of blast disease, Proline content (µmoles/g). Phenological Traits: Days to 50% flowering, Days to maturity. Physiological Traits: Chlorophyll content. Yield and Yield-Attributing Traits: 1000-seed weight (g), Seed yield per hill (g), Seed yield per hectare (t/ha).

Statistical Analysis was done using a Completely Randomized Design (CRD) for the laboratory standardization work, while the field evaluation was carried out using a Randomized Block Design (RBD) with three replications for each rice variety (RNR 15048, JGL 24423, and Tellahamsa).

Super oxide dismutase Activity (units /mg of protein)

The super oxide dismutase activity was estimated as per the procedure given by [44]. 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 µM), 0.3 ml of nitro blue tetrazolium (NBT) (300 µM) and 2.8 ml of water. Reaction was initiated by adding 0.2 ml of NADH (780 µM) 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).

$$\text{S. O. D Activity} = \frac{\text{A0} - \text{A1}}{\text{A1}} \div 50\% \times \frac{\text{Total reaction volume}}{\text{Volume of enzyme extract}} \times \text{Dilution Factor}$$

Where, A0 = Initial value, A1 = Final value

Peroxidase activity (units/min/mg of protein)

The peroxidase activity was estimated using the procedure given by [45]. 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.

Catalase activity (n moles of H_2O_2 used/ min/ g weight of sample)

The catalase activity was estimated by using H2O2 as given by [46]. 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.

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

Proline content (µmoles of proline/g of tissue)

The proline content was estimated as per the protocol developed by [47]. 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 100ºC 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.

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

Declarations

Competing Interests

The authors declare no competing interests.

Dual Publication

This manuscript has not been published previously and is not under consideration for publication elsewhere, in whole or in part.

Third-Party Material

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Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Source of variance	d.f	Germination per cent at normal temperature	Germination per cent at cold stress	Seedling dry weight at normal temperature (mg)	Seedling dry weight at cold stress (mg)	Seedling vigour index-II at normal temperature	Seedling vigour index -II at colds tress
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
C.V (%)	27	4.184	3.799	0.446	11.141	5.263	8.684

Table 1: Analysis of variance of seed physiological parameters in RNR15048 of rice treatedwith plasma treatment, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds, P: Plasma treatments, S*P: interaction between types of seeds and treaments,d.f: degrees of freedom,C.V: coefficient of variation.

Tables:

Source of variance	d.f	seed infection(%) at normal temperature	seed infection(%)at cold stress	Germination index at normal temperature	Germination index at cold stress
S	1	234.321 ***	1.286	0.401	36.161 ***
P	6	4.286 *	5.810 **	71.067 ***	73.897 ***
S*P	6	2.905 *	2.619 *	0.285	0.902
Error (P)	14	0.964	0.857	1.287	0.422
C.V(%)		17.781	12.739	7.584	6.270

Table 2: Analysis of variance of seed physiological parameters in RNR15048 of rice treated with plasma treatment, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds, P: Plasma treatments, S*P: interaction between types of seeds and treaments,d.f: degrees of freedom,C.V: coefficient of variation.

Types of seeds	Treatments	Germination percentage (%)		Seedling dry weight (mg)		Seedling vigour index- II		seed infection (%)		Germination index	
		Normal temperature	cold stress	Normal temperature	cold stress	Normal temperature	cold stress	Normal temperature	cold stress	Normal temperature	cold stress

Dry seed (S1)	P1:10 Kv for 15 min treated with plasma	89	84	5.15	3.05	456	256	1.50	3.00	16.06	15.11
	P2:10 Kv for 20 min treated with plasma	89	83	4.95	2.90	438	240	2.50	4.00	15.40	12.93
	P3:20 Kv 15 min treted with plasma	91	87	5.85	3.70	532	323	1.50	1.50	22.86	16.50
	P4:20 Kv for 20 min treaated with plasma	85	79	4.80	3.10	408	245	2.00	4.00	9.48	8.06
	P5:30 Kv for 15 min treated with plsma	88	80	5.05	3.20	442	257	2.50	5.50	10.33	5.07

	P6:30 Kv for 20 min treaated with plasma	89	80	5.10	3.30	451	265	2.00	7.00	15.33	10.60
	P7:control with out plsma treatment	87	82	4.80	3.45	415	282	2.50	5.00	16.10	12.21
Sprouted seed (S2)	P1:10 Kv for 15 min treated with plasma	80	63	3.80	3.05	303	194	7.50	3.00	15.14	12.61
	P2:10 Kv for 20 min treated with plasma	78	64	3.95	2.10	308	136	6.50	3.50	15.28	12.07
	P3:20 Kv 15 min treted with plasma	83	69	4.55	4.20	376	290	4.50	2.50	22.25	14.58

	P4:20 Kv for 20 min treaated with plasma	71	61	3.80	2.40	270	146	9.50	5.50	10.17	4.70
	P5:30 Kv for 15 min treated with plsma	75	59	4.00	1.70	302	101	9.00	4.50	10.42	1.61
	P6:30 Kv for 20 min treaated with plasma	69	47	4.15	2.60	287	120	9.50	3.50	14.74	8.27
	P7:control with out plsma treatment	78	41	3.95	3.40	308	139	8.50	4.50	15.86	10.73
	GRAND MEAN	82.17	70.17	4.56	3.01	378	214	4.96	4.07	14.96	10.35
	CD	2.43	1.885	0.243	0.231	23.248	13.932	0.694	0.654	2.433	1.393
	SE(m)	7.374	5.717	0.738	0.719	70.51	42.26	2.10	1.98	0.802	0.459
	CV (%)	4.18	3.79	7.54	10.14	8.68	9.20	9.78	9.73	7.584	6.270

Table 3:Mean comparision effect of cold plasma treatment on both normal temperature and cold stress conditions of rice variety RNR15048 seed physiological parameters seed germination(%) ,seedling dry weight(mg), seedling vigour index-II, seed infection percent and germination index(The discharge voltages are of three different types 10, 20, 30Kvand plasma treatment time is 15,20 min). and the data produced were of interaction effect between the types of seeds and treatments.

Source of variance	d. f	Superoxide dismutase activity (units/mg) at normal temperature	Superoxide dismutase activity (units/ mg) at cold stress	Peroxidase activity (units/ min/mg) at normal temperature	Peroxidase activity (unit/ min/ mg) at cold stress	Catalase (n moles/ min/g) at normal temperatu re	Catalase (n moles/ min/g) at cold stress	Proline content (µ moles of proline/ g) at normal temperatur e	Proline content (µ moles of proline/g) at cold stress conditions
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
C.V(%)		12.956	11.675	15.988	15.960	16.881	16.886	13.282	13.430

Table 4: Analysis of variance of seed biochemical and antioxidant parameters in RNR15048 of rice treated with plasma treatment, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds, P: Plasma treatments, S*P: interaction between types of seeds and treaments,d.f: degrees of freedom,C.V: coefficient of variation.

Types of seeds	Treatments	Superoxide dismutase activity		Peroxidase activity		Catalase activity		Proline content	
		Normal temperature	cold stress	Normal temperature	cold stress	Normal temperature	cold stress	Normal temperature	cold stress
Dry seed (S1)	P1:10 Kv for 15 min treated with plasma	4.30	21.37	0.036	0.107	129	389	0.027	0.207
	P2:10 Kv for 20 min treated with plasma	8.30	20.12	0.069	0.207	120	360	0.017	0.132
	P3:20 Kv 15 min treted with plasma	8.10	19.67	0.059	0.178	140	421	0.027	0.206
	P4:20 Kv for 20 min treaated with plasma	8.70	21.09	0.040	0.122	121	363	0.014	0.111
	P5:30 Kv for 15 min treated with plsma	8.70	21.14	0.036	0.108	121	363	0.020	0.150
	P6:30 Kv for 20 min treaated with plasma	8.50	20.72	0.058	0.173	141	424	0.019	0.143

	P7:control with out plsma treatment	9.00	21.88	0.058	0.174	142	433	0.030	0.225
Sprouted seed (S2)	P1:10 Kv for 15 min treated with plasma	6.30	15.37	0.019	0.059	124	372	0.016	0.121
	P2:10 Kv for 20 min treated with plasma	6.70	16.23	0.052	0.156	101	305	0.014	0.111
	P3:20 Kv 15 min treted with plasma	8.70	21.09	0.045	0.135	96	287	0.015	0.115
	P4:20 Kv for 20 min treaated with plasma	7.90	19.28	0.022	0.065	115	346	0.023	0.177
	P5:30 Kv for 15 min treated with plsma	8.00	19.43	0.014	0.042	144	439	0.017	0.130
	P6:30 Kv for 20 min treaated with plasma	8.80	21.42	0.018	0.055	124	372	0.022	0.170
	P7:control with out plsma treatment	9.10	21.97	0.032	0.096	118	354	0.033	0.250
	GRAND MEAN	7.96	20.05	0.040	0.120	124.5	373.5	0.021	0.160

	CD (5%)	NS	NS	NS	0.169	NS	NS	NS	NS
	SE(m)	1.340	1.650	0.018	0.055	14.8	44.6	0.004	0.035
	CV (%)	8.900	8.600	9.900	8.500	8.88	7.88	7.28	9.43

Table 5: Mean comaprision effects of cold plasma treatment on both normal temperature and cold stress conditions of rice variety RNR15048 seed antioxidant and biochemical parameters Superoxide dismutase activity Peroxidase activity ,Catalase activity ,Proline content (The discharge voltages are of three different types 10, 20, 30Kvand plasma treatment time is 15,20 min). and the data produced were of interaction effect between the types of seeds and treatments.

Source of variance	d.f	Radicle emergence at normal temperature	Radicle emergence at cold stress

S	6	17.905	547.810***
Error (S)	7	29.714	30.857
C.V (%)		5.889	22.654

Table 6: Analysis of variance of seed biochemical and antioxidant parameters in RNR15048 of rice treated with plasma treatment, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds, P: Plasma treatments, S*P: interaction between types of seeds and treaments,d.f: degrees of freedom,C.V: coefficient of variation

S. No	Treatments	Radicle emergence (%) at normal temperature	Radicle emergence (%) at cold stress
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

Table 7: mean comaprision effects of cold plasma on radicle emergence at both normal and cold stress conditions of rice variety RNR15048. (The discharge voltages are of three different types 10, 20, 30Kvand plasma treatment time is 15,20 min). and the data produced were of interaction effect between the types of seeds and treatments.

Source of variance	d.f	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	0.005 ***	0.85 ***	7.049 ***	0.001 ***	0.024	2.18 ***
S	2	0.000 *	0.17	0.356	0.000	0.042 *	1.05 **
V*S	4	0.000	0.23 *	0.595 *	0.000	0.041 *	1.37 ***
Error (V)	16	0.000	0.06	0.171	0.000	0.010	0.14
C.V(%)		13.770	20.656	17.624	16.662	18.067	23.271

Table 8: Analysis of variance of field parameterss of rice treated with plasma treatment under Rabi cold stress conditions, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds,V:Varieties, S*V: interaction between types of seeds and Varieties,d.f: degrees of freedom,C.V: coefficient of variation

Types of seeds	Varieties	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)
S1: Normal seed	V1: RNR15048	0.047	0.319	0.343	0.014	0.352	0.172
S2: Sprouted seed		0.032	0.316	0.512	0.013	0.190	0.376
S3: Untreated control		0.041	0.398	0.853	0.014	0.229	0.532
S1: Normal seed	V2: JGL24423	0.070	0.458	1.734	0.028	0.232	0.837
S2: Sprouted seed		0.049	0.533	1.883	0.022	0.242	1.361
S3: Untreated control		0.069	0.490	1.145	0.026	0.222	0.564

S1: Normal seed	V3:Tellahamsa	0.092	1.439	2.909	0.031	0.364	2.434
S2: Sprouted seed		0.087	0.557	2.259	0.030	0.497	1.215
S3: Untreated control		0.082	0.807	1.829	0.031	0.132	0.379
GRAND MEAN		87	87	87	0.024	0.274	0.87
CD		6.696	6.696	6.696	0.006	0.175	0.655
SE(m)		2.233	2.233	2.233	0.002	0.058	0.218
CV (%)		4.42	4.42	4.42	16.66	15.06	13.27

Table 9: mean comaprision effects of cold plasma treatment in nursery field under Rabi cold stress conditions (The discharge voltages is only one 20Kv 15 min which is the best treatment obtained from experiemnt 1s). and the data produced were of mean comparision effect between the interactions of types of seeds and varieties.

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 m^2	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
C.V(%)		26.965	2.300	1.778	22.116	18.816	15.820

Table 10: Analysis of variance of field parameterss of rice treated with plasma treatment under Rabi cold stress conditions, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds,V:Varieties, S*V: interaction between types of seeds and Varieties,d.f: degrees of freedom,C.V: coefficient of variation

Types of seeds	Varieties	Chlorophyll content of leaf	Days to 50% flowering	Days to maturity	Number of tillers/ hill	Number of productive tillers per m^2	Proline content(µmoles/ g)
S1: Normal seed	V1: RNR15048	43.59	93.33	121.33	25.3	379	0.061
S2: Sprouted seed		40.21	90.67	118.67	20.0	372	0.021
S3: Untreated control		38.65	96.67	124.67	18.0	405	0.067
S1: Normal seed	V2: JGL24423	38.75	95.33	123.33	17.3	394	0.027
S2: Sprouted seed		40.28	96.00	124.00	19.0	396	0.020
S3: Untreated control		24.72	100.33	128.33	18.3	361	0.032

S1: Normal seed	V3:Tellahamsa	37.43	95.00	123.00	23.6	379	0.020
S2: Sprouted seed		36.45	94.00	122.00	21.0	447	0.018
S3: Untreated control		39.05	96.00	124.00	21.3	415	0.027
GRAND MEAN		37.68	95.25	123.25	20.44	394.33	0.030
CD		NS	3.792	3.792	NS	NS	NS
SE(m)		5.866	1.265	1.265	7.820	12.830	0.014
CV (%)		16.96	2.30	1.77	12.12	10.82	15.82

Table 11: mean comaprision effects of cold plasma treatment of field parameters under Rabi cold stress conditions (The discharge voltages is only one 20Kv 15 min which is the best treatment obtained from experiemnt 1s). and the data produced were of mean comparision effect between the interactions of types of seeds and varieties.

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
C.V(%)		4.151	23.145	10.581	4.997

Table 12 : Analysis of variance of field parameterss of rice treated with plasma treatment under Rabi cold stress conditions, NOTE: *, **, *** represents significance at 5%, 1% and 0.1 % probability levels, respectively,S: Type of seeds,V:Varieties, S*V: interaction between types of seeds and Varieties,d.f: degrees of freedom,C.V: coefficient of variation

Types of seeds	Varieties	Plant height (cm)	Seed yield per hill (g)	Seed yield (t/ha)	1000 seed weight (g)
S1: Normal seed	V1: RNR15048	90.27	28.55	9.68	11.52
S2: Sprouted seed		88.10	31.86	9.63	11.38
S3: Untreated control		81.93	27.99	9.01	12.49
S1: Normal seed	V2: JGL24423	86.30	46.31	11.37	23.24
S2: Sprouted seed		84.00	58.85	10.13	22.67
S3: Untreated control		81.53	43.55	10.04	21.41

S1: Normal seed	V3:Tellahamsa	99.67	32.51	12.94	22.10
S2: Sprouted seed		97.30	35.59	11.74	23.72
S3: Untreated control		93.07	37.75	10.70	22.27
GRAND MEAN		89.12	38.11	10.92	18.98
CD		6.403	15.266	2.000	1.642
SE(m)		2.135	5.092	0.667	0.547
CV (%)		4.15	13.14	6.27	4.99

Table 13: mean comaprision effects of cold plasma treatment of field parameters under Rabi cold stress conditions (The discharge voltages is only one 20Kv 15 min which is the best treatment obtained from experiemnt and the data produced were of mean comparision effect between the interactions of types of seeds and varieties.

Figures :

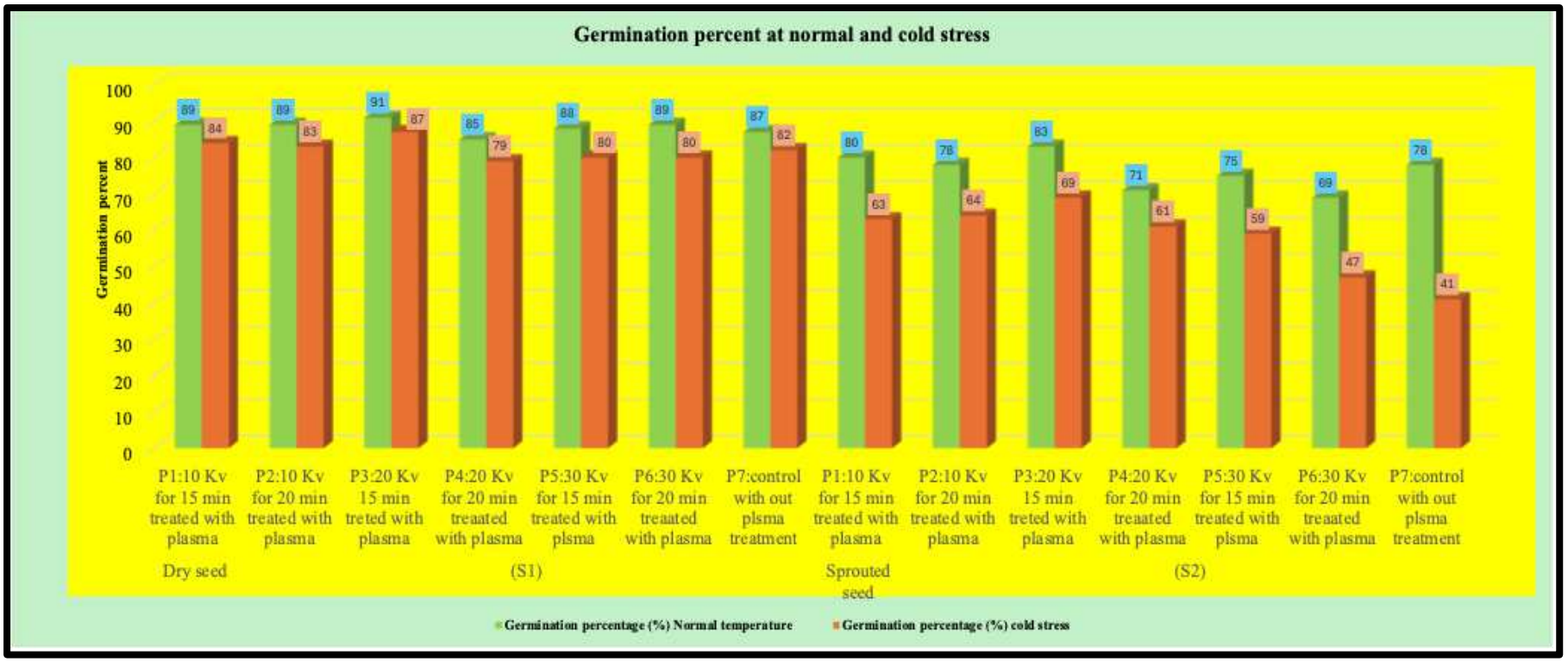


Figure 1: Germination percentage of RNR15048 variety at both normal and cold stress conditions

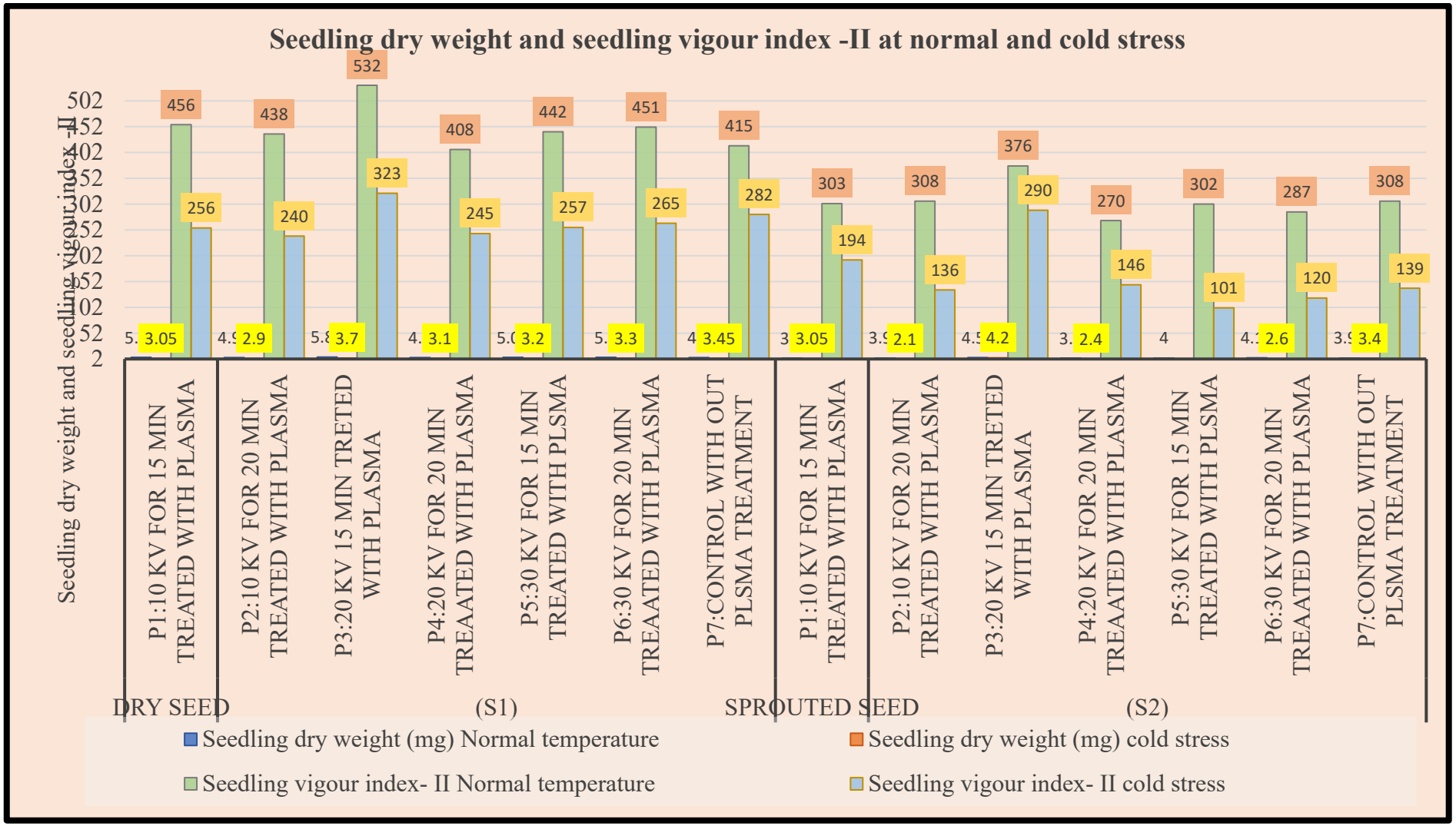


Figure 2: seedling dry weight and seedling vigour index -II at both normal and cold stress conditions.

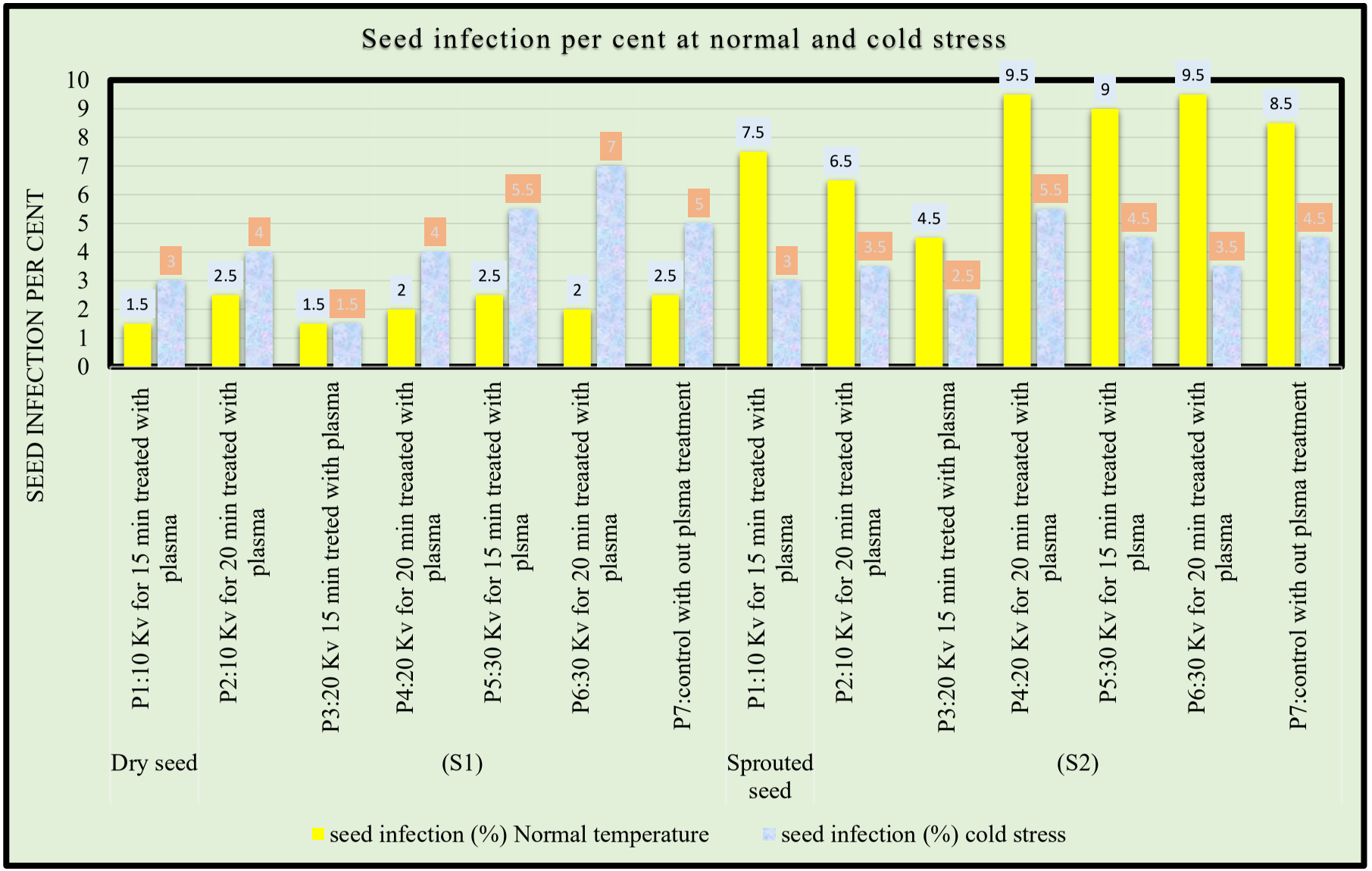

Seed infection per cent at normal and cold stress
SEED INFECTION PER CENT
10
9
8
7
6
5
4
3
2
1
0
1.5
3
2.5
4
1.5
1.5
2
4
2.5
5.5
2
7
2.5
5
7.5
3
6.5
3.5
4.5
2.5
9.5
5.5
9
4.5
9.5
3.5
8.5
4.5
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
Dry seed
(S1)
Sprouted seed
(S2)
seed infection (%) Normal temperature
seed infection (%) cold stress

Figure 3. seed infection percentage of RNR15048 variety at both normal temperature and cold stress conditions.

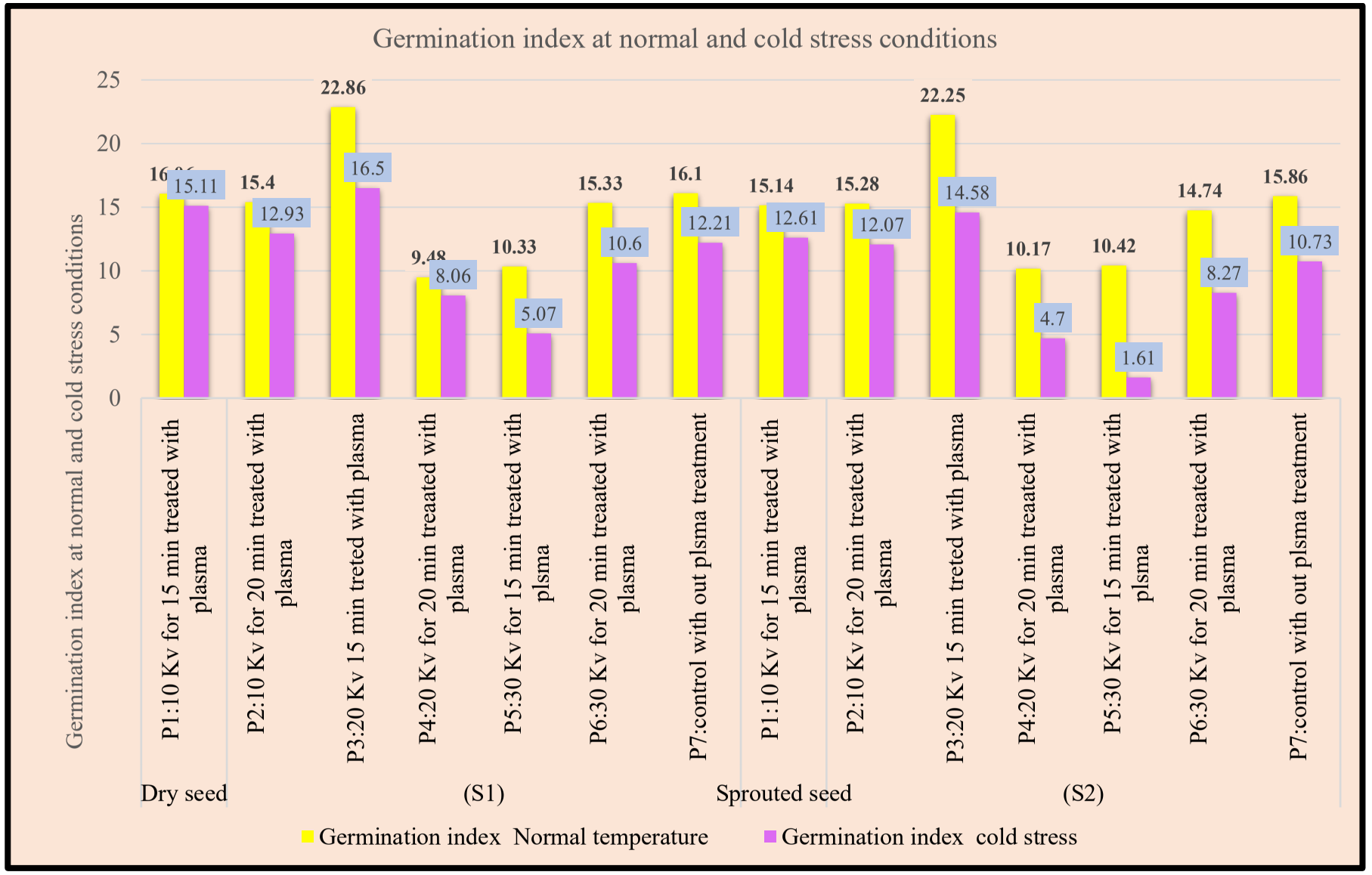

Germination index at normal and cold stress conditions
Germination index at normal and cold stress conditions
25
20
15
10
5
0
15.11
15.4
12.93
22.86
16.5
9.48
8.06
10.33
5.07
15.33
10.6
16.1
12.21
15.14
12.61
15.28
12.07
22.25
14.58
10.17
4.7
10.42
1.61
14.74
8.27
15.86
10.73
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
Dry seed
(S1)
Sprouted seed
(S2)
Germination index Normal temperature
Germination index cold stress

Figure 4. Germination index of RNR15048 variety at both normal temperature and cold stress conditions.

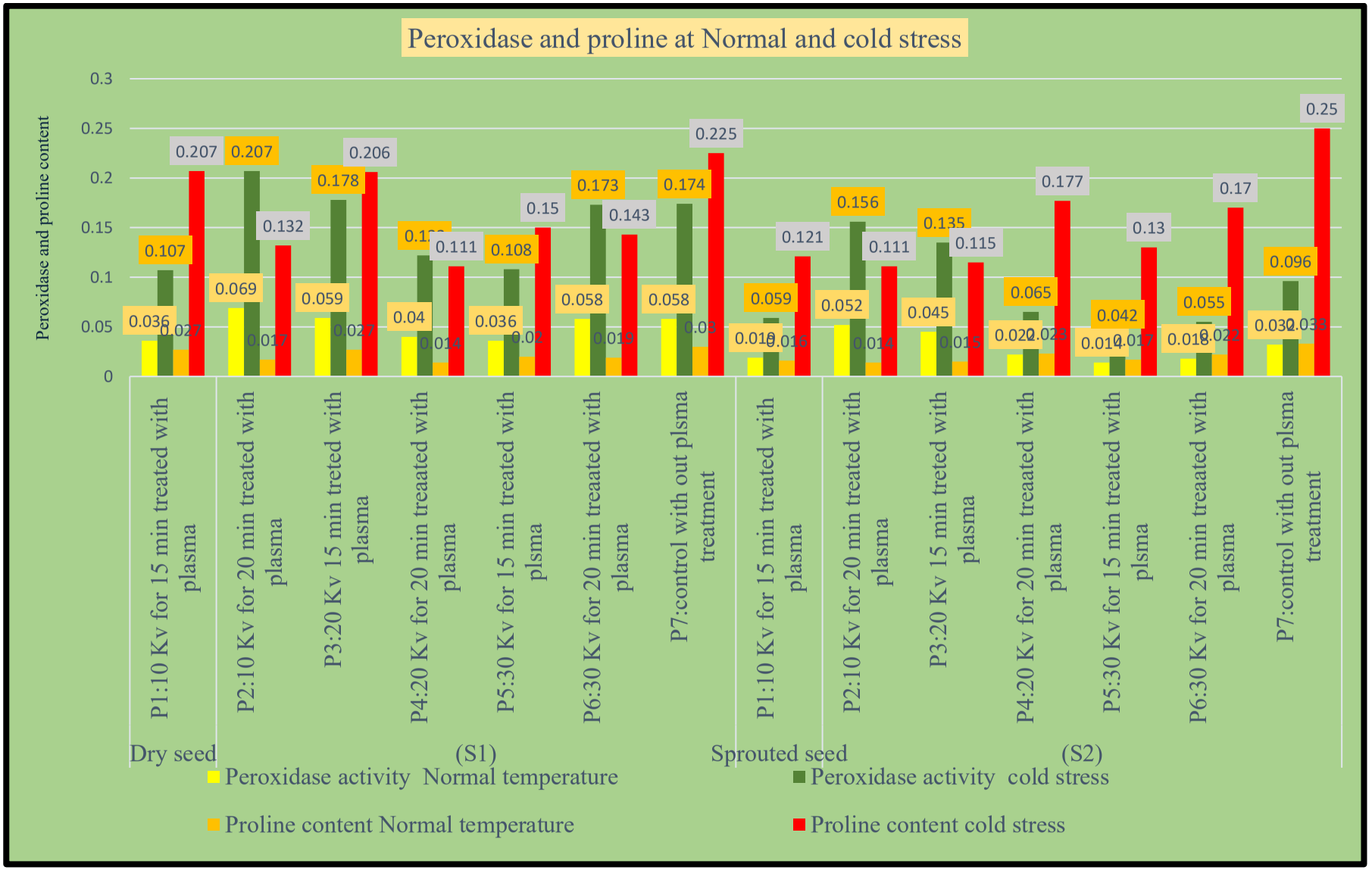

Peroxidase and proline at Normal and cold stress
Peroxidase and proline content
0.3
0.25
0.2
0.15
0.1
0.05
0
0.036
0.027
0.107
0.207
0.069
0.017
0.207
0.132
0.059
0.027
0.178
0.206
0.04
0.014
0.111
0.036
0.02
0.108
0.15
0.058
0.019
0.173
0.143
0.058
0.03
0.174
0.225
0.018
0.016
0.059
0.121
0.052
0.014
0.156
0.111
0.045
0.015
0.135
0.115
0.022
0.023
0.065
0.177
0.014
0.017
0.042
0.13
0.018
0.022
0.055
0.17
0.032
0.033
0.096
0.25
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
Dry seed
(S1)
Sprouted seed
(S2)
Peroxidase activity Normal temperature
Peroxidase activity cold stress
Proline content Normal temperature
Proline content cold stress

Figure 5.peroxidase and proline content at both normal temperature and cold stress conditions.

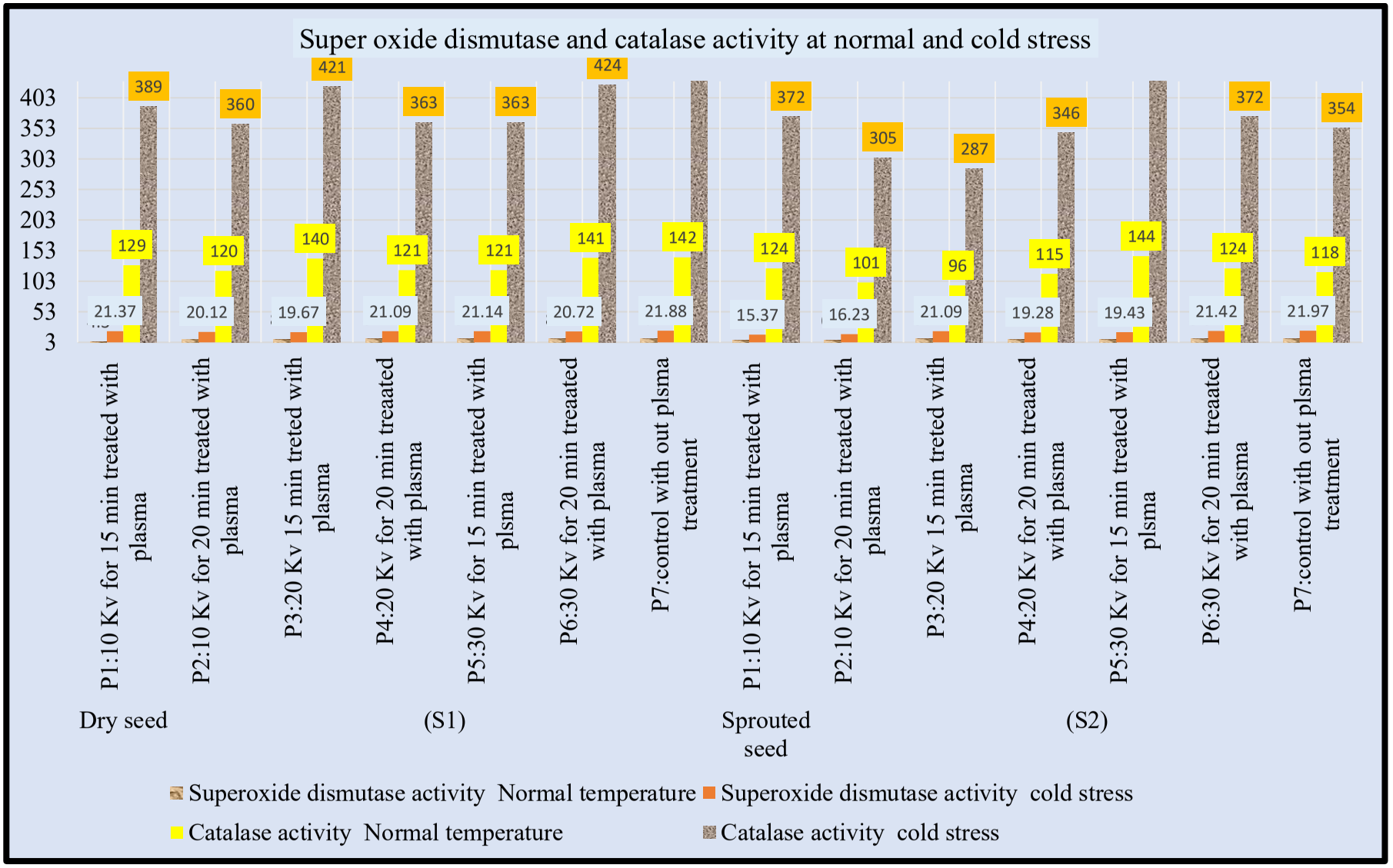

Super oxide dismutase and catalase activity at normal and cold stress
403
353
303
253
203
153
103
53
3
389
129
21.37
360
120
20.12
421
140
19.67
363
121
21.09
363
121
21.14
424
141
20.72
142
21.88
372
124
15.37
305
101
16.23
287
96
21.09
346
115
19.28
144
19.43
372
124
21.42
354
118
21.97
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
P1:10 Kv for 15 min treated with plasma
P2:10 Kv for 20 min treated with plasma
P3:20 Kv 15 min treted with plasma
P4:20 Kv for 20 min treaated with plasma
P5:30 Kv for 15 min treated with plsma
P6:30 Kv for 20 min treaated with plasma
P7:control with out plsma treatment
Dry seed
(S1)
Sprouted seed
(S2)
Superoxide dismutase activity Normal temperature
Superoxide dismutase activity cold stress
Catalase activity Normal temperature
Catalase activity cold stress

Figure 6. super oxide dismutase and catalse actvity at normal and cold stress conditions.

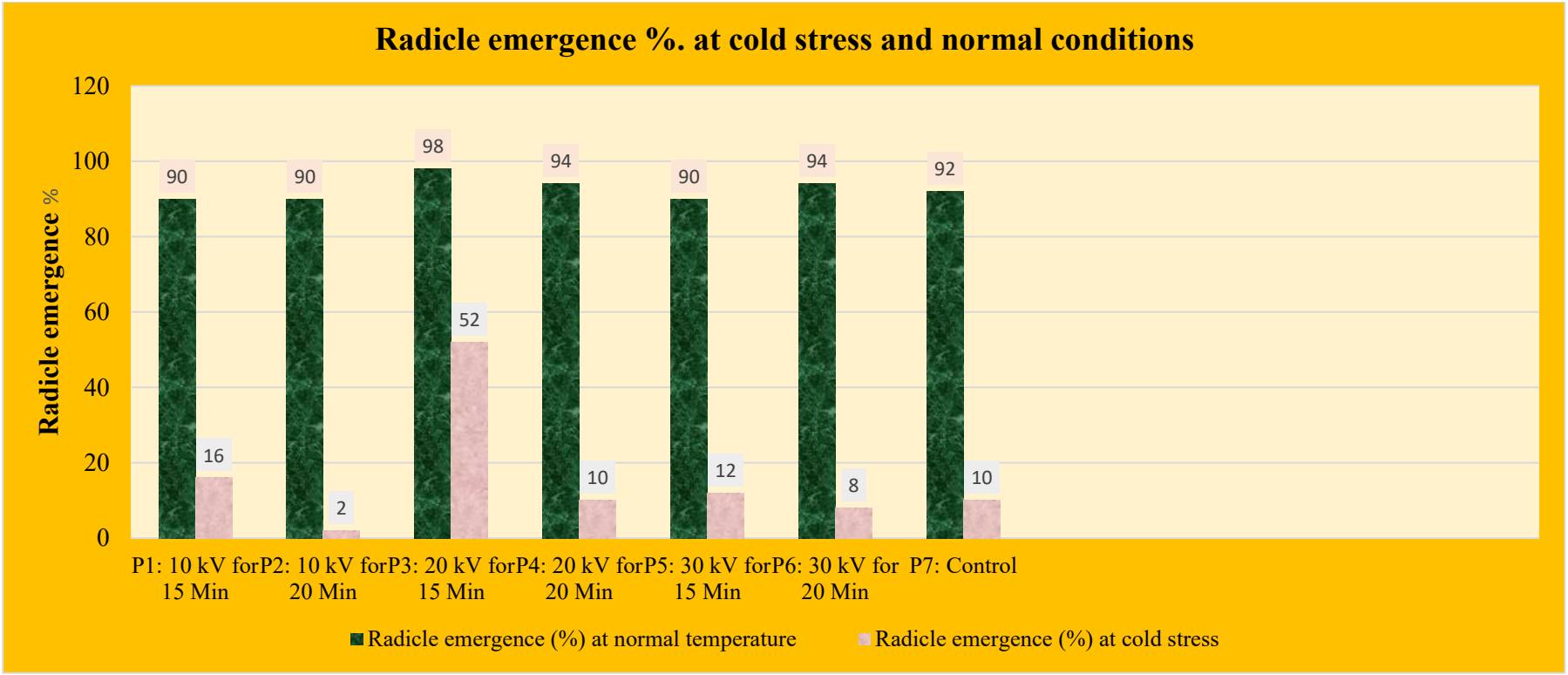


Figure 7.Dry matter for root and shoot at 15,30,45 days in Rabi season 203-2024

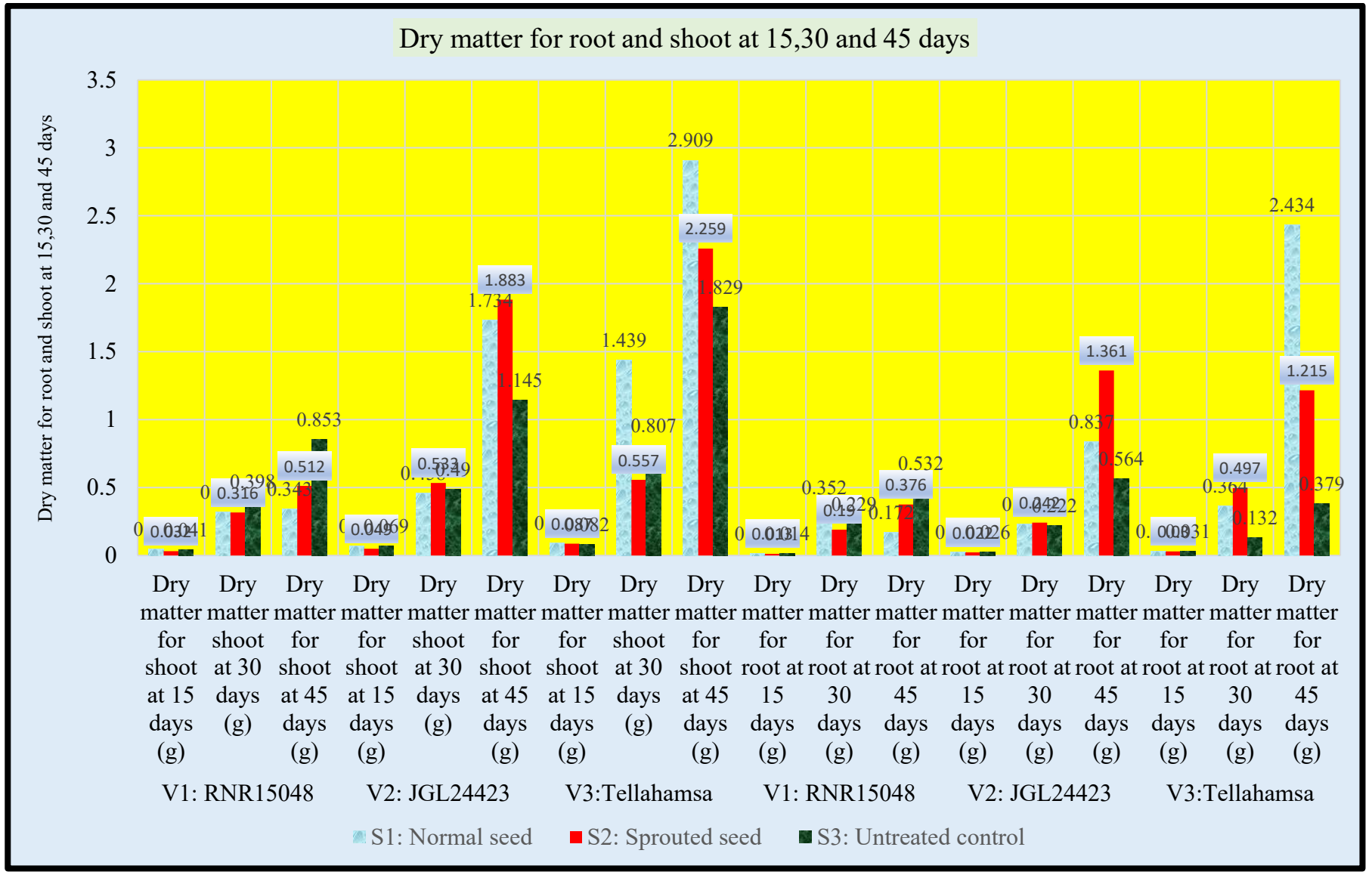

Dry matter for root and shoot at 15,30 and 45 days
Dry matter for root and shoot at 15,30 and 45 days
3.5
3
2.5
2
1.5
1
0.5
0
0.316
0.398
0.512
0.853
0.557
0.807
2.909
2.259
1.829
1.883
1.734
1.145
1.439
0.352
0.376
0.532
1.361
0.564
0.497
0.364
0.132
2.434
1.215
0.379
Dry matter for shoot at 15 days (g)
Dry matter shoot at 30 days (g)
Dry matter for shoot at 45 days (g)
Dry matter for shoot at 15 days (g)
Dry matter shoot at 30 days (g)
Dry matter for shoot at 45 days (g)
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)
Dry matter for root at 15 days (g)
Dry matter for root at 30 days (g)
Dry matter for root 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)
V1: RNR15048
V2: JGL24423
V3:Tellahamsa
V1: RNR15048
V2: JGL24423
V3:Tellahamsa
S1: Normal seed
S2: Sprouted seed
S3: Untreated control

Figure 8.Dry matter for root and shoot at 15,30,45 days in Rabi season 203-2024.

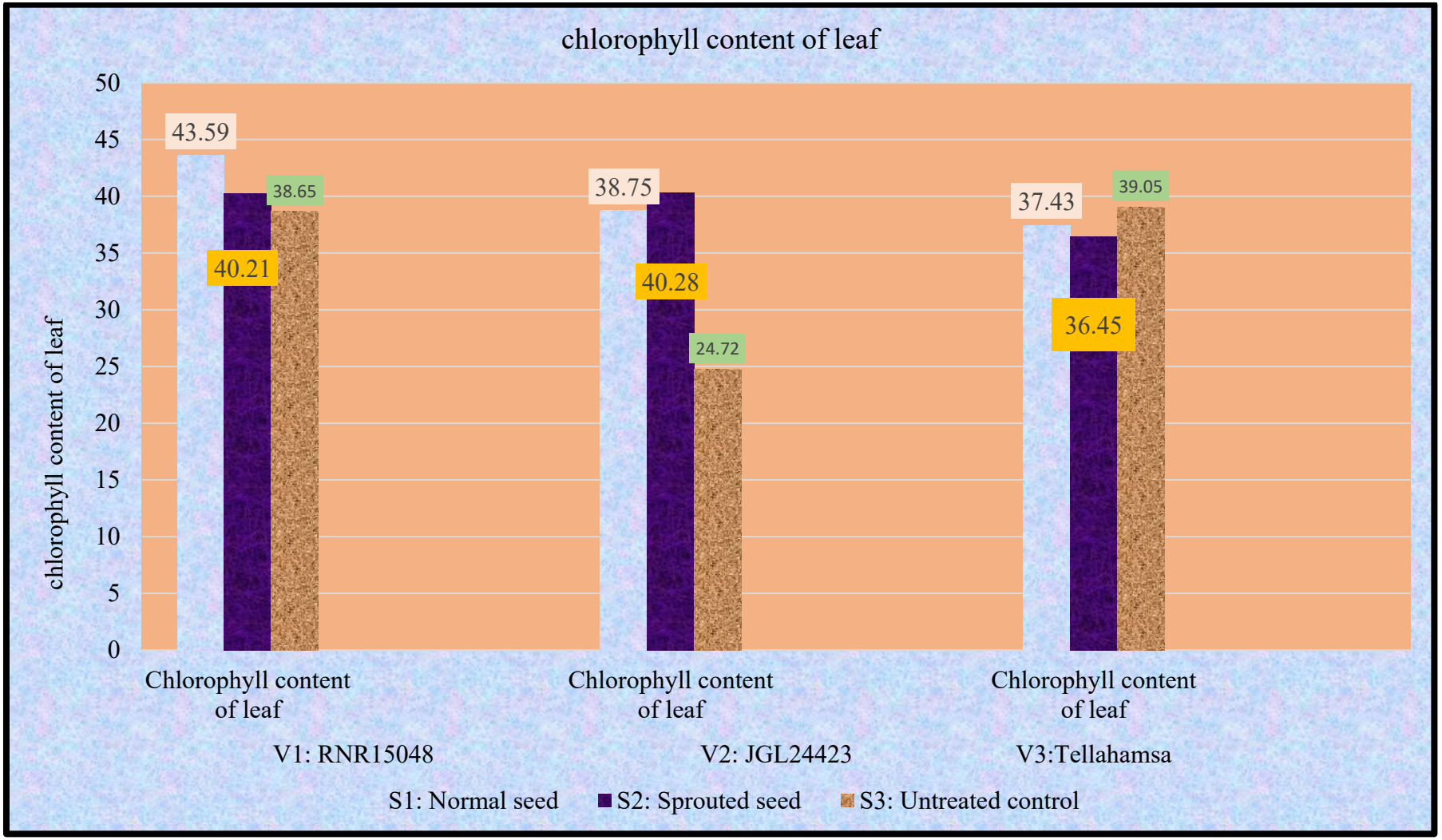
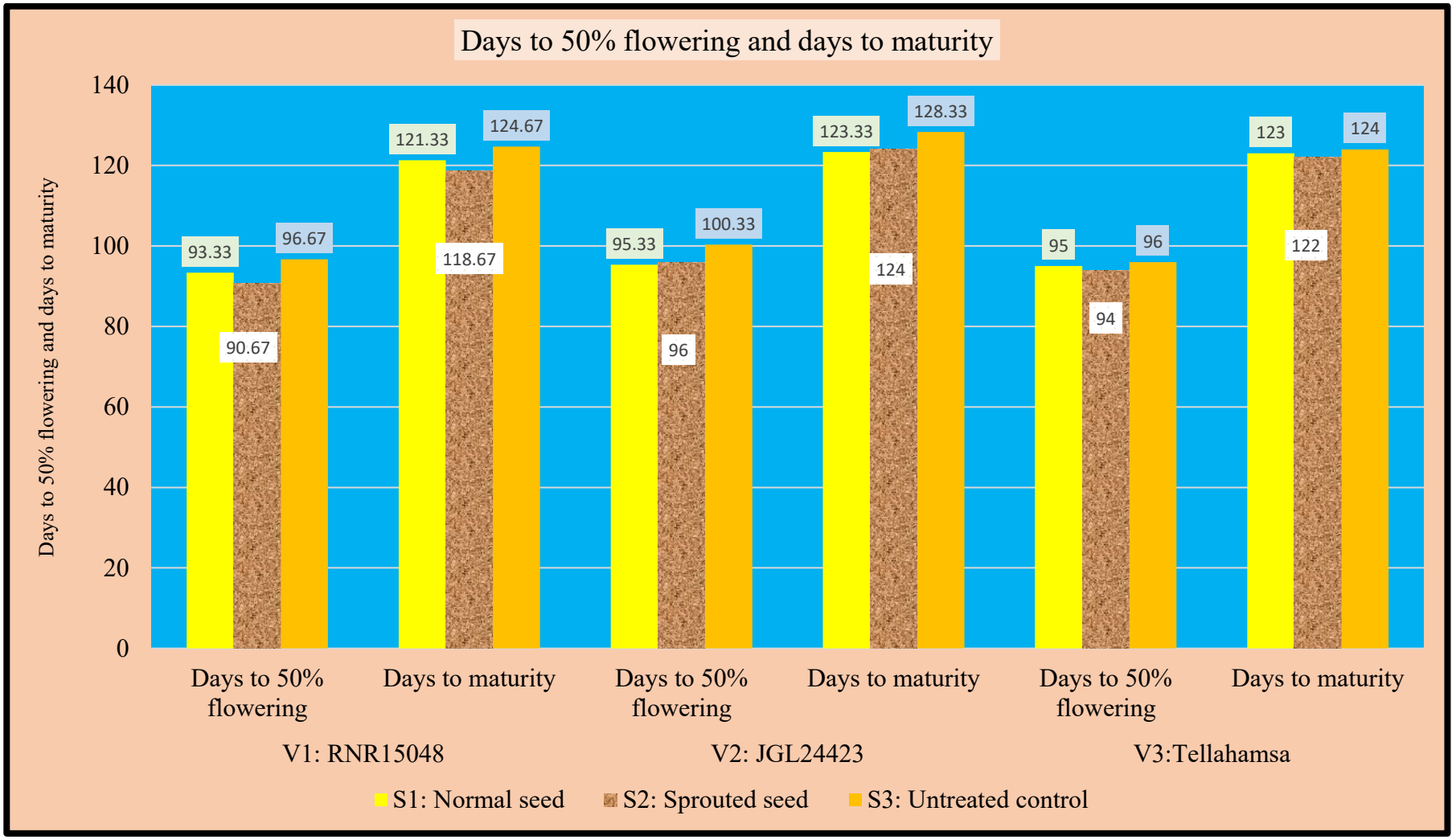


Figure 9. chlorophyll content of leaf in Rabi season 2023-2024.



Fugure 10.Days to 50% flowering in Rabi season 2023-24.

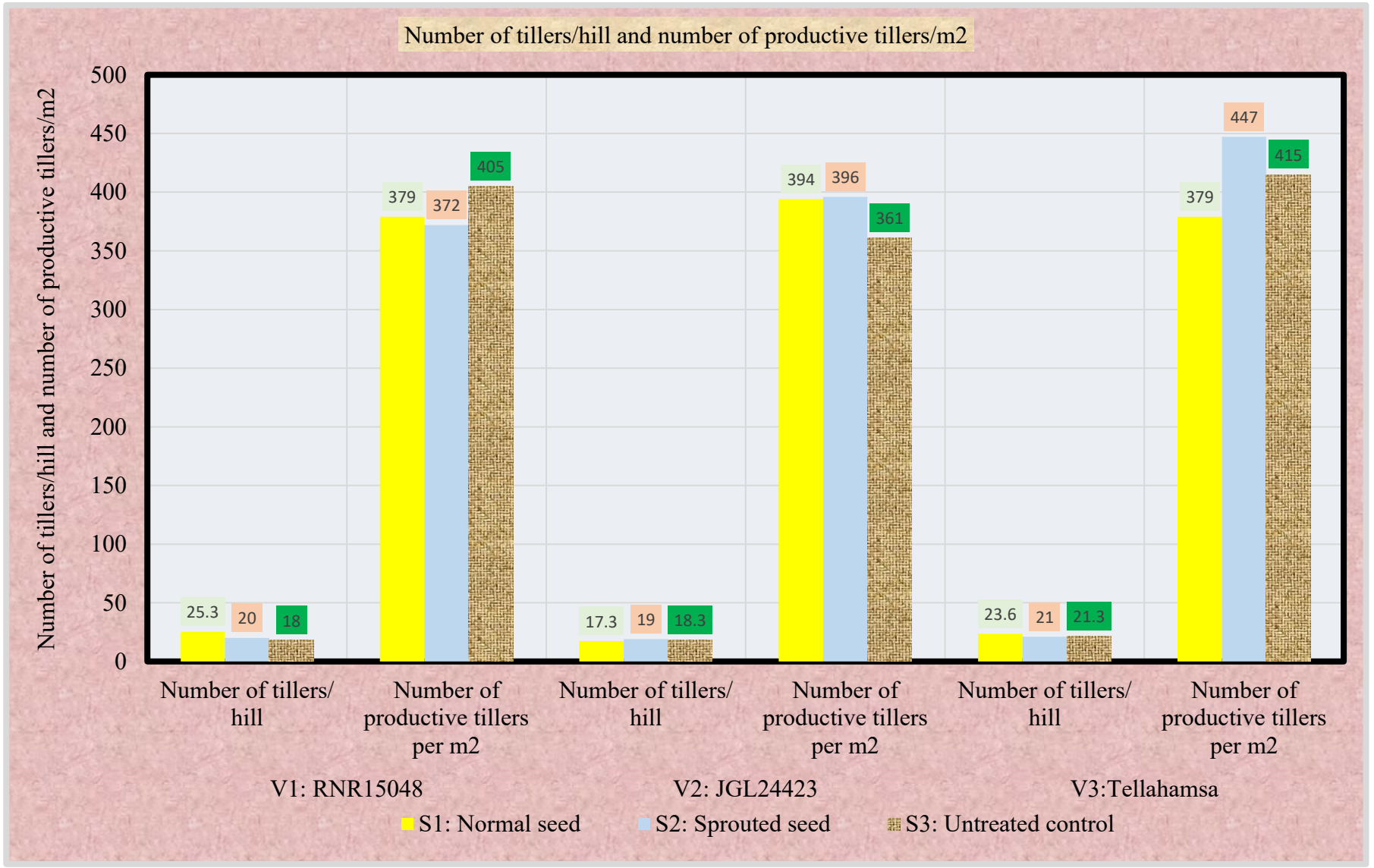

Number of tillers/hill and number of productive tillers/m2
Number of tillers/hill and number of productive tillers/m2
500
450
400
350
300
250
200
150
100
50
0
25.3
20
18
379
372
405
17.3
19
18.3
394
396
361
23.6
21
21.3
379
447
415
Number of tillers/ hill
Number of productive tillers per m2
Number of tillers/ hill
Number of productive tillers per m2
Number of tillers/ hill
Number of productive tillers per m2
V1: RNR15048
V2: JGL24423
V3:Tellahamsa
S1: Normal seed
S2: Sprouted seed
S3: Untreated control

Figure 11.Number of tillers per hill and number of productive tillers per m^2 in Rabi season 2023-2024

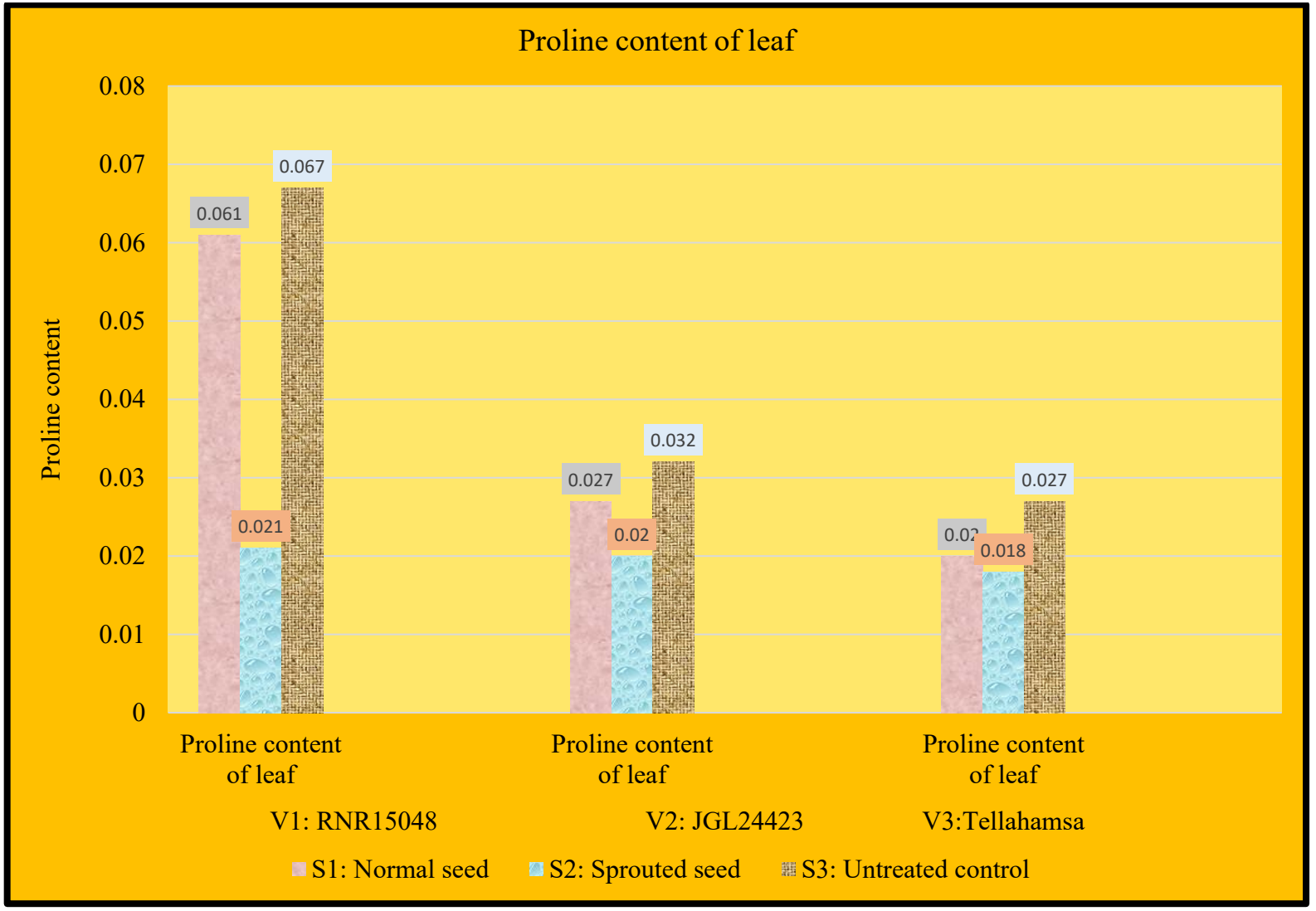


Figure 12.proline content of leaf in Rabi season 2023-2024.

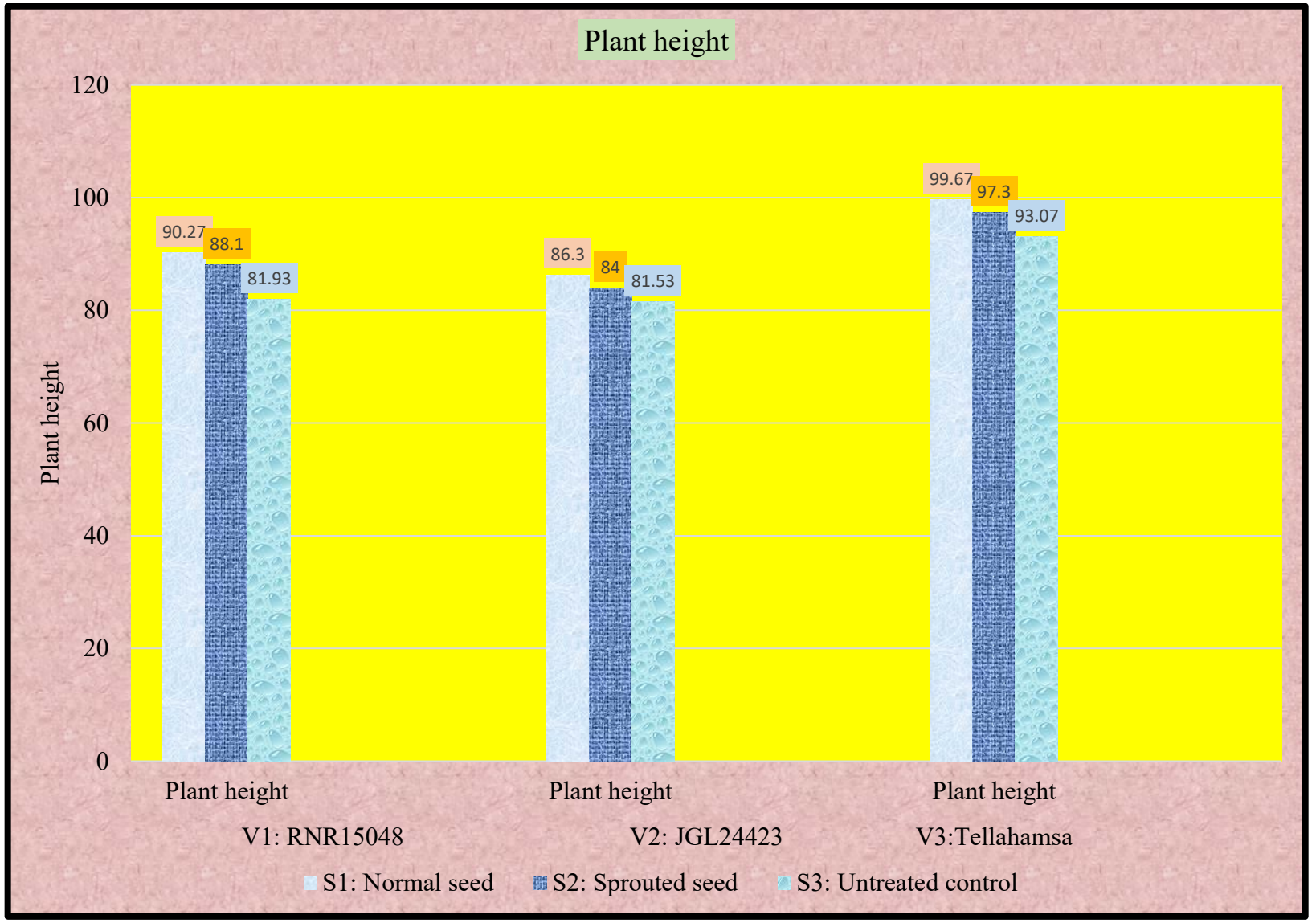

Plant height
120
100
80
60
40
20
0
Plant height
90.27
88.1
81.93
86.3
84
81.53
99.67
97.3
93.07
Plant height
Plant height
Plant height
V1: RNR15048
V2: JGL24423
V3:Tellahamsa
S1: Normal seed
S2: Sprouted seed
S3: Untreated control

Figure 13.Plant height under Rabi season 2023 -2024

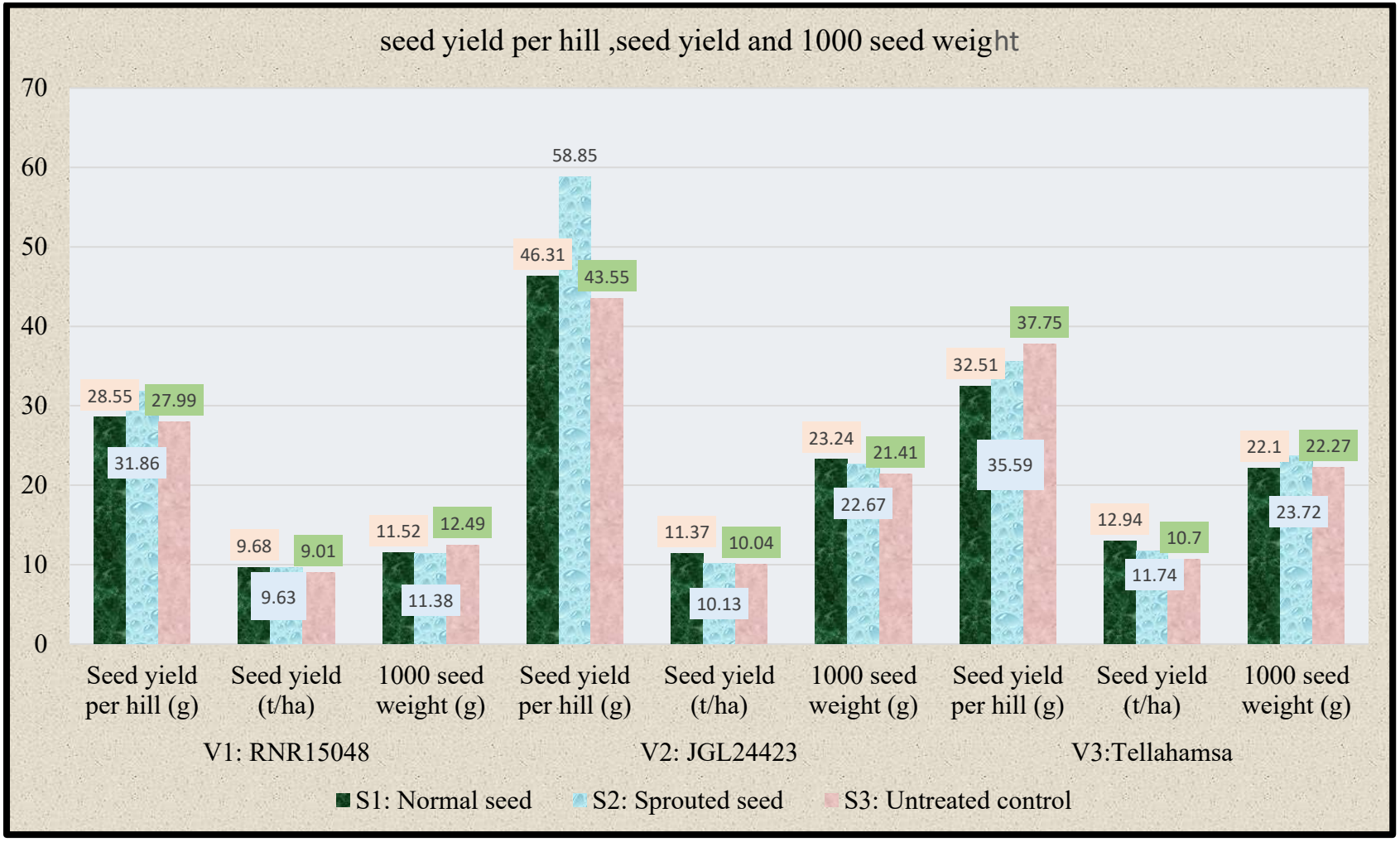


Fig.14 seed yield and grain parameters during *Rabi* season,20223-24

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- sairamfinalmergedthesis30102024compressed1.pdf
- radicleemergencedataanalysis.xlsx
- coldplasmafieldvalidationanalysis.csv
- coldplasmastandardizationdata.xlsx