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BIOPRIMING OF *INDIGOFERA TINCTORIA* L. SEEDS FOR IMPROVEMENT IN SEED GERMINATION CHARACTERISTICS

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ABSTRACT

Indigofera tinctoria seed possesses hard seed coat and thick integument which hampers uniform seed germination and establishment. In this study the effectiveness of dormancy breaking treatments combined with microbial bio-priming for improving germination and seedling vigour in indigo plant was evaluated. Seed was subjected to dormancy breaking treatments using sand, boiling water and con. sulphuric acid followed by microbial pre-treatments with *B. pumilus*, *B. amyloliquefaciens*, *B. velezensis* and a consortium of these three bacteria. Results revealed that seed pre-treatment using the bacterial strain *B. pumilus* VLY 17 consistently enhanced shoot quality characteristics *viz*., shoot length (7.35 ± 0.21 cm), number of branches (4.00 ± 0.00), nodes (5.50 ± 0.71) and leaves (14.50 ± 0.71) along with moderate root length (17.15±0.49 cm) and germination percentage (62.00±1.41). This study emphasizes the role of microbial bio-priming in improving seed quality characteristics.

Key words: *Bacillus*, Bacterial consortium, Indian Indigo, Pre-treatments, Scarification.

Introduction

The true indigo plant, *Indigofera tinctoria* L., holds dual significance as a commercially vital dye source and a powerful medicinal plant (Marquiafavel *et al*., 2009). Across various traditional medical systems, its leaves and roots are prized, notably for their hepatoprotective qualities (Renukadevi and Kumar, 2011). The plant is utilized for a wide spectrum of bioactivities, acting as an antipyretic, anti-inflammatory, antiviral, and antimicrobial agent. Systemically, it addresses conditions such as asthma, bronchitis, and disorders affecting the liver, stomach, and nervous system (Gerometta *et al*., 2020). Despite its declining use as a commercial dye, the sustained scientific focus on *I. tinctoria*'s medicinal attributes underscores its unique and enduring value proposition. However, its commercial cultivation is hindered by several seed-related challenges such as seed dormancy, poor quality and germination, non- uniform germination and poor establishment of seedlings. Inherently, the hard seed coat of indigo seeds restricts germination, while the thickness of integument affects seedling emergence and early establishment (Somrug *et al*., 2021).

Developing effective seed pre-treatment techniques is therefore essential to enhance germination, breaking dormancy, and improve early seedling vigour. According to Bahrasemani *et al*., (2023) hydropriming of *I. tinctoria* with putrescine (0.5 mM) effectively mitigated the negative effects of salinity stress by improving germination (31%), weight vigour index (36%), and seedling root and shoot length (27 and 19 % respectively) compared to the control. Kumaran *et al*. (2025) reported that sandpaper-based mechanical scarification for 15 minutes greatly improved *I. tinctoria* seed performance, resulting in the highest germination (~80%) and enhanced seedling biomass with greater shoot length (4.36 cm), root length (2.71 cm), and vigour index (566.4).

Nursery-based seedling production followed by transplantation offers a practical strategy to shorten the period required for field establishment, mitigate adverse climatic effects, and reduce the cost of operations such as irrigation, gap filling, and weeding. Furthermore, such approaches may help minimize early season pest and disease incidence, thereby improving crop survival and growth. Integrating physical or chemical dormancy breaking methods with microbial pre-treatments can substantially improve seed and seedling performance (Taylor and Harman, 1990). The present study evaluated the combined effectiveness of integrating conventional dormancy breaking methods with microbial bio-priming strategies to optimize germination and seedling vigour in *I. tinctoria*.

Materials and Methods

Indigo plant seed was subjected to seed dormancy breaking treatments *viz.*, mechanical scarification using sand (S_1), boiling water dip for one second (S_2) and concentrated sulphuric acid dip for four minutes (S_3) followed by bacterial pre-treatments with *Bacillus pumilus* VLY 17(T_1), *B. amyloliquefaciens* VLY 24 (T_2), *B. velezensis* PCSE 10(T_3) and bacterial consortium [*B. pumilus* VLY17+ *B. amyloliquefaciens* VLY 24+ *B. velezensis* PCSE 10] (T_4). Seed without dormancy breaking treatment (S_4) and bacterial pre-treatment (T_4) was maintained as control.

It was sown in protrays filled with potting media composed of coirpith compost and farmyard manure (1:1). The protrays were maintained in open conditions for 30 days and adequately irrigated as and when required. Germination percentage of bio-primed seed and quality characteristics of indigo seedlings *viz.*, shoot length, root length, number of nodes, number of branches and number of leaves were observed from the treated seedlings and control.

Germination Percentage

Germination percentage is an estimation of number of viable seed within a population (Kuntal, 2014). Seed germination percentage was calculated by counting the number of seeds that produced normal seedlings out of the total seeds sown after 14 days. It was computed using the following formula and expressed in percentage (%).

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds initially sown}} \times 100$$

Shoot Length of Seedling (cm)

The shoot length was measured from the collar region of the seedling (at the soil surface) to the tip of fully opened young leaf using a ruler or measuring tape. The mean length was calculated and recorded as shoot length per plant in centimeter (cm).

Root Length of Seedling (cm)

The seedlings were uprooted without causing any root damage, and length of the longest root was measured from the collar region of seedling using a ruler or tape. The mean root length was calculated and represented in centimeter (cm).

Number of Nodes per Seedling

Number of nodes per seedling was determined by counting the total number of distinct nodes present along the main stem of each seedling from the base to the apex. The mean value was recorded as the number of nodes per seedling.

Number of Branches per Seedling

Branches emerged from main stem of the observational plant was counted and its mean value was recorded.

Number of Leaves per Seedling

Number of leaves was recorded by counting all fully

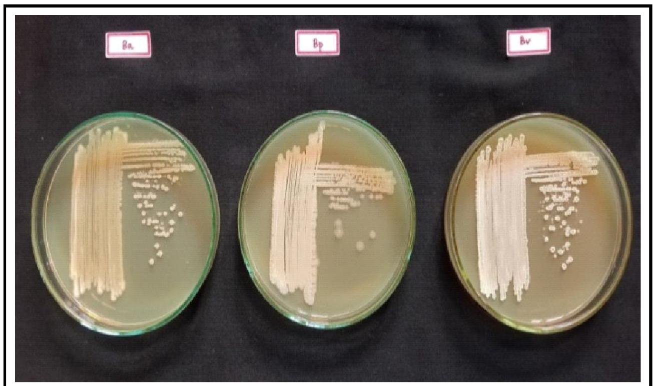

Plate 1. Pure culture of *Bacillus amyloliquefaciens* VLY 24, *B. pumilus* VLY 17 and *B. velezensis* PCSE 10.

Plate 2. *I. tinctoria* seeds after dormancy breaking methods and bio-priming treatments

Table 1: Effect of seed dormancy and *Bacillus* spp. pre-treatments on germination percentage, shoot length, root length, number of branches, number of nodes, and number of leaves of *I. tinctoria* at 30 DAS.

	Germination percentage	Shoot length	Root length	No. of branches	No. of nodes	No. of leaves
$S_1 \times T_1$	77.00 ± 1.41^{a}	6.40 ± 0.14^{bcd}	11.75 ± 0.49^{m}	4.00 ± 0.00^{a}	6.00 ± 0.00^{a}	13.00 ± 1.41^{ab}
$S_1 \times T_2$	79.00 ± 1.41^{a}	6.30 ± 0.14^{cde}	11.75 ± 0.21^{m}	3.50 ± 0.71^{ab}	5.50 ± 0.71^{ab}	13.00 ± 1.41^{ab}
$S_1 \times T_3$	76.50 ± 0.71^{a}	5.95 ± 0.21^{efg}	14.05 ± 0.21^{kl}	2.50 ± 0.71^{cd}	4.50 ± 0.71^{bcd}	10.00 ± 2.83^{cdef}
$S_1 \times T_4$	79.50 ± 2.12^{a}	6.50 ± 0.42^{bc}	$16.50 \pm .57^{ghi}$	3.00 ± 0.00^{bc}	4.50 ± 0.71^{bcd}	12.00 ± 1.41^{abc}
$S_1 \times T_5$	78.00 ± 1.41^{a}	6.55 ± 0.21^{bc}	19.95 ± 1.20^{ab}	2.50 ± 0.71^{cd}	4.50 ± 0.71^{bcd}	8.00 ± 0.00^{ef}
$S_2 \times T_1$	59.00 ± 2.83^{de}	5.40 ± 0.14^{hij}	$18.50 \pm .28^{cde}$	3.00 ± 0.00^{bc}	4.50 ± 0.71^{bcd}	12.00 ± 1.41^{abc}
$S_2 \times T_2$	67.00 ± 1.41^{b}	6.80 ± 0.14^{b}	$18.15 \pm .35^{def}$	3.50 ± 0.71^{ab}	4.50 ± 0.71^{bcd}	13.00 ± 0.00^{ab}
$S_2 \times T_3$	67.00 ± 2.83^{b}	4.95 ± 0.21^{k}	14.40 ± 0.42^{kl}	2.00 ± 0.00^{d}	3.50 ± 0.71^{d}	8.00 ± 0.00^{ef}
$S_2 \times T_4$	65.00 ± 2.83^{bc}	5.75 ± 0.21^{fgh}	$18.95 \pm .49^{bcd}$	3.00 ± 0.00^{bc}	4.00 ± 0.00^{cd}	13.00 ± 0.00^{ab}
$S_2 \times T_5$	53.00 ± 2.83^{fg}	5.20 ± 0.14^{ijk}	17.55 ± 0.35^{ef}	2.00 ± 0.00^{d}	3.50 ± 0.71^{d}	7.50 ± 0.71^{f}
$S_3 \times T_1$	$64.00 \pm .41^{bcd}$	5.40 ± 0.14^{hij}	16.40 ± 0.14^{hi}	2.00 ± 0.00^{d}	3.50 ± 0.71^{d}	8.00 ± 0.00^{ef}
$S_3 \times T_2$	63.00 ± 2.83^{bcd}	5.15 ± 0.07^{ijk}	12.50 ± 0.42^{m}	2.00 ± 0.00^{d}	4.00 ± 0.00^{cd}	10.00 ± 0.00^{cdef}
$S_3 \times T_3$	63.50 ± 3.54^{bcd}	6.60 ± 0.14^{bc}	20.95 ± 0.21^{a}	3.50 ± 0.71^{ab}	5.00 ± 0.00^{abc}	13.00 ± 0.00^{ab}
$S_3 \times T_4$	66.50 ± 3.54^{b}	6.00 ± 0.28^{def}	13.85 ± 0.49^{l}	2.50 ± 0.71^{cd}	4.50 ± 0.71^{bcd}	11.50 ± 2.12^{bcd}
$S_3 \times T_5$	55.00 ± 2.83^{ef}	5.95 ± 0.21^{efg}	15.55 ± 0.49^{ij}	2.00 ± 0.00^{d}	4.00 ± 0.00^{cd}	10.50 ± 0.71^{bcde}
$S_4 \times T_1$	62.00 ± 1.41^{bcd}	7.35 ± 0.21^{a}	17.15 ± 0.49^{fgh}	4.00 ± 0.00^{a}	5.50 ± 0.71^{ab}	14.50 ± 0.71^{a}
$S_4 \times T_2$	63.50 ± 2.12^{bcd}	6.25 ± 0.21^{cde}	19.20 ± 0.57^{bc}	3.50 ± 0.71^{ab}	4.00 ± 0.00^{cd}	10.50 ± 0.71^{bcde}
$S_4 \times T_3$	60.00 ± 2.83^{cde}	5.10 ± 0.00^{jk}	17.45 ± 0.49^{fg}	2.00 ± 0.00^{d}	3.50 ± 0.71^{d}	10.00 ± 0.00^{cdef}
$S_4 \times T_4$	60.50 ± 2.12^{cd}	5.55 ± 0.21^{ghi}	18.65 ± 0.49^{cd}	2.50 ± 0.71^{cd}	4.50 ± 0.71^{bcd}	11.50 ± 2.12^{bcd}
$S_4 \times T_5$	48.00 ± 2.83^{g}	4.90 ± 0.00^{k}	15.00 ± 0.28^{jk}	3.00 ± 0.00^{bc}	3.50 ± 0.71^{d}	9.00 ± 1.41^{def}
CD (A×B)	5	0.41	1.01	0.93	1.23	2.51
SE(m)	1.70	0.14	0.34	0.32	0.42	0.85
CV(%)	3.67	3.31	2.96	15.97	13.60	11.05

expanded leaves present on each seedling at 30 days after sowing. The mean count was taken as the number of leaves per seedling.

Statistical Analysis

The experimental design used for the study was completely randomized design (CRD) and the analysis of variance was calculated using the web application Kerala Agricultural University (KAU) GRAPES Agri1 package based on R programming language (Gopinath *et al.*, 2021; R core team, 2024).

Results

Germination percentage, shoot length, root length, number of branches, number of nodes, and number of leaves in *I. tinctoria* seedlings with response to seed dormancy and *Bacillus* spp. pre-treatments at 30 days after sowing (DAS) are found to be significantly different and presented in Table 1.

Germination Percentage

Germination percentage of *I. tinctoria* in response to seed dormancy breaking methods and bacterial pre-treatments at 30 DAS differed significantly (Graph 1). The highest germination percentage (79.50± 2.12) was recorded in sand-scarified seeds treated with the bacterial consortium (S_1T_4). This treatment was on par with sand-scarified seed treated with *B. amyloliquefaciens* VLY 24 (S_1T_2) (79.00 ± 1.41), sand scarification without bacterial pre-tretment (S_1T_5) (78.00 ± 1.41), sand scarification with *B. pumilus* VLY 17 (S_1T_1) (77.00 ± 1.41) and sand scarification with *B. velezensis* PCSE10 (S_1T_3) (76.50 ± 0.71). The lowest germination percentage was observed in untreated control seeds (S_4T_5) (48.00 ± 2.83) which was on par with seed scarified using boiling water for one second (S_2T_5) (53.00 ± 2.83).

Shoot Length of Seedlings

Shoot length of indigo seedling showed significant

Plate 3. *I. tinctoria* seedlings in portrays 30DAS.

difference in response to the interaction effect of seed dormancy breaking methods and biopriming treatments (Graph 2). The maximum shoot length was recorded in seed treated with *B. pumilus* VLY 17 (S_4T_1) (7.35 ± 0.21 cm) followed by seed dipped in boiling water for one second and then treated with *B. amyloliquefaciens* VLY 24 (S_2T_2) (6.80 ± 0.14 cm), sand scarified seed (S_1T_5) (6.55 ± 0.21 cm), sand scarified seed treated with bacterial consortium (S_1T_4) (6.50 ± 0.42 cm), sulphuric acid treated seed followed by *B. velezensis* PCSE10 (S_3T_3) (6.60 ± 0.14 cm) and sand scarified seed treated with *B. pumilus* VLY 17 (S_1T_1) (6.40 ± 0.14 cm). The minimum shoot length was noticed in untreated control plants (S_4T_5) (4.90 ± 0.00 cm) and in seed dipped in boiling water for one second followed by *B. velezensis* PCSE10 (S_2T_3) (4.95 ± 0.21 cm). These treatments were on par with boiling water dip for one second (S_2T_5) (5.20 ± 0.14 cm), sulphuric acid treated seed pre-treated with *B. amyloliquefaciens* VLY 24 (S_3T_2) (5.15 ± 0.07 cm) and seed treated with *B. velezensis* PCSE10 (S_4T_3) (5.10 ± 0.00 cm).

Root length of seedlings

A significant interaction effect of seed dormancy-breaking methods and biopriming treatments was observed on root length of indigo seedlings. Seed scarified using con. sulphuric acid for four minutes and sprayed with *B. velezensis* PCSE10 (S_3T_3) was found to be significantly superior (20.95 ± 0.21 cm). This treatment was on par with sand scarified seed (S_1T_5) (19.95 ± 1.20 cm). Sulphuric acid treated seed pre-treated with *B. amyloliquefaciens* VLY 24 (S_3T_2), sand scarified seed treated with *B. pumilus* VLY 17 (S_1T_1) and sand-scarified seed treated with *B. amyloliquefaciens* VLY 24 (S_1T_2) showed significantly lower values, 12.50 ± 0.42, 11.75 ± 0.49 and 11.75 ± 0.21 respectively.

Number of branches per seedling

The number of branches of *I. tinctoria* in response to seed dormancy breaking methods and bacterial pre-treatments at 30 DAS differed significantly (Graph 3). Seed scarified using sand and sprayed with *B. pumilus* VLY 17 (S_1T_1) as well as untreated seed sprayed with *B. pumilus* VLY 17 (S_4T_1), were found to be significantly superior (4.00 ± 0.00). These were on par with S_1T_2 (sand scarification + *B. amyloliquefaciens* VLY 24), S_2T_2 (boiling water dip + *B. amyloliquefaciens* VLY 24), S_3T_3 (H_2SO_4 scarification + *B. velezensis* PCSE10) and S_4T_2 (*B. amyloliquefaciens* VLY 24 alone) which recorded 3.50 ± 0.71 branches. Significantly lower number of branches, 2.00 ± 0.00 were observed in treatments S_2T_3 (boiling water dip + *B. velezensis* PCSE10), S_2T_5 (boiling water dip), S_3T_1 (H_2SO_4 scarification + *B. pumilus* VLY 17), S_3T_2 (H_2SO_4 scarification + *B. amyloliquefaciens* VLY 24), S_3T_5 (H_2SO_4 scarification) and S_4T_3 (*B. velezensis* PCSE10).

Plate 4. *I. tinctoria* seedling 30 DAS.

Number of nodes per seedling

The number of nodes of indigo seedling showed significant difference due to the interaction effect of seed dormancy breaking methods and biopriming treatments. Seed scarified using sand and sprayed with *B. pumilus* VLY 17 (S_1T_1) was found to be significantly superior (6.00 ± 00). This was on par with S_1T_2 (sand scarification + *B. amyloliquefaciens* VLY 24), S_4T_1 (*B. pumilus* VLY 17 treated seed) which recorded 5.50 ± 0.71number of nodes and with S_3T_3 (conc. sulphuric acid + *B. velezensis* PCSE10), 5.00 ± 0.00 number of nodes. All other treatments recorded significantly lower or comparable values with lower means.

Number of leaves per seedling

Seed without scarification and sprayed with *B. pumilus* VLY 17 (S_4T_1) (14.50 ± 0.71) was found to be significantly superior and on par with S_1T_1 (sand scarification + *B. pumilus* VLY 17), S_1T_2 (sand scarification + *B. amyloliquefaciens* VLY 24) (13.00 ±

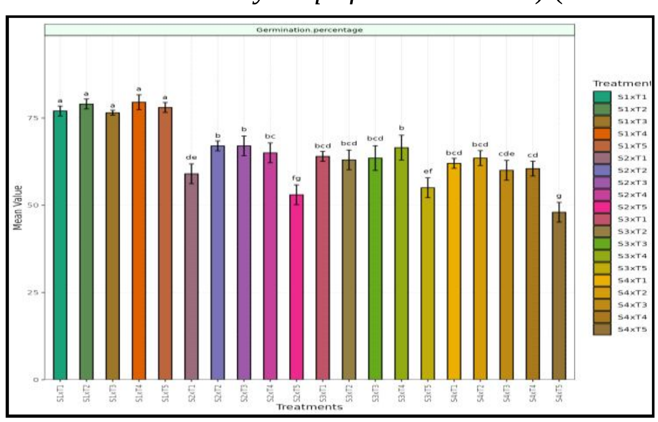

Fig. 1: Effect of seed dormancy breaking treatments and microbial biopriming on germination percentage of *I. tinctoria* seed.

1.41), S_2T_2 (boiling water dip + *B. amyloliquefaciens* VLY 24), S_2T_4 (boiling water dip + bacterial consortium), S_3T_3 (conc. sulphuric acid + *B. velezensis* PCSE10) (13.00 ± 0.00), S_1T_4 (sand scarification + bacterial consortium) and S_2T_1 (boiling water dip + *B. pumilus* VLY 17) (12.00 ± 1.41). Seed scarified using boiling water for one second recorded significantly lower values (7.50 ± 0.71). This was on par with S_1T_5 (sand scarification alone), S_2T_3 (boiling water dip + *B. velezensis* PCSE10), S_3T_1 (conc. sulphuric acid + *B. pumilus* VLY 17) (8.00 ± 0.00), S_1T_3 (sand scarification + *B. velezensis* PCSE10) (10.00 ± 2.83), S_3T_2 (conc. sulphuric acid + *B. amyloliquefaciens* VLY 24) (10.00 ± 0.00), S_4T_3 (*B. velezensis* PCSE10 alone) (10.00 ± 0.00) and S_4T_5 (Untreated control) (9.00 ± 1.41).

Discussion

The scope of using endospore-forming bacteria is considered to be worthwhile for quality seedling production. Endophytes could enhance host plant growth, nutrient gain, improve abiotic and biotic stress tolerance through production of secondary metabolites and disease suppression (Gouda *et al*., 2016; Khan *et al*., 2020).

Integration of dormancy breaking treatments and microbial bio-priming significantly influenced germination and early seedling vigour in *I. tinctoria*. In the present study, the highest germination percentage was recorded in sand-scarified seeds treated with the bacterial consortium (79.5± 2.12). Maximum root length was recorded (20.95 ± 0.21cm) in sulphuric acid-scarified seed pre-treated with *B. velezensis*. Enhanced shoot length (7.35 ± 0.21 cm) and leaf number (14.50 ± 0.71) were noted in non-scarified seed treated with *B. pumilus*, whereas the highest number of branches (4.00 ± 0.00) and nodes per seedling (6.00 ± 0.00) was observed in sand-scarified seed treated with *B. pumilus*. However, for number of branches and nodes, non scarified seed with *B. pumilus was statistically on par with the highest values. This* indicates that non-scarified seed treated with *B. pumilus* provide significant enhancement in shoot characteristics *viz*., shoot length, number of branches, nodes and leaves.

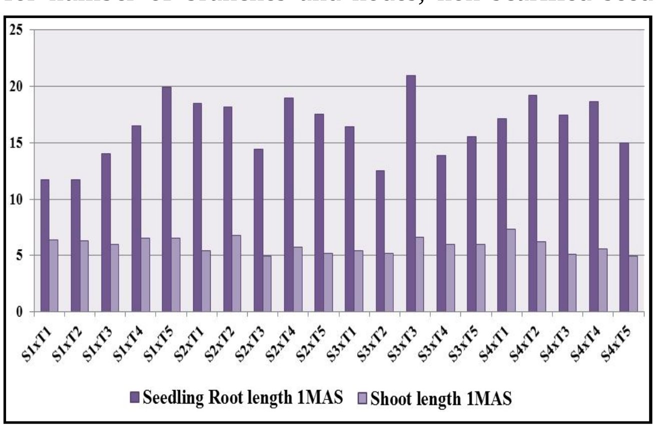

Fig. 2: Effect of seed dormancy breaking treatments and microbial biopriming on shoot length (cm) and root length (cm) of *I. tinctoria* seedlings.

The bacterial endophyte *B. pumilus* was demonstrated to promote growth in *Ocimum sanctum*, resulting in increased root (3.8±0.483 cm) and shoot length (13.6±0.658 cm) along with a greater number of leaves (8.0±0.408) (Murugappan *et al*., 2013). Moreover, the efficacy of *B. pumilus* has been established to support seedling growth in tomato (Anith *et al*., 2015). *B. pumilus* was demonstrated to significantly enhance the overall growth, root development, and nutrient absorption capacity in 21 days old rice seedlings, performing notably better than the untreated control group (Ngo *et al*., 2019). It is suggested that *B. pumilus* can enter through the root epidermis, traverse the cortical tissues to reach the vascular system, and subsequently migrate and colonize in the leaves as an endophyte. Meanwhile, it produces indole-3-acetic acid (IAA) which facilitates improved nutrient absorption and promotes growth.

Seed dormancy breaking treatments combined with the *Bacillus* consortium resulted in improved germination percentage compared to untreated seed [Graph 1]. Sand scarification irrespective of the microbes used for pre-treatment was statistically on par for germination percentage, highlighting the advantage of physical abrasion coupled with the possible synergistic microbial influence on hormonal activity, nutrient mobilization, and enzyme secretion. Neema *et al*., (2018) also opinied that sand scarification is the most effective mechanical scarification treatment for breaking dormancy in *I. tinctoria*. *Bacillus* species have advantages over other microorganisms due to their endospore-forming ability, which ensures survival under adverse conditions. Moreover, it produce plant growth–promoting hormones such as gibberellic acid (GA) and indole-3-acetic acid (IAA), along with other

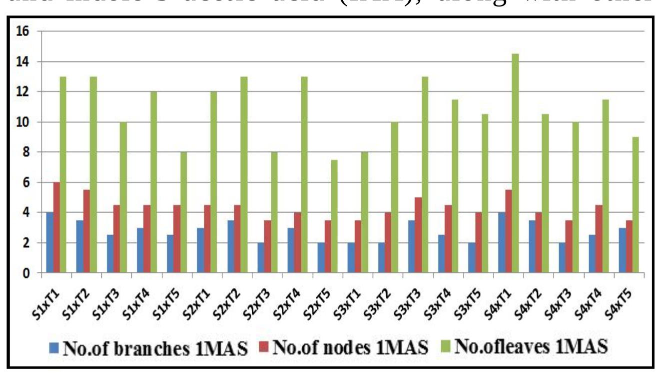

Fig. 3: Effect of seed dormancy breaking treatments and microbial biopriming on number of branches, nodes and leaves of *I. tinctoria* seedlings.

secondary metabolites that may enhance α-amylase activity, promoting early germination through improved starch mobilization and resulting in better seed germination and radicle growth.

Effect of scarification methods and bacterial pre-treatment on root length of seedlings in *I. tinctoria* is represented in Graph 2. It shows that sulphuric acid-scarified seed pre-treated with *B. velezensis* exhibited the higher root length. Studies proved that *B. velezensis* promotes root stimulation, siderophore production, and efficient rhizosphere colonization (Chowdhury *et al*., 2015; Huang *et al*., 2023). Priming sorghum seeds with *B. amyloliquefaciens* improved germination from 83% to 95%, enhanced shoot and root length (16.9 cm and 16.6 cm), boosted overall seedling vigour (seedling vigour index-I (3183), seedling vigour index-II (32234)), and reduced seed infection (pathogen infection- 0.6%) than control seeds. Moreover, those seeds showed lower electrical conductivity than the control, as the bacterium protects the seed coat and thus limits seed infection and electrolyte leakage (Koradhanyamath *et al*., 2024). Additionally, Sharma *et al*., (2015) confirmed that microbial bio-priming improves uniform seedling emergence, seedling robustness, and shoot–root equilibrium in legumes too.

Scientific studies have confirmed that seedling shoot growth characteristics of I. tinctoria have positive influence on overall vegetative performance and biomass yield. In the present study, seed treated with B. pumilus found to exhibit superior shoot characteristics. In addition, it moderately improved germination and root length of indigo seedlings. Hence, it can be concluded that good quality I. tinctoria seedlings can be raised using B. pumilus bioprimed seed. The field evaluation on yield performance of this superior treatment is presently under investigation in the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellayani, Kerala Agricultural University.

Conclusion

The present research examined for the synergistic effect of seed dormancy breaking methods and microbial biopriming in improving seed quality characteristics of *I. tinctoria*. The findings demonstrated that specific microbial bio-priming serves as a highly effective approach to enhance germination and early seedling performance in *I. tinctoria* when compared to the untreated control. The results proved that microbial biopriming using the single strain *B. pumilus* VLY 17 consistently enhanced shoot quality characteristics *viz*., shoot length (7.35 ± 0.21 cm), number of branches (4.00 ± 0.00), nodes (5.50 ± 0.71) and leaves (14.50 ± 0.71) along with moderate root length (17.15±0.49 cm) and germination percentage (62.00±1.41).

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