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Synergistic Effects of Kinetin and Magnesium Nitrate Seed Priming on Enzymatic Activity and Reserve Mobilization in Rice var. MTU-7029 during Seed Germination

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Abstract

Seed germination in rice (*Oryza sativa* L.) depends on the mobilization of storage reserves, a process regulated by hydrolytic enzymes and influenced by seed priming techniques. Enhancing reserve mobilization through eco-friendly priming strategies can improve seedling establishment under variable conditions. This study aimed to evaluate the effects of kinetin and magnesium nitrate [$Mg(NO_3)_2$] seed priming, individually and in combination, on enzymatic activity, reserve mobilization, and seedling vigor in rice variety MTU-7029. Seeds of rice variety MTU-7029 were primed with varying concentrations of kinetin (2.5–30 ppm), $Mg(NO_3)_2$ (2–50 mM), and their combinations. Germination assays were conducted under controlled conditions. Parameters assessed included germination percentage, Timson index, α- and β-amylase activity (free and bound form), protease activity, soluble and insoluble sugar content, nitrogen dynamics, and FESEM micrographs. Observations were recorded from 12 h to 96 h, depending on the parameter studied. Statistical analyses were performed to determine treatment significance at 1% and 5% levels. Results revealed that priming significantly enhanced germination rate and reserve mobilization compared to control. $Mg(NO_3)_2$ priming accelerated early germination, while kinetin priming improved germination velocity. Combined priming treatment [2.5 ppm kinetin + 4 mM $Mg(NO_3)_2$] synergistically enhanced α- and β-amylase, and protease activity, alongside increased soluble sugar and nitrogen accumulation and reduced insoluble reserves; validated by FESEM micrograph. These findings highlight the potential of hormonal and nutrient-based priming as eco-friendly strategies to strengthen rice seedling establishment under various climatic conditions.

Keywords Reserve mobilization, Seed germination, Seedling vigor, Rice, Seed priming

Running Title Eco-Friendly Priming Improves Rice Seedling Establishment

Introduction

Rice (*Oryza sativa L.*) is one of the most important cereal crops worldwide, feeding more than half of the world's population. India, as the largest rice exporter, contributed over 40% of global rice trade in 2022, highlighting its strategic role in sustaining food supply chains (FAS USDA 2023). Nutritionally, seeds of rice contain fiber, resistant starch, proteins, and

lipids (Nascimento et al. 2022). The life cycle of rice begins with seed germination, a critical developmental stage that determines crop establishment, vigor, and yield potential.

Mobilization of storage reserve and seed germination are highly controlled processes regulated by environmental factors like light and temperature, as well as developmental phase of seeds (Bewley and Black 1994). Whereas, desiccated seeds enter into dormancy phase where germination will not take place, even under favourable conditions followed by imbibition. The seed dormancy was released by endogenous changes, and the emergence of the radicle from the seed coat was noted by Bewley (1997). The beginning of a new lifecycle starts with seed germination, it involves both physical and biological process including water uptake, DNA repair, protein synthesis, cell wall loosening, and ultimately the mobilization of storage reserves takes place which helps the radicle to protrude. In this process, transport of nutrients to the embryo provides essential energy for growth and development of the seedlings until they become photoautotrophic.

Reserve mobilization is the central part of rice seed germination. In rice, starch and seed storage proteins (SSPs) are the most abundant storage reserves. Moreover, hydrolytic enzymes like α-amylase, β-amylase, and proteases, secreted from the aleurone layer and scutellum, degrade these macromolecules into simple sugars and amino acids. Gibberellic acid (GA), released from the embryo, plays a pivotal hormonal role in stimulating enzyme secretion, thereby accelerating reserve mobilization and sustaining seedling growth (Bose et al. 2018; Xiong et al. 2021).

Seed priming has emerged as an eco-friendly and sustainable pre-germination technique to enhance germination physiology. By partially hydrating seeds under controlled conditions, priming pre-activates metabolic pathways, and improves speed, synchronization and seedling vigor which requires very short or no activation time during germination (Bose et al. 2018; Anwar et al. 2021; Srivastava et al. 2021; Mondal and Bose 2022). Henceforth, in seed priming, the imbibition should be halted precisely at a right time; but it depends upon various factors like species, genotype and the types of seed and has to be dried carefully under the fan/ forced air up to retain its original weight. Hormonal priming, particularly with GA, brassinosteroids, or salicylic acid, modulates signaling pathways, enhances antioxidant defense, and stimulates reserve mobilization (Sarmin et al. 2025; Qian et al. 2025). Nutri-priming with essential nutrients like magnesium, potassium, calcium, zinc, boron, sulphur, silicon and many more improves morpho-physiological, biochemical and gene expression, leading to better seedling establishment under adverse environmental condition, with stress resilience (Mondal and Bose 2019; Monajjem et al. 2023; Yadev et al. 2023; Zhang et al. 2025).

The priming process is typically divided into three phases: (i) activation of priming memory, DNA and mitochondrial repair, and initiation of stress-responsive gene expression like LEA, DHY, AQP and hormone signaling; (ii) germination phase, where transcription and translation resume, culminating in radicle protrusion; and (iii) post-germination reserve mobilization, which supports rapid radicle elongation and seedling establishment (Khan et al.

2020; Mondal and Bose 2022). Recent studies confirm that priming enhances rice seedling establishment under stress conditions like salinity, drought, and low temperature, by improving reserve mobilization efficiency and seedling vigor (Monajjem et al. 2023; Zhang et al. 2025).

In this context, α-amylase causes endo-cleavage of substrates and hydrolyses α-1,4-linkages in a random manner, having the ability to bypass α-1, 6-branch points. In addition, β-amylase hydrolyses alternate bonds from the non-reducing end of the substrate. The enzyme degrades amylose, amylopectin or glycogen in an exo- or stepwise fashion by hydrolysing alternate glycosidic bonds; and the end product is β-maltose. Similarly, proteases namely endopeptidases are present in the aleurone layer and the scutellum, where they are secreted to perform their hydrolytic functions. However, they present low levels of metabolic activity during the initial hours of germination, becoming evident from the first day of germination (Lemmens et al. 2021). Their activity is observed more efficiently under specific conditions.

Literature suggested that the impact of rice seed priming on reserve mobilization under controlled condition promotes hydrolytic enzymes activity which break down the storage form of complex starch and protein into simple monomeric form in a faster way, signifies uniform seed germination and seedling establishment (Bose et al. 2018; Xiong et al. 2021). This study aimed to investigate the individual and combined effects of kinetin and magnesium nitrate seed priming on germination physiology and reserve mobilization in rice (*Oryza sativa* L. var. MTU-7029), with emphasis on enzymatic activity, sugar and nitrogen dynamics, and seedling vigor.

Methods and materials

The experiment, were carried out in the Laboratory of Seed Physiology, Department of Plant Physiology, Institute of Agricultural Sciences, B.H.U, Varanasi, and Department of Crop Physiology, Institute of Agriculture, V.B. at a temperature of 30-35^{0}C. The experiment was deals with the germination physiology of rice variety MTU-7029.

Seed priming

Healthy bold seeds were surface sterilized by deeping them in 0.1% $HgCl_2$ (Mercuric chloride) solution for 5 minutes and then thoroughly washed with distilled water for 5-6 times. For seed priming, the sterilized seeds were soaked either in distilled water or in different concentrations/ combinations of Kinetin (ranging from 0 to 30 ppm) and $Mg(NO_3)_2$ (ranging from 0 to 50 mM) for 20h. The seeds were taken out and gently washed with distilled water once and then dried back to its initial weight at the room temperature by placing them under fan forced air. The seeds without any treatment referred as control.

On the basis of the above priming treatments, the details were as follows – Non primed seeds (control) (T_1), Seeds primed with distilled water (T_2), Kinetin primed seeds [2.5 (T_3), 5 (T_4), 7.5 (T_5), 10 (T_6), 12.5 (T_7), 15 (T_8), 20 (T_9), 25 (T_{10}), 30 (T_{11}) in ppm], magnesium nitrate primed seeds ($Mg(NO_3)_2$) [2 (T_{12}), 4 (T_{13}), 6 (T_{14}), 8 (T_{15}), 10 (T_{16}), 12.5 (T_{17}), 15 (T_{18}), 20 (T_{19}), 30 (T_{20}), 40 (T_{21}), 50 (T_{22}) in mM], Kinetin + $Mg(NO_3)_2$ primed seeds [2.5 ppm + 2 mM

(T_{23}), 2.5 ppm + 4 mM (T_{24}), 2.5 ppm + 6 mM (T_{25}), 2.5 ppm + 8 mM (T_{26}), 5 ppm + 2 mM (T_{27}), 5 ppm + 4 mM (T_{28}), 5 ppm + 6 mM (T_{29}), 5 ppm + 8 mM (T_{30})].

The primed and non-primed seeds were used to study the reserve mobilization during rice seed germination from the time period of 24 to 96h by visualization under the Field Emission Scanning Electron microscopy (FE-SEM) along with associated biochemical parameters. The total duration of the experiment was 0 to 168h. Petri dishes of 3-inch diameter were used to conduct germination studies. The air-dried seeds were then placed equidistantly (25 seeds in each Petri dish) and 5 mL of distilled water was poured in each Petri dish containing control, hydro-primed, Kinetin primed, $Mg(NO_3)_2$ primed, and combination of both, in different concentrations. The germination percentage and Timson Index was recorded upto 42h, α & β-amylase activity (free and bound), protease activity, soluble and insoluble sugar and nitrogen contents were noted at 48, 72 and 96h.

Germination percent

i) On the expiry of different studied hours, the number of germinated seeds were noted from Petri dishes. The seeds were germinated when the radical was visible through a scar in the seeds. This was calculated based on following formula

Germination percent = Number of seeds germinated × 100% / Number of seeds present in the petridish.

ii) The rate of germination was estimated using a modified formula of Timson Index (Patade et al. 2009):

Timson index $=\sum G/t$

Where G is total germination percentage at the interval of 6h for 2 days (i.e. % of germinated seeds of all seeds that germinated finally); t, total period of germination (days). The higher value of Timson's Index indicated the rapid rate of germination.

α -Amylase Activity

The estimation of α-amylase (EC 3.2.1.1) enzyme in the germinating endosperm of rice seeds, Bernfield (1955) method was followed. For assay, 100mg endosperm was taken out aseptically from the 48, 72 and 96h germinated seeds and homogenized in 5mL phosphate buffer (0.2 M, pH 6.9) in a pre-chilled mortar and pestle. The homogenate was centrifuged (Model: C-24 Remi revolutionary table top high speed cooling centrifuge) at 5,000g at 4^0C for 10 minutes. Supernatant represented the enzyme (α-amylase) extract. Then supernatant was collected and volume was maintained up to 5mL by adding phosphate buffer. 1mL of starch solution (1%) prepared in phosphate buffer (0.2 M, pH 6.9) was taken in a test tube and 1mL of enzyme extract was added to it. The same was kept for incubation at 20^0C for 10 minutes. After proper incubation 2mL of 1% DNS (3, 5-dinitrosalicylic acid) solution was added for the suppression of enzyme activity. Test tube containing reaction mixture was kept in boiling water bath for 5 minutes then cooled with the help of running tap water and final volume raised up to 50 mL by adding distilled water. For blank preparation in place of enzyme extract 1mL of distilled water was used.

Absorbance was read at 510 nm and standard curve of maltose was used for calculating the activity of α-amylase in rice endosperm.

β-Amylase Activity:

Estimation of β-amylase enzyme (free and bound form) activity in germinating endosperm of rice seeds, method of Bernfield (1955); Kruger (1972) and Paavola et al (1972) were followed with some modification.

Extraction of β-amylases (free and bound): The free β-amylase was extracted from acetone defatted [Homogenized the sample in cold (-20ºC) acetone 7mL and centrifuge at 1800g X 10min and the defatted pellets were air dried] sample material (100mg endosperm was taken out aseptically from the 48, 72 and 96h germinated seeds) dissolved in 66mM phosphate buffer (pH-7.0) containing 0.5M NaCl. The extract was centrifuged at 20,000rpm for 15 min. The supernatant was used as a source of free β-amylase. Further the remaining pellet was then extracted with phosphate buffer (66mM, pH 7) containing 0.5% 2-mercaptoethanol. The clear extract was used as source of bound β-amylase. Then supernatant was collected and volume was maintained up to 5mL by adding phosphate buffer. The experiment was carried out at 4ºC.

Assay: In a test tube 1mL of starch solution (1%) prepared in acetate buffer pH 7 was taken and 1mL of enzyme extract was added to it. The same was kept for incubation at 20^0C for 10 minutes. After proper incubation 2mL of 1% DNS (3, 5-dinitrosalicylic acid) solution was added for the suppression of enzyme activity. Test tube containing reaction mixture was kept in boiling water bath for 5 minutes then cooled with the help of running tap water and final volume raised up to 50 mL by adding distilled water. For blank preparation, in the place of enzyme extract 1mL of distilled water was used. Absorbance was read at 560nm and standard curve of maltose was used for calculating the activity of β-amylase in rice endosperm.

Alcohol soluble and insoluble sugar content

The alcohol soluble and insoluble sugar contents in endosperm were determined by the method of Dubios et al (1956). 100mg finally powdered dried sample of 48, 72 and 96h dehusked endosperm were taken in a centrifuge tube and 10 mL of 80% alcohol was added to it, and each treatment contains 3 replications. It was then kept in a hot water bath, temperature maintained upto 80 to 85ºC for 30 minutes; the same was centrifuged for 10 minutes at 5000g. After cooling, supernatant was decanted in a beaker. The process repeated for 3 times and decant were collected in the same beaker; the alcohol of extract was removed by heating it on a water bath maintained at 80-85ºC. Then 25mL distilled water was added to it and now this was known as soluble sugar extract.

Residue left in centrifuge tube was dried by keeping it in an oven at 80ºC for insoluble sugar extraction. Little amount of distilled water (2mL) was added to dried residue and then the tube was kept in a boiling water bath for 15 minutes and after cooling 2mL 9.2N $HClO_4$

was added to it and stirred for 15 minutes. This mixture was made upto 10mL by adding distilled water and centrifuged for 10 minutes before collecting the supernatant. 2mL of 4.6N $HClO_4$ was added to the left residue and the same procedure was repeated. The supernatant was collected in the same beaker mentioned above. This extract was finally raised upto 200mL by adding distilled water. 1ml of sugar extract was pipetted to a test tube and 1mL of 4% phenol was added to it. Then 2.5mL of concentrated H_2SO_4 was added rapidly for good mixing; the test tube was allowed to stand for 10 minutes and then it was shaken and placed for 10 to 20 minutes in water bath at 25 to 30ºC before taking the reading of the finally developed yellow colour, at 490nm for hexose in spectrophotometer (Model-UV-VIS Spectrophotometer, Universal-Bio).

To measure the mg sugar g^{-1} dry weight of endosperm, a calibration curve was prepared from the glucose solution of different concentration.

Protease activity

Extraction of the crude enzyme: To assay the activity of the protease, the method of Harvey and Oaks (1974a & b) with slight modification, was adopted.

Germinated seeds of 48, 72 and 96h were dissected out aseptically to take out the endosperm for enzyme extraction. Those were quickly frozen (-10ºC), for one hour, then taken out and weighed. 500mg was crushed with 2mL of 0.2M acetate buffer (pH 7.5) containing 5.0mM β-mercaptoethanol in chilled mortar and pestle (0-4ºC). The homogenate was centrifuged at 14,000g for 40 min at 4ºC. The supernatant was collected for the assay of protease.

Assay: The assay of this enzyme was done by extracting the enzyme from germinated rice endosperm. The assay mixture consisted of 20mg substrate (Bovine serum albumin (BSA)) dissolved in 1.0mL of 0.2M phosphate buffer (pH 7.5), 1mL of 2.5mM EDTA solution prepared in the same buffer and 0.5 mL of enzyme in a total volume of 2.5 mL. The mixture was incubated for 1h at 40ºC. The enzyme activity was calculated by estimating the protein content from the reaction mixture after adding the enzyme with zero time and at end of the reaction (after 1h incubation time) by the method of Bradford (1976). Bradford protein assay was done by taking 20µL of enzyme extract + 80µL of doubled distilled water + 3mL of dye was added and the reading was taken at 595 nm and in the blank double distilled water was taken 100µL in place of enzyme. The extent of hydrolysis of BSA (proteolytic activity of the enzyme) was calculated by subtracting the final value (incubated for 1h with enzyme) from the first one (incubated for zero time with enzyme).

For preparation of standard curve 10mg of BSA was added in 100mL of water and arranged in a series of test tubes from 50µL to 300µL of BSA/ 300µL. The rest of the procedure is same as above. The readings obtained and plotted on a graph paper by taking BSA on X-axis and absorbence on Y-axis.

Determination of soluble and insoluble nitrogen content

The soluble and insoluble nitrogen was determined from dried samples of rice endosperm at various time periods like 48, 72 and 96h after germination by employing the modified micro kjeldahl (Lang 1958) method.

Preparation of Nessler's reagent

To prepare Nessler's reagent the following were required. (i) Potassium Iodide (KI): The KI (50g) was dissolved in 35mL of distilled water. (ii) Mercuric Chloride ($HgCl_2$): The $HgCl_2$ (22g) was dissolved in 350mL of distilled water. (iii) Sodium hydroxide (NaOH): NaOH (40g) was dissolved in 200mL of distilled water and was cooled.

The mercuric chloride was gradually mixed up in KI solution with constant stirring until a slight red precipitate remained. Thereafter, the NaOH solution was introduced with stirring to get either yellow or a light golden yellow colour solution and it was made up to 1000mL by adding distilled water. Then the solution was allowed to stand overnight in dark and the clear supernatant was filtered and stored in amber colour bottle.

Assay: To extract of soluble and insoluble part of nitrogen oven dried endosperm (100mg) was taken into mortar-pestle and soluble part was extracted with 80%, 10mL ethanol and centrifuged it in 5000g for 10 minute and supernatant was decanted into kjeldahl flask, the same process was repeated twice. The residue which was left in the tube was the insoluble part of the nitrogen. After that both the soluble and insoluble part was kept in the water bath at 60^{o}C until the ethanol evaporated. After evaporation the insoluble portion was digested with 1-2mL of salicylic acid (5%) in presence of a pinch of sodium thiosulphate to avoid the bumping of solution. About 1-2mL of perchloric acid (PA) (60%) was added and then heated till to get a colourless solution. The colourless solution made up to 50mL by adding distilled water. Then 1mL of prepared sample aliquot was pipetted in a test tube to which 2mL of distilled water was added. The colour was developed by adding 2mL of Nessler's reagent. After 15 minutes, the optical density of the coloured solution was read at 440 nm by using the spectrophotometer (UV-VIS Spectrophotometer, Universal-Bio). The soluble and insoluble nitrogen content of endosperm was calculated by preparing a standard curve of ammonium sulphate [$(NH_4)_2SO_4$, (1mM)].

Field Emission Scanning Electron microscopy (FE-SEM) sample preparation

Vertical sectioning of the rice endosperm for Field Emission Scanning Electron Microscopy (FESEM) analysis to expose the internal structure of the starch granules and protein bodies without causing significant damage; were mounted on both side carbon tape, pasted on aluminium stabs. Mounted samples were gold coated in Quorum made SC7620 Sputter Coater and the timing of gold coating was 150seconds. The samples were analysed in ZEISS GeminiSEM 450 (Gemini 2) FESEM; made in UK/GB (Serial No. 8216010130).

Statistical Analysis

The experimental data were analyzed using a Completely Randomized Design (CRD). The significance of treatment effects was tested using the F-test, and the differences among treatment means were compared using Critical Difference (CD) at 1% and 5% levels of significance. Mean values were calculated from three independent replications for each treatment. All statistical analyses and graphical representations were performed using R software (version 4.5.2). Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to evaluate treatment differences. Principal Component Analysis (PCA) was conducted to examine the relationships among biochemical parameters and treatments. PCA and biplot visualization were performed using the stats package (base R; version 4.5.2) along with FactoMineR (version 2.13) and factoextra (version 1.0.7). Graphs and plots were generated using ggplot2 (version 4.0.2). Heatmap analysis of biochemical parameters was performed using pheatmap (version 1.0.12). Variable Importance in Projection (VIP) plots were generated using mixOmics (version 6.26.0) to determine the contribution of variables in the multivariate analysis. Radar (spider) plots showing time-wise enzyme dynamics were constructed using fmsb (version 0.7.5).

Results

Seed germination percentage

Data presented in Fig 1a show the effect of seed priming with distilled water (D.W.) and different concentrations of $Mg(NO_3)_2$/ kinetin alone or in combination on germination percentage of rice variety MTU-7029 at 12, 18, 24, 30, 36 and 42h. Percent germination was found to increase with increasing hours in control and primed seeds. Controlled seeds, showed no germination after 12h. Among all the treatments when studied in respect to the performance of different concentrations (0 to 30ppm) of kinetin primed seeds at different hours of germination it was observed that at 12h except T_{11} (30 ppm) (1.33%) all the kinetin primed seeds showed statistically at par result at 1% and 5% level of significance. Whereas, at 18h, T_4 (73.33%), T_5 (73.33%), T_6 (72%), T_7 (72%) and T_8 (74.67%); at 24h, T_7 (94.67%) and T_{30} (94.67%) and at 30h, T_3 (98.67%), T_4 (97.33%), T_5 (97.33%), T_6 (98.67%) T_7 (97.33%) and T_{11} (99%) noted statistically significant results at 1% and 5% level of significance followed by the other kinetin primed, hydroprimed (T_2) and controlled sets (T_1). At 36 and 42h both hydro and kinetin primed sets showed a static response as compared to T_1.

Data regarding germination percentage of different concentrations of $Mg(NO_3)_2$ primed seeds at 12 to 42h depicted that $Mg(NO_3)_2$ primed seeds has taken much less time for germination as compared to control and kinetin primed seeds. At 12h, 5.33% germination was shown by T_{12}, T_{13} and T_{18} which represented the best performance among all the treatments. At 18h, T_{14} (66.67%), T_{16} (70.67%), T_{17} (68%), T_{18} (70.67%), T_{19} (68%), T_{20} (66.67%) and at 24h, T_{14} (88%), T_{18} (85.33%), T_{19} (86.67%), T_{20} (86.67%) showed statistically at par results at both 1 and 5% level of significance followed by other $Mg(NO_3)_2$ primed seeds. All the treatments showed a stagnancy in the germination percentage except for T_1 (69.33, 90 and

96%), T_{21} (86.67 and 93.33 and 98.67%) and T_{22} (84, 93.33 and 94.67%) treatments at 30, 36 and 42h a dynamism in data was noticed (Fig 1b).

Fig 1c depicts the germination percentage of combined priming of $Mg(NO_3)_2$ and kinetin. At 12h, 4% germination was noted by T_{23} to T_{28} whereas at 18h, T_{25} (77.33%), T_{28} (80%) and T_{30} (78.67%), at 24h, T_{23} to T_{27} (89.33, 92, 88, 88 and 89.33% respectively) and T_{29} (88%) and at 30h, T_{23} (97.33%), T_{26} (97.33%) and T_{27} (98.67%) showed best performance. At 36 and 42h except T_1 all the primed seeds showed at par and static results.

Timson Index

The rate/ velocity of germination measured in terms of Timson index was also higher in response to the priming treatments. Among the different priming sets, the Timson index was maximum in kinetin primed set in the treatment number T_6 followed by the combined set (primed with kinetin and $Mg(NO_3)_2$) and $Mg(NO_3)_2$ primed set (Fig 2a, b and c). The non-primed control one has shown very poor performance.

α-amylase activity (mg maltose g^{-1} h^{-1} fresh weight of endosperm)

Seed priming treatments had a highly significant effect on α-amylase activity in rice (MTU-7029) during the study periods (48h, 72h, and 96h), as confirmed by the computed F-statistics (231.72 at 48h, 450.83 at 72h, and 102.15 at 96h). These values were far greater than unity, indicating that treatment effects were not due to random variation but were statistically significant.

At 48 h, the highest α-amylase activity was observed in treatment T_{13}–T_{14} (303.83 in 4–6mM of $Mg(NO_3)_2$). The differences between treatments exceeded the CD at 1% (15.96), confirming strong statistical significance. At 72h, activity peaked in T_{24} (2.5ppm + 4mM) with (578.65), compared to control (266.72), was highly significant with CD at 1% (15.18). Other treatments like T_{25} (554.09) and T_{14} (513.64) also showed significantly higher activity than control, confirming the stimulatory effect of seed priming. At 96h, control (408.46) showed the highest activity among all treatments. Most primed treatments showed reduced activity, with values significantly lower than control. Hydropriming (T_2) enhanced activity at 48h (173.82 vs. 88.72 in control) and 72h (464.54 vs. 266.72), both differences exceeding the CD at 1%, but activity declined sharply at 96h (235.48), significantly lower than control. Combined treatments of kinetin + $Mg(NO_3)_2$ also showed synergistic effects at 72h.

β-amylase activity (free and bound form) (mg maltose g^{-1} h^{-1} fresh weight of endosperm)

Seed priming treatments exerted a highly significant effect on β-amylase activity (both free and bound forms) in rice (MTU-7029) during the study periods (48h, 72h, and 96h), as confirmed by the computed F-statistics which were far greater than unity (free form: 1526.65 at 48h, 500.14 at 72h, and 1583.22 at 96h; bound form: 145.84 at 48h, 187.85 at 72h, and 650.08 at 96h). These values indicate strong treatment effects well beyond random variation. At 48h, free β-amylase activity ranged from (54.82 in control, T_1) to (184.14 in 6mM $Mg(NO_3)_2$, T_{14}). The difference between treatments exceeded the CD at 1% (4.48), confirming statistical significance. Bound activity also varied significantly, with T_{24} (2.5ppm + 4mM) recording the

highest (140.06) compared to control (85.37), exceeding the CD at 1% (5.98). At 72 h, free β-amylase activity peaked in T_{19} (325.17) compared to control (192.93) with a CD at 1% (6.45). Bound activity was maximized in T_{17} (264.91) and T_5 (264.49), both significantly higher than control (176.19), again exceeding the CD at 1% (7.20). At 96 h, free β-amylase activity reached its maximum in T_{15} (290.86), significantly higher than control (136.85), with differences well above the CD at 1% (6.15). Bound activity was highest in T_{15} (171.59), compared to control (77.42), again surpassing the CD at 1% (6.27). Hydropriming (T_2) and lower kinetin concentrations (2.5–7.5ppm; T_3–T_5) showed moderate improvements in free activity (61.10–65.70 at 48h; 211.34–228.92 at 72h), but their effects were less pronounced at 96h. Bound activity under these treatments peaked at 72h (238.55–264.49), significantly higher than control, but declined sharply thereafter. Combined treatments of kinetin + $Mg(NO_3)_2$ showed synergistic enhancement at lower concentrations. T_{24} (2.5ppm + 4mM) produced significantly higher free activity (310.25 at 72h) and bound activity (266.17 at 72h), both exceeding the CD at 1% thresholds. However, higher combinations (e.g., T_{30}: 5ppm + 8mM) reduced activity (101.28 free, 43.11 bound at 96h), suggesting antagonistic effects at elevated levels.

Soluble and insoluble sugar content (mg g^{-1} dry weight of endosperm)

Fig 3(a, b & c) represented the performance of primed rice germinated endosperm, on alcohol soluble and insoluble sugar contents at 48, 72 and 96h. In kinetin primed seeds, at all the studied hours the soluble sugar content were represented the highest value in treatment T_4; those are 13.99, 17.14 and 31.43 respectively. However, at 48h T_3 (14.12) and T_4 (13.99) noted to have higher values and shows statistically at par results in this context followed by the other treatments of primed seeds and control. At 72h the similar situation was visualised where T_5 (16.93) showed at par result with T_4. While studying the insoluble sugar content control (T_1) represented the highest value i.e., 566.06, 401.45 and 343.58 values at 48, 72 and 96h of germination respectively. Whereas, the treatment T_4 (426.25, 338.83 and 268.25 respectively) showed significantly less insoluble sugar content in comparison to others with differences well above the CD at 1% (4.14, 4.03 and 6.66).

Similarly, in Fig. 3b treatment T_{15} (11.0), T_{16} (16.71) & T_{17} (31.74) showed highest soluble sugar content at 48, 72 and 96h respectively. However, at 48h T_{16} (10.77) and T_{17} (10.68) showed at par result with T_{15}; at 72h, T_{15} (16.22) showed at par values with T_{16}; at 96h T_{13} to T_{16} (13.04 to 31.54) noted at par data with T_{17} with differences well above the CD at 0.01 (0.65, 0.63 and 1.22). Furthermore, in insoluble sugar content it was found that T_{14} (343.13, 280.24 and 219.17) and T_{16} (345.13, 276.31 and 217.83) had the lowest amount of insoluble sugar content in the germinating endosperm at all the three studied hours with a CD value at 1% (3.71, 4.57 and 4.00).

Combined form of kinetin/ $Mg(NO_3)_2$ while used for priming purpose showed an increasing trend of soluble sugar content for 48 to 96h and simultaneously the content of insoluble sugar was found to show a declining trend. Treatment number T_{24} (19.81) at 48h, T_{25} and T_{26} (38.79 and 39.05 respectively) at 72h and T_{26} (45.69) at 96h have shown the higher level of soluble sugar content and statistically significant with a CD value at 0.01 (0.63, 1.19

and 1.55) in comparison to other treatments. The lowest level of insoluble sugar content was attained by T_{24} (386.36, 262.16 and 208.53) at all three studied hours and it were statistically significant CD value at 1% level (6.06, 5.58 and 2.37). In each and every set the control non-primed seeds showed the lowest level of soluble sugar content and highest level of insoluble sugar (Fig 3c).

Protease activity (mg g^{-1} fresh weight of endosperm)

Seed priming treatments significantly influenced protease activity in rice (MTU-7029) across study periods (48h, 72h, and 96h). At 48h, the highest protease activity was observed in T_{22} (2.38). The differences between treatments exceeded the CD at 1% (0.0888), confirming strong statistical significance. In this regard, control (1.02) was the poor performer. At 72h, activity peaked in T_{24} (2.5ppm + 4mM) with (5.40), followed by T_{22}, T_{25}-T_{26} and other priming treatments (4.24, 5.17 respectively) in comparison to control. At 96h, control (T_1) (3.61) maintained the highest activity among all treatments. Most primed treatments showed reduced activity, with values significantly lower than control. However, moderate kinetin concentrations (20–25ppm; T_9–T_{10}) sustained relatively higher activity (3.43–3.44), significantly above hydropriming (2.81) and $Mg(NO_3)_2$ alone at higher concentrations (2.62–2.95). Hydropriming (T_2) enhanced activity at 48h (1.27 vs. 1.02 in control) and 72h (3.39 vs. 2.29), both differences exceeding the CD at 1%, but activity declined at 96h (2.81), significantly lower than control. Combined treatments of kinetin + $Mg(NO_3)_2$ showed synergistic effects at 72h.

Soluble and insoluble nitrogen content (mg g^{-1} dry weight of endosperm)

The data of Fig 4(a, b & c) presented the effect of seed priming on soluble and insoluble nitrogen contents (SNC and INC) in germinating rice seed's endosperm var. MTU-7029 at 48, 72 and 96h.

Fig 4a represented the kinetin primed sets, where T_3 showed highest SNC i.e. 15.12 and 23.13 at 48 and 72h and statistically significant CD at 1% (0.38 and 0.48 respectively). Whereas, at 96h, treatment T_3 to T_6 (26.35, 27.18, 27.48 and 26.59) showed higher value and statistically significant CD at 1% (1.06) with rest of the treatments. For INC, the control seeds (T_1) showed the highest level which was found statistically significant in respect to other treatments. However, at 48h T_3 (30.64) and T_4 (30.59), at 72h T_5 to T_7 (26.63, 26.5 and 26.6) and at 96h T_3 to T_6 (19.08, 19.77, 19.9 and 19.66) showed low INC as compared to other treatments with a CD at 1% (0.26, 0.31 and 1.02).

Similar trend was noted in $Mg(NO_3)_2$ primed sets; at 48h treatment T_{12} (15.09) and T_{13} (15.09), and at 72 & 96h treatment T_{13} to T_{15} (23.78, 23.36, 23.2 and 30.07, 30.10, 29.71) reported higher SNC and statistically significant with other treatments, with CD at 1% (0.52, 0.31 and 0.53). Study regarding INC revealed that T_{13} to T_{16} (29.56, 29.56, 29.45 and 29.81) at 48h; T_{14} (26.15) and T_{15} (25.96) at 72h and T_{13} (17.15) at 96h had the lower INC in comparison to the other treatments (Fig. 4b).

In combined primed set, T_{24} (17.05, 25.03, 32.31) for SNC and (28.13, 26.22, 17.8) for INC have maximum and minimum values 24, 72 and 72h of germination. However, the lowest and highest levels of ethanol SNC and INC respectively were attended by the non-primed control seeds at every studied period i.e. at 48, 72 and 96h. The values were statistically significant with other treatments with CD at 1% (0.26, 0.26, 0.36 for SNC and 0.36, 0.34 and 0.32 for INC respectively) (Fig. 4c).

Correlation Heatmap

The heat map illustrates the pairwise Pearson correlation coefficients (r) among six biochemical parameters: α-amylase activity (AAA), β-amylase activity (bound form) (BABE), β-amylase activity (free form) (BAA), protease activity (PA), soluble sugar content (SSC) and soluble nitrogen content (SNC) in Fig 5. A highly significant positive correlation network was observed among all measured enzymatic activities and all were mutually correlated (r = 0.65 to 0.77, $p < 0.001$). Cell colours encode the strength and direction of associations, with dark blue indicating strong positive correlations, red denoting negative correlations, and lighter shades representing weak or negligible relationships. Statistical significance is highlighted by yellow asterisks (* $p < 0.05$, *** $p < 0.001$), allowing rapid identification of robust associations.

Furthermore, the primary metabolites soluble sugar content (SSC) and soluble nitrogen content (SNC) exhibited a strong positive correlation with each other (r = 0.83, $p < 0.001$). The relationships between these metabolites and the enzyme activities were more variable. SNC showed moderate positive correlations with both BAA (r = 0.51, $p < 0.001$) and PA (r = 0.50, $p < 0.001$), while SSC exhibited weaker, yet significant, associations with PA (r = 0.35, $p < 0.001$) and BAA (r = 0.25, $p < 0.05$). Notably, neither SSC nor SNC demonstrated significant correlations with AAA or BABE, indicating that the overall accumulation of these soluble metabolites is largely uncoupled from these specific enzymatic fractions. Overall, the correlation structure indicates two principal metabolic clusters: (i) an enzymatic cluster (α-amylase, β-amylase, protease) representing active hydrolysis, and (ii) a metabolite cluster (soluble nitrogen and soluble sugars) representing the products of these reactions. The moderate positive relationship between amylase and protease enzymes further supports their complementary roles in mobilizing carbohydrates and amino acids during germination (Fig 5).

Principal Component Analysis (PCA)

PCA at 48 Hours

The PCA at 48 hours explained approximately 71% of the total variability (PC1 = 43.6%, PC2 = 27.6%). The first principal component (PC1) was primarily driven by α-amylase activity and protease activity, indicating that these enzymes were the major contributors differentiating treatments at this early stage. Treatments located toward the positive PC1 side (e.g., 2–6 mM) exhibited higher enzyme activities, suggesting rapid activation of hydrolytic processes. In contrast, combined ppm + mM treatments and the Hydro/control group clustered near the origin, reflecting moderate to low enzymatic induction. The protease and α-amylase vectors dominated PC1, whereas β-amylase and soluble nitrogen content contributed moderately along

PC2. These results suggest that early germination responses were characterized by the activation of amylolytic and proteolytic enzymes, especially in $Mg(NO_3)_2$ priming treatments (Fig 6a).

PCA at 72 Hours

At 72 hours, the PCA accounted for approximately 68% of total variance (PC1 = 47.9%, PC2 = 19.9%). Similar to the 48-hour profile, PC1 was dominated by α-amylase and protease activity. However, longer vectors for soluble sugar and nitrogen content indicated an increasing contribution of carbohydrate and nitrogen metabolism to the overall variation. Treatments such as 2.5 ppm + 4 mM and 2.5 ppm + 6 mM clustered near the high enzyme quadrant, indicating strong induction of metabolic activity. The Hydro and control groups separated distinctly, showing lower biochemical activity. In contrast, higher concentrations of $Mg(NO_3)_2$ (10–15 mM) shifted toward the negative PC1 region, suggesting saturation or inhibitory effects on enzyme activity. Thus, by 72 hours, metabolic processes transitioned from enzyme induction to an integrated sugar and nitrogen response (Fig 6b).

PCA at 96 Hours

The PCA at 96 hours explained 72% of total variance (PC1 = 46.4%, PC2 = 25.7%), indicating strong separation of treatments based on soluble metabolite accumulation. α-amylase, β-amylase (bound enzymes), and protease activity loaded positively along PC1, while soluble sugar and nitrogen content dominated PC2. Lower ppm + mM combinations (5 ppm + 2–8 mM) showed high PC2 scores, reflecting greater accumulation of soluble sugars and nitrogen. The control group remained separated with minimal projection on PC1, signifying limited enzyme activity. Hydro and moderate $Mg(NO_3)_2$ treatments clustered centrally, showing steady but moderate metabolic responses. These observations confirm that, at 96 hours, germination metabolism reached completion with accumulated soluble metabolites (Fig 6c).

Variable Importance in Projection (VIP) Plot

The PLS-DA–derived VIP scores reveal that protease activity, β-amylase activity, and α-amylase activity were the most discriminant biochemical traits differentiating the germination time points. These variables showed the highest contribution to model separation, indicating that protein and starch hydrolysis dominated the biochemical transition during germination. The high importance of protease activity suggests that protein degradation and mobilization of amino acids were early and critical events. Simultaneously, the prominence of both β-amylase (bound and free forms) and α-amylase reflects enhanced enzymatic breakdown of starch into soluble sugars to support seedling energy demands. In contrast, soluble nitrogen and soluble sugar contents ranked lower, signifying that these represent secondary metabolic outcomes of the hydrolytic enzyme cascade rather than direct discriminant drivers. Collectively, the VIP profile indicates a strong enzymatic coordination during mid to late germination, where proteolysis and carbohydrate catabolism jointly regulate nutrient release (Fig 7).

Radar (Spider) Plot of Time-wise Enzyme Dynamics

The radar visualization provides a comparative overview of mean biochemical parameter intensities across 48 h, 72 h, and 96 h of germination. At 48 h, the polygon area is smallest, indicating low enzymatic activity and minimal soluble metabolite accumulation representing the initiation phase. By 72 h, the polygon expands significantly, particularly along α- and β-amylase and protease axes, marking the peak hydrolytic phase of germination. At 96 h, although enzyme activity begins to plateau or decline, soluble nitrogen and sugar contents reach their maximum, signifying the metabolite accumulation phase. This progressive expansion and shape shift confirm a temporal metabolic cascade from enzyme induction to substrate mobilization and finally to nutrient availability (Fig 8).

Field Emission Scanning Electron Microscopy (FESEM)

FESEM micrographs of germinating rice endosperm section represents the breakdown of starch granules of non-primed control seeds (a-h), 2.5ppm kinetin primed seeds (a_1-h_1), 4mM magnesium nitrate primed seeds (a_{11}-h_{11}), and combination of 2.5ppm kinetin + 4mM magnesium nitrate primed seeds (a^0_{11}-h^0_{11}) from a time duration of 24h to 96h with a scale bar of 100µM, 20µM and 10 µM (Fig. 9). The starch degradation in endosperm cells facing the scutellum of the embryo clearly depicted the effect of seed priming from the initiation of seed germination to the completion of the process. During the studied hours (24 to 96h) the considered primed and non-primed germinated rice endosperm where primed germinated seeds showed better enzymatic activity, and the breakdown/ hydrolysis of starch granule was much faster in comparison to control one. The micrographs again validates the priming regulated prior activation of hydrolytic enzymes i.e., α- amylase, β-amylase (free and bound form) and protease activity which is responsible for faster reserve mobilization from the complex form into monomeric one and provide nutrition to the germinating embryo until it becomes photoautotropic. As per the literature, there is a time gap between the germination of non-primed and primed seeds; in FESEM micrograph of the present study proved the time gap between the non-primed and primed rice endosperm's patterns of starch hydrolysis. The photograph of control seeds from 24h to 96h represents the less hydrolysis of starch in comparison to other primed seeds. In these micrographs, the combination of kinetin and magnesium nitrate primed seeds showed better reserve mobilization pattern from the period of 24-96h.

Discussion

Rapid, uniform seed germination and early seedling establishment are crucial for modern agriculture to ensure robust root systems before soil moisture depletes or temperatures become sub-optimal (Reed et al. 2022). Seed priming is a restrictive hydration treatment of seeds, mostly used to improve crop performance (Bose et al. 2018). Primed seeds thus reach a 'germinating state' but without radicle protrusion. Due to this 'head-start', they typically exhibit improved germination rate and uniformity. The priming-led enhanced germination performance under both optimal and adverse environments has been reported in diverse crop plants, including cereals, pulses, oilseeds and many more. (Mondal and Bose 2019).

The primary objective of employing seed priming is to improve the germination and seedling establishment. Osmoconditioning, osmopriming or halopriming synonymous seed priming methods are used to describe the soaking of seeds in aerated low-water potential solutions to control water uptake and prevent radicle protrusion (Bray 1995). Osmopriming or halopriming with calcium chloride, potassium nitrate, sodium chloride, magnesium nitrate, calcium nitrate and polyethylene glycol-8000 improved the energy of germination and lowered mean germination time in rice (Mondal et al. 2011; Srivastava and Bose 2012 and Mondal and Bose 2012; Anwar et al. 2021). To improve the performance of seed, various salts, osmolytes, plant growth regulators, polyamines and certain other organic sources are incorporated during priming and as presowing soaking treatments in many vegetables and field crops, including rice (Kim et al. 1993; Jeong et al. 1994; Lee et al. 1999). Of the phytohormones, gibberellic acid is well known to activate β-amylase for the breakdown of starch stored in seeds to be utilized by growing embryos during germination (Taiz and Zeiger 2006; Xiong et al. 2021). In a study when kinetin and gibberellins applied to the dehusked seeds of indica and japonica rice under aerobic conditions, both of these hormones stimulated the germination of rice (Miyoshi and Sato 1997).

In the present investigation the popular rice Var. MTU-7029 while primed with various concentrations of kinetin and nitrate salt [$Mg(NO_3)_2$] separately and in combination were found to improve not only the germination percentage but also the velocity of germination (Timson Index) as compared to control one. An increase in germination percentage/ velocity/ rate was observed by various workers time to time with a number of field crops, vegetable and ornamental plants (Yadav et al. 2023). However, concentrating upon the result it was observed that the combined form of kinetin and $Mg(NO_3)_2$ was better in respect to their separate application during priming. This may happen due to the action of particular essential element within the cell system and that has been added with the action of particular PGR like kinetin, specifically required for the accumulation of source in proper side for enhancing the performance of sink (Banerji and Laloraya 1965 and Sugiura 1963). Hence, the high velocities of germination resultantly supposed to induce the growth of the emerging seedlings.

Pre-treating seeds with protective agents such as phytohormones, antioxidants, and essential nutrients has been shown to enhance seed performance under both favourable and challenging environmental conditions (Rady et al. 2019; Ren et al. 2020; Wu and Wang 2012). The use of synthetic cytokinins (CKs) has been particularly effective, as they promote nutrient mobilization, increase photosynthetic protein and chlorophyll levels, delay leaf senescence,

and optimize plant growth under normal as well as stress conditions (Hamayun et al. 2015; Kaya et al. 2018). The improvement in seed germination following priming treatments is often linked to the earlier initiation and intensification of metabolic processes, which accelerate reserve mobilization and seedling establishment (Wang et al. 2016; Zhang et al. 2020).

Reserve mobilization during germination and growth of cereals requires a set of coordinate events which includes hydrolytic enzyme induction by GA_3 (Chrispeels and Varner 1967; Jacobsen and Varner 1967; Xiong et al. 2021), enzyme activation, enzymic hydrolysis of reserve materials in the starchy endosperm (Harvey and Oaks 1974; Bose and Srivastava 1980 and Macgregor and Matsuo 1982), and transport of products to the embryo (Biuggs 1968; Sopanen 1979; Sopanen et al. 1980 and Bose et al. 1982; Subedi and Bhattari 2003; Jaybhaye et al. 2024). The aleurone layer has a central role in these events providing hydrolytic enzymes, such as α-amylases and proteases (Chrispeels and Varner 1967; Jacobsen and Varner 1967; Xiong et al. 2021). Another aleurone layer function, which has not received much attention, is the capacity of this tissue to acidify the starchy endosperm (Mikola and Virtanen 1980). This acidification could have a regulatory or permissive role in the reserve mobilization process. It has been shown that some of these phenomena require acidic pH between 4 to 5, like the enzymic activity of several proteases (Jacobsen and Varner 1967; Mikola and Mikola 1980; Bose et al. 1982; Mondal and Bose 2012), and several active transport systems in the scutellar epithelium (Sopanen 1979; Sopanen et al. 1980; Mondal and Bose 2012). During germination protease and α-amylase increase in activity at a time when the storage reserves are being lost at a rapid rate (Mondal and Bose 2012). Increasing hydrolytic enzyme activity throughout the periods of protein, starch, phytin and RNA breakdown has been observed in oat (Paulis and Wall 1971).

Rice grains are composed of three genetically distinct tissues: the diploid embryo, the triploid endosperm, and the diploid maternal tissue. The embryo, situated at the ventral base, is largely made up of the scutellum, which serves as the interface between the embryo and the endosperm (Wang et al. 2020). The endosperm itself consists of two regions the starchy endosperm and the aleurone layer with starch concentrated in the central portion. During germination, gibberellic acid (GA) released from the embryo stimulates the aleurone layer to secrete starch-degrading enzymes such as α-amylase (Zeeman 2015). The GA-mediated activation of α-amylase (*AMY I*) is closely linked to nutrient remobilization (Sreenivasulu et al. 2010); by producing relatively large dextrins, and then slowly small oligosaccharides, indicating a binding preference for polysaccharides over oligosaccharides. However, β-amylase (exoamylase that attacks the nonreducing ends of starch molecules) produces only maltose as reported by Mathewson and Seabourn (1983). In rice, β-amylase have both free and bound forms (Evans et al. 1997) responsible for hydrolysis process. These findings highlight the role of distinct hormonal signals in regulating starch breakdown during cereal germination. Supporting this, pit-like structures observed on starch granules confirm hydrolytic activity by α-amylase (Lin et al. 2016; Nakata et al. 2017). These supports the present experiment's results i.e., the enhanced activity of hydrolytic enzymes due to seed priming and these supporting data from the literatures along with the micrographs of FESEM also validates the synergistic activation of amylolytic activity and reserve mobilization with no time lag phase during rice

seed germination was the fulfilment due to seed priming with lower doses of kinetin and magnesium nitrate alone or in combination form.

In the present piece of work the hydrolysing enzyme α & β-amylase and proteases all were found to have higher activity in primed sets in respect to non-primed control seeds during germination. The best concentrations which have shown the highest activity/ better performance for all the studied parameters is 2.5ppm kinetin and 4mM $Mg(NO_3)_2$ priming in combination followed by individually as priming agent. When both concentrations of the chemicals were combined, the rate of activity of all these enzymes were increased further and performed best among all the combinations. This depicts that both the chemical in combined form in lower doses as priming agent act synergistically; which has been well correlated with results of Lee and Kim (2000). The enhanced activity of the hydrolytic enzymes also increased the solubilization of reserve products in the endosperm as has been observed in the present study that the increment in ethanol soluble sugar and nitrogen were directly correlated with the increase in activities of α & β-amylase and protease enzymes respectively at different studied hours of germination. The breakdown of insoluble sugar and nitrogen were facilitated with the higher activities of these hydrolytic enzymes and not lost during the redrying process, representing a part of priming of seeds and upon rehydration during germination it represented higher activities as compared to non-primed directly sown controlled seeds (Xiong et al. 2021). These results are in support with the findings of Lee and Kim (1999 and 2000), Basra et al (2005), Anaytullah and Bose (2007), Mondal et al (2011) who reported increased activity of protease and α-amylase ultimately resulted in higher level of germination in various cereals like wheat, maize and rice. In 1982, Bose et al. reported that during rice seed germination the biosynthesis of protease was noted; responsible for breakdown of storage proteins. They have proved it by using inhibitors like cycloheximide and chloramphenicol. Furthermore, Mondal and Bose (2012) while working with wheat variety Malviya 234 primed with $Ca(NO_3)_2$ observed that for α-amylase the activity and synthesis both were increased during priming. However, the detail kinetic studies of α-amylase and application of inhibitors of protein synthesis cycloheximide and chloramphenicol proved this fact also. Hence, in the present investigation it may be suggested that during priming the synthesis as well as activity of all the studied hydrolytic enzymes were found to be induced in case of rice variety MTU 7029. However, the higher range of concentrations of kinetin and $Mg(NO_3)_2$ inhibited the activities of these hydrolytic enzymes. This may be due to the toxicity generated from the higher accumulation of these chemicals in cellular organelles (Bose et al. 1993); during priming when along with these chemicals water entered in the cell, injured the organelles and membranes due to their presence in higher concentrations. Beside this the higher concentration of kinetin and salt declined the water potential of the outer solution which simultaneously restricts the entry of the water in seed at the time of germination as and when required. This might reduce the activity of hydrolytic enzymes and breakdown of storage materials, which has been recorded in the present study where the activity of α & β-amylase and protease reduced and that was well correlated with the reduction in the breakdown of storage products like starch (insoluble sugar represents in Fig 3(a, b & c)) and protein (insoluble nitrogen Fig 4(a, b & c)) respectively. Similarly, Basra et al (2005) also suggested that lower activity of amylase is responsible for salt toxicity (KNO_3) in rice seeds during germination. They further stated that the decreased α-amylase activity and reduced starch conversion into simple sugar resulted in delayed poor

germination and emergence in rice. Water potential is another contributing factor during germination if it is restricted due to the development of lower water potential for the presence of higher concentration of PGR or salt may also inhibit germination. In addition, seed priming is responsible for improved germination and seedling stand establishment by osmoconditioning (Basra et al. 2006; Corbineau and Come 2006; Anwar et al. 2021).

Conclusion

The present study demonstrates that seed priming with kinetin and magnesium nitrate, individually and in combination, significantly enhances reserve mobilization in rice (var. MTU-7029) by stimulating hydrolytic enzyme activity, accelerating germination rate, and improving seedling vigor. The synergistic treatment of 2.5 ppm kinetin with 4 mM $Mg(NO_3)_2$ proved most effective, promoting α- and β-amylase as well as protease activity, while simultaneously increasing soluble sugar accumulation and reducing insoluble reserves. These biochemical shifts highlight the capacity of priming to reprogram early metabolic events, ensuring rapid and uniform seedling establishment.

Looking ahead, such eco-friendly priming strategies hold promises for sustainable rice cultivation under diverse agro-ecological conditions. By fine-tuning hormonal and nutrient inputs, seed priming could be optimized germination physiology and early seedling establishment which increase the tolerance against various biotic and abiotic stresses. Thus, kinetin and magnesium nitrate priming not only strengthen the physiological basis of rice germination but also provide a futuristic framework for climate-smart agriculture and food security.

Author contributions

S.M. Conceptualization, Laboratory Experiment, original draft preparation, Visualization, Validation, Supervision, Methodology, Data curation. B.B. review & editing, Supervision, Conceptualization. D.S. Writing – review & editing, Data Analysis. S.D. Data Analysis, review & editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Conflict of interest

The author declares that they have no conflict of interest.

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872 Saf 188:109901. https://doi.org/10.1016/j.ecoenv.2019.109901.

873 **Table 1: Effect of seed priming with distilled water (D.W.) and different concentrations of**
874 **Kinetin/ $Mg(NO_3)_2$ alone or in combination on α-amylase activity (mg maltose g^{-1} h^{-1} fresh weight**
875 **of endosperm) of rice var. MTU-7029 at different study periods**

Treatments	α-amylase activity		
	48h	72h	96h
Control (T_1)	88.72^{l}	266.72^{o}	408.46^{a}
Hydro primed (T_2)	173.82^{g}	464.54^{hi}	235.48^{fg}
2.5 ppm (T_3)	288.21^{b}	543.21^{bc}	188.05^{jkl}
5 ppm (T_4)	261.70^{c}	538.75^{c}	179.40^{l}
7.5 ppm (T_5)	227.11^{de}	503.60^{de}	195.02^{ijkl}
10 ppm (T_6)	186.93^{f}	482.95^{fg}	199.76^{ij}
12.5 ppm (T_7)	145.64^{i}	479.04^{g}	205.62^{hi}
15 ppm (T_8)	124.15^{j}	473.46^{gh}	221.53^{gh}
20 ppm (T_9)	114.39^{jk}	456.17^{i}	219.29^{gh}
25 ppm (T_{10})	118.02^{jk}	389.21^{l}	230.45^{fg}
30 ppm (T_{11})	109.65^{k}	347.91^{n}	263.38^{cde}
2 mM (T_{12})	292.95^{ab}	495.50^{e}	137.55^{m}
4 mM (T_{13})	303.83^{a}	474.86^{gh}	141.73^{m}
6 mM (T_{14})	303.83^{a}	513.64^{d}	180.79^{kl}
8 mM (T_{15})	287.09^{b}	492.16^{ef}	225.71^{fg}
10 mM (T_{16})	229.90^{de}	507.78^{d}	253.89^{e}
12.5 mM (T_{17})	239.94^{d}	412.64^{k}	271.19^{cd}
15 mM (T_{18})	239.38^{d}	427.43^{j}	275.09^{c}
20 mM (T_{19})	260.59^{c}	427.15^{j}	268.12^{cde}
30 mM (T_{20})	258.35^{c}	363.54^{m}	264.49^{cde}
40 mM (T_{21})	255.56^{c}	277.05^{o}	238.27^{f}
50 mM (T_{22})	252.49^{c}	240.22^{p}	198.09^{ij}
2.5 ppm + 2 mM (T_{23})	162.38^{gh}	472.07^{gh}	206.74^{hi}
2.5 ppm + 4 mM (T_{24})	225.15^{e}	578.65^{a}	196.97^{ijk}
2.5 ppm + 6 mM (T_{25})	216.78^{e}	554.09^{b}	208.69^{hi}
2.5 ppm + 8 mM (T_{26})	217.90^{e}	533.17^{c}	195.02^{ijkl}
5 ppm + 2 mM (T_{27})	154.85^{hi}	342.05^{n}	308.85^{b}
5 ppm + 4 mM (T_{28})	159.59^{h}	419.89^{jk}	306.90^{b}
5 ppm + 6 mM (T_{29})	150.38^{hi}	432.17^{j}	256.40^{de}
5 ppm + 8 mM (T_{30})	152.89^{hi}	424.08^{jk}	257.24^{de}
SEm(±)	4.24	4.04	5.32

CD at 0.05	12.00	11.42	15.05
CD at 0.01	15.96	15.18	20.02
F Statistic (Computed)	231.72	450.83	102.15

Table 2: Effect of seed priming with distilled water (D.W.) and different concentrations of Kinetin/ $Mg(NO_3)_2$ alone or in combination on β-amylase activity (free and bound form) (mg maltose g^{-1} h^{-1} fresh weight of endosperm) of rice var. MTU-7029 at different study periods

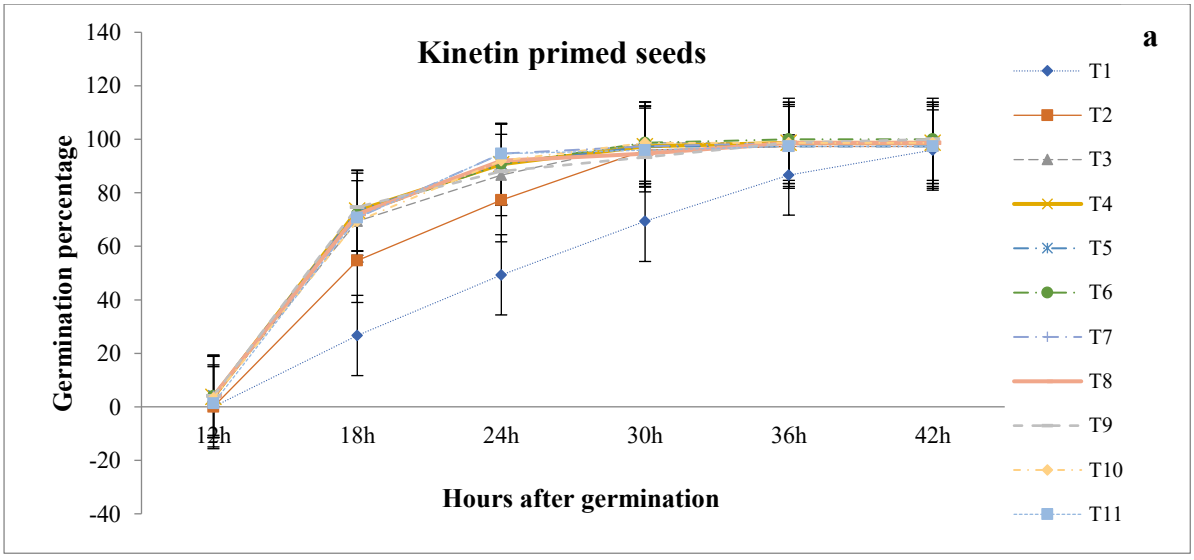
Treatments	β-amylase activity (free form)			β-amylase activity (bound form)		
	48 h	**72 h**	**96 h**	**48 h**	**72 h**	**96 h**
Control (T_1)	54.82^{p}	192.93^{q}	136.85^{kl}	85.37^{k}	176.19^{m}	77.42^{h}
Hydro primed (T_2)	61.10^{no}	211.34^{p}	105.88^{q}	102.95^{efg}	238.55^{cd}	47.71^{mn}
2.5 ppm (T_3)	64.87^{lm}	215.11^{op}	120.53^{op}	105.46^{ef}	251.52^{b}	50.22^{lm}
5 ppm (T_4)	65.29^{lm}	220.55^{mn}	128.90^{mn}	107.55^{e}	255.70^{b}	56.50^{k}
7.5 ppm (T_5)	65.70^{klm}	228.92^{l}	131.83^{m}	113.83^{d}	264.49^{a}	63.61^{j}
10 ppm (T_6)	69.05^{jk}	234.36^{k}	138.52^{k}	101.28^{fgh}	238.96^{cd}	69.47^{i}
12.5 ppm (T_7)	68.22^{kl}	236.45^{k}	146.89^{j}	107.55^{e}	235.20^{de}	64.03^{j}
15 ppm (T_8)	72.40^{ij}	247.75^{j}	140.62^{k}	97.09^{hij}	225.57^{fg}	56.50^{k}
20 ppm (T_9)	75.33^{i}	235.62^{k}	131.41^{m}	95.42^{ij}	215.11^{ij}	53.99^{kl}
25 ppm (T_{10})	81.19^{h}	220.55^{mn}	115.92^{p}	83.28^{k}	223.06^{gh}	57.33^{k}
30 ppm (T_{11})	63.61^{mn}	210.92^{p}	99.60^{r}	74.49^{l}	197.53^{k}	55.24^{k}
2 mM (T_{12})	130.99^{f}	280.40^{g}	212.60^{f}	87.47^{k}	176.61^{m}	121.78^{f}
4 mM (T_{13})	164.05^{c}	285.84^{f}	246.50^{e}	93.74^{j}	182.88^{l}	143.96^{c}
6 mM (T_{14})	184.14^{a}	286.67^{f}	250.26^{e}	99.60^{ghi}	196.70^{k}	156.10^{b}
8 mM (T_{15})	174.10^{b}	274.12^{h}	290.86^{a}	97.93^{hij}	200.46^{k}	171.59^{a}
10 mM (T_{16})	164.05^{c}	294.62^{e}	263.66^{d}	104.63^{ef}	253.61^{b}	141.45^{cd}
12.5 mM (T_{17})	170.75^{b}	298.39^{de}	274.12^{c}	107.97^{e}	264.91^{a}	145.64^{c}
15 mM (T_{18})	166.98^{c}	313.04^{b}	290.44^{a}	99.60^{ghi}	235.20^{de}	138.52^{d}
20 mM (T_{19})	164.05^{c}	325.17^{a}	279.14^{b}	83.7^{0k}	218.88^{hi}	133.08^{e}
30 mM (T_{20})	159.87^{d}	261.98^{i}	283.32^{b}	73.66^{l}	211.76^{j}	124.29^{f}
40 mM (T_{21})	153.59^{e}	245.24^{j}	208.41^{f}	85.79^{k}	221.39^{gh}	104.21^{g}
50 mM (T_{22})	97.09^{g}	216.78^{no}	161.12^{h}	71.98^{l}	175.77^{m}	71.98^{i}
2.5 ppm + 2 mM (T_{23})	59.99^{o}	307.74^{c}	177.72^{g}	129.18^{b}	243.57^{c}	39.76^{o}
2.5 ppm + 4 mM (T_{24})	83.42^{h}	310.25^{bc}	154.57^{i}	140.06^{a}	266.17^{a}	40.18^{o}
2.5 ppm + 6 mM (T_{25})	64.73^{lmn}	300.76^{d}	152.33^{i}	133.08^{b}	236.03^{de}	44.78^{no}
2.5 ppm + 8 mM (T_{26})	63.89^{mn}	296.02^{de}	145.92^{j}	130.57^{b}	230.45^{ef}	47.29^{mn}

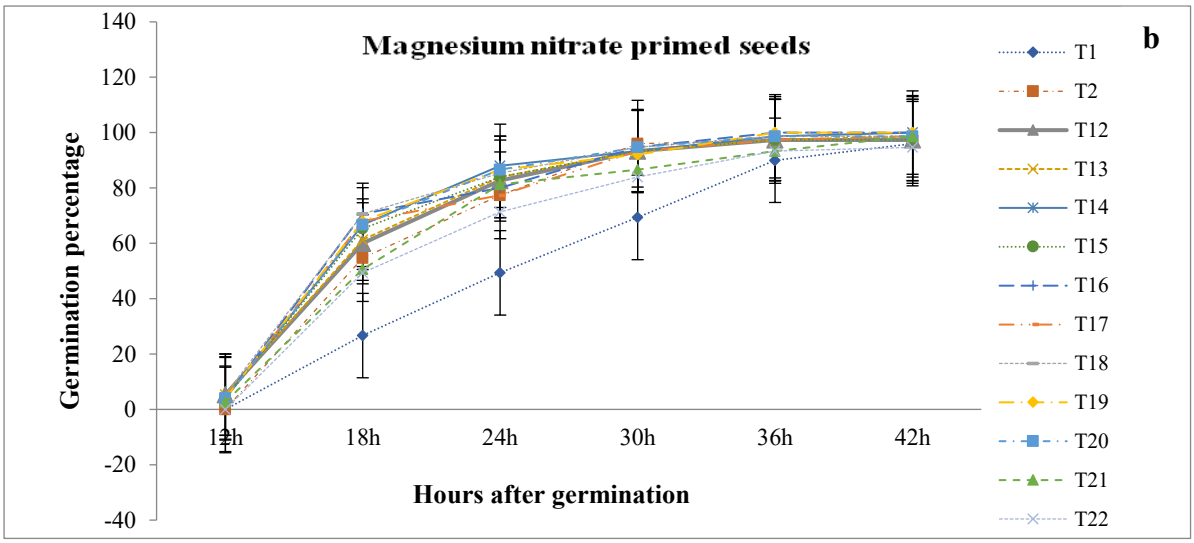
5 ppm + 2 mM (T_{27})	73.66^{i}	235.76^{k}	132.53^{lm}	132.53^{b}	232.97^{de}	46.87^{mn}
5 ppm + 4 mM (T_{28})	74.49^{i}	232.97^{kl}	124.43^{no}	133.36^{b}	233.80^{de}	48.13^{mn}
5 ppm + 6 mM (T_{29})	69.19^{jk}	222.36^{m}	120.81^{o}	115.51^{d}	221.81^{gh}	46.45^{mn}
5 ppm + 8 mM (T_{30})	55.24^{p}	220.13^{mno}	101.28^{qr}	122.20^{c}	221.81^{gh}	43.11^{no}
SEm(±)	1.19	1.71	1.63	1.59	1.91	1.67
CD at 0.05	3.37	4.85	4.62	4.50	5.42	4.72
CD at 0.01	4.48	6.45	6.15	5.98	7.20	6.27
F Statistic (Computed)	1526.65	500.14	1583.22	145.84	187.85	650.08

Table 3: Effect of seed priming with distilled water (D.W.) and different concentrations of Kinetin/ $Mg(NO_3)_2$ alone or in combination on protease activity (mg g^{-1} fresh weight of endosperm) of rice var. MTU-7029 at different study periods

Treatments	**Protease Activity**		
	48h	**72h**	**96h**
Control (T_1)	1.02^{t}	2.29^{p}	3.61^{a}
Hydro primed (T_2)	1.27^{rs}	3.39^{o}	2.81^{ijk}
2.5 ppm (T_3)	1.27^{rs}	3.59^{n}	2.88^{ghi}
5 ppm (T_4)	1.32^{r}	3.56^{n}	2.63^{l}
7.5 ppm (T_5)	1.40^{q}	3.86^{lm}	3.04^{def}
10 ppm (T_6)	1.59^{nop}	4.03^{j}	3.05^{def}
12.5 ppm (T_7)	1.68^{klm}	4.42^{hi}	3.25^{c}
15 ppm (T_8)	1.94^{ef}	4.46^{h}	3.15^{d}
20 ppm (T_9)	2.07^{d}	4.77^{f}	3.44^{b}
25 ppm (T_{10})	2.11^{cd}	4.87^{e}	3.43^{b}
30 ppm (T_{11})	2.14^{c}	4.91^{e}	3.42^{b}
2 mM (T_{12})	1.23^{s}	3.83^{lm}	2.85^{hijk}
4 mM (T_{13})	1.56^{op}	3.83^{lm}	2.76^{jk}
6 mM (T_{14})	1.63^{mno}	3.81^{m}	2.62^{l}
8 mM (T_{15})	1.71^{kl}	3.95^{k}	2.85^{hijk}
10 mM (T_{16})	1.74^{jk}	3.90^{kl}	2.95^{fgh}
12.5 mM (T_{17})	1.88^{fgh}	4.35^{i}	2.78^{ijk}
15 mM (T_{18})	1.95^{e}	4.42^{hi}	3.06^{def}
20 mM (T_{19})	2.10^{cd}	4.74^{f}	2.87^{ghij}
30 mM (T_{20})	2.13^{cd}	5.00^{d}	2.83^{ijk}
40 mM (T_{21})	2.24^{b}	5.16^{c}	2.75^{k}
50 mM (T_{22})	2.38^{a}	5.24^{b}	2.74^{k}
2.5 ppm + 2 mM (T_{23})	1.84^{hi}	4.89^{e}	3.12^{d}

2.5 ppm + 4 mM (T_{24})	1.97^{e}	5.40^{a}	3.00^{ef}
2.5 ppm + 6 mM (T_{25})	1.53^{p}	5.17^{c}	3.06^{def}
2.5 ppm + 8 mM (T_{26})	1.65^{lmn}	5.17^{c}	3.27^{c}
5 ppm + 2 mM (T_{27})	1.92^{efg}	4.49^{h}	2.99^{ef}
5 ppm + 4 mM (T_{28})	1.95^{e}	4.62^{g}	3.09^{de}
5 ppm + 6 mM (T_{29})	1.86^{ghi}	4.64^{g}	2.97^{fg}
5 ppm + 8 mM (T_{30})	1.80^{ij}	4.78^{f}	2.97^{fg}
SEm(±)	0.0236	0.0238	0.0346
CD at 0.05	0.0668	0.0673	0.0980
CD at 0.01	0.0888	0.0895	0.1303
F Statistic (Computed)	200.26	837.31	51.59





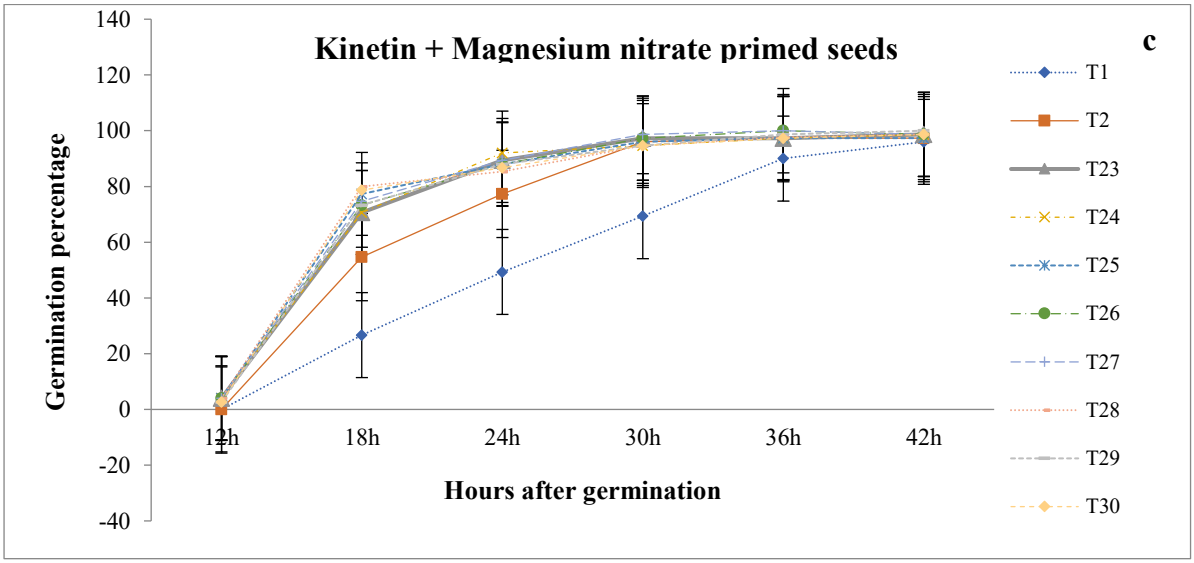
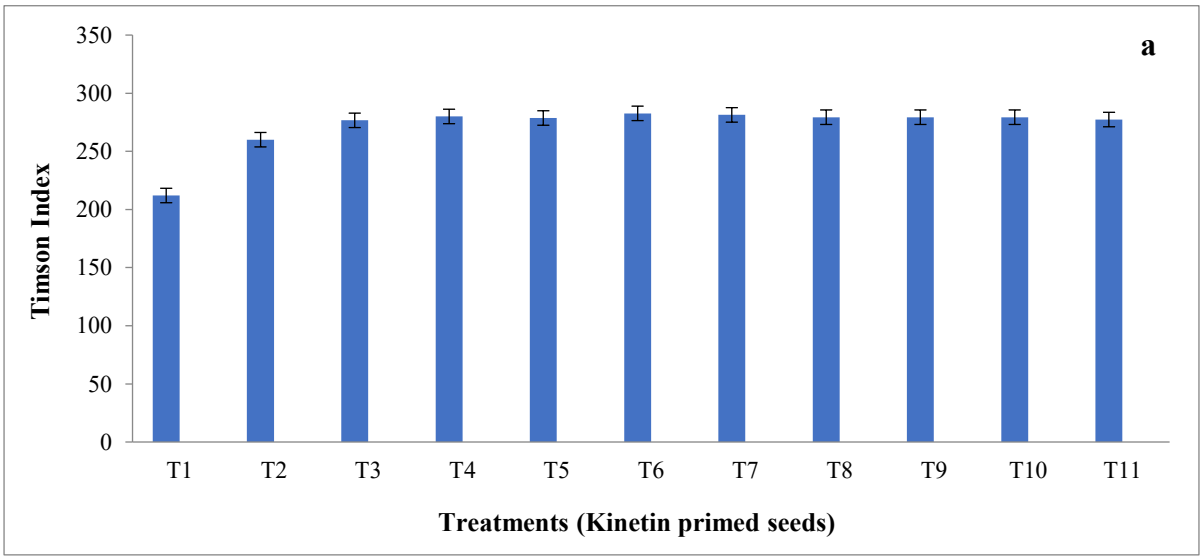
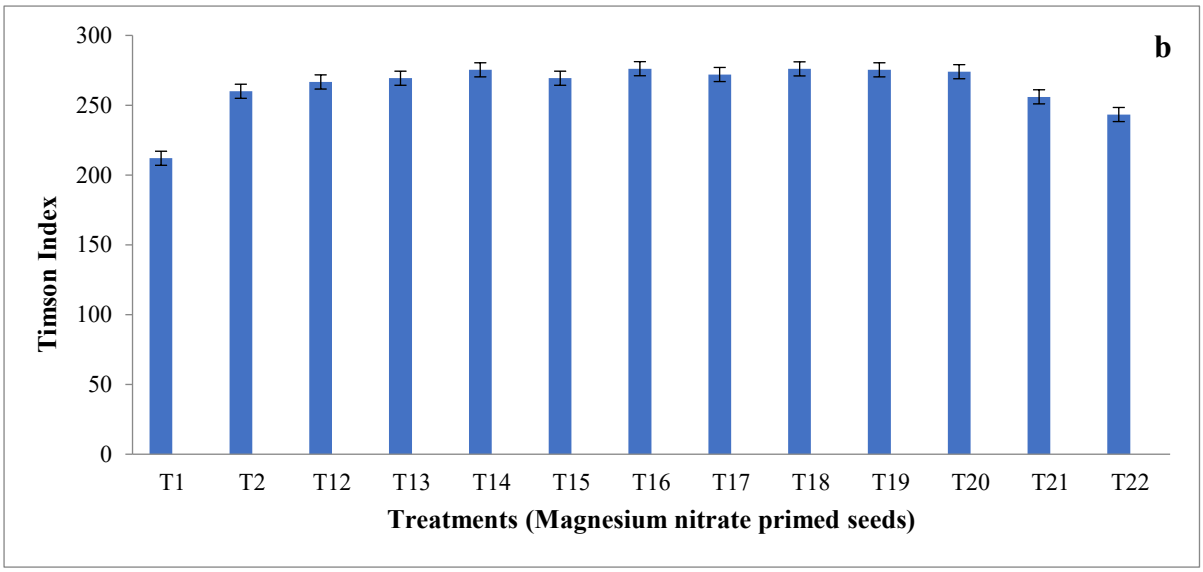
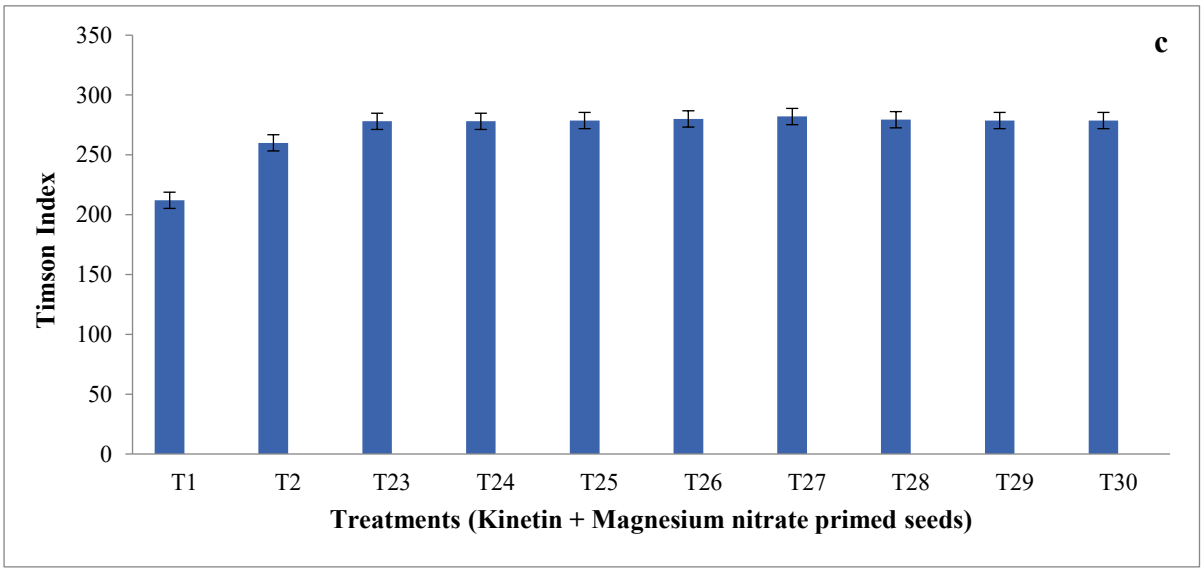


Fig 1(a, b & c): Effect of seed priming with distilled water (D.W.) and different concentrations of Kinetin/ $Mg(NO_3)_2$ alone or in combination on germination percentage of rice var. MTU-7029 at different study periods



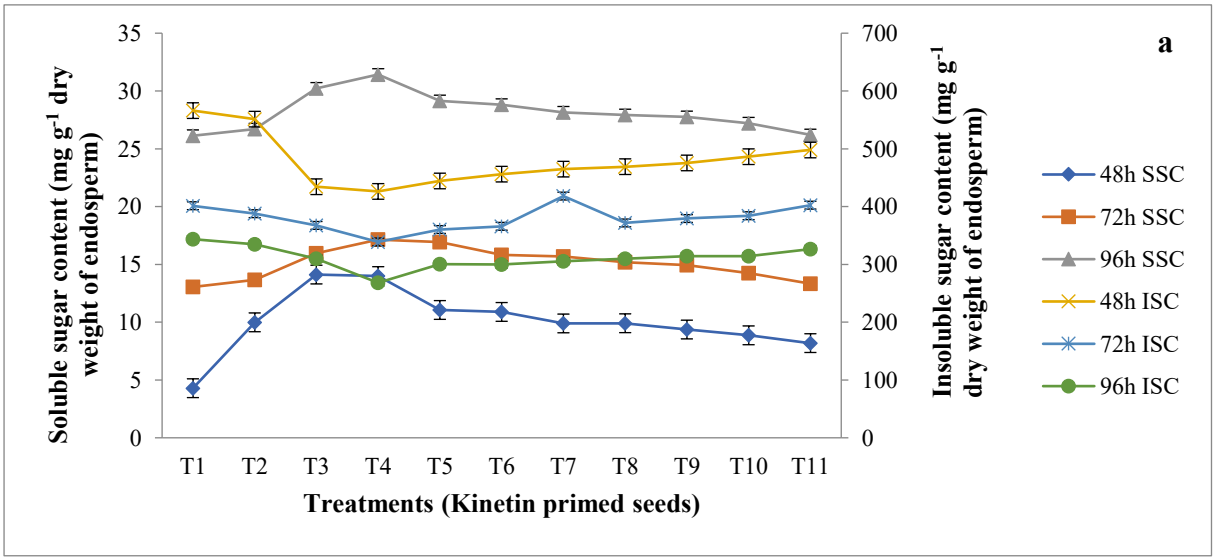


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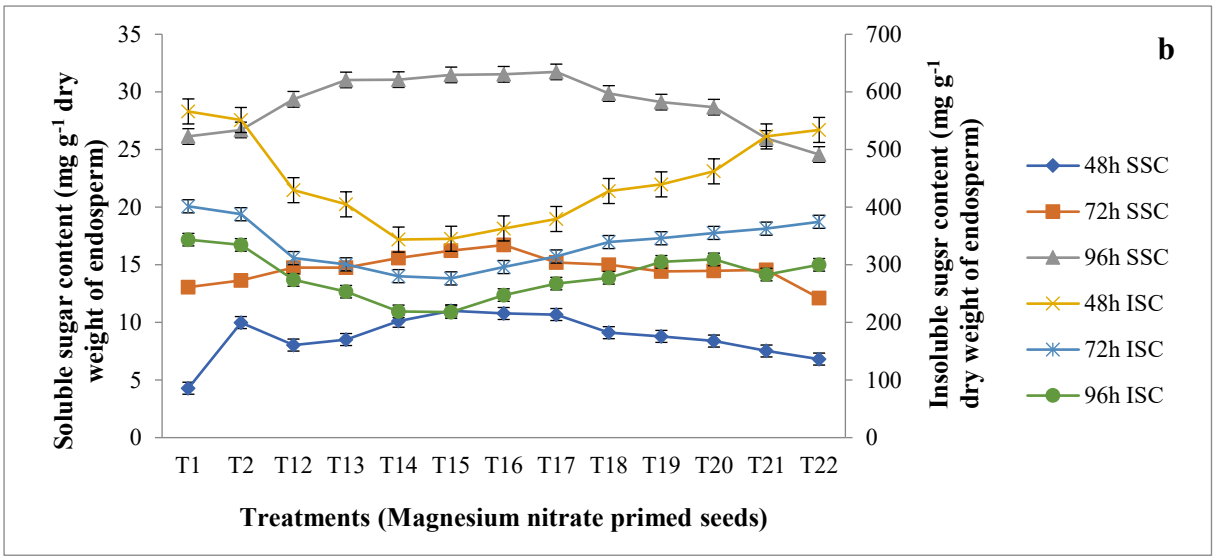


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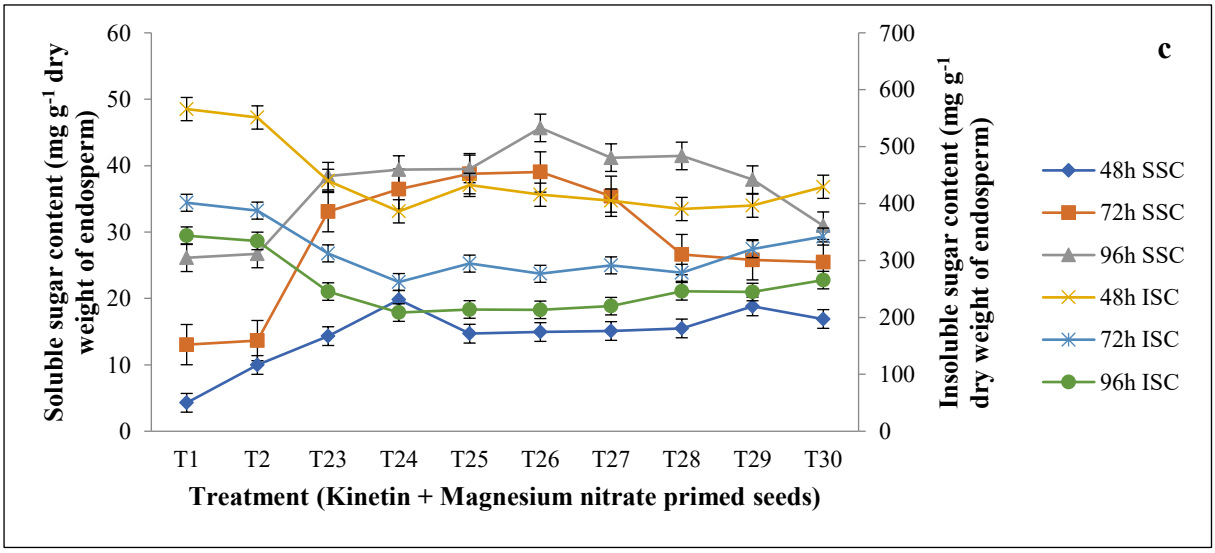
902 **Fig 2(a, b & c): Effect of seed priming with distilled water (D.W.) and different concentrations of**
903 **Kinetin/ $Mg(NO_3)_2$ alone or in combination on Timson Index of rice var. MTU-7029**



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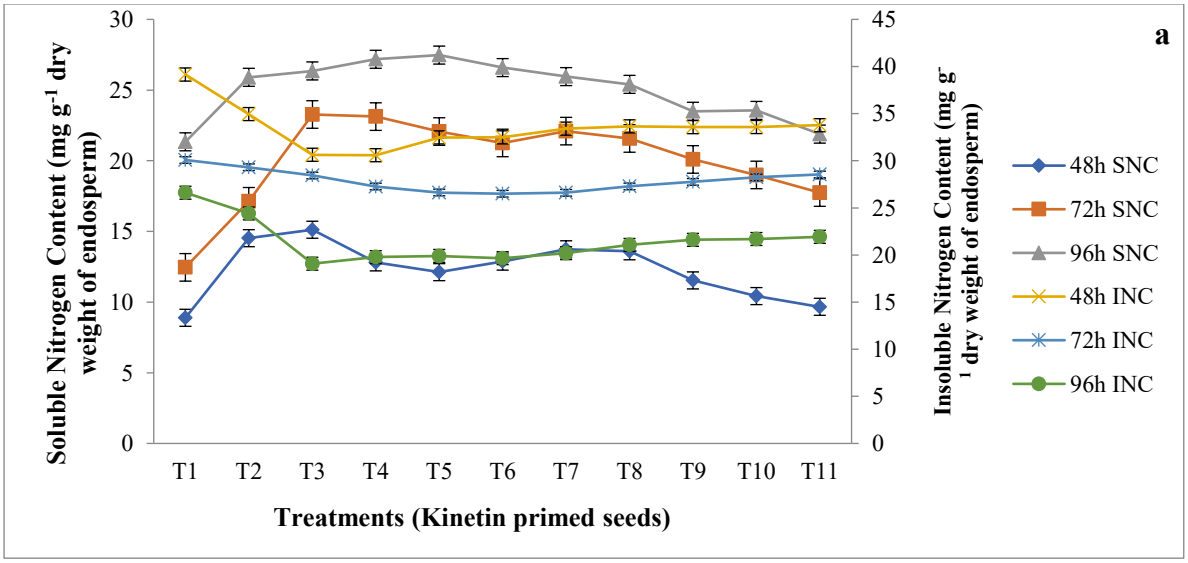


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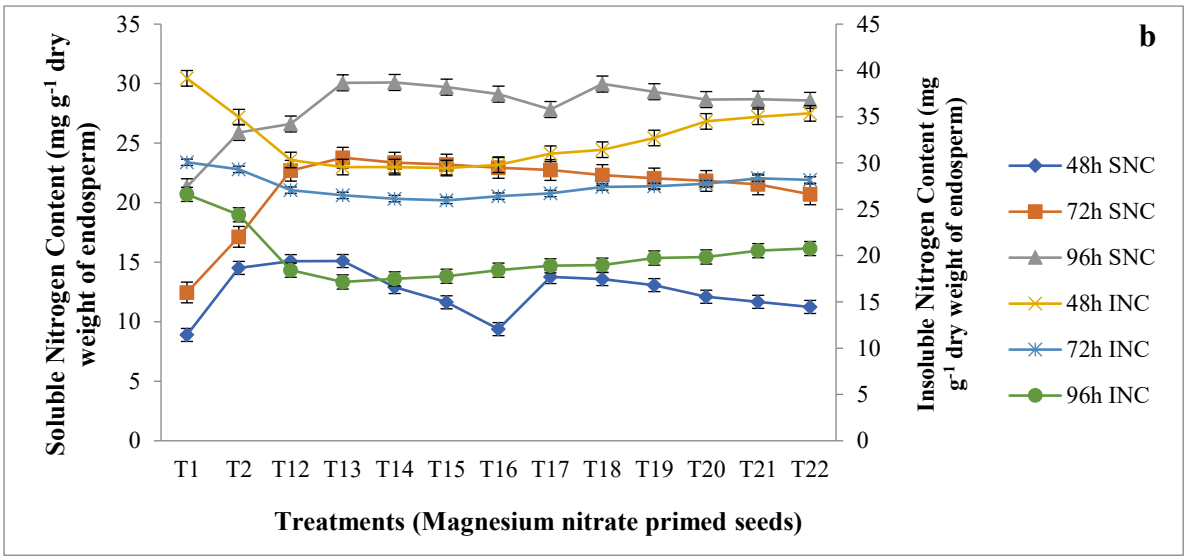


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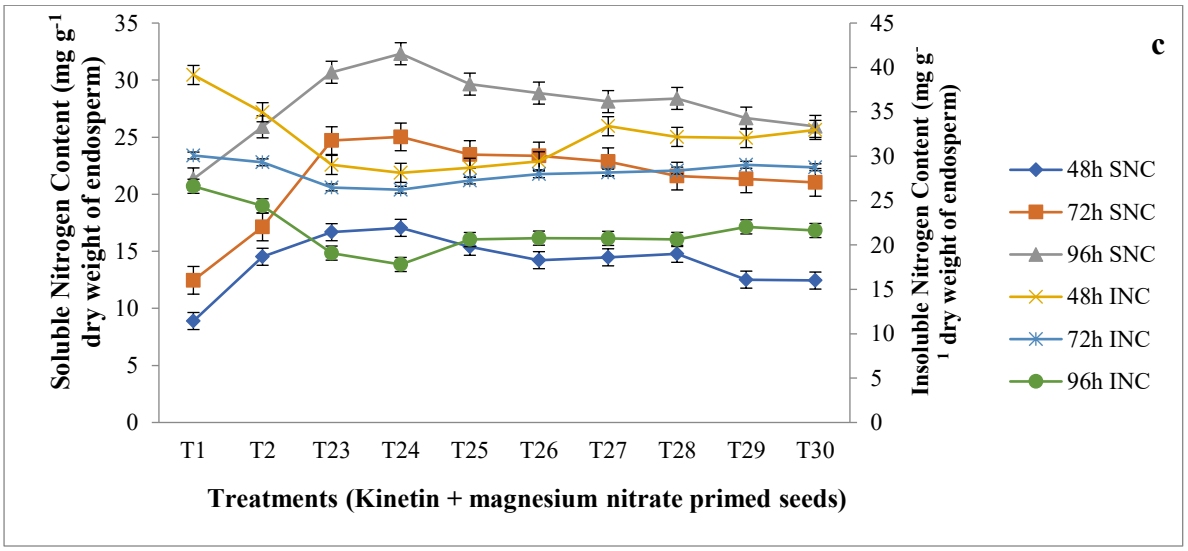
907 **Fig 3(a, b & c): Effect of seed priming with distilled water (D.W.) and different concentrations of**
908 **Kinetin/ $Mg(NO_3)_2$ alone or in combination on sugar content (soluble and insoluble form) of rice**
909 **var. MTU-7029 at different study periods**



910



911

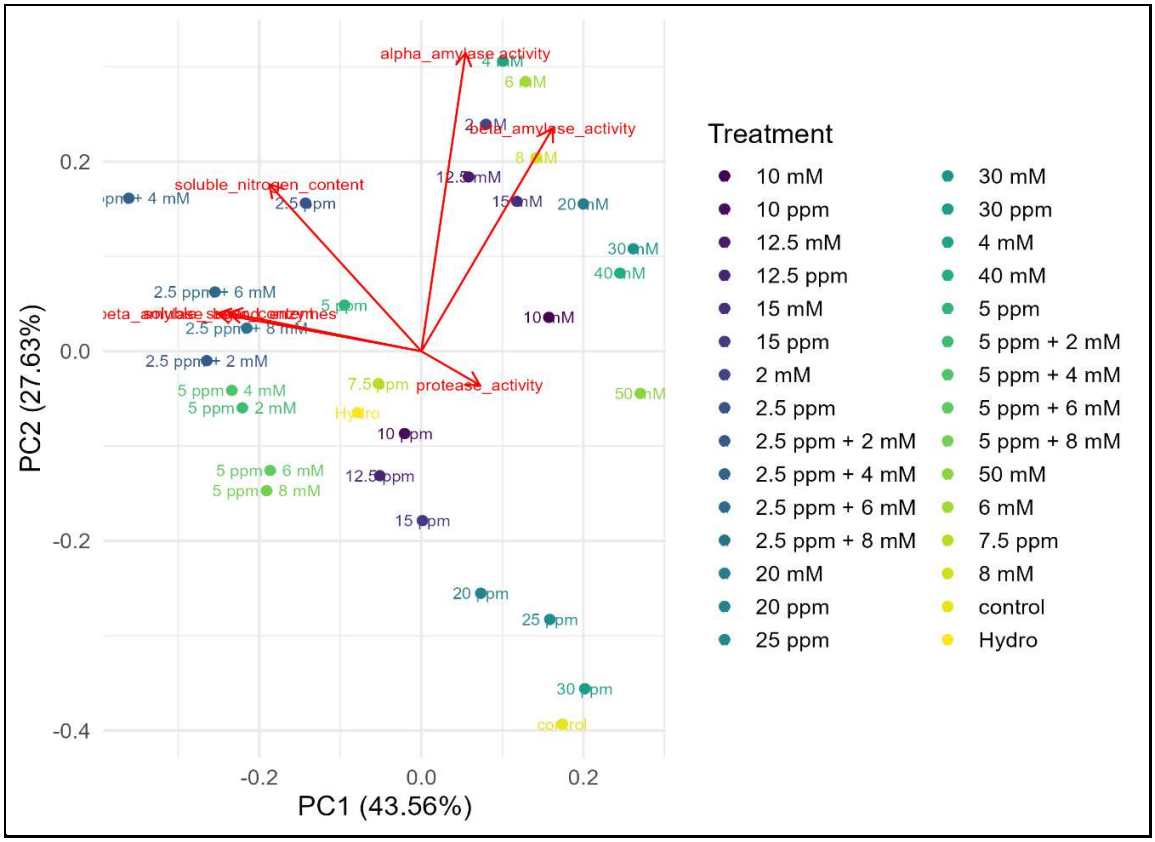


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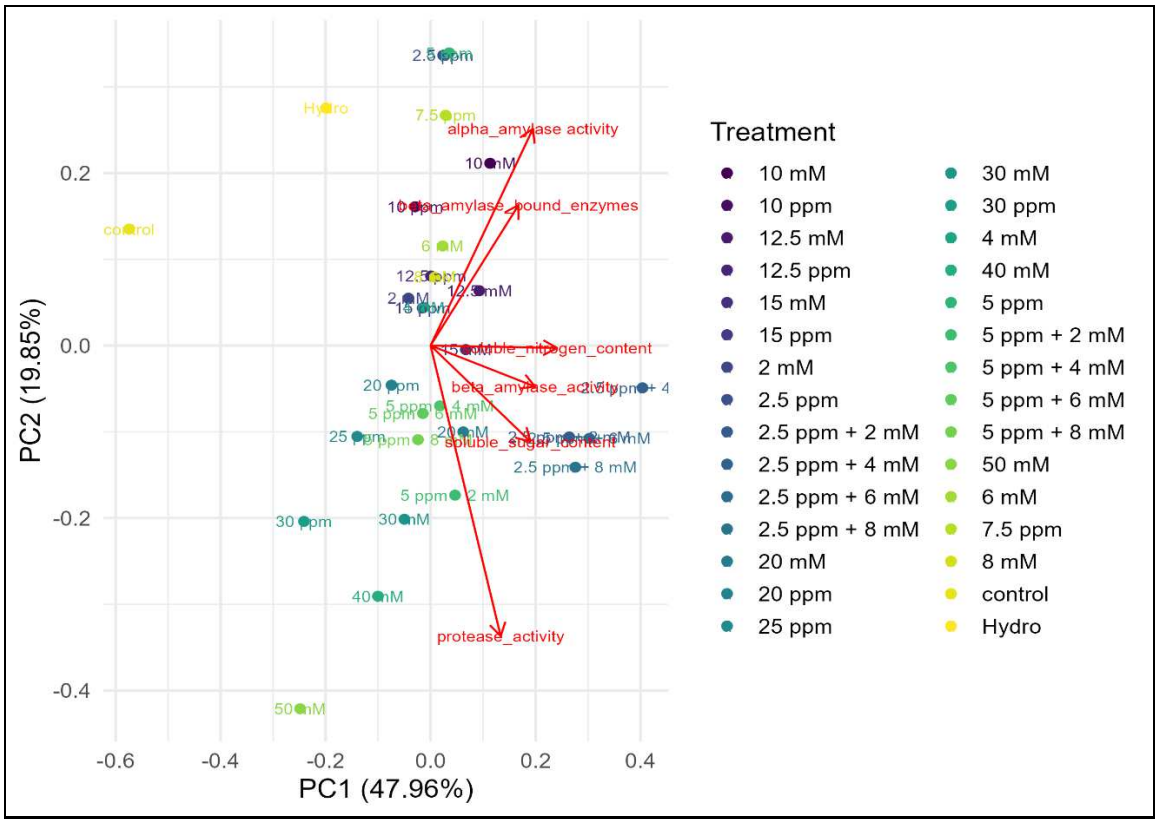
913 **Fig 4(a, b & c): Effect of seed priming with distilled water (D.W.) and different concentrations of**
914 **Kinetin/ $Mg(NO_3)_2$ alone or in combination on nitrogen content (soluble and insoluble form) of**
915 **rice var. MTU-7029 at different study periods.**



Figure 5. Pearson correlation matrix (Heat Map) of biochemical parameters. Cell colours indicate the strength and direction of the relationships, with dark blue representing strong positive correlations and light colours representing weak or no correlations. Values within the cells are the Pearson correlation coefficients (*r*). Statistical significance is denoted by yellow asterisks (* $p < 0.05$, *** $p < 0.001$). Abbreviations: SNC, soluble nitrogen content; SSC, soluble sugar content; BAA, β-Amylase activity (free form); PA, protease activity; AAA, α-Amylase activity; BABE, β-Amylase bound enzymes.

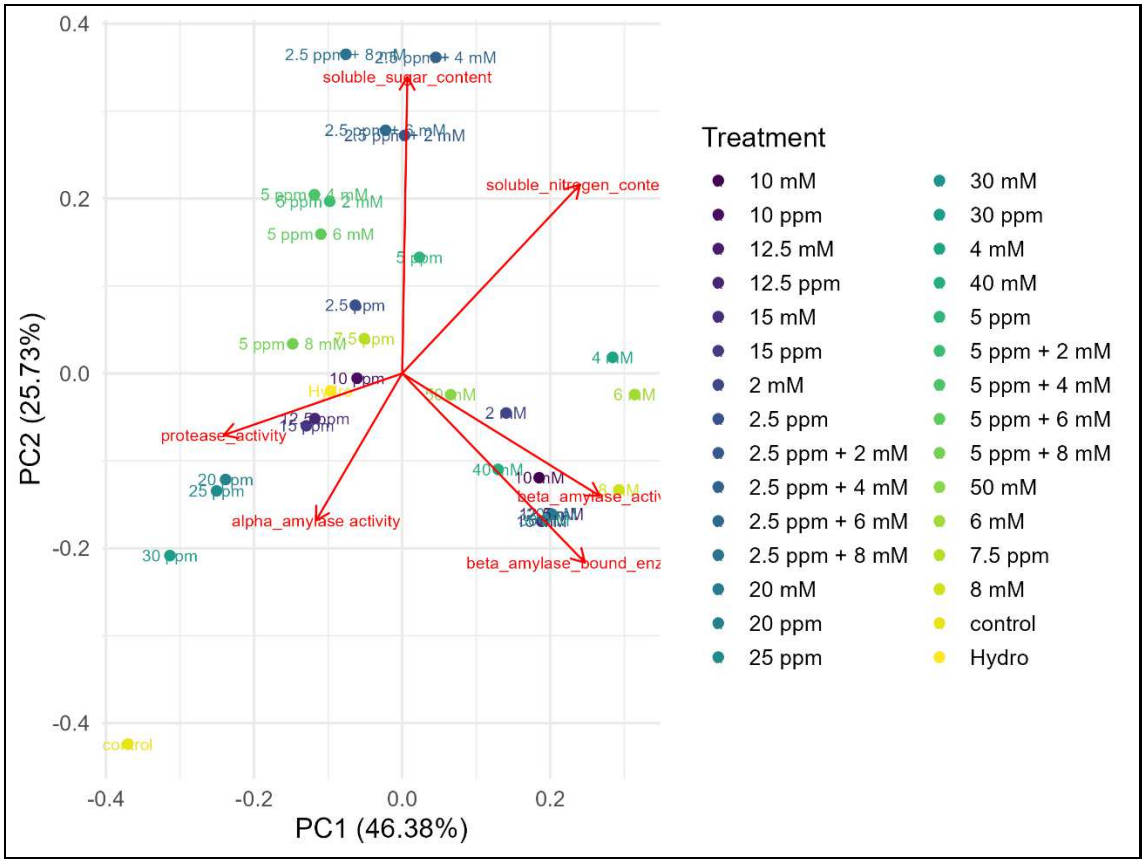

alpha_amylase activity
beta_amylase_activity
soluble_nitrogen_content
protease_activity
PC2 (27.63%)
PC1 (43.56%)
0.2
0.0
-0.2
-0.4
-0.2
0.0
0.2
Treatment
10 mM
10 ppm
12.5 mM
12.5 ppm
15 mM
15 ppm
2 mM
2.5 ppm
2.5 ppm + 2 mM
2.5 ppm + 4 mM
2.5 ppm + 6 mM
2.5 ppm + 8 mM
20 mM
20 ppm
25 ppm
30 mM
30 ppm
4 mM
40 mM
5 ppm
5 ppm + 2 mM
5 ppm + 4 mM
5 ppm + 6 mM
5 ppm + 8 mM
50 mM
6 mM
7.5 ppm
8 mM
control
Hydro

931


alpha_amylase activity
beta_amylase_bound_enzymes
soluble_nitrogen_content
soluble_sugar_content
protease_activity
PC2 (19.85%)
PC1 (47.96%)
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
Treatment
10 mM
10 ppm
12.5 mM
12.5 ppm
15 mM
15 ppm
2 mM
2.5 ppm
2.5 ppm + 2 mM
2.5 ppm + 4 mM
2.5 ppm + 6 mM
2.5 ppm + 8 mM
20 mM
20 ppm
25 ppm
30 mM
30 ppm
4 mM
40 mM
5 ppm
5 ppm + 2 mM
5 ppm + 4 mM
5 ppm + 6 mM
5 ppm + 8 mM
50 mM
6 mM
7.5 ppm
8 mM
control
Hydro

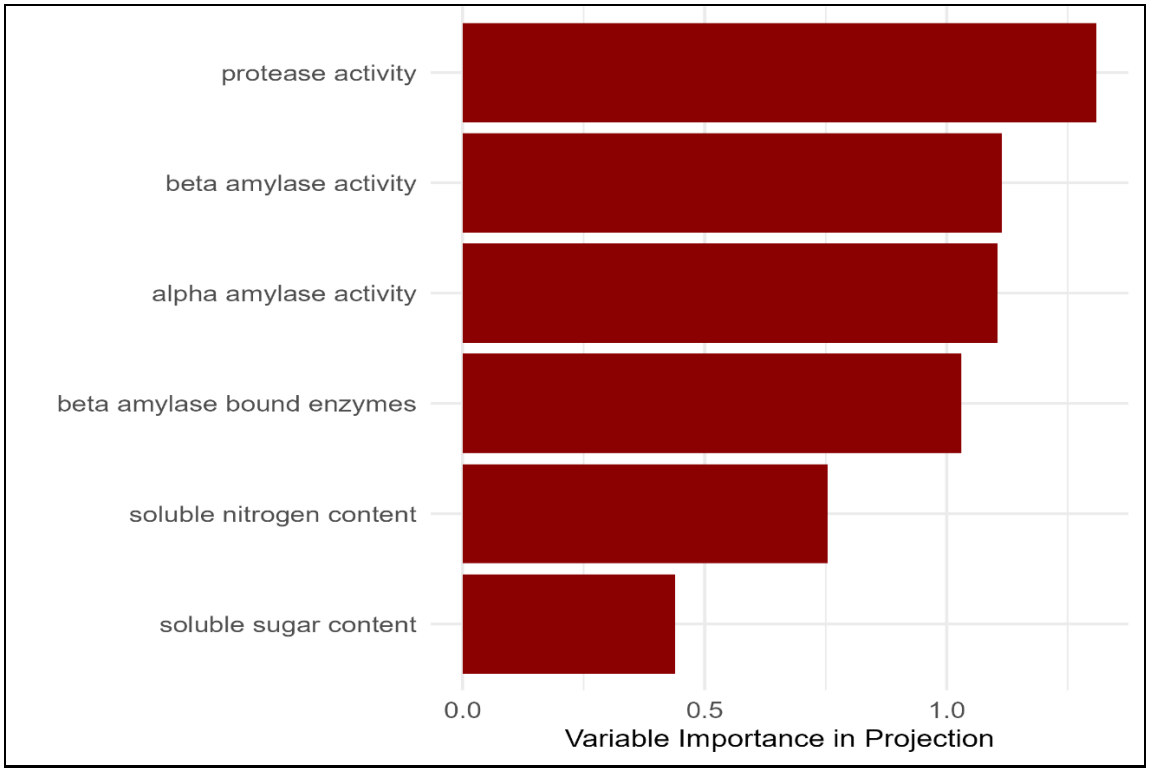
932

933



934

935 **Fig. 6 (a, b and c). Principal Component Analysis (PCA) of biochemical parameters**
936 **during germination of rice variety MTU-7029 under different seed priming treatments at**
937 **various studied hours (48, 72 and 96 Hrs).**



938

Fig. 7. PLS-DA–derived VIP scores plot showing the percentage of variance explained by each principal component derived from PCA of biochemical parameters and germination traits of rice variety MTU-7029

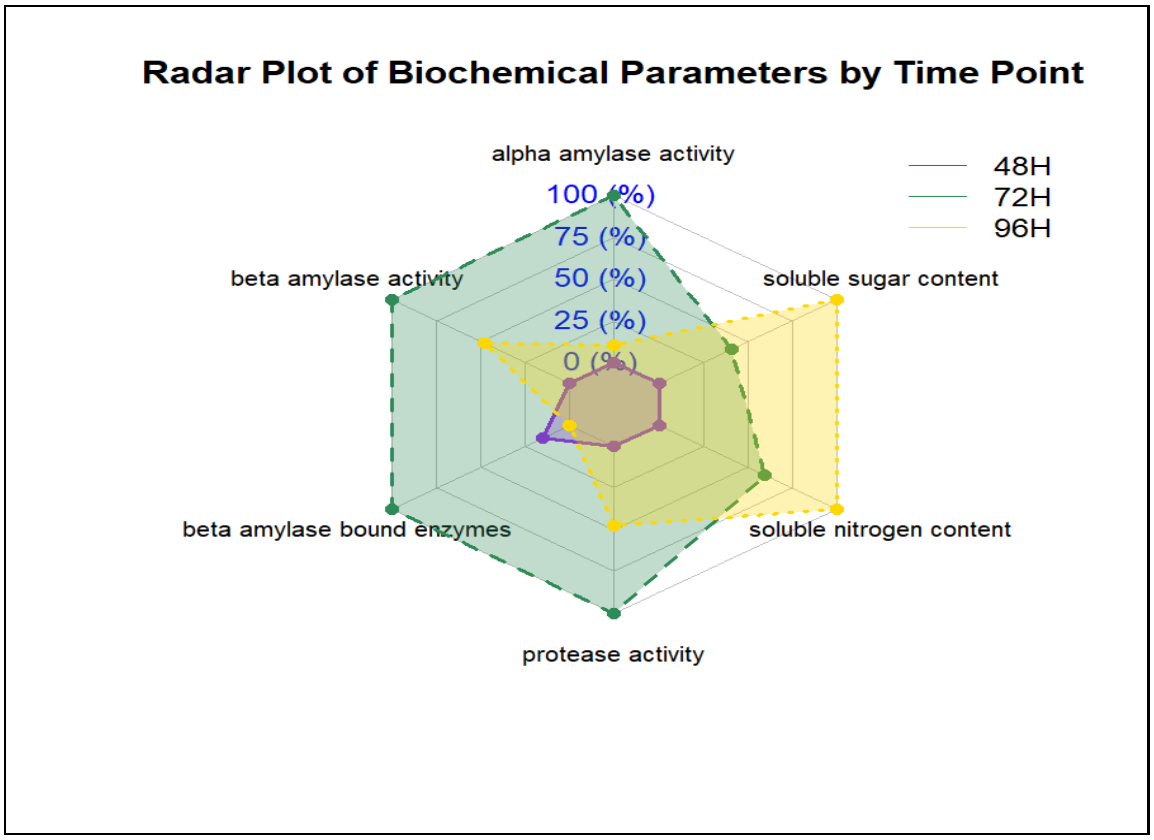
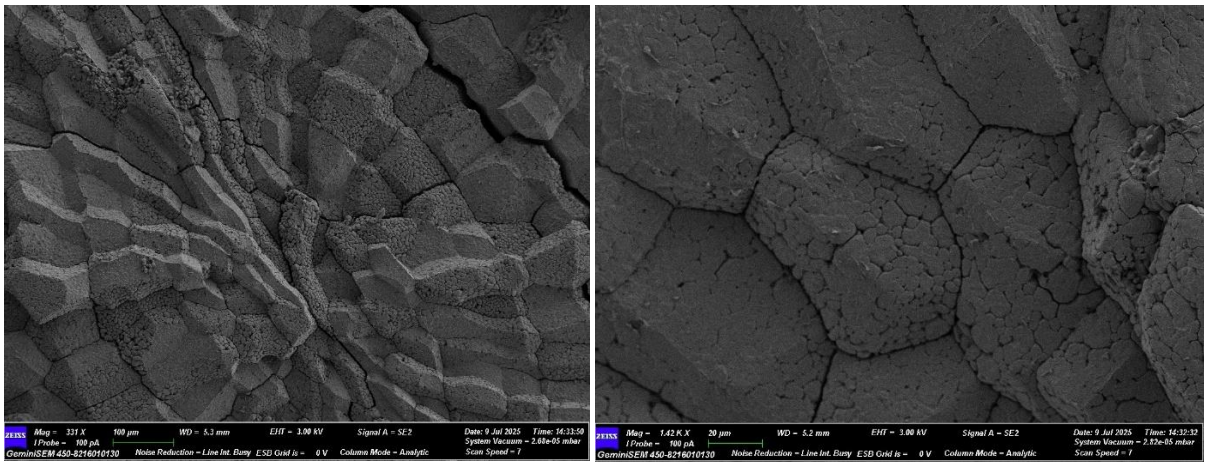
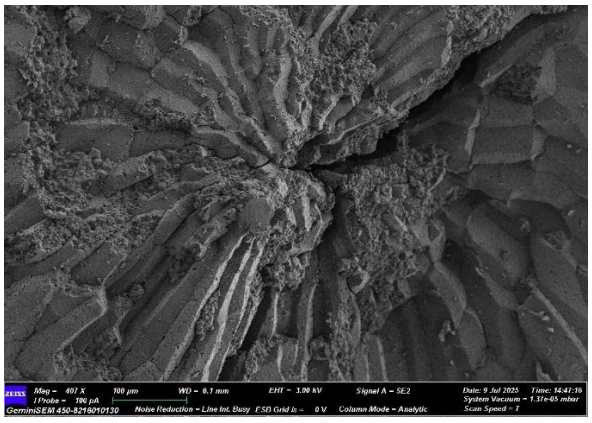
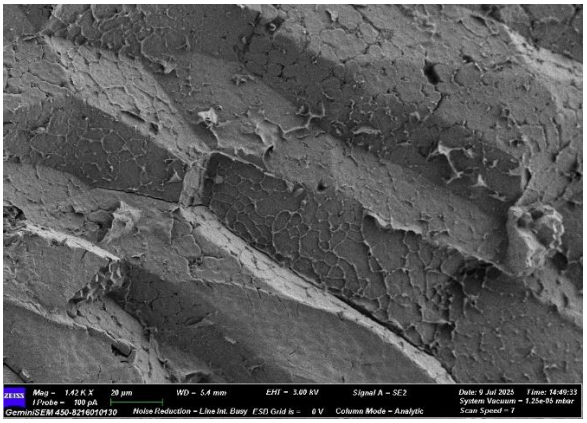


Fig. 8. Radar Plot depicted the enzymatic activities and metabolites in germinated rice endosperm var. MTU-7029 subjected to different priming treatments and in different studied hours.

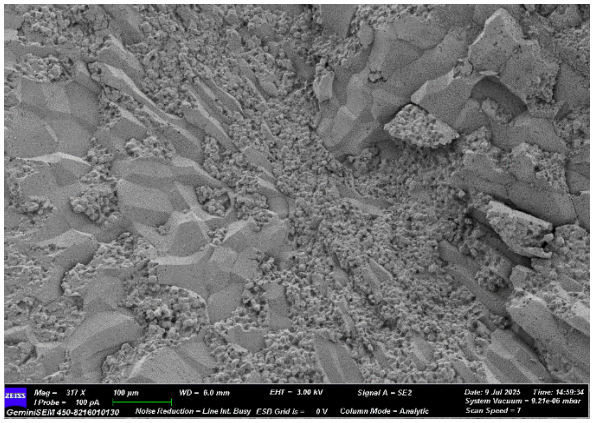


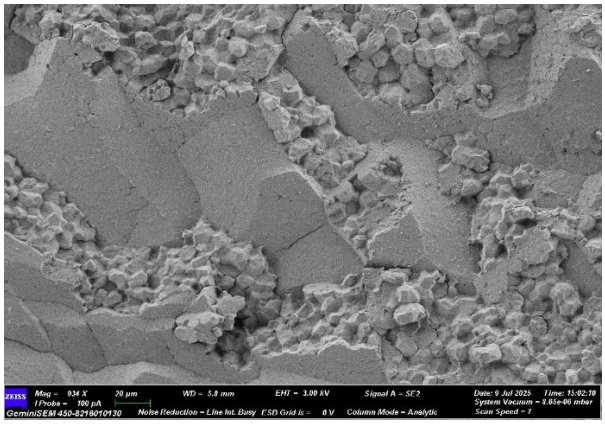
a [24h; Scale-100µm] **b [24h; Scale-20µm]**





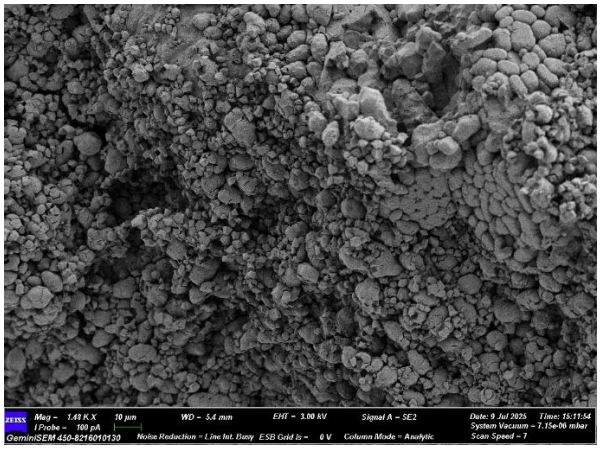
c [48h; Scale-100µm] **d [48h; Scale-20µm]**



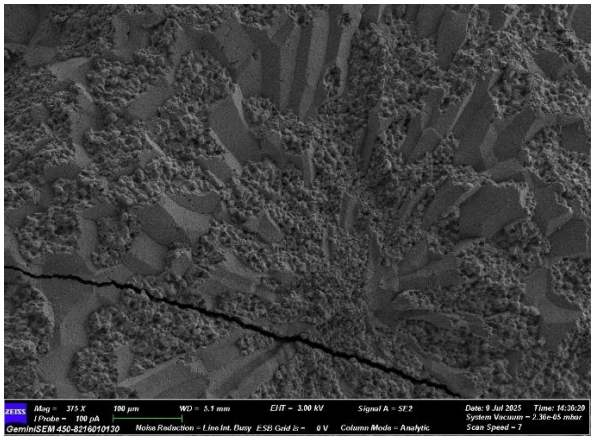


e [72h; Scale-100µm] **f [72h; Scale-20µm]**

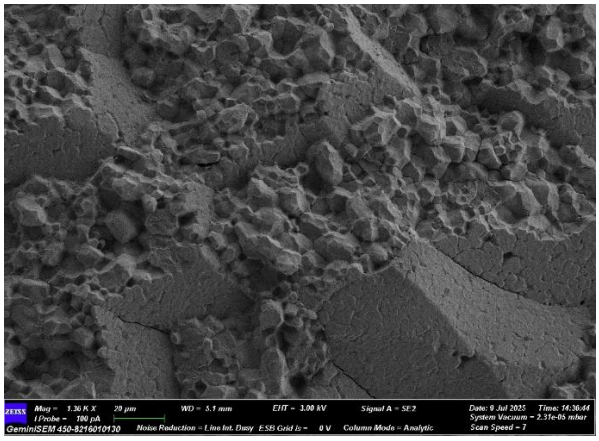




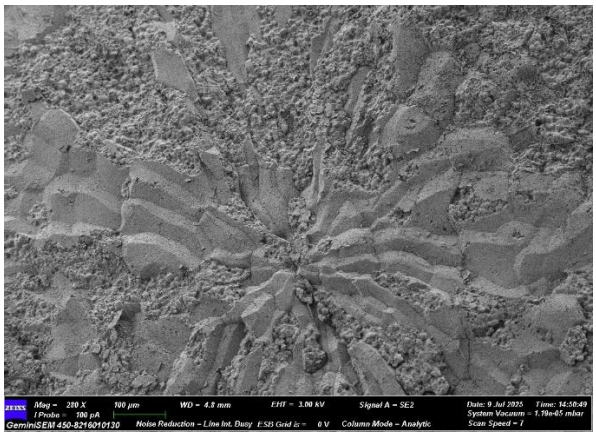
g [96h; Scale-100µm] **h [96h; Scale-10µm]**



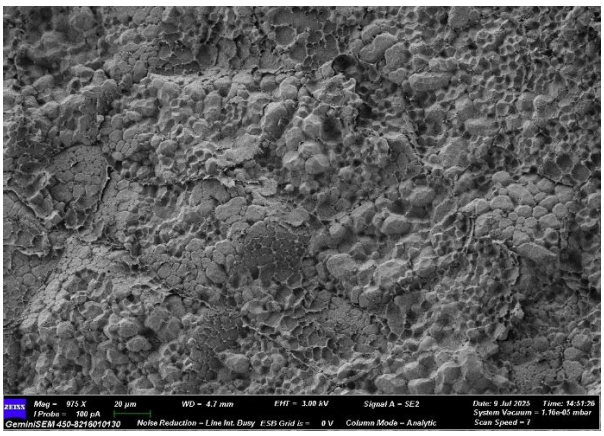
a_1 [24h; Scale-100µm]



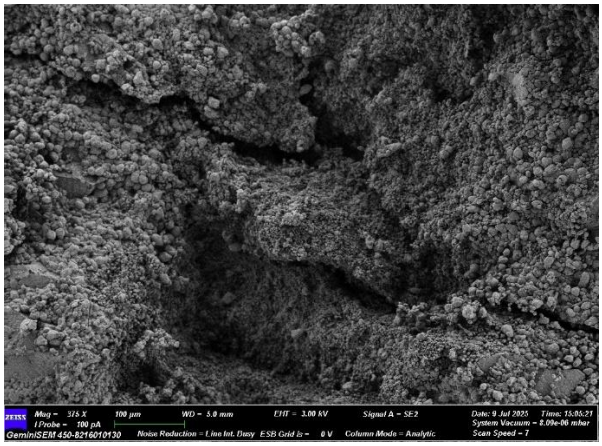
b_1 [24h; Scale-20µm]



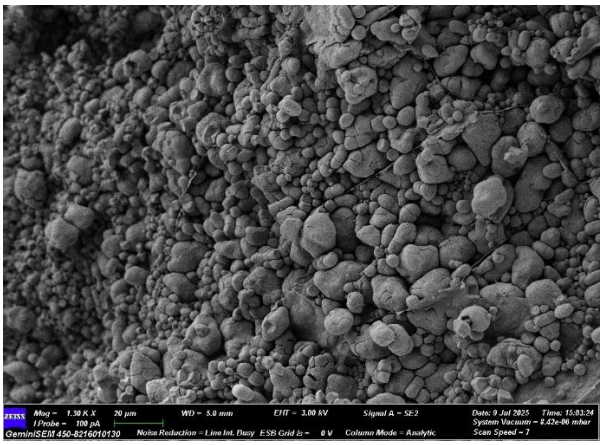
c_1 [48h; Scale-100µm]



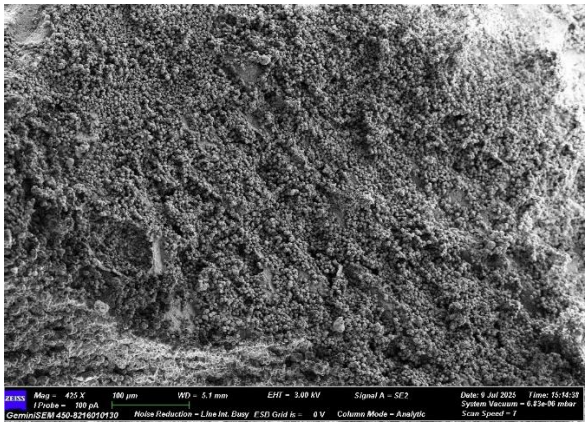
d_1 [48h; Scale-20µm]



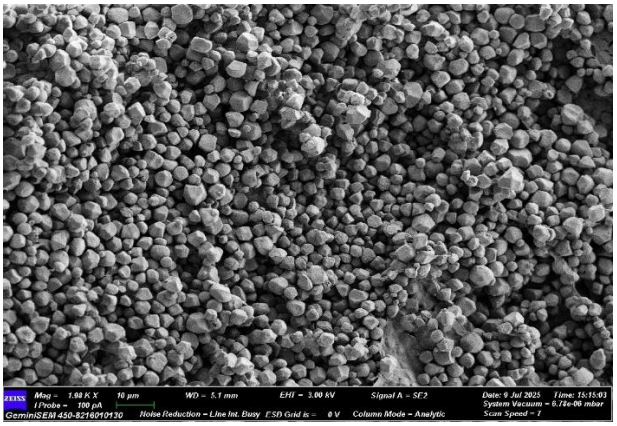
e_1 [72h; Scale-100µm]



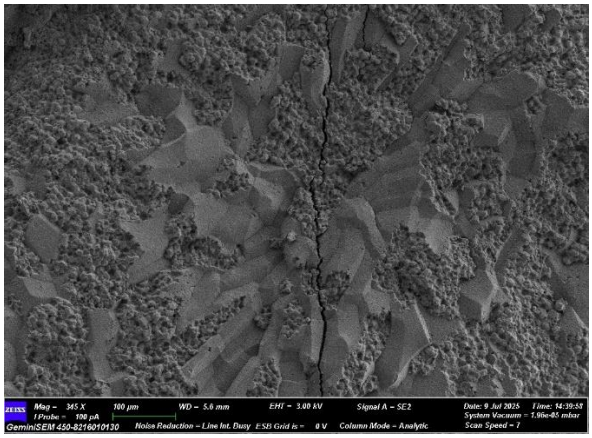
f_1 [72h; Scale-20µm]



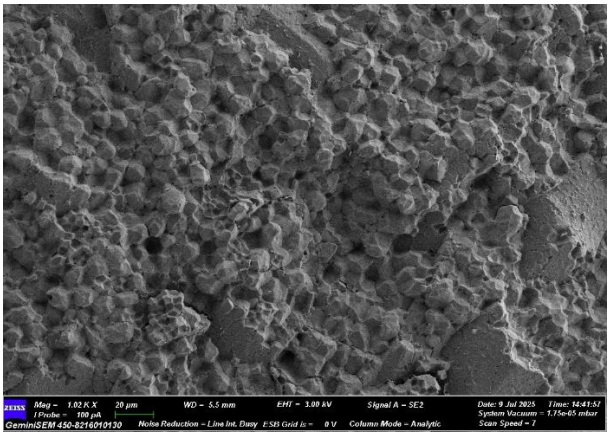
g_1 [96h; Scale-100µm]



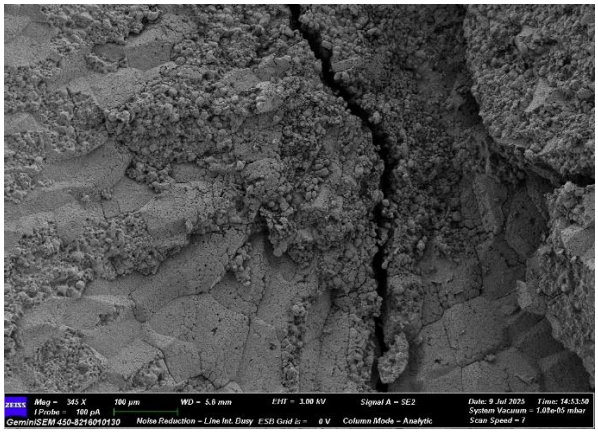
h_1 [96h; Scale-10µm]



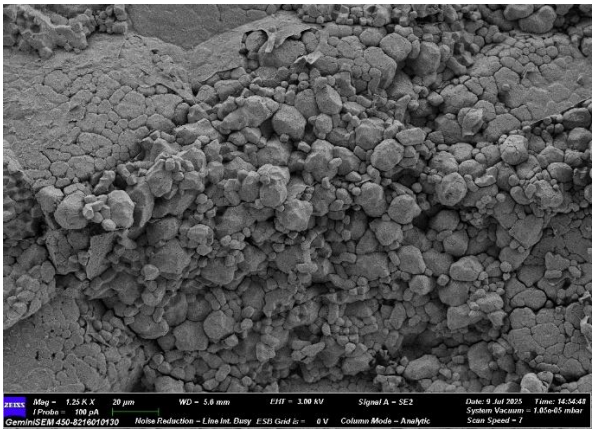
a_{11} [24h; Scale-100µm]



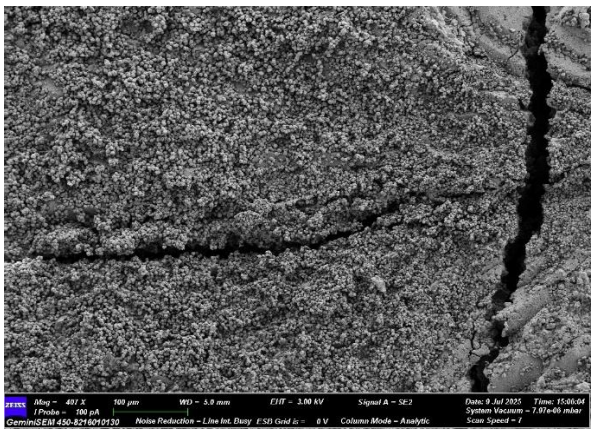
b_{11} [24h; Scale-20µm]



c_{11} [48h; Scale-100µm]

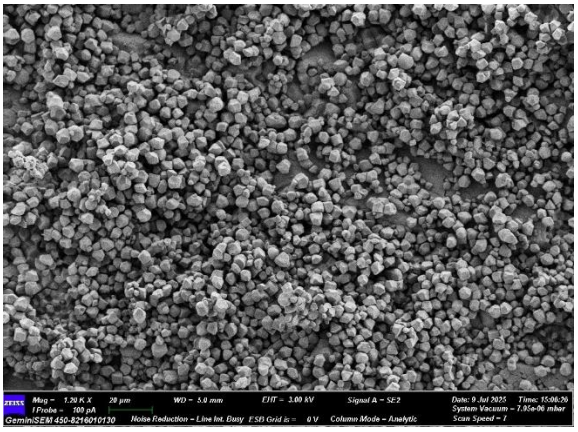


d_{11} [48h; Scale-20µm]

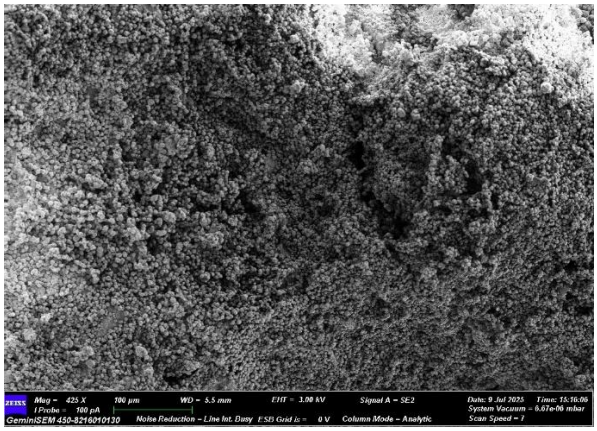


973

974 **e_{11} [72h; Scale-100µm]**

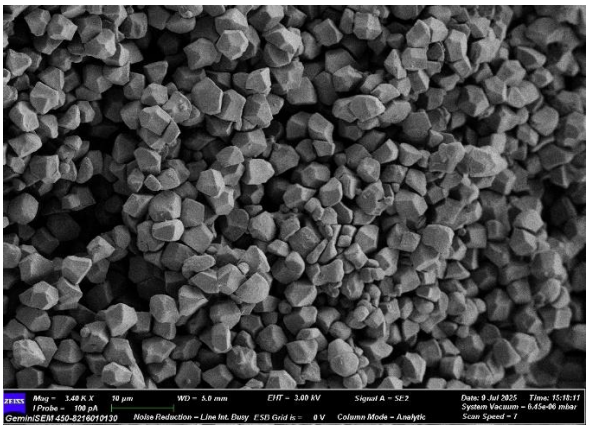


f_{11} [72h; Scale-20µm]

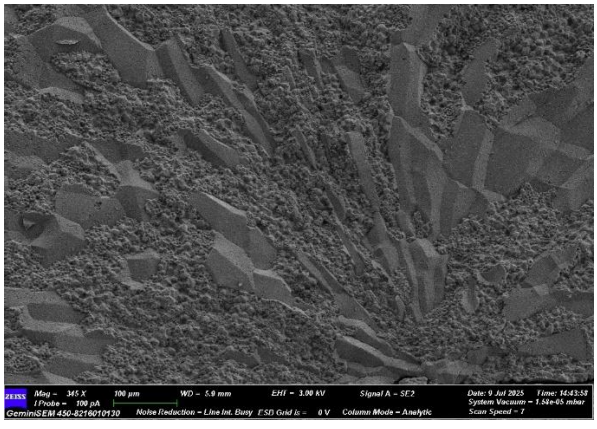


975

976 **g_{11} [96h; Scale-100µm]**

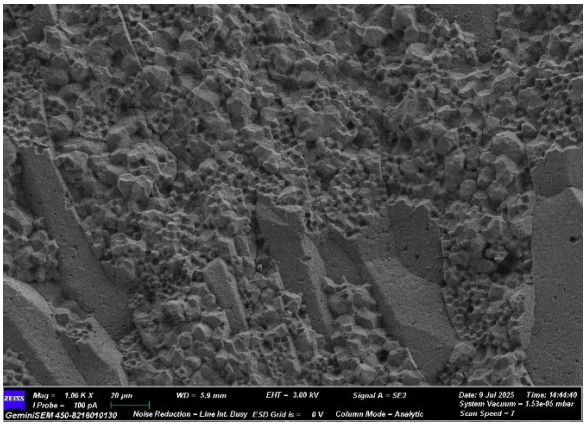


h_{11} [96h; Scale-10µm]

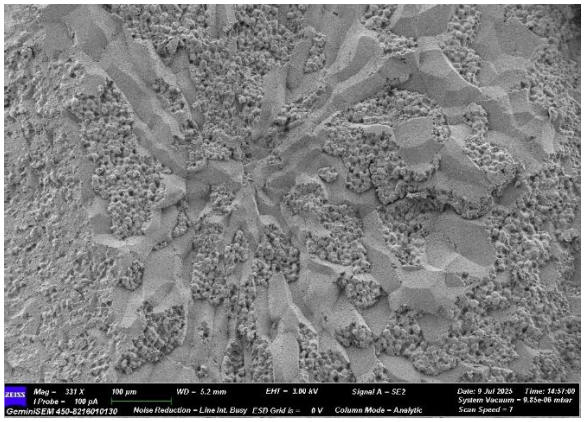


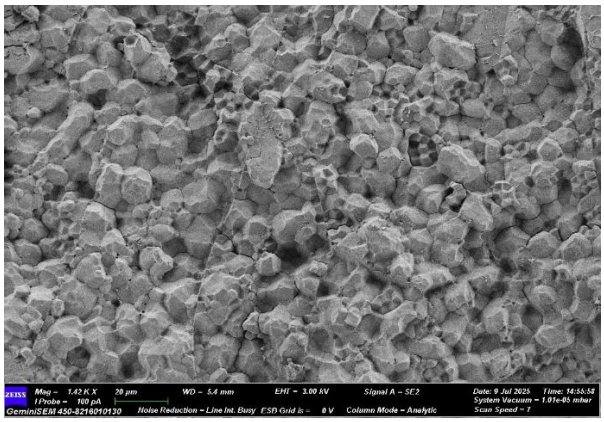
977

978 **a^0_{11} [24h; Scale-100µm]**

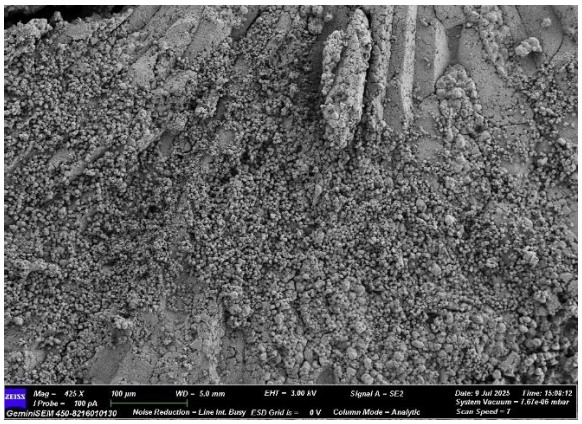


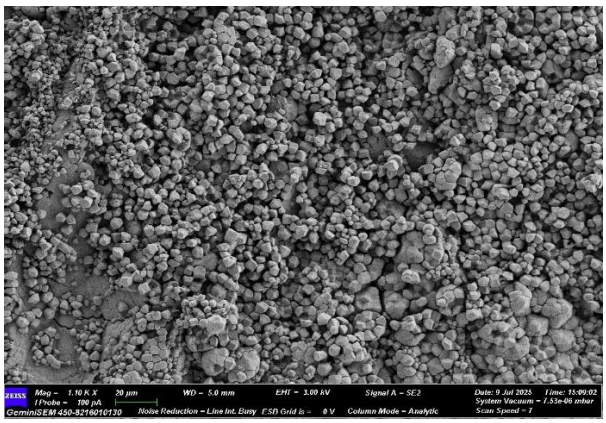
b^0_{11} [24h; Scale-20µm]



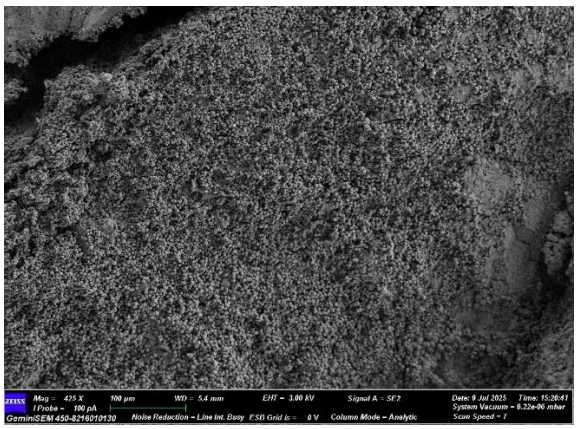


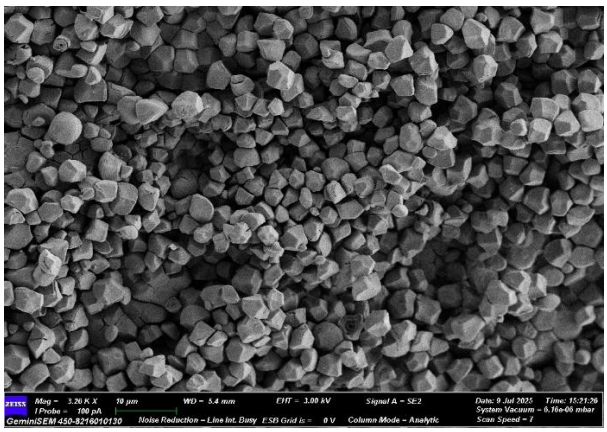
c^0_{11} [48h; Scale-100µm] **d^0_{11} [48h; Scale-20µm]**





e^0_{11} [72h; Scale-100µm] **f^0_{11} [72h; Scale-20µm]**





g^0_{11} [96h; Scale-100µm] **h^0_{11} [96h; Scale-10µm]**

Fig. 9: FeSEM micrographs of germinating rice endosperm section represents the breakdown of starch granules of non-primed control seeds (a-h), 2.5ppm kinetin primed seeds (a_1-h_1), 4mM magnesium nitrate primed seeds (a_{11}-h_{11}), and combination of 2.5ppm kinetin + 4mM magnesium nitrate primed seeds (a^0_{11}-h^0_{11}) from a time duration of 24h to 96h with a scale bar = 100µM, 20µM and 10 µM for better representation.