Original Research Article

Cultivating Success- Optimizing Pre-Sowing Techniques for Enhanced Growth, Fruit Yield, and Seed Quality in Okra (*Abelmoschus esculentus* L. Moench)

Abstract

This research investigated diverse pre-sowing techniques' impact on the growth, fruit, and seed yield of okra variety Arka Anamika. From fourteen treatments, seven were selected based on germination and vigor index for a subsequent field experiment. The Randomized Block Design trial with three replications revealed significant variations in growth and yield among pre-sowing treatments.The treatment employing PEG 6000-13.5% (-0.25MPa) for 6 hours and Trichoderma viride @4g/kg of seed exhibited the highest germination percentages. Pseudomonas fluorescens @10g/kg of seed also displayed notable performance. Treatment variations were observed in plant height, branches, and flowering timings. Pseudomonas fluorescens @10g/kg of seed demonstrated the most branches and quickest flowering.Fruit-related parameters were highest in Pseudomonas fluorescens (10g/kg of seed), followed by Trichoderma viride (4g/kg of seed). Pseudomonas fluorescens @10g/kg of seed also achieved maximum seed yield parameters. Pseudomonas fluorescens @ 10g/kg of seed ranked highest overall, and Trichoderma viride @ 4g/kg of seed was the second most effective treatment.Recommendations for preserving seed quality included KNO3 2% for 6 hours and PEG 6000-13.5% (-0.25MPa) for 12 hours. Application of biocontrol agents, especially Pseudomonas fluorescens and Trichoderma viride, markedly improved okra variety Arka Anamika's fruit and seed yield characteristics. The study concludes by emphasizing the benefits of biocontrol agents on overall performance and suggesting targeted treatments for optimal seed quality.

Key words: Okra (variety- Arka Anamika), pre sowing treatments, Biocontrol agents

Introduction

Okra (Abelmoschus esculentus L. Moench), commonly known as lady's finger or bhindi, is a prized warm-season vegetable appreciated for its tender and nutritious pods. Rich in protein, vitamins, minerals (especially iron), and dietary fiber, okra stands out as a vegetable uniquely beneficial for vegetarians, contributing significantly to the mitigation of goiter disease (Sindhumole, 2003). Moreover, its noteworthy antioxidant properties, attributed to flavonoids, carotene, and vitamin C, have led to its medicinal use in conditions like diabetes and hyperlipidemia (Dantas et al., 2021).

Okra is a commonly cultivated traditional vegetable in Kerala, albeit on a limited scale, as mentioned by Pradheep et al. in 2021. It holds a unique status as a year-round cultivar in the state, primarily integrated into homestead farming systems rather than large-scale commercial endeavors. The distinctive cultivation practices in Kerala reflect the challenges and opportunities inherent in the local agricultural landscape. Traditional okra varieties in the region are characterized by long and white fruits; however, the prevalence of the yellow vein mosaic virus has prompted a shift toward cultivating varieties with green and small fruits, despite consumer preferences (Rajamony,1999).

India stands as the global leader in okra production, contributing nearly 60% of the world's output (FAOSTAT,2022). In contrast, Kerala's share is modest, comprising only 0.04% of the total production (GOK,2022). Challenges persist in the region, with issues like hardseedness affecting germination and stand establishment (Purquerio et al., 2010), contributing to suboptimal crop productivity. Despite India's prominent role in global okra production, challenges persist, with commercial cultivars experiencing only 66% initial germination (Balla et al., 2011; Sharma et al., 2011), highlighting the need for effective solutions. Addressing issues like hardseedness becomes imperative for sustaining okra's importance as a valuable vegetable crop.

Balancing agricultural production with environmental sustainability is paramount. Sustainable technologies, particularly pre-sowing methods like seed priming, offer solutions for improving stand establishment without compromising ecological integrity. Seed priming involves controlled hydration, enhancing germinability and mitigating issues of delayed and non-synchronous germination. This approach aligns with broader agricultural sustainability goals by optimizing stand establishment while minimizing adverse environmental impacts (Bradford, 1986; Ashraf and Foolad, 2005; Venkatasubramanian and Umarani, 2007).

Based on the priming materials /agents used seed priming is categorized into different types, , namely, hydropriming (water), solid matrix priming (SMP) or matripriming (hydrated sand,peat and vermiculite), osmopriming (soaking in osmotic solutions such as PEG or inorganic salts),thermopriming (treatment with low or high temperatures),plant growth inducers(hormonal priming) and biopriming (bioagents +hydration). Biological treatment is an example of environmental-friendly alternatives, and for vegetable crops, growers are inclined to adopt the seed treatment approach (via seed priming, biopriming) due to its low cost (Chin et al.,2021).

The most prominent fungi and bacteria which are used extensively in bio-priming include Trichoderma, Pseudomonas, Glomus, Bacillus, Agrobacterium, and Gliocladium. A mycorrhiza-like endophytic Agaricomycetes fungus called *Piriformospora indica* has drawn a lot of attention in recent years due to its outstanding capacity to effectively boost plant development, protection, and stress tolerance. *P. indica*-induced seed germination and development have been reported in several crop plants including vegetables (Varma *et al*., 2012a,b, 2013).However, there are no reports on use of *P.indica*as biopriming agent in okra

In this context, this article explores pre-sowing interventions in okra cultivation, focusing on the Arka Anamika variety, known for its resistance to the yellow vein mosaic virus. By investigating the efficacy of interventions such as seed priming and biological seed treatments, the study aims to provide insights into sustainable practices that enhance okra cultivation in Kerala, contributing to both local food systems and broader agricultural sustainability objectives.

Materials and methods

Experimental Design

Five months old seeds (variety – Arka Anamika) were subjected to 14 treatments mentioned in Table 3. Initial seed quality parameters were assessed immediately after the treatments. Seven best treatments were selected based on germination percentage and vigor index. Seeds from these treatments were sown in the field along with the control from January 2023 to April 2023, as per Package of Practice (POP) recommendations (KAU,2016). The experimental design was Randomized Block Design (RBD) in three replications with ten plants per replication. The plot size was 3m × 2m with the spacing of 60cm×45cm.

Environmental variables

The experiment comprising field studies was conducted in the field located at the Department of Seed Science and Technology, during January (2023) and April (2023). The soil's pH was recorded as 5.6. This place lies between 13^{o} 32'N latitude and 76o 26'E longitude with an elevation of about 40m from MSL. The monthly meteorological data collected for the study period October 2022 to August 2023

Data Analysis

Five months old okra seeds were treated with fourteen pre sowing treatments such as control, hydration -dehydration 12 hours, hydration - dehydration 24 hours, PEG 6000 13.5 % (-0.25MPa) 6 hours, PEG 6000 13.5 % (-MPa) 12 hours, KNO_3 2% (6 hours) KNO_3 2% (12hours), Sandmatric (60% WHC -3 hours), Sandmatric (60% WHC -6 hours) Trichoderma viride 4g/kg of

seed, Pseudomonas fluorescens 10g/kg of seed, T.viride 4g/kg+ P.fluorescens 10g/kg of seed, Piriformospora indica 5x10^5 spores mL^{-1} 5ml/kg, Piriformospora indica 5x105 spores mL^{-1} 10 ml/kg.

Germination (%)

The germination test was conducted as prescribed by International Seed Testing Association (ISTA) (2010). From each replication of treatment, four sets of hundred seeds were drawn and placed on wet sand for germination. The sand trays were kept in walk-in germination room at a constant temperature of 25 ^{0}C and 90±3 per cent relative humidity. The number of normal seedlings at the 7th day of germination was counted and germination per cent was worked out using the formula as given below.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total no of seeds sown}} \times 100$$

Vigor index I

The seedling vigor index I was calculated using the formula suggested by Abdul- Baki and Anderson (1973).

Vigor index I = Germination (%) x seedling length (cm)

Vigor Index II

Vigor index II was computed as suggested by Abdul- Baki and Anderson (1973).

Vigor index-II = Germination (%) x Seedling dry weight (mg)

The statistical analysis of the data recorded and performed using General R-shiny based Analysis Platform Empowered by Statistics (GRAPES) developed by Kerala Agricultural University and the ranking of treatments was done by using Duncan's Multiple Range Test (DMRT) for Randomized Block Design (RBD). The data obtained were subjected to the analysis of variance (ANOVA).

Result

Initially, all the seeds were subjected to fourteen pre sowing treatments and seven best treatments were selected based on germination percentage, vigour index I and vigour index II.

Germination (%)

Maximum germination percentage obtained in (PEG 6000-13.5 % (-0.25MPa) 6 hours -50) and it was on par with (*Trichoderma viride* @4g/kg of seed), (PEG 6000-13.5 % (-MPa) 12 hours), (KNO_3 2% (6 hours)), (Hydration-Dehydration (24 hours) and (Sandmatric (60% WHC -6 hours) and which was followed by (KNO_3 2% (12hours) (Figure 1), whereas non-primed seeds displayed lowest germination (97.16 %).

Vigour index

The seedling vigor Index, a complex trait influenced by both germination percentage and average seedling length, exhibited a distinct pattern in the performance of different varieties. A careful examination of the overall performance of each treatment based on this derived seedling parameter could help in identifying specific characteristics unique to Arka Anamika. Maximum vigour index I was recorded from *Trichoderma viride* @4g/kg of seed (2871.90) and which was on par with Sandmatric (60% WHC -3 hours), Sandmatric (60% WHC -6hours), Pseudomonas fluorescens 10g/kg of seed, hydration -dehydration 12 hours and PEG 6000 13.5 % (-0.25MPa) 6 hours. Vigour index II was exhibited maximum in Pseudomonas fluorescens 10g/kg of seed (4.81), *Trichoderma viride* @4g/kg (4.60), PEG 6000 13.5 % (-0.25MPa) 12 hours (4.13), hydration – dehydration 24 hours (3.89).

The impact of seed priming on crop development and various growth characteristics.

Plant height (cm)

There were no significant differences among the treatments at 25DAS (days after sowing). Analysis of variance revealed that seed priming treatment had a significant effect on plant height at 45 DAS and 75DAS. (Table 1) The results showed that all seed priming treatments increased the plant height in the field compared to unprimed seeds. Okra seeds primed in PEG 6000-13.5 % (-0.25MPa) 6 hours had the highest (75.75, 115.65cm) plant highest at 45 and 75 DAS, which was statistically comparable to seeds primed in *Pseudomonas fluorescens* @ 10g/kg of seed (73.33,110.90cm). In contrast, control had a significantly lowest plant height at 45 and 74 DAS (70.94, 94.82), which was statistically similar (71.23) to hydration – dehydration 24 hours at 45 DAS.

Branches per plant

The number of branches on each plant was significantly influenced by seed priming(Table 1). Seeds treated with *Pseudomonas fluorescens* @10g/kg of seed (T_8 – 3.40) had a higher number of branches per plant, and they exhibited statistical similarity with treatments T_2 (Hydration-Dehydration (24 hours) - 3.20) and T_5 (Sandmatric (60% WHC -3 hours) – 3.20), followed by T_4 (KNO_3 2% -12hours). Whereas seeds without priming had a minimum number of branches recorded in T3 (PEG 6000-13.5 % (-0.25MPa) 6 hours – 2.86) and it was on par with control (T_1).

Days to 50 % flowering

Days to 50% flowering and fruit maturity were significantly influenced by pre sowing seed treatments. Among the treatments, seed treated with T_8*(Pseudomonas fluorescens @10g/kg* of seed) had the earliest (48) days to 50 % flowering and it was statistically on par with T_7 (*Trichoderma viride* @4g/kg of seed – 49) and T_3 (PEG 6000-13.5 % (-0.25MPa) 6 hours -50). Unprimed seeds showed a delayed 50 per cent flowering (60.33).

Impact of seed priming on yield-related parameters and fruit yield

Fruits per plant and Fruit length

Treatments for okra (variety Arka Anamika) seed priming significantly affected the number of fruits produced per plant. Overall, the findings proved that compared to osmo-primed and halo-primed seeds, the seeds treated with biocontrol agents produced a greater number of fruits per plant. Treatment T8 *(Pseudomonas fluorescens @10g/kg* of seed) was recorded maximum fruits per plant which was on par with treatment T7 (*Trichoderma viride* @4g/kg of seed). Minimum fruits per plant recorded - 8.62 in T_1 (control). The results revealed that when compared to unprimed seeds, all seed priming treatments increased the number of fruits produced per plant. Fruit length was significantly affected by seed priming (Table 2). Among the treatments, maximum fruit length was recorded in treatment T_8 (20.42cm - *Pseudomonas fluorescens* @10g/kg of seed). In contrast, the unprimed seed has significantly shorter fruit length -T_1 (control- 11.46cm) followed by treatments T_4 (KNO_3 2% 12 hours) which was statistically similar with T_2 (Hydration-Dehydration 24 hours).

Fruit weight (g) at maturity and fruit yield t/ha

Okra (variety- Arka Anamika) seeds treated with various pre sowing treatments significantly affected the fruit weight and fruit yield per hectare (Table 3). Among the treatments highest fruit weight was determined 22.36g T_8*(Pseudomonas fluorescens @10g/kg* of seed. And which was on par with T_6 (Sandmatric 60% WHC 6 hours- 21.68g). Minimum fruit weight was observed in T_4 (KNO3 2% 12 hours -18.69g) and in T_1 (control-17.69g). Highest seed yield was observed in T8- *(Pseudomonas fluorescens @10g/kg* – 11.08t/ha), which was followed by T7 (*Trichoderma viride* @4g/kg of seed – 9.334t/ha) and T6 (Sandmatric 60% WHC 6 hours - 8.85t/ha). Lowest fruit yield was recorded in control (5.31t/ha).

Impact of seed priming on seeds per fruit, seed yield per plant and 100 seed weight(g)

Okra (variety- Arka Anamika) seeds treated with various pre sowing treatments significantly affected with seeds per fruit, seed yield per plant and 100 seed weight (g). It was revealed that from the statistical analysis (Table 3), the highest number of seeds per fruit, seed yield per plant and 100 seed

weight (g) were observed in treatment T8 *(Pseudomonas fluorescens @10g/kg*) and following of T7 (*Trichoderma viride* 4g/kg of seed – 47) and T3 (PEG 6000 13.5 % (0.25MPa) 6 hours compared to all other treatments. Minimum seeds per fruit, seed yield per plant and 100 seed weight (g) were observed in treatments T1 (control), T2 (Hydration-Dehydration 24 hours) respectively.

Discussion

The most important elements to guarantee efficient crop establishment and productivity are uniform germination, seedling emergence, seedling vigour, plant growth, and maturity (Savage et al., 2016). By enhancing the qualities of the seed and increasing its vigour, seed priming treatments promote seed germination and crop establishment in the field (Paparella et al., 2015). The study investigated the impact of different treatments on the growth and flowering of okra plants, focusing on plant height, number of branches, time to first flower, and days to 50 per cent flowering. At 45 and 75 days after sowing (DAS), significant differences in plant height were observed. Treatment T3 (PEG 6000 13.5% (-0.25MPa) 6 hours) resulted in the maximum plant height (115.65cm), followed by T8 (Pseudomonas fluorescens @ 10g/kg of seed) with 110.90cm. Similar findings were reported in okra by Ali et al. (2016) with higher osmotic potential PEG. The number of branches per plant varied significantly among treatments, with the control having the least (2.86), and T8 showing the highest (3.40). T8, involving Pseudomonas fluorescens, exhibited the earliest first flower emergence (33.40), followed by T7 (Trichoderma viride @ 4g/kg of seed - 35.66). Delayed flowering was noted in T1 (39.80), in line with T2. These results align with previous studies using liquid formulations of bioagents. Dry formulations of bioagents in the present study, consistent with Rai et al. (2019) and Bindu (2020), were found effective for seed treatment when considering subsequent seasons. The application of Pseudomonas fluorescens positively impacted germination and overall plant growth, supporting the findings of Pal et al. (2018). Days to 50 percent flowering were significantly influenced by treatments, with T8 (Pseudomonas fluorescens @ 10g/kg of seed) exhibiting the shortest period (48 days), similar to T7 and T3. The control took the maximum time (60.33 days). Pseudomonas fluorescens at 10g/kg positively influenced flowering in okra, consistent with previous studies (Sivasankari and Devi, 2016; Chaurasia and Bara, 2018). In conclusion, the study suggests that applying Pseudomonas fluorescens and other bioagents, especially in dry formulations, positively influences the growth and flowering of okra, with potential implications for seed storage and subsequent seasons.

Pseudomonas fluorescens, identified as a plant growth-promoting rhizobacteria (PGPR), demonstrates versatility in promoting growth and nutrient absorption in the rhizosphere, according to David et al. (2018). Its effectiveness is often plant-specific, as highlighted by studies from Bashan (1998), Gupta et al. (2000), and Lucy et al. (2004), emphasizing the influence on particular plant species, cultivars, and genotypes. PGPR impacts plant growth through direct and indirect mechanisms, elucidated by Glick (1995) and Gupta et al. (2000). These mechanisms include: (1) enhancing mineral nutrient solubilization and nitrogen fixation, improving nutrient accessibility; (2) suppressing soilborne pathogens through hydrogen cyanide, siderophores, antibiotics, and nutrient competition; (3) increasing plant stress tolerance to conditions like drought, salinity, and metal toxicity; and (4) synthesizing phytohormones such as indole-3-acetic acid (IAA). Some PGPR also possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase, breaking down ACC, the immediate precursor of ethylene in plants. This action reduces ethylene concentration in seedlings, mitigating its inhibitory effect and promoting seedling root length.

Treatment T8 (Pseudomonas fluorescens @ 10g/kg) exhibited the highest number of seeds per fruit, followed by T7 (Trichoderma viride 4g/kg of seed - 47) and T3 (PEG 6000 13.5% (0.25MPa) 6 hours). The precise mechanisms by which plant growth-promoting rhizobacteria (PGPR) enhance plant growth are not fully elucidated but may involve hormone production (auxins, cytokinins, and gibberellins), inhibition of ethylene production, symbiotic N2 fixation, nutrient solubilization, antagonism against pathogens through siderophore production, synthesis of antibiotics and fungicidal compounds, and competition with harmful microorganisms (Caballero-Mellado et al., 2007). This

comprehensive mechanism accelerated overall plant growth and fruit yield, aligning with the findings of Kaur et al. (2015).In terms of seed yield per plant, T8 (Pseudomonas fluorescens @ 10g/kg) recorded the maximum, followed by T7 (Trichoderma viride 4g/kg of seed), while the control (T1) had the least yield. Enhanced plant growth and yield were associated with delayed leaf senescence, increased photosynthetic efficiency, and the buoyant effects of PGPR, consistent with Rafique et al. (2018). Analysis of 100-seed weight revealed that T8 had the maximum weight (6.83g), followed by T7. The lowest weight was in T2 (Hydration-Dehydration 24 hours), on par with T4 (KNO3 2% 12 hours). This may be attributed to a higher percentage of bold seeds and improved nutrient transfer. The positive effects of biocontrol agents align with the findings of Kaur et al. (2015) and Rafique et al. (2018), emphasizing the favorable impact on okra pod yield and growth.

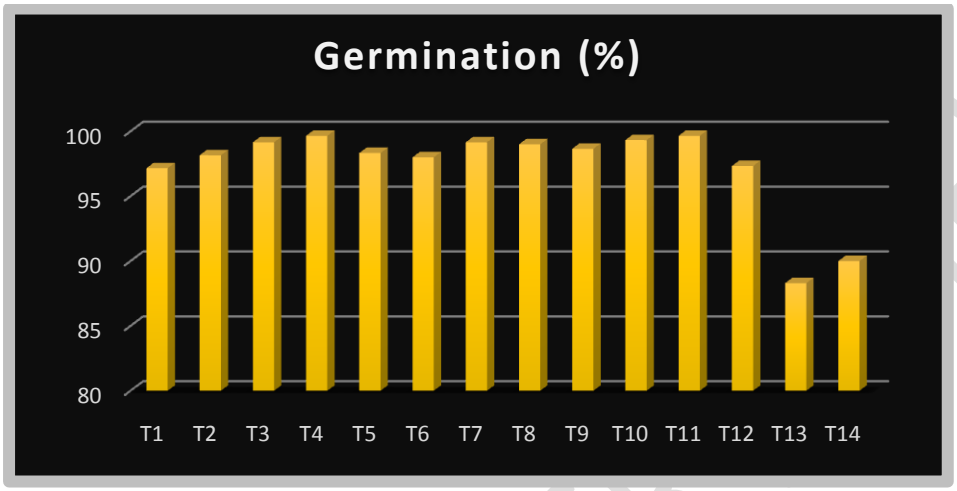

Figure 1. Germination %

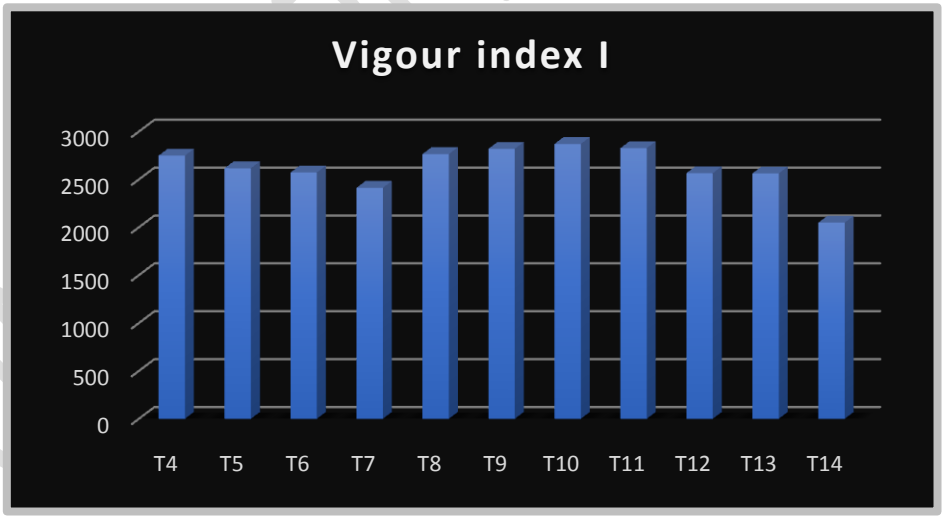

Figure 2. Vigour indexI

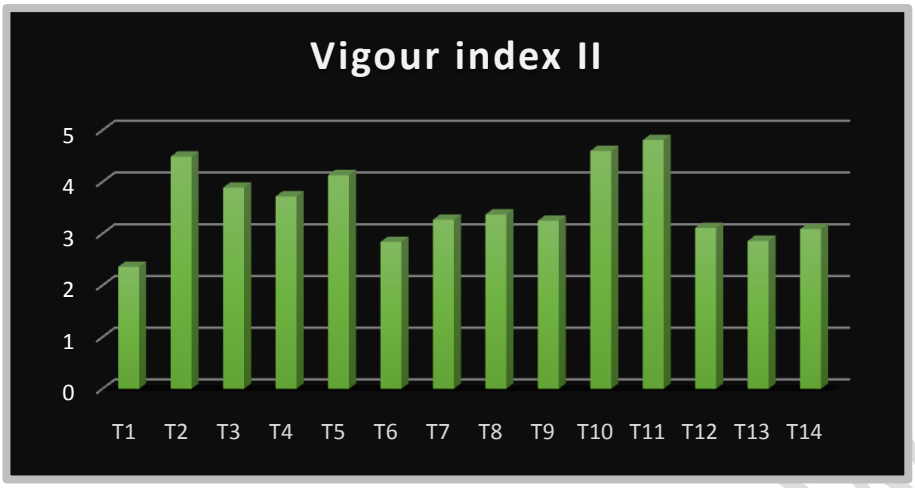

Figure 3. Vigour index II

Treatments	**Plant height**			**Branches per plant**	**Days to 50 % flowering**
	25DAS	45DAS	75DAS		
T1- Control	42.92	70.94	94.82	2.73	60.33
T2- Hydration – dehydration 24 h	41.11	71.23	102.18	3.20	59.33
T3- PEG 6000 13.5 % (0.25MPa) 6h	43.85	75.75	115.65	2.86	50.00
T4- KNO_3 2% 12h	43.83	74.31	103.84	3.06	54.00
T5- Sandmatric 60% WHC- 3h	43.26	72.56	104.36	3.20	51.33
T6- Sandmatric 60% WHC- 6h	42.16	71.96	103.60	2.93	51.33
T7- Trichoderma viride 4g/kg	43.97	73.24	104.52	3.00	49.00
T8- Pseudomonas fluorescens 10g/kg	43.03	73.33	110.90	3.40	48.00
SE(m)	NS	0.406	1.038	0.109	1.221
CD (0.05)	NS	1.23	3.148	0.33	3.703

Table 1. The impact of seed priming on crop development and various growth characteristics

Treatments	Fruits per plant	Fruit length(cm)	Fruits weight(g)	Fruit yield (t/ha)
T1- Control	8.62	11.46	17.69	5.311
T2- Hydration – dehydration 24 h	10.17	16.49	20.04	7.212
T3- PEG 6000 13.5 % (0.25MPa) 6h	10.89	17.08	18.78	7.760
T4- KNO_3 2% 12h	9.82	16.00	18.69	7.030
T5- Sandmatric 60% WHC- 3h	10.00	16.89	19.21	7.519
T6- Sandmatric 60% WHC- 6h	10.08	16.97	21.68	8.858
T7- Trichoderma viride 4g/kg	11.77	18.36	20.07	9.334
T8- Pseudomonas fluorescens 10g/kg	12.31	20.42	22.36	11.081
SE(m)	0.241	0.263	0.535	0.353
CD (0.05)	0.731	0.798	1.624	1.071

Table 2.Impact of seed priming on yield-related parameters and fruit yield

Treatments	Seeds per fruit	Seed yield per plant(g)	100 seed weight(g)
T1- Control	35.06	16.84	5.07
T2- Hydration – dehydration 24 h	35.53	22.94	5.26
T3- PEG 6000 13.5 % (0.25MPa) 6h	43.93	27.04	6.00
T4- KNO_3 2% 12h	36.86	23.6	5.43
T5- Sandmatric 60% WHC- 3h	39.13	21.66	5.53
T6- Sandmatric 60% WHC- 6h	40.86	24.94	5.82
T7- Trichoderma viride 4g/kg	47.00	30.29	6.36
T8- Pseudomonas fluorescens 10g/kg	49.20	32.76	6.73
SE(m)	0.336	0.431	0.06
CD (0.05)	1.018	1.307	0.182

Table 3. **Impact of seed priming on seeds per fruit, seed yield per plant and 100 seed weight(g)**

Conclusion

In conclusion, seed priming treatments, particularly Pseudomonas fluorescens, enhance okra growth. The study demonstrates the versatility of Pseudomonas fluorescens in promoting nutrient absorption and stresses its positive impact on plant parameters, seed yield, and 100-seed weight. These findings signify a promising strategy for sustainable okra production with potential agricultural benefits.

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