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Seed Nanopriming and Nanosilica Combined with Field Capacity Treatments: Impacts on Chlorophyll and Morphological Traits of Inpari 32 HDB

Rika Rismayang[1]*, Ida Retno Moeljani[1], Rossyda Priyadarshini[1], Juli Santoso[1]

[1] East Java Veterans National Development University, Indonesia.

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Corresponding Author:
Rika Rismayang
rismayangrikaa@gmail.com

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Abstract: Drought stress can cause reduced rice production. One effort that can be made is seed nanopriming. This study aims to examine the effect of nanosilica seed nanopriming and field capacity on the chlorophyll content and morphological growth of the Inpari 32 HDB rice variety. The study was designed using a completely randomized factorial design with two factors, namely nanosilica concentration (0, 600, 900, and 1,200 mg/L) and field capacity (3 cm, 100%, 75%, and 50% flooding). The results showed that the treatment combinations produced different responses in physiological and morphological parameters. Chlorophyll content increased with increasing nanosilica concentration, with the highest value found in the treatment with 1,200 mg/L of nanosilica and 3-cm flooding. Plant height and leaf width parameters did not show significant differences, but there was a tendency for better growth at higher nanosilica concentrations. For root length and root wet weight parameters, a very significant interaction was found, where the 1,200 mg/L of nanosilica treatment under flooding conditions produced the longest roots and the highest root biomass. Conversely, a 50% field capacity caused a significant decrease in all observed parameters. Overall, nanosilica seed nanopriming, especially at a concentration of 1,200 mg/L, can increase rice tolerance to drought stress by increasing chlorophyll content, root growth, and root biomass.

Keywords: Chlorophyll content; Field capacity; Morphological traits; Nanosilica; Seed

Introduction

Rice is a major food commodity in Indonesia, so increasing its productivity is an important aspect of supporting national food security. However, rice production is often hampered by various abiotic factors, one of which is drought stress (Chaniago et al., 2021). Drought stress is the most common limiting factor for growth and yield, often causing a significant decline in productivity (Hassan et al., 2023). Water deficit disrupts cell turgor, reduces tissue water potential, and inhibits photosynthesis due to decreased stomatal opening. This condition triggers the accumulation of reactive oxygen species (ROS), disrupts chloroplast function, and reduces plant chlorophyll content (Sarma et al., 2023). The subsequent impact of this stress manifests in the form of stunted vegetative growth, including plant height, leaf width, root length, and decreased biomass accumulation such as root wet weight. In the generative phase, drought also inhibits panicle formation and grain filling, resulting in a drastic decrease in grain yield (Salleh et al., 2022).

Various strategies have been developed to increase plant tolerance to drought stress, one of which is

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through seed priming techniques. Seed priming is a seed treatment method that utilizes the initial imbibition stage to trigger physiological processes before the seeds germinate completely. Some commonly used priming methods include hydropriming, halopriming, osmopriming, biopriming, and nanopriming. Among these techniques, nanopriming is considered more effective because it utilizes very small nanoparticles (1–100 nm) that have a large surface area, high reactivity, and optimal penetration ability into seed tissue (Nile et al., 2022).

Nanosilica (SiO_2) is one of the nanoparticles widely applied in seed nanopriming. Nanosilica (silica nanoparticles, SiO_2 NPs) is available in various sizes, shapes, and with modifiable surfaces. Common synthesis methods include sol-gel processes, hydrothermal techniques, and biological or green synthesis routes. The size of nanosilica particles is typically 5–200 nm, with porosity and surface charge determining relative solubility, water or molecule adsorption capacity, and interaction with seed walls and cell membranes (Li et al., 2025).

Several studies have shown that nanosilica plays a role in improving water use efficiency, stabilizing cell membranes, and reducing oxidative damage caused by ROS accumulation. Nanosilica can improve the initial resistance of plants to drought through several pathways, namely reducing leaf water loss, improving tissue water status, and increasing osmoregulation capacity. Physiologically, SiO_2-NPs are known to help maintain turgor and reduce membrane destabilization effects during water deficit. In addition, nanosilica can increase chlorophyll content and maintain photosynthesis during water shortage, so vegetative growth such as plant height, leaf width, root length, and root wet weight can remain optimal.

The effectiveness of nanosilica-based seed nanopriming has been reported in various commodities. Hussain et al. (2019) showed that nanosilica nanopriming at a concentration of 1,200 mg/L was able to increase chlorophyll content and wheat plant growth and reduce oxidative stress due to drought. However, plant response to nanopriming is highly dependent on seed type, nanosilica concentration, and environmental conditions, including water availability in the growing medium. Based on this, this study aims to examine the effect of nanosilica concentration on seed nanopriming and its interaction with various field capacities on the early growth of the Inpari 32 HDB rice variety. The evaluation was carried out through physiological and morphological parameters, including chlorophyll content, plant height, leaf width, root length, and root wet weight, so the optimal concentration and environmental conditions for increasing rice tolerance to drought stress could be obtained.

Method

The research was conducted at the Land Resources Laboratory of the Veteran National Development University of East Java, the PT Advanta Seeds Indonesia Laboratory, and the Komboh Hamlet, Sambirejo Village, Wonosalam Subdistrict, Jombang Regency, East Java. Sambirejo Village has an altitude of 600 m above sea level and an average temperature of 24–30°C. Stomatal density testing was conducted at the Plant Production Management Laboratory of Gadjah Mada University, chlorophyll testing was conducted at the Plant Physiology Laboratory of Brawijaya University, and proline content testing was conducted at the Biotechnology Laboratory of Brawijaya University. The research was conducted from March to July 2025.

The tools used in this study were measuring cups, analytical scales, digital scales, microscopes, specimen slides, dishes, tweezers, ovens, desiccators, sieves, rings (cylinders), mortars, test tubes, centrifuges, spectrophotometers, cuvettes, spoons, trays, germination trays, 30 x 25 cm buckets, shovels, scoops, watering cans, rulers, and cameras. The materials used in this study were Inpari 32 HDB rice seeds, nanosilica (particle size 10 nanometers), deionized water, alcohol, sterile sand, soil, compost, urea fertilizer, SP-36 fertilizer, KCl fertilizer, nail polish, 80% acetone, insulation, gauze, polybags, plastic wrap, and label paper.

The research was a factorial experiment based on a completely randomized design (CRD) with two factors (Aboellail et al., 2023). The first factor was the concentration of nanosilica, which consisted of four levels. The second factor was the field capacity, which consisted of four levels. The two factors were coded as follows:

Factor I: Nanosilica Concentration (N), consisting of 4 levels, namely: N0: Without nanosilica nanopriming seed (Control); N1: Nanosilica concentration of 600 mg/L; N2: Nanosilica concentration of 900 mg/L; and N3: Nanosilica concentration of 1,200 mg/L

Factor II: Field Capacity (K) consisting of 4 levels, namely: K0: Optimal water supply (3 cm flooding) (Control); K1: Field capacity 100%; K2: Field capacity 75%; and K3: Field capacity 50%.

The combination of these two factors resulted in 16 treatment combinations that were repeated three times, resulting in 48 experimental units. Each experimental unit consisted of one bucket, so there was a total of 48 buckets in the experimental plot, and each bucket contained one plant.

Seed Preparation

The seeds used were Inpari 32 HDB rice seeds. Before being treated with nanopriming seeds, the seeds were identified and selected. Seed selection was based on large seed size, no damage, no holes, no emptiness, and no mold. A total of 1,000 rice seeds were prepared for each experimental unit, or 3,000 seeds for each seed nanopriming treatment with 600 mg/L, 900 mg/L, 1,200 mg/L nanosilica, and without seed nanopriming.

Seed Nanopriming Process

Seed nanopriming began with the preparation of priming materials in the form of nanosilica (NP Si) at 600, 900, and 1,200 mg/L. Each nanosilica concentration was dissolved in 1 L of deionized water for 30 minutes to obtain the priming solution concentration for each treatment. Two hundred rice seeds were soaked in 100 mL of the prepared nanosilica solution in a dark room at room temperature of approximately 25–27°C for 20 hours (Hussain et al., 2019). The seeds resulting from nanopriming were placed in Petri dishes and air-dried until their moisture content met the Indonesian National Standard (SNI) and were stored at 4°C for further research.

Preparation for Planting Media and Determination of Field Capacity

Preparation of planting media was carried out one week before planting. Planting media were made by mixing soil with compost (1:1). Planting media materials were mixed and leveled using a hoe. Field capacity was determined by weighing 6 kg of soil and watering the soil medium in a polybag until saturated, then leaving it for 24 hours. The moisture content of the soil at field capacity was measured using the gravimetric method. This method was used to determine the initial soil moisture content before treatment, so that the amount of water that must be added to achieve field capacity for each treatment could be determined. Gravimetric water content determination was carried out by drying 10 g of soil sample in an oven at 105°C for 24 hours. Soil moisture content was calculated using the formula (Haridjaja et al., 2013).

$$\text{Water Content (WC) (\%)} = \frac{\text{BTKU} - \text{BTKO}}{\text{BTKO}} X\ 100\% \quad (1)$$

Description:
BTKU = Air-Dried Soil Weight
BTKO = Oven-Dried Soil Weight

The field capacity moisture content value obtained was then used to calculate the weight of the soil after adding water according to the field capacity treatment. The weight of the soil for each field capacity treatment is shown in Table 1.

Table 1. Soil weight in field capacity treatment

Water Content %	Field Capacity %	Weight of Air-Dried Soil (kg)	Weight of Soil after Treatment Field Capacity (kg)
8.1	100	6	11.03
	75	6	9.65
	50	6	8.27

Result and Discussion

Chlorophyll Content

The results showed that there were variations in leaf chlorophyll content for each treatment combination of nanosilica concentration and field capacity. The average leaf chlorophyll content affected by the combination of nanosilica concentration and field capacity treatments is presented in Table 2.

Table 2. Chlorophyll Content of Rice Plants in Combination Treatments of Nanosilica Concentration and Field Capacity

Treatment	Ca (µg/ml)	Cb (µg/ml)	CT (µg/ml)
N_0K_0	211.69	55.66	267.34
N_0K_1	164.54	38.08	202.62
N_0K_2	133.60	30.53	164.13
N_0K_3	111.25	24.94	136.19
N_1K_0	210.03	58.42	268.46
N_1K_1	158.27	46.74	205.01
N_1K_2	198.95	50.47	249.42
N_1K_3	117.51	24.64	142.14
N_2K_0	182.50	47.95	230.45
N_2K_1	181.21	45.64	226.84
N_2K_2	147.23	35.16	182.39
N_2K_3	142.87	30.88	173.76
N_3K_0	259.53	92.10	351.64
N_3K_1	213.30	59.73	273.04
N_3K_2	166.63	50.31	216.94
N_3K_3	143.77	36.01	179.78

Description:
Ca = chlorophyll a content (µg/ml)
Cb = chlorophyll b content (µg/ml)
CT = total chlorophyll content (µg/ml)

Table 2 shows that the combination of nanosilica concentration treatments in seed nanopriming and field capacity affects the chlorophyll content of rice plants. Chlorophyll content plays an important role in plant growth and development. Kumar et al. (2025); Meharg & Meharg (2015); Putri et al. (2017) stated that silica accumulated in rice leaves causes the leaves to stand upright, thereby helping sunlight absorption for chlorophyll biosynthesis. The highest chlorophyll a content was obtained in the N3K0 treatment (259.53 μg/ml). This indicates that under optimal water conditions (3 cm flooding) (K_0), an increase in nanosilica concentration can increase chlorophyll a formation. The chlorophyll b content showed a similar pattern, indicating that seed nanopriming with high concentrations of nanosilica can support chlorophyll b accumulation. The N3K0 treatment also produced the highest total chlorophyll (351.64 μg/ml). This shows that nanosilica-based seed nanopriming, especially at high concentrations, is effective in increasing total chlorophyll content when plants are under normal to moderate field capacity conditions. Conversely, the lowest value was found in the N0K3 treatment (136.19 μg/ml), indicating the negative effect of low field capacity stress without the help of nanosilica-based seed nanopriming. The higher the chlorophyll a and b content, the higher the photosynthesis rate. Nanosilica functions to increase photosynthetic efficiency by stimulating the formation of photosynthetic pigments and helping plants adapt to drought stress.

Drought stress affects chlorophyll levels in leaves. Based on the results of the study, it can be seen that the lower the field capacity, the lower the chlorophyll content. At 50% field capacity, the chlorophyll content produced was the lowest compared to other treatments. The low chlorophyll content at 50% field capacity is thought to be due to plants producing reactive oxygen species (ROS) that can cause lipid peroxidation, resulting in chlorophyll damage (Ahmadikhah & Marufinia, 2016).

Plant Length

The results of the analysis of variance of the effect of the combination of nanosilica concentration and field capacity on rice plant length showed that there was no significant interaction, nor did the single factors have a significant effect. The average rice plant length under the combined influence of nanosilica concentration and field capacity is presented in Table 3.

Table 3. Average Rice Plant Length under Nanocilica Concentration and Field Capacity Treatments.

Nanosilica	Average Length of Rice Plants (cm)								
Concentration (mg/L)	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	63 DAP
0	42.88	45.49	57.56	63.75	71.13	71.78	73.43	79.19	81.94
600	47.00	49.46	62.89	70.56	77.58	79.11	82.06	85.70	89.05
900	46.28	48.23	57.43	61.45	70.43	74.36	79.44	84.81	87.35
1.200	49.57	50.92	65.85	74.68	82.71	85.53	88.78	94.16	97.93
DMRT 5%	tn	tn	tn	tn	tn	tn	tn	tn	tn
Field Capacity (%)									
Flooding 3 cm	49.00	51.16	64.43	71.02	79.93	80.96	84.69	94.08	101.00
100	47.20	49.43	64.48	71.53	79.62	80.03	84.46	86.38	88.30
75	47.04	48.96	60.54	67.35	76.52	79.91	83.00	86.33	87.86
50	42.48	44.54	54.28	60.54	65.79	69.18	71.56	76.17	83.77
DMRT 5%	tn	tn	tn	tn	tn	tn	tn	tn	tn

Note: n.s. = not significantly different.

Table 3 shows that the average length of rice plants increased with age from 7 to 63 days after sowing in all treatments. Seed nanopriming with nanosilica showed a positive trend in plant length growth, with the highest concentration of 1,200 mg/L resulting in a plant length of 97.93 cm at 63 days after sowing. Physiologically, nanosilica has the ability to strengthen cell walls, increase water absorption, and improve photosynthetic efficiency in the vegetative phase. Silica is known to accumulate in the epidermal tissue of leaves and stems, providing structural support that helps maintain plant turgor and improve photosynthetic activity (Huang & Ma, 2024). Nanosilica measuring 1–100 nm is able to enter plant tissue faster than conventional silica, thereby optimizing cell division and elongation processes in the early stages of stem growth (Suriyaprabha et al., 2012).

In terms of water availability, field capacity shows a clear pattern in that the lower the field capacity, the shorter the plant height. The 3 cm flooding treatment resulted in the highest plant height of 101.00 cm, while 50% field capacity only reached 83.77 cm 63 days after planting (DAP). This is in line with plant physiology theory, which states that water deficit reduces turgor pressure, inhibits cell elongation, and slows stem growth (Taiz & Zeiger, 2010). Rice plants are highly dependent on water to support nutrient transport and photosynthesis; thus, water deficiency, especially at low field capacity, inhibits vegetative growth. Although the effect of nanosilica was not statistically significant, the tendency for increased plant length at concentrations of 600–1,200 mg/L indicates that nanosilica still contributes physiologically to increased plant vigor. Several studies support this phenomenon.

Leaf Width

The analysis of variance results showed that there was no significant interaction between the combination of nanosilica concentration and field capacity on rice leaf width, nor did the single factors have a significant effect. The average rice leaf width by nanosilica concentration and field capacity treatments is presented in Table 4.

Table 4. Average Leaf Width of Rice Plants under Nanocrystalline Silica Concentration and Field Capacity Treatments

Nanosilica Concentration (mg/L)	Average Rice Leaf Width (cm)
0	1.09
600	1.24
900	1.26
1.200	1.36
DMRT 5%	tn
Field Capacity (%)	
Flooding 3 cm	1.33
100	1.26
75	1.23
50	1.13
DMRT 5%	tn

Note: ns = not significantly different.

Table 4 shows that although the treatment of nanosilica concentration and field capacity was not significantly different, there was a tendency for rice leaf width to increase up to a nanosilica concentration of 1,200 mg/L. This indicates that nanosilica can promote leaf morphology development by increasing nutrient availability, strengthening cell walls, and improving photosynthetic efficiency. Meanwhile, in the field capacity treatment, there was a tendency for rice leaf width to decrease up to a field capacity of 50%.

Nanosilica absorbed by seeds during the seed nanopriming process can increase cell metabolic activity and stimulate the production of plant hormones such as cytokinin and gibberellin. In addition to playing an important role in water absorption by seeds, these hormones play a role in cell division and differentiation, including in the epidermal tissue where stomata are formed. (Wei et al., 2021) stated that cytokinin plays an important role in regulating cell proliferation and differentiation during leaf growth, vascular pattern, and stomata production. Nanosilica plays a role in cell differentiation, which is one form of plant adaptation to adjust its function to the environment.

Root Length and Wet Weight

The analysis of variance showed a very significant interaction between the treatment factors of nanosilica concentration and field capacity on the root length of rice plants. The average root length of rice plants under the combined treatment of nanosilica concentration and field capacity is presented in Table 5.

Table 5. Average root length of rice plants under combined treatment of nanosilica concentration and field capacity

Nanosilica Concentration (mg/L)	Flooding 3 cm	Field Capacity (%)		
		100	75	50
0	43.27cd	29.60abc	16.70a	16.17a
600	30.03bc	30.37abc	30.40abc	16.23a
900	23.13ab	21.90ab	24.10ab	26.90ab
1.200	56.17d	24.17ab	33.67bc	21.10ab
DMRT 5%				-

Note: Average values followed by the same letter in the same column are not significantly different based on the 5% DMRT test.

Table 5 shows that the 1,200 mg/L nanosilica treatment (N3) produced good results in increasing root length, especially under 3 cm flooding conditions (K0) with root lengths reaching 56.17 cm. This indicates that the application of nanosilica at high concentrations can stimulate longer root growth, especially when combined with optimal water availability. Conversely, the treatment without nanopriming seeds (N0) also produced fairly long roots under flooding conditions (43.27 cm). However, this treatment showed a drastic decrease in low field capacity (K2 and K3). A similar result was observed in the 600 mg/L (N1) and 900 mg/L (N2) treatments, where root length was shorter than in the N3K0 combination. These results indicate that lower nanosilica concentrations are insufficient to compensate for drought stress. Longer roots are very important in supporting the efficiency of water and nutrient absorption, as well as plant resistance to environmental fluctuations, which ultimately contribute to the overall growth and yield of rice plants.

Figure 1. Root Length of Rice Plants Treated with a Combination of Seed Nanopriming Concentrations of Nanosilica and Field Capacity.

The visual appearance of rice roots can be seen in Figure 1, which shows that root length varies significantly between treatments, reflecting the response of plants to the treatments given. The N3K0 treatment produced longer and denser roots, indicating that nanopriming can increase plant resistance to drought stress by stimulating root system growth. Conversely, treatments without nanopriming, such as N0K3, produced shorter roots, indicating that plants were less able to develop optimally under water stress.

The results of the analysis of variance showed a very significant interaction between the factors of nanosilica concentration treatment and field capacity on the wet weight of rice roots. The average wet weight of rice roots under the influence of the combination of nanosilica concentration and field capacity treatments is presented in Table 6.

Table 6. Average wet weight of rice plant roots in the combined treatment of nanosilica concentration and field capacity

Nanosilica Concentration (mg/L)	Flooding 3 cm	Field Capacity (%)		
		100	75	50
0	14.60b	3.90a	3.23a	3.17a
600	14.07b	5.40a	4.77a	3.27a
900	3.90a	3.90a	3.47a	5.13a
1.200	17.00b	4.40a	4.33a	3.33a
DMRT 5%				-

Note: Average values followed by the same letter in the same column are not significantly different based on the 5% DMRT test.

Table 6 shows that the combination treatment of 1,200 mg/L nanosilica concentration (N3) with flooding conditions (K0) produced the highest root wet weight of 17.00 grams. This indicates that the application of nanosilica at high concentrations can significantly increase root biomass growth, especially under optimal water conditions. Conversely, at lower field capacities, root wet weight tends to decrease at all nanosilica concentration levels. The effectiveness of nanosilica concentration in seed nanopriming depends on water availability, and the interaction between these two factors is very important in determining plant response. The increase in root wet weight reflects improved physiological activity of the roots in absorbing water and nutrients, and contributes to plant resistance to environmental stress.

Seed nanopriming increases antioxidant levels, alters and enhances defense system activity, making plants more resistant to biotic and abiotic stresses in the field (Imtiaz et al., 2023; Salam et al., 2022). Seed nanopriming also allows nanosilica particles to penetrate the outer layer of seeds, which increases water imbibition capacity, accelerates seed metabolism, and activates enzymes that are important for early plant growth (do Espirito Santo Pereira et al., 2021; B. Kumar et al., 2022; Salve et al., 2025). Nanoparticles accelerate the activity of antioxidant enzymes that reduce stress on plants by reducing the negative impact of reactive oxygen species (ROS), resulting in increased growth and production. Nanosilica can enhance plant growth and development by improving gas exchange, photosynthesis rate, transpiration rate, stomatal electrical phenomena, and effective photochemical potential (Hao et al., 2023; Surendar et al., 2024). According to Suriyaprabha et al. (2012), nanosilica can strengthen plant cell walls and improve vascular tissue formation, thereby supporting more efficient water and nutrient absorption. Thus, plants are able to form longer roots and higher root wet weight, which can support canopy growth and the formation of tillers and panicles.

Rooting is closely related to drought stress. Under drought conditions, plant roots will be longer. However, based on research, the lower the field capacity, the shorter the root size and the lighter the wet weight of the roots. Rice roots under drought stress are influenced by the expression of aquaporins, which regulate water transport capacity from the root system. Extreme water deficits limit root growth and development due to increased soil resistance and low water availability (Parent et al., 2010). According to (HanBastian, 2013), this can occur because the determining factor for root development is the nutritional status of the plant as a whole. Suboptimal nutrition in plants causes low root growth and development. Low root wet weight will also disrupt the efficiency of nutrient uptake, such as nitrogen, phosphorus, and potassium. As a result, rice plants become more susceptible to other stresses and are unable to support important phases such as flowering and grain filling.

Conclusion

Nanosilica-based seed nanopriming has a positive effect on chlorophyll content and root growth in Inpari 32 HDB rice varieties, especially when applied at a concentration of 1,200 mg/L and combined with optimal water availability conditions. This treatment resulted in the highest chlorophyll content and significantly increased root length and root wet weight. Although plant height and leaf width parameters did not show significant differences, there was a tendency for better vegetative growth at high nanosilica concentrations. Conversely, low field capacity (50%) reduced chlorophyll content, root length, and root wet weight in all treatments. These findings indicate that nanosilica-based seed nanopriming has the potential to improve plant resistance to drought stress, but its effectiveness is highly dependent on water availability in the growing medium.

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The authors declare that they have no conflict of interest related to this study.

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