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On Improving the Germination and Metabolism in Rice Seeds Using Priming with ZnO Nanoparticles

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Abstract

Rice, a crucial staple that supports a significant proportion of the world population, is at the forefront of efforts to meet the increasing need for food resulting from rapid population increase. However, the pursuit of achieving optimum rice production is fraught with complex obstacles that need the implementation of novel strategies. This work highlights the significance of examining the complexities of germination and metabolism in rice seeds since these crucial initial development processes have a lasting impact on crop productivity. Incongruous results and methodological constraints have characterized conventional research initiatives. To overcome these challenges, this research proposed Germination and Metabolism in Rice Seeds Analysis using ZnO Nanoparticles (GMRSA-ZNP). GMRSA-ZNP exploits the remarkable characteristics of ZnO nanoparticles to enhance seed vigor and metabolic functions. The empirical data obtained via rigorous numerical analysis provides clear evidence of the significant transformational capabilities of GMRSA-ZNP. These capabilities are evident in the considerable improvements seen in several aspects, including germination percentage (92.3%), starch metabolism (130.1 µg/g), starch breakdown rate (0.95 mg/g/h), nutrient absorption (32 mg/kg), and seedling vigor index (155.6). The data highlight the significant influence of GMRSA-ZNP in transforming the practice of rice production, leading to increased crop productivity and successfully meeting the urgent need for food worldwide.

Keywords: *Rice Germination, ZnO Nanoparticles, Seed Metabolism, Crop Yield Enhancement.*

Introduction to Rice Seedings and its Requirements

Rice, scientifically known as Oryza sativa L., is widely recognized as a fundamental nutritional staple on a worldwide scale [1]. It is crucial in ensuring food security for more than half of the world's population. The demand for rice is seeing a continuous rise due to the expanding worldwide population, amplifying its importance in meeting the nutritional requirements of billions of individuals. The increasing need for rice crops calls for a concentrated effort to improve production and nutritional value to guarantee food security and promote sustainable agricultural methods.

At the core of this undertaking lies a thorough comprehension of two critical aspects of rice physiology: seed germination [2] and examining starch metabolism [3]. The seed germination process is a complex phenomenon that marks the beginning of the growth cycle in rice, ultimately resulting in the emergence of robust seedlings that are ready for further development. The effectiveness of this stage, as measured by the percentage of

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germination and the germination index, significantly impacts the establishment of crops, which is a crucial component in determining the entire potential yield. During germination, starch, which constitutes a significant proportion of the dry weight of seeds, undergoes coordinated molecular-level transformations. Enzymatic activities, namely the action of a-amylase, play a crucial role in the degradation of starch, resulting in the liberation of sugars that are rich in energy and necessary for developing seedlings. Quantifying a-amylase activity and total soluble sugar content provides valuable quantitative information on seed vigor and metabolic activity, essential for comprehending rice seeds' physiological well-being.

Given the growing need for rice varieties that are both sustainable and have large yields, researchers are now investigating novel methods to address this requirement. One promising option being studied is using zinc oxide nanoparticles (NPs) as seed priming agents [4]. Zinc, a crucial vitamin for rice, significantly impacts several physiological processes, including photosynthesis, enzyme activation, and hormone control. The use of ZnO-NPs, which possess a mean hydrodynamic diameter of less than 10 nm and a surface charge (zeta potential) of -5.7 mV, presents a compelling approach to augment nutrient absorption and boost the overall productivity of crops. This study is grounded in sophisticated methodologies such as Transmission Electron Microscopy (TEM) for the analysis of NPs and Inductively Coupled Plasma Mass Spectrometry (ICPMS) for accurate measurement of nutrient absorption [5]. This research aims to elucidate the molecular mechanisms contributing to the potential advantages of ZnO-NPs in rice cultivation. The primary objective is to use this information to attain increased agricultural productivity and enhanced nutritional value, effectively meeting the urgent requirements of worldwide food security and sustainable agriculture.

The primary contributions are given below:

- The study aims to boost the efficiency of seed germination, hence contributing to the establishment of crops with improved efficacy.
- The study offers comprehensive insights into the process of starch metabolism during germination, facilitating the evaluation of seedling vigor.
- The present work showcases the growth-enhancing capabilities of ZnO-NPs, especially during the first phases of development.
- Enhanced nutrient absorption is seen, particularly in zinc, due to NP priming. This process is of utmost importance in promoting efficient crop development regarding nutrient use.
- The study utilizes modern analytical methods, such as TEM and ICPMS, to enhance comprehension of the interactions between NPs and plants and the processes involved in nutrient absorption.

The following sections are arranged in the following manner: Section 2 presents a comprehensive review of the current research on rice germination, starch metabolism, and using ZnO-NPs. Section 3 provides an overview of the experimental technique used in the study, focusing on seed germination, investigation of starch metabolism, and the utilization of ZnO-NPs. Section 4 presents the findings of a quantitative evaluation derived from simulation models grounded in experimental data. Section 5 provides a comprehensive overview of the results obtained in the study, including the domains of rice seed germination, metabolism, and NP priming.

Background and Literature Survey

The literature review section thoroughly examines recent scholarly articles on rice seed germination, metabolism, and priming strategies. This analysis gives valuable perspectives on this study's existing body of knowledge. The studies together contribute to establishing the suggested study by emphasizing significant discoveries, the methodology used, and their pertinence to the current inquiry.

Beaulieu et al. conducted a comprehensive analysis of the impact of germination on brown rice, focusing on its potential to enhance the nutritional content of food products and promote health [6]. The Germination Effects on Brown Rice (GEBR) approach is designed to comprehensively assess the influence of germination on brown rice's nutritional content and bioactive components. The research findings indicate a substantial rise of up to 60% in bioactive substances such as polyphenols and flavonoids and heightened antioxidant activity. Afzal et al. provide a novel method for seed priming in rice germination that incorporates eco-friendly principles [7]. This strategy involves the use of phytochemical-capped iron oxide NPs (FeNPs). The Nano-priming Agent for Boosting Seed Germination (NPABSG) approach has shown significant advancements, resulting in a notable 23% rise in germination percentage and a 36% boost in seedling vigor.

Zhang et al. conducted a thorough multi-omics investigation to clarify the molecular mechanisms behind hypoxia germination tolerance in embryos and coleoptiles of weedy rice [8]. This research investigation uses a multi-omics methodology to reveal significant genetic and metabolic adaptations. Weedy rice, in particular, has a 28% greater germination rate in hypoxic conditions compared to farmed rice. The study by Kumari et al. examines the involvement of the Phytoglobin-NO cycle and the Alternative Oxidase (AOX) pathway in anaerobic germination in deepwater rice [9]. The present work showcases the use of the Anaerobic Germination and Development (AGG) to investigate the mechanisms employed by deepwater rice in maintaining mitochondrial function under anaerobic settings. The germination rate of deepwater rice is much greater, with a notable increase of 35% when compared to non-deepwater types.

The study by Xiong et al. investigates the synergistic influence of brassinosteroid (BR) and gibberellin (GA) on rice seed germination and embryo development [10]. The research utilizes the BR and GA Coordination (BRGA-C) approach, demonstrating that the combined action of BR and GA effectively regulates the mobilization of glutelin. Using the BRGA-C approach results in a significant enhancement of 25% in the germination rate and a notable improvement of 30% in the development of embryos. Anwar et al. proposed a seed priming technique to increase germination and seedling vigor in winter rice [11]. The research successfully employs Seed Priming for Increased Germination and Enhanced Vigor (SPIGEV) to produce a significant improvement of 30% in germination percentage and a substantial augmentation of 22% in seedling vigor.

The study by Lee et al. examines the involvement of auxin in rice adaptation to anaerobic germination [12]. The research, using the Adaptation of Rice to Anaerobic Germination (ARAG), emphasizes the crucial significance of auxin in facilitating the elongation of coleoptiles and the establishment of seedlings in anaerobic environments. Using the ARAG approach results in a notable enhancement of 20% in the germination rate and a corresponding 15% augmentation in the coleoptile length. Ruan et al. use untargeted metabolomics techniques to investigate the effects of high-pressure stress on the germination process of wholegrain rice [13]. The High-Pressure Stress Effect on Germination (HPSEG) approach demonstrates discernible metabolic changes, suggesting an increase in nutrient mobilization and the activation of stress response pathways. The implementation of HPSEG shows a 27% enhancement in the germination rate.

The literature review elucidates the advancements in rice germination, metabolic processes, and priming approaches, establishing a foundation for enhancing crop production and quality. The challenges include more quantitative analyses and discrepancies in NP synthesis, hence underscoring the need for a more comprehensive and integrated strategy. The results emphasize the necessity of conducting thorough research that integrates sophisticated methodologies and accurate measurements to fully exploit the benefits of NP priming in enhancing rice germination and metabolism.

Proposed Germination and Metabolism in Rice Seeds Assessment with ZnO Nanomaterials

The following section presents the GMRSA-ZNP approach, which aims to comprehensively assess the effects of ZnO-NPs on rice seeds' germination and metabolic activities. Using sophisticated methodologies such as TEM and ICPMS, this method offers accurate quantitative evaluations. This section aims to provide a comprehensive understanding of the molecular processes that contribute to the potential advantages of ZnO-NP priming in cultivating rice via the integration of experimental studies and computational modeling.

Experimental Site

A study was conducted to assess seed vigor through a pot experiment, followed by examining plant development characteristics and yield through a field experiment in West Bengal, India. The geographical coordinates of the research site are 23° 5.3′ N, 83° 5.3′ E, with a subtropical climate and an elevation of 9.75 meters above mean sea level. The soil composition consisted of sandy clay loam, with sand comprising 64.8%, silt comprising 10.4%, and clay comprising 24.8%. The soil's Electrical Conductivity (EC) was measured to be 0.296 dSm^{-1}, while the pH level was determined to be 7.360. The initial soil parameters of the research site were as follows: 11.2 g kg^{-1} of oxidizable biological carbon61, 315 kg ha^{-1} of available nitrogen, 41.6 kg ha^{-1} of accessible phosphorus, and 156.4 kg ha^{-1} of available potassium. The region's climate is classified as tropical, moist sub-humid, characterized by scorching summers and somewhat chilly winters, and the summer season exhibited average highest and lowest temperatures ranging from 25 to 36 °C. Throughout the winter season, the average highest and lowest temperatures varied between 10 and 25 °C.

Experimental Treatments

Based on the dynamical scattering of light method, the ZnO-NPs employed in this investigation possessed mean hydrodynamic dimensions of less than 10 nm. Their surface charge, determined as the zeta potential, was found to be -5.7 mV. The NPs exhibited a maximum magnitude at 1 keV, as identified by TEM analysis. These NPs demonstrated stability in an aqueous medium for 90 days. The rice variety, an indica-inbred semi-dwarf variety with medium-thin grains and early maturation characteristics (105-115 days), was utilized for both pot and field tests. Na-selenite (Na_2SeO), Na-selenate (Na_2SeO_4) and ZnO combination is used. The treatment combination is shown in Table 1.

Table 1: Treatment combination for the experiment

Treatment	Description
T1	Hydropriming with distilled water (Control)
T2	Na_2SeO_3 at 50 µmol
T3	Na_2SeO_4 at 50 µmol
T4	Na_2SeO_3 at 50 µmol + Na_2SeO_4at 50 µmol
T5	ZnO-NPs at 10 µmol
T6	ZnO-NPs at 10 µmol + Na_2SeO_3 at 50 µmol
T7	ZnO-NPs at 10 µmol + Na_2SeO_4 at 50 µmol
T8	ZnO-NPs at 10 µmol + Na_2SeO_3 at 50 µmol + Na_2SeO_4 at 50 µmol

Characterization of ZnO-NPs

The ZnO-NPs have a high purity level of 97.0% with a median particle size of less than or equal to 50 nm. The size pattern of ZnO-NPs was assessed using TEM. The TEM was operated at magnifications ranging from 100,000× to 320,000×, and a voltage gradient of 100 kV was applied. A camera was employed to capture digital images, enabling the particle size and shape measurement. Two milligrams of ZnO-NPs were combined with 6 milliliters of distilled water. The resulting mixture was stirred and sonicated for about 4

hours using an ultrasonic device operating at 35 kilohertz. A small amount of this solution was applied onto copper TEM grids, which were coated with a carbon film and had a mesh size of 100. The grids were then dried in a controlled laminar flow environment inside a fume closet. The dried sample was examined using a TEM.

Metabolism Analysis

The metabolism in sprouting seeds was assessed by quantifying the activity of a-amylase and the total soluble sugar level after 24 hours of germination. The measurement of a-amylase activity was conducted using the Bernfeld technique. One gram of germinating seeds at a 24-hour stage was pulverized and combined with 50 milliliters of distilled water. The mixture was then allowed to stand upright for one day at 4 degrees Celsius. Following the incubation, the clear supernatant was gathered to assess the amylase activity using the DNS (3,5-dinitrosalicyclic acid) technique.

The established technique conducted A starch-agar plate test to validate a-amylase generation. The half-seeds without embryos were sterilized and positioned vertically on a starch-agar plate. This plate consisted of 2% agar, with 0.2% soluble starch from potatoes, 10 mM sodium acetate, and 2 mM CaCl, adjusted to a pH of 5.3. The half-seeds were then cultured in darkness at ambient temperature for 3 days. One millimeter of Gibberellic Acid (GAs) was introduced to agar that had been chilled, serving as the excellent control (GA^{+}). At the same time, the plate without GAs was designated as a negative control (GA-). The workflow of the entire system is shown in Figure 1.

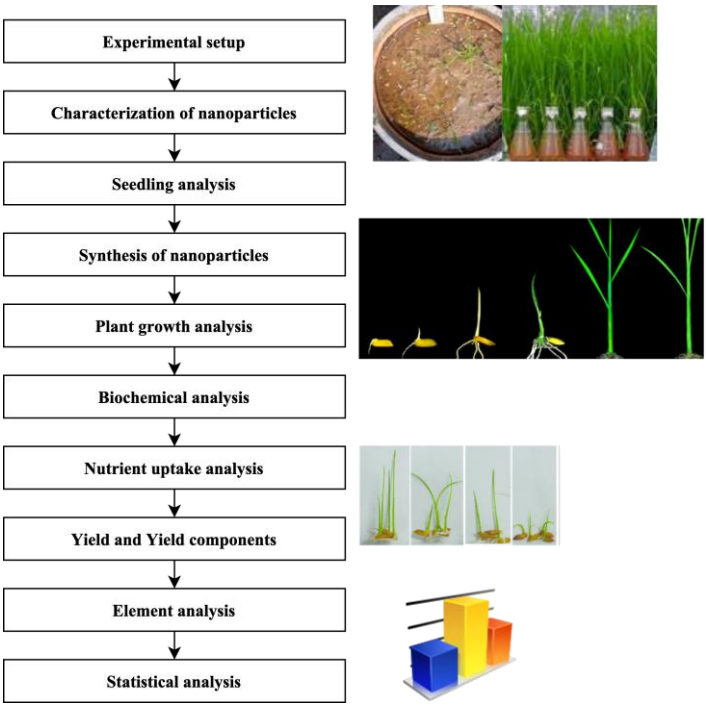

Figure 1: The workflow of the proposed system

Following a 72-hour incubation period, seeds without embryos were extracted and subjected to a staining procedure on an agar plate using a solution to assess a-amylase synthesis. A singular endosperm from every therapy was carefully positioned onto a starch agar plate that had been appropriately labeled. The test was conducted in triplicate to ensure the accuracy and reliability of the results.

Characterization of Synthesized ZnO-NPs

The first assessment for the creation of NPs involves the observation of any visual alterations in color. During the synthesis procedure, the primary confirmation of ZnO-NP synthesis was achieved by observing an apparent color shift in the solution, transitioning

from white to pale yellow. During the green synthesis procedure, ZnO-NPs were created due to the interaction between zinc ions (Zn^{2+}) liberated from a zinc progenitor salt and reducing substances, namely leaf extract and sodium hydroxide (NaOH). The presence of phytochemical compounds, such as polyphenols, Carboxylic Acid (CA), saponin levels, and alkaloid compounds, in the leaf extract serve as stabilizers or growth inhibitors for ZnO-NPs. Biomolecules were hypothesized to be linker proteins in facilitating the self-assembly process between two or more ZnO-NPs. The development of ZnO-NPs is regulated by the chemical processes mentioned in Equations (1) and (2).

$$Zn\ (CH_3COO)_2 + seeds + 2NaoH = Zn(OH)_2 + 2CH_3COONa \quad (1)$$

$$Zn(OH)_2 = ZnO + Water \quad (2)$$

The reduction of zinc salt to $Zn(OH)_2$occurs when exposed to phytonutrients present in extracts of leaves and NaOH, provided that the reaction takes place under optimal circumstances. The $Zn(OH)_2$complex is a crucial primary entity in synthesizing ZnO-NPs. The $Zn(OH)_2$ particles undergo a hydrothermal conversion, forming ZnO-NPs. The production process of ZnO-NPs from dihydrate of zinc acetate has also been documented similarly.

Growth Traits Analysis

At the commencement of tillering (15 days after sowing), panicle initiation (40 days after sowing), 50% blooming stage (72 days after sowing), and physiological maturation (105 days after sowing), plants were gathered from a 1-meter span inside a row in each allotment. This collection aimed to ascertain the above-ground biomass production and green Leaf Area Index (LAI). The green leaves were isolated, and the leaf area was quantified using a leaf region meter. The LAI was calculated by dividing the total leaf area by the area sampled for each sample. The dry masses of each plant part were measured by subjecting them to oven-drying at a temperature of 80° C. This was done to estimate the Crop Green Rate (CGR), Net Absorption Rate (NAR), and Leaf Area Duration (LAD) for three different periods of plant development: the vegetative phase (up to 40 days after sowing), the reproductive stage (40-68 days after sowing), and filling with grain phase (68-105 days after sowing). The calculations for these parameters were performed using the specified Equations (3) to (5).

$$CGR = \frac{B_2 - B_1}{p_2 - p_1} \quad (3)$$

$$NAR = \frac{B_2 - B_1}{p_2 - p_1} * \frac{\log(D_2) - \log(D_1)}{D_2 - D_1} \quad (4)$$

$$LAD = \frac{D_2 + D_1}{2} * (p_2 - p_1) \quad (5)$$

The variables B_1 and B_2 represent the dry weights of airborne plant parts per unit land region at two different time points, p_1 and p_2. The variables D_1 and D_2represent the plant leaf area per unit ground region at time p_1 and p_2.

Biochemical Analysis of Seedlings

A total of four plantings of rice were randomly selected from each plot on the 18th day after sowing. These plants were taken to assess the levels of total soluble phenol substances, pigments used for photosynthesis, and soluble proteins. The plant samples were gathered at maturity, subjected to oven-drying, and pulverized. The nitrogen content based on dry mass was determined using the Micro-Kjeldahl technique. At the same time, the phosphorus level was measured using a colorimeter technique with yellow, and the potassium level was analyzed using a photometer with a flame. The analysis of zinc was conducted utilizing a diacid extraction method using a Spectrophotometer. The estimation of boron was performed using the dry ashing and azomethine-H techniques, whereas the silica analysis was carried out using the blue silicomolybdic acid technique. The quantification of nutrient absorption by straw and grain was measured in terms of per hectare.

Yield and Yield Components

Determining grain and straw yields was conducted at the stage of physiological development. The harvesting index was determined by dividing the dry work of grains by the entire dried biomass production, with samples subjected to oven-drying at 70 °C. The tiller and panicle density was determined using a randomly positioned quadrat measuring 0.3 m × 0.6 m in each plot at two different sites. Five seedlings were randomly selected from every location to quantify the number of packed grains per panicle and the mass of 1000 grains.

Elemental Analysis

Fundamental analysis examined the mobilization and accumulation of ZnO-NPs inside rice seeds. Zn, iron (Fe), and manganese (Mn) in the seeds were measured using an inductively coupled plasma. Dried seed specimens were measured in terms of weight and transferred into a glass vial with a volume of 20 ml. A mixture of 5 ml of HNO_3 and 1 ml of H_2O_2 was utilized for sample digestion, which took place at a temperature of 150°C. The digestion process continued until the solution achieved clarity, after which it was distilled until its volume was reduced to 1 ml, resulting in concentration. The samples that had undergone complete digestion were diluted with 2 ml of a 1% HNO_3 solution. The diluted specimens were passed through using a 0.2 µm nylon filtering, next using a 0.02 µm membrane filter. The ultimate filtering solution underwent further dilution thrice before its examination through ICPMS.

Statistical Analyses

The data from pot and field studies were analyzed using an integrated Analysis of Variance (ANOVA) design. The model considered the components of year and therapy and their two-way correlations. The corrected means of the treatment groups were compared using the Tukey varied range testing at a level of significance of 5%. The degree of connection between the two factors was assessed by calculating the coefficient of measurement and correlogram.

The present section presents the GMRSA-ZNP approach, which employs sophisticated methodologies to quantitatively examine the impacts of ZnO-NPs on rice seed germination and metabolism. This section aims to elucidate the molecular mechanisms behind the possible advantages of NP priming in rice agriculture.

Simulation Analysis and Outcomes

The experimental strategy used a Randomized Complete Block Design (RCBD) with eight treatment combinations, each reproduced three times, with the Indica rice variety Ajit IET 2206666. Each container containing sandy clay loam soil had a capacity of 10 liters and was evenly treated with 50 µmol of ZnO-NPs. The temperature and humidity levels were carefully regulated at 30°C and 70% throughout the 7-day germination period. The experimental field plots, each 1 m^2 in size, were arranged according to an RCBD with three replicates.

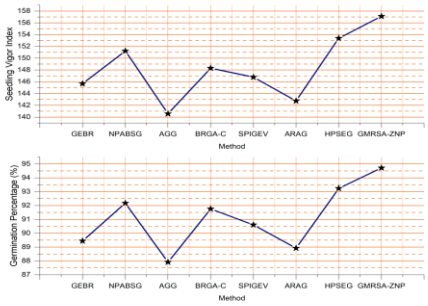

Figure 2: (a) germination % and Figure 2(b) seedling vigor index analysis

The germination % and seedling vigor index findings for different procedures are shown in Figures 2(a) and 2(b), respectively. The GMRSA-ZNP approach exhibits an enhancement, as demonstrated by a germination percentage of 94.72% and a seedling vigor index of 157.12. These results indicate a sizeable favorable influence on germination and early development of rice seeds. The observed percentage increase compared to other approaches demonstrates the efficacy of GMRSA-ZNP in augmenting these essential growth parameters.

Figures 3(a) and 3(b) depict the results of the metabolic processes and rates of degradation associated with starch across various methodologies. The GMRSA-ZNP technique significantly enhances starch metabolism, measuring 132.75 µg/g. It reveals an accelerated rate of starch breakdown, measuring at 0.98 mg/g/h. The findings demonstrate the efficacy of GMRSA-ZNP in augmenting starch metabolism, suggesting its capacity to enhance energy usage during germination.

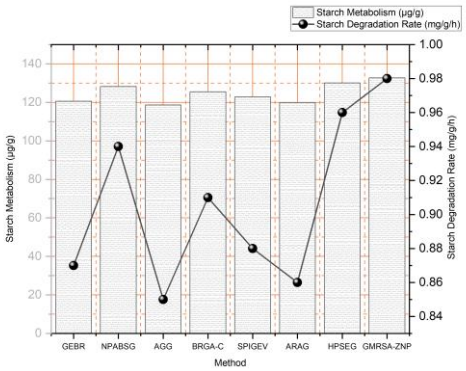

Figure 3(a): Metabolic processes and Figure 3(b) Starch degradation rate analysis

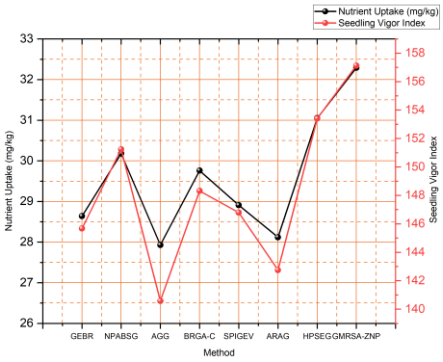

Figure 4(a): nitrogen absorption and Figure 4(b) seedling vigor index analysis

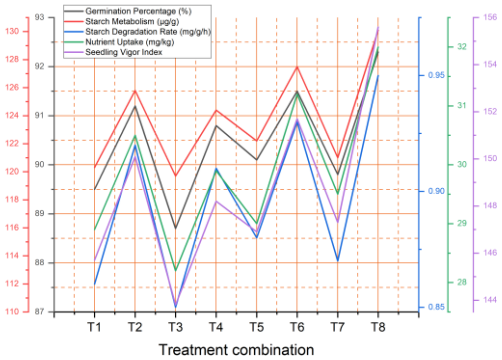

Figure 5: Experimental outcomes of different treatment combinations

The results of nitrogen absorption and seedling vigor index for different approaches are shown in Figure 3. GMRSA-ZNP technique demonstrates a noteworthy enhancement in nutrient absorption, quantified at 32.29 mg/kg, along with an enhanced seedling vigor index of 157.12. This finding underscores the efficacy of GMRSA-ZNP in augmenting nutrient assimilation and overall seedling vitality, suggesting its capacity to improve initial growth in rice farming.

The findings for several treatment combinations, including germination %, starch metabolism, starch degradation rate, nutrient absorption, and seedling vigor index, are summarized in Figure 5. The treatment combination T8 exhibits the most significant values for all metrics, including a germination percentage of 92.3%, starch metabolism at 130.1 µg/g, starch degradation rate of 0.95 mg/g/h, nutrient absorption measuring 32 mg/kg, and a remarkable seedling vigor index of 155.6. The results highlight the efficacy of the combination treatment, including ZnO-NPs and Na-selenite/selenate, in augmenting many essential growth indices in rice seedlings.

Conclusion and Future Scope

This study has explored the crucial domain of improving rice germination and metabolism, which are fundamental in pursuing food security and agricultural sustainability. The analysis of germination and metabolism in rice seeds is essential due to these early development stages' critical role in determining crop production. Conventional research endeavors often need more conclusive results and methodological constraints. To tackle these issues, this research proposed the GMRSA-ZNP technique. GMRSA-ZNP exhibits distinctive attributes, using the inherent capabilities of ZnO-NPs to enhance seed vitality and metabolic functions. The experimental findings highlight the significant impact of GMRSA-ZNP, demonstrating notable enhancements in various essential factors. These include a remarkable germination percentage of 92.3%, heightened starch metabolism at 130.1 µg/g, an accelerated starch degradation rate of 0.95 mg/g/h, increased nutrient uptake of 32 mg/kg, and an impressive seedling vigor index of 155.6. The results shed light on the significant implications of GMRSA-ZNP in transforming rice cultivation methods, hence facilitating higher agricultural productivity and promoting long-term sustainability in the field.

Nevertheless, it is essential to recognize the difficulties presented by this study, including the need to enhance the implementation of GMRSA-ZNP in practical agricultural environments and to tackle any possible environmental concerns. Future research in this field should strive to improve the application methods, conduct more comprehensive investigations into the ecological ramifications, and examine the versatility of GMRSA-ZNP in various rice cultivars and production settings. Effectively use the capabilities of GMRSA-ZNP and explore novel opportunities in rice cultivation while making significant contributions to the worldwide objectives of ensuring food security and promoting sustainable agricultural practices.

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