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Optimizing Pre-Sowing Treatments for the Enhanced Growth, Fruit Yield, and Seed Quality in *Abelmoschus esculentus* L. Moench

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 vigour 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 (Poly ethylene glycol) 6000-13.5% (-0.25MPa) for 6 hours exhibited the highest germination percentages. *Pseudomonas fluorescens*-10g/kg of seed

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also displayed notable performance. Treatment variations were observed in plant height, branches, and flowering timings. The results suggest that in terms of plant growth and flowering time, *P. fluorescens* was more effective than *Trichoderma viride*. *P. fluorescens* -10g/kg of seed, demonstrated superior results in achieving maximum seed yield parameters, ranking highest overall. *T. viride*-4g/kg of seed, emerged as the second most effective treatment. Recommendations for preserving seed quality included KNO_3 2% for 6 hours and PEG 6000-13.5% (-0.25MPa) for 12 hours. Application of biocontrol agents, especially *P. fluorescens and T. 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.

Keywords: Okra; Pseudomonas fluorescens; Trichoderma viride; seed quality; fruit; seed yield.

1. 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 [1]. Moreover, its noteworthy antioxidant properties, attributed to flavonoids, carotene, and vitamin C, have led to its medicinal use in conditions like diabetes and hyperlipidemia [2].

Okra is a commonly cultivated traditional vegetable in Kerala, albeit on a limited scale, as mentioned by Pradheep et al. in [3]. 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 [4].

India stands as the global leader in okra production, contributing nearly 60% of the world's output [5]. In contrast, Kerala's share is modest, comprising only 0.04% of the total production [6]. Challenges persist in the region, with issues like hardseedness affecting germination and stand establishment [7], contributing to suboptimal crop productivity. Despite India's prominent role in global okra production, challenges persist, with commercial cultivars experiencing only 66% initial germination [8,9], 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 [10,11,12].

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 inosmotic solutions such as PEG orinorganic 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 [13].

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 [14,15].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.

2. MATERIALS AND METHODS

2.1 Experimental Design

Five months old seeds of okra variety – Arka Anamika were subjected to 14 treatments. Initial seed quality parameters were assessed immediately after the treatments using Completely Randomized Design (CRD). Seven best treatments were selected based on germination percentage and vigour index. Seeds from these treatments along with control wereraisedin the field experiment spanning from January 2023 to April 2023. The experimental layout followed a Randomized Block Design (RBD) comprising three replications, each containing ten plants. The individual plot dimensions were 3m × 2m, with a spacing of 60cm × 45cm between plants.The crop management procedures adhered to the prescribed guidelines outlined in the package of practices [16].

2.2 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 76^{o} 26'E longitude with an elevation of about 40m from MSL (Mean Sea Level). The monthly meteorological data collected for the study period October 2022 to August 2023

2.3 Treatment Variables

Five months old okra seeds were treated with fourteen pre sowing treatments such as control(T1), hydration -dehydration 12 hours(T2), hydration – dehydration 24 hours(T3), PEG 6000 13.5 % (-0.25MPa) 6 hours(T4), PEG 600013.5 % (-0.25MPa) 12 hours(T5), KNO_3 2% (6 hours)(T6), KNO_3 2% (12hours)(T7), Sandmatric (60% WHC (water holding capacity) -3 hours)(T8), Sandmatric(60% WHC -6 hours)(T9), *T.viride*-4g/kg of seed(T10), *P. fluorescens* 10g/kg of seed(T11), *T.viride* 4g/kg+ *P.fluorescens* 10g/kg of seed(T12), *Piriformospora indica* 5x10^{5} spores ml^{-1}-5ml/kg(T13), *Piriformospora indica*5x10^{5}spores ml^{-1}-10 ml/kg(T14).

2.4 Seed quality Analysis

Initially, all the seeds of okra variety were subjected to fourteen pre sowing treatments and the seed quality was assessed based on three important parameters such as germination percentage, seed vigour index I and seed vigour index II as detailed below.

2.4.1 Germination (%)

The germination test was conducted as prescribed by International Seed Testing Association (ISTA) [17]. 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.

Germination (%) = (Number of seeds germinated / Total no of seeds sown) × 100

2.4.2 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)

2.4.3 Vigor Index II

Vigor index II was computed as suggested by Abdul- Baki and Anderson [18].

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

2.5 Plant Performance and Yield Analysis

From seed quality analysis, seven best treatments based on germination percentage,

vigour index I and vigour index II were selected for field studies. Selected seven treatments were reassigned treatment nos. *viz*.; T1 (control), T2(hydration-dehydration 24 hours), T3 (PEG 6000 13.5 % (-0.25MPa) 6 hours), T4($KNO_3$2%(12hours), T5(Sandmatric- 60% WHC -3 hours), T6(Sandmatric-60% WHC -6 hours), T7 (*Trichoderma viride*-4g/kg of seed), T8 (*Pseudomonas fluorescens*-10g/kg of seed) and are mentioned in the same order in Tables 1.,2 and 3 in the results section of field trial.

Table 1. Impact of pre-sowing seed treatments on growth attributes of okra (Variety Arka Anamika)

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- *T. viride*-4g/kg	43.97	73.24	104.52	3.00	49.00
T8- *P. 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 2. Impact of pre-sowing seed treatments on fruit characteristics and yield in okra (Variety Arka Anamika)

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- *T. viride*- 4g/kg	11.77	18.36	20.07	9.334
T8- *P. 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 3. Impact of pre-sowing seed treatments on seed attributes and seed yield of okra (Variety Arka Anamika)

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- *T. viride*-4g/kg	47.00	30.29	6.36
T8- *P. 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

In the field experiment, five plants were randomly chosenand labeled in each plot to facilitate observation recording. All pertinent observations were documented during the respective growth stages of the tagged plants and the average value determined.

Plant height (cm) was recorded on 25, 45 and 75 days after sowing (DAS) using a metric scaleandexpressed in centimeter.The total number of branches was recorded on 90DAS by counting the number of branches arising from the main stem.The days taken from sowing to50 % plants in a plot to flower were recorded to determine days to 50% flowering.The total number of fruits harvested from each five tagged plants was counted and average worked out.Fruit length (cm) was measured in ten fruits per plant by using a meter scale from the point of proximal end (stalk end) to the distal end of each fruit. Fruit weight (g) was measured by taking the weight of ten fresh green fruits per plant harvested separately at harvest/commercial maturity.For determining fruit yield in t/ha,the weight of fruits harvested per plot per replication from each harvest was documented and then combined. In each replication, seeds from the fruit of tagged plants were carefully removed and counted to determine seeds per fruit.The seed yield was determined by extracting seeds from the harvested pods of the five tagged plants of each replication, these were weighed, mean computed and expressed in grams.By selecting a random sample of all the seeds from each treatment replication, the 100-seed weight (g) was calculated. One hundred seeds were randomly selected from each seed sample, and their weight was noted in grams.

2.6 Statistical Analysis

The statistical analysis of the data recorded, wasperformed using General R-shiny based Analysis Platform Empowered by Statistics (GRAPES) developed by Kerala Agricultural University [19]. The data obtained from seed quality analysis and field performance were subjected to analysis of variance (ANOVA).According to the design RBD, the pre-sowing treatments were ranked in relation to plant performance in the field trial using Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

The most important elements to guarantee efficient crop establishment and productivity are uniform germination, seedling emergence, seedling vigour, plant growth, and maturity [20]. By enhancing the qualities of the seed and increasing its vigour, seed priming treatments promote seed germination and crop establishment in the field [21]. The present study investigated the impact of different pre-sowing treatments on seed quality, growth and yield of okra var.Arka Anamika and the results are discussed below.

3.1 Impact of Pre-sowing Seed Treatments on Seed Quality

The results of the analysis of variance for seed quality parameters revealed significant variation and the outcomes of seed quality analysis are visually depicted in Figs. 1, 2, and 3, respectively. The details are as follows

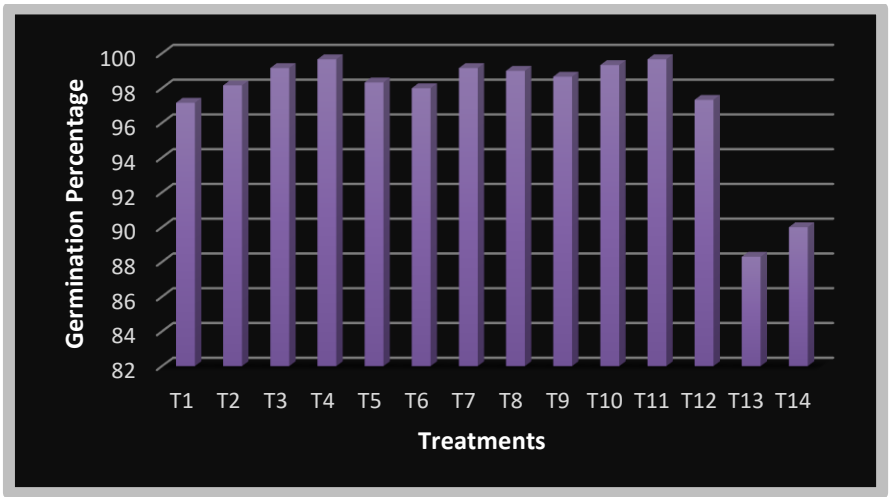

Fig.1. Germination percentage of okra (variety Arka Anamika) after 14 pre- sowing treatments

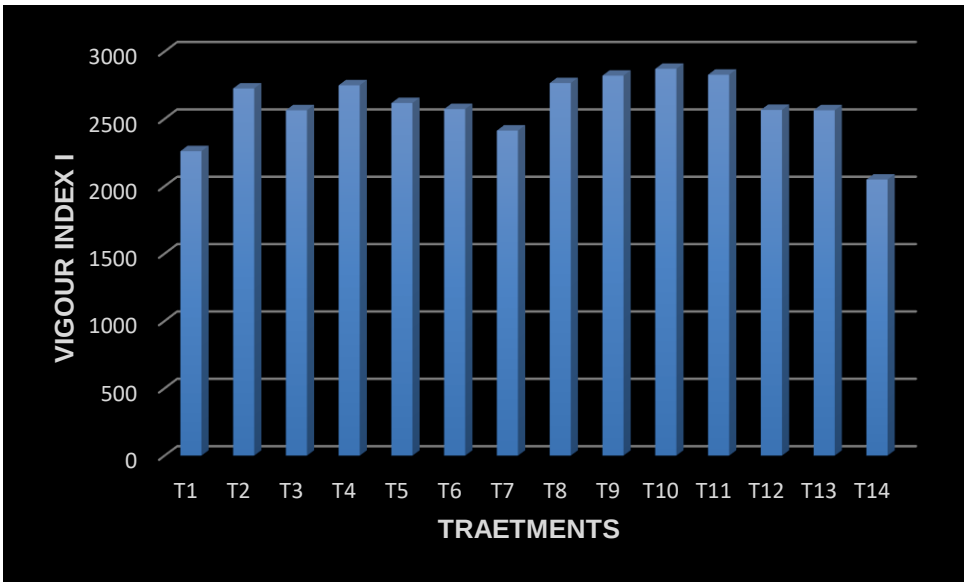

Fig. 2. Vigour indexIof okra (variety Arka Anamika) after 14 pre- sowing treatments

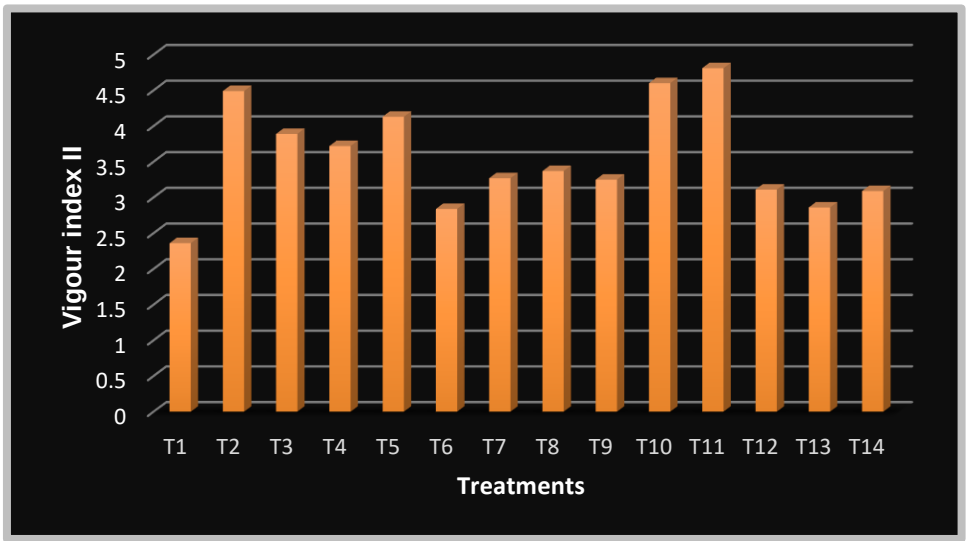

Fig. 3. Vigour index I of okra (variety Arka Anamika) after 14 pre- sowing treatments

The highest germination percentage was observed in seeds treated with PEG 6000 at 13.5% concentration under -0.25MPa for 6 hours (T4), along with favorable outcomes from treatments involving *T. viride* at 4g/kg (T10), KNO3 at 2% for 6 hours (T6), hydration-dehydration for 24 hours (T3), and Sandmatric at 60% water holding capacity (WHC) for 3 (T8) and 6 hours (T9). Additionally, KNO3 at 2% for 12 hours (T7) demonstrated promising results. These findings align with previous research by Sharma et al. [22] in okra for hydropriming and osmopriming. Umarani and Kuppusamy [23] reported similar findings for sandmatric priming in their study .Conversely, the seeds treated with *Piriformospora indica* at $5x10^5$ spores mL-1 with 5ml/kg (T13) and 10ml/kg (T14), exhibited the lowest germination rates, of 88.3% and 90%, respectively, compared to the untreated seeds(Fig. 1).

In terms of vigour indices, *T. viride* at 4g/kg (T10) exhibited the maximum vigour index I (2871.90), comparable to treatments with Sandmatric at 60% WHC for 3 hours (T8) and 6 hours (T9), *P. fluorescens* at 10g/kg of seed, hydration-dehydration for 12 hours(T2), and PEG 6000 at 13.5% (-0.25MPa) for 6 hours(T4). The highest expression of vigour Index II was observed with *P. fluorescens* at 10g/kg of seed (4.81- T11),

followed by *T. viride* at 4g/kg (4.60- T10), PEG 6000 at 13.5% concentration (-0.25MPa) for 12 hours (T5- 4.13), and hydration-dehydration for 24 hours (T3) (3.89), as illustrated in Fig. 2 and Fig 3. Collectively, these results identify seven treatments with the most promising outcomes in both germination and vigour indices.

3.2 Impact of Pre-sowing Seed Treatments on Plant Performance and Yield

Following the seed quality analysis, seven pre-sowing seed treatments demonstrating superior performance were selected for subsequent field analysis. The analysis of variance for these seven treatments, focusing on ten plant characteristics of the okra variety Arka Anamika, revealed noteworthy variations. The impacts of these treatments on growth attributes, fruit yield attributes, and seed yield attributes are detailed in Tables 1, 2, and 3, respectively. The specifics are outlined.

All seed priming treatments enhanced field plant height compared to unprimed seeds (Table.1). Okra seeds primed with PEG 6000-13.5% (-0.25MPa) for 6 hours (T3) showed the highest plant height at 45 and 75 DAS (75.75cm and 115.65cm), statistically similar to *P. fluorescens*-10g/kg (T8) treatment (73.33cm and 110.90cm). In contrast, the control (T1) had significantly lower plant height at 45 and 75 DAS (70.94cm and 94.82cm), akin to hydration-dehydration for 24 hours (T2) at 45 DAS (71.23cm).Comparable findings were reported in okra by Ali et al. [24], where treatments involving PEG with higher osmotic potential demonstrated similar effects on plant height.Seed priming significantly influenced the number of branches per plant, with *P. fluorescens*-10g/kg of seed (T8) displaying the highest count (3.40), statistically similar to hydration-dehydration for 24 hours (T2) and Sandmatric (60% WHC - 3 hours) (T5). Following closely was KNO3 2% for 12 hours (T4). Conversely, the lowest number of branches was observed in PEG 6000-13.5% at -0.25MPa for 6 hours (T3), which was comparable to the control (T1). These findings are consistent with earlier research by Rai et al. [25] and Bindu [26], which focused on liquid formulations of bioagents. Notably, our current investigation deviates by utilizing dry formulations of bioagents. For seeds intended for storage and subsequent season sowing, the efficacy of seed treatment in the form of dry powder coating with bioagents has been found to be more effective, as reported by Afzal et al. [27]. The application of *P. fluorescens* positively impacted germination and overall plant growth, supporting the findings of Pal et al. [28].

In the treatments, T8 (*P. fluorescens*-10g/kg of seed) displayed the earliest 50% flowering at 48 days, akin to T7 *(T. viride*-4g/kg of seed - 49) and T3 (PEG 6000-13.5% (-0.25MPa) for 6 hours - 50). Unprimed seeds exhibited delayed 50% flowering at 60.33 days. *P. fluorescens*-10g/kg positively influenced okra flowering, aligning with prior studies [29].

Pre-sowing treatments significantly impacted all the fruit attributes and fruit yield per hectare in okra (Arka Anamika variety) Compared to unprimed seeds, all seed priming treatments increased the number of fruits per plant. Fruit length was notably influenced by seed priming (Table 2). Treatment T8 (*P. fluorescens*-10g/kg of seed) recorded the highest number of fruits per plant, similar to T7 (*T. viride*-4g/kg of seed). The control (T1) had the lowest number of fruits per plant at 8.62. Additionally, T8 exhibited the maximum fruit length at 20.42cm. Unprimed seeds (T1) had significantly shorter fruit length at 11.46cm, followed by T4 (KNO3 2% 12 hours) which was statistically similar to T2 (hydration-dehydration 24 hours). T8 (*P. fluorescens*-10g/kg of seed) exhibited the highest fruit weight at 22.36g, similar to T6 (Sandmatric 60% WHC 6 hours - 21.68g). The minimum fruit weight was observed in T4 (KNO3 2% 12 hours - 18.69g) and T1 (control - 17.69g). Similar outcomes for fruit attributeswere reported by Adersh [30], Nishitha [31], Rai et al. [32], and Bindu [33].

In this study, T8 (*P. fluorescens* at 10g/kg) exhibited the highest early fruit yield at 11.27t/ha, with T7 (*Trichoderma viride* at 4g/kg) and T3 (PEG 6000 at 13.5%) following closely. The study, emphasizing seed production, concurs with the Indian Institute of Horticultural Research's reported yield of 20 tons per hectare for Arka Anamika in terms of fruit production.

Various pre-sowing treatments, significantly impacted seeds per fruit, seed yield per plant, and 100-seed weight (g). As evident in Table 3, the highest values for seeds per fruit, seed yield per plant, and 100-seed weight (g) were observed in T8 *(P. fluorescens*-10g/kg), followed by T7 (T. viride-4g/kg of seed - 47) and T3 (PEG 6000 13.5% (-0.25MPa) for 6 hours), when compared to all other treatments. Conversely, the minimum values for seeds per fruit, seed yield per plant, and 100-seed weight (g) were

noted in T1 (control) and T2 (hydration-dehydration for 24 hours). This may be attributed to a higher percentage of bold seeds and improved nutrient transfer. The positive effects of biocontrol agents on seed attributes and seed yield align with the findings of Kaur et al. [32] and Rafique et al. [33].

Therefore, the aforementioned findings indicate that pre-sowing seed treatments significantly impacted the seed quality, plant performance, and yield of the okra variety Arka Anamika. Notably, the major outcome underscores the substantial influence of biological seed treatments involving Pseudomonas fluorescens and *T. viride. P. fluorescens* consistently demonstrated superior performance across various attributes throughout the growth stages of okra in the present study, suggesting its efficiency in nutrient utilization.

Pseudomonas fluorescens, recognized as a plant growth-promoting rhizobacteria (PGPR), exhibits adaptability in promoting growth and nutrient absorption in the rhizosphere, as noted by David et al. [34]. Its effectiveness is often plant-specific, as highlighted by studies from Bashan [35], Gupta et al. [36], and Lucy et al. [37], emphasizing the influence on particular plant species, cultivars, and genotypes. While the precise mechanisms by which PGPR enhance plant growth are not fully understood, studies by Glick [38] and Gupta et al. [35] have elucidated both direct and indirect mechanisms. These mechanisms encompass enhancing mineral nutrient solubilization and nitrogen fixation to improve nutrient accessibility, suppressing soilborne pathogens through hydrogen cyanide, siderophores, antibiotics, and nutrient competition, increasing plant stress tolerance to conditions like drought, salinity, and metal toxicity, and synthesizing phytohormones such as indole-3-acetic acid (IAA). Furthermore, some PGPR possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks 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 [39].

4. CONCLUSION

The comprehensive findings from the study on okra seeds (variety- Arka Anamika) subjected to various pre-sowing treatments reveal significant impacts on several growth and yield parameters. Among the treatments, PEG 6000 at 13.5% concentration under -0.25MPa for 6 hours (T3), *P. fluorescens*-10g/kg of seed (T8), and *T. viride* at 4g/kg (T10) consistently showed positive effects across multiple parameters. In conclusion, the adoption of biological seed treatments and priming, particularly utilizing eco-friendly and cost-effective options such as *P. fluorescens* and *T. viride,* emerges as a promising approach for farmers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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