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AGRONOMY | ORIGINAL ARTICLE

Bio-priming with *Trichoderma viride* and *Pseudomonas fluorescens* Improves Crop Performance in Nepalese Rice Landraces

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ABSTRACT

This study evaluated agronomic responses of indigenous rice landraces to seedling-mediated microbial biopriming in an inner-Terai agroecosystem of western Nepal. A triply replicated experiment in split-plot design comprised four main plots of microorganism treatments – pre-transplant root-dipping of seedlings with individual and combined inoculums of *Trichoderma viride* Pers. ex S.F. Gray and *Pseudomonas flouresens* Migula and unprimed control, and three subplots of rice landraces - Anadi, Simtharo and Tilki. Simtharo bioprimed with the duel microbial culture exhibited the highest grain yield. Anadi and Tilki showed greater response to sole *T. viride* biopriming with 39% and 111% increase in seed yields, respectively, compared to their corresponding controls. The sole biopriming with *P. fluorescens* did not produce a clear effect on the growth and yield of any rice landraces. Simtharo, Anadi and Tilki with increasing maturity days, had mean grain yield of 2.68 t ha^{-1}, 2.14 t $ha^{-1,}$ and Tilki 0.77 t $ha^{-1,}$ respectively. Later maturity was linked to higher grain sterility with a strong negative correlation with grain yield ($r = -0.93$, $p < 0.01$). Further studies should integrate the use of rice landraces of similar maturity class for comparative studies, and utilization of locally isolated microbial strains under organic direct seeding or system of rice intensification (SRI) in the cropping systems approach.

1. Introduction

Indigenous landraces are crop or animal populations evolved under distinct climatic, soil and cultural contexts as components of traditional farming systems. Native landraces are crucial for crop improvement and breeding purposes due to their genetic diversity of desirable traits (Marone et al., 2021). Although modern varieties are high yielding, landraces are adaptive to extreme biotic and abiotic stress (Jonge et al., 2021) and lower soil fertility conditions (Rao et al., 2018). Similarly, landraces exhibit special culinary, medicinal and aromatic characteristics which make them unique than modern hybrids (Villa et al., 2005) and also contribute agroecological balance and supports food security under changing climatic conditions (Maathew et al., 2023). About 2,500 different indigenous rice landraces with different agro-ecological adaptations and quality traits are reported from Nepal, of which 8389 rice accessions have been conserved in nine different gene banks in the world, and sixty countries have received Nepalese rice accessions from the International Rice Research Institute (Bhandari et al., 2017). However, the mismatch of agronomic practices with agroecological context including inappropriate use of fertilizers have engendered soil degradation, acidification and decline in productivity affecting food security in Nepal (Krupnik et al., 2021). Application of excessive synthetic fertilizers to indigenous landraces can lead to luxurious vegetative growth, resulting in lodging, and reduction in grain yield with low harvest index (Hossain et al., 2008). Moreover, the nutrient application misaligned with the crop nutrient uptake results in soil degradation, nutrient losses, profitability decline and negative environmental consequences (Kumar et al., 2022). In the fertilizer-intensive cropping systems, the first crop utilizes about 30 to 50% of applied N and subsequent six consecutive crops can recover less than 7% of the residual N (Ladha et al., 2005). A meta-analysis of 1000 observations from tropical

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field studies showed that tripling of fertilizer N input from 50 kg ha^{-1} to 150 kg ha^{-1} would increase ammonia volatilization, nitrate leaching, nitrous oxide emissions and nitric oxide emissions by 74%, 30%, 30% and 66% respectively (Huddell et al., 2020). Therefore, concerted efforts and strategies have been essential to enhance crop nutrient uptake, recycling and use efficiency in the production systems.

Extensive studies have elucidated the beneficial effects of plant bio-inoculants based on various *Trichoderma* (Woo et al., 2023) and *Pseudomonas* species (Zboralski & Filion, 2023). The genus *Trichoderma* is a ubiquitous, opportunistic fungi belonging to the Ascomycota phylum. These group of fungi have ability to colonize in diverse substrates and pervade in the diverse terrestrial, fresh water and marine ecosystems. With their ability to interact with plants as endophytes as well as rhizosphere colonizers, these fungi exert competition and antagonism against harmful organisms, while eliciting biochemical pathways of phytohormonal and bio-stimulatory secondary metabolites and defense responses against biotic and abiotic stresses (Woo et al., 2023). The trichodermal activation of diverse biochemical pathways also enhances plant photosynthetic capability (Boat et al., 2022), growth vigour (Subramaniam et al., 2022) and root proliferation leading to increased uptake of nutrients (Mehmood et al., 2023). Unlike the *Trichoderma* as fungi, *Pseudomonas* are bacterial genus, of which some species are recognized as plant growth-promoting rhizobacteria (PGPR). The *Pseudomonas* PGPR form the components of rhizospheric and endophytic micro-biomes. They are also known to activate biochemical pathways of phytohormones, lytic enzymes, volatile organic compounds, antibiotics, and secondary metabolites imparting plant stress tolerance, suppression of plant pathogens, and improving growth through fixation of atmospheric nitrogen, solubilization of phosphorus and potassium, and protection from heavy metals and soil pollutants (Rajkumar et al., 2017).

Previous studies have reported beneficial effects of inoculation (biopriming) of rice with *Trichoderma viride* Pers. ex S.F. Gray *Pseudomonas flouresens* Migula. The major reported benefits of *T. viride* inoculant include suppression of brown spot disease caused by *Bipolaris oryzae* by way of gliotoxin production (Harish et al., 2008) and significant increase in rice grain yield (Khadka & Uphoff, 2019). Similarly, biopriming with *P. flouresens* controlled sheath blight caused by *Rhizoctonia solani* Kühn, and increased tiller growth and grain yield (Mathivanan et al., 2005). The biopriming with the combined inoculum of both *T. viride* and *P. flouresens* was as effective as systemic fungicide, Carbendazim in controlling the sheath blight disease of rice (Kabdwal et al., 2023). Only few studies have been done with the use of beneficial micro-organisms on Nepalese landraces under the prevailing bunded, puddled-tillage systems in the hot sub-tropical environment. This study attempted to bridge this gap, and thereby contributing to the scientific knowledge base for utilizing the beneficial micro-organisms as a low-input, cost-effective options under small-holder rice-based farming systems.

It was hypothesized that biopriming with *T. viride* and P. *flouresens* have differential effects on the growth, yield parameters of rice landraces in the puddled rice cultivation systems.

2. Materials and Methods

2.1. Site description

A field experiment was conducted in Tulsipur 13 Fulbari of Dang district in western Nepal from June to November, 2021. Situated around 28.1199° N, 82.2969° E with about 600 m elevation from mean sea level, a sub-tropical agro-climate prevail in the area. The site has adequate irrigation facility, but no risk of flooding.

2.2. Rice landraces

Three rice landraces namely Anadi, Tilki and Simtharo collected from the study area were used for the study. These landraces possess preferable agronomic and culinary values. Anadi has light reddish colored seed with high flour quality for preparing various cuisine and traditional medicinal use in curing factures (Sthapit et al., 2008) and is anecdotally beneficial for diabetic patients. Simtharo is brownish black in seed color, beneficial for diabetes, hypertension and gastritis problems (Sthapit et al., 2008). In author's own experience, Tilki is sweet in taste and used for pudding.

2.3. Strains collection and quality determination

Two microbial inoculants *T. viride and P. flourescens* were obtained from Ilameli biotechnological laboratory, Kathmandu, Nepal. The quality of the inoculums was verified prior to their application.

2.4. Crop management practices

The crop management practices and data collection activities were performed at different dates are illustrated in the Table 1.

Table 1. Dates of various field operations and data collection from the experiment

Field operations	Date	DAS
Nursery establishment	15 Jun 2021	-
Land preparation and transplanting	30 Jun 2021	15
First data collection	20 Jul 2021	35
First weeding	30 Jul 2021	36
Second data collection	10 Aug 2021	56
Second weeding	15 Aug 2021	68
Third data collection	30 Aug 2021	76
Harvest		
Anadi	11 Oct 2021	118
Simtharo	1 Nov 2021	139
Tilki	14 Nov 2021	152

DAS: day after seeding

2.4.1. Nursery establishment

Seedlings of all three landraces were raised under modified dapog method. First, admixture of cocopeat and well rotten farm yard manure (1:1) was spread over the cemented floor up to 15 cm of height followed by levelling. Seeds soaked for 12 hours were spread over the growing media and then covered about 3 cm with the medium. Proper growing media moisture was maintained through frequent irrigation.

2.4.2. Land preparation

Three months fallow land was ploughed using animal powered mould-board plough ensuring the depth of 15 cm followed by harrowing to break the clods and left for one week to for solarization. Secondary tillage was also performed by same implements to obtain desirable tilth. Puddling was performed using hand manual implements such as hoe and spade.

2.4.3. Seedling biopriming

The liquid media with individual and combined cultures of *T. viride* and *P. flourescens* were poured in different plastic troughs. The roots of 14-day old seedlings were dipped into the solution for 20 minutes followed by immediate transplanting to the field.

2.4.4. Seedling transplanting

The seedlings bioprimed with different inoculants and un-primed controls were transplanted into the bund-separated plots manually ensuring the two seedlings per hill. Crop geometry was maintained at 20 cm by 20 cm row to row and plant to plant respectively.

2.4.5. Fertilizer application

Basal dose of macronutrients at the rate of 40:60:60 kg NPK/ha in the form of Urea, Diammonium Phosphate and Muriate of Potash fertilizers was broadcast on the puddled soil before rice seedling transplantation.

2.4.6. Weed management

Manual weeding operations were performed at 30 and 45 days of transplantation. Cross mixing of micro-organisms during weeding was controlled by proper sanitation of weeding implements. Separate individuals were assigned to weed each combination of micro-organisms throughout the replicates.

2.4.7. Irrigation

Alternate wetting and drying cycles of irrigation were practiced. After each irrigation, field plots were allowed to dry until the appearance of fine visible cracks on the soil surface. Irrigations were scheduled depending upon the soil moisture and rainfall to improve the soil aeration and root growth.

2.4.8. Crop harvesting

Crop was harvested from 1 m^2 area from net area plot using sickle. Harvested plants were threshed manually followed by cleaning. Due to varied maturity requirements, three cultivars were harvested in different dates (Anadi: 118, Simtharo: 139 and Tilki: 152 DAT).

2.4.9. Crop protection measures

Neem oil (Nibecidine at 3 ml per liter of water) was sprayed at 45 DAT to suppress the infestation of rice stem borer (*Scirpophaga innotata*).

2.5. Experimental design

The two-factor experiment were carried out in split plot design. Four biofertilizer treatments (Control, *T. viride, P. flourescens* and equal combination of both strains) made main plot factors, while three local rice varieties namely Anadi, Tilki and Simtharo constituted sub-plot factors comprising a total of 12 different treatment combinations (Table 2), each replicated three times. Each plot had dimensions of 3.2m x 2m=6.4m^2 consisting of 20 plant rows of 2 m length spaced 20 cm apart (2 border rows, 4 sampling rows, 4 invasive sampling rows and remaining rows for net area plot for yield sampling).

Table 2. The main plot and sub-plot factors constituting 12 treatment combinations in the experiment

S.N.	Main plot factor: Biopriming microbes	Sub-plot factor: Rice landraces
T_1		Anadi
T_2	*T. viride*	Simtharo
T_3		Tilki
T_4		Anadi
T_5	*P. fluorescens*	Simtharo
T_6		Tilki
T_7		Anadi
T_8	*T. viride + P. fluorescens*	Simtharo
T_9		Tilki
T_{10}		Anadi
T_{11}	*Control*	Simtharo
T_{12}		Tilki

2.6. Sampling, data collection and analysis

Plant phenotypic properties such as root length, shoot length, and leaf area at peak vegetative stage at 20, 40 and 60 days after transplanting (DAT), biomass at maturity, plant height and grain yield were determined. The time course phenotypic data were utilized to calculate secondary growth parameters such as net assimilation rates and relative growth rates under each treatment as described by (Sudhakar et al., 2016).

2.6.1. Measurement of shoot length

Ten plants including tillers i.e., hills were randomly selected from predetermined four sampling rows for plant height determination in each treatment. Three randomly selected tillers per hill were measured from the plant base to the tip of the longest leaf was measured at 60 DAT by using metric scale and average shoot length was computed.

2.6.2. Measurement of leaf area

From the predetermined sampling rows, 10 hills of plants were randomly selected out of which three randomly selected leaves were measured making altogether 30 leaf measurements per plot. Length of leaves were measured from lamina joint to tip of the leaf while breadth was measured across the leaf blade using metric scale. The average leaf area per leaf is multiplied with the product of number of leaves per plant and number of plants per plot to calculate the leaf area per unit area.

2.6.3. Measurement of root length

Randomly selected 10 plants were dug out to recover roots by using deep digging shovel and carried to the laboratory. Roots were cleaned gently in running water to remove the soil from root mass and measurement was taken from root-shoot junction to the root tip using metric scale.

2.6.4. Measurement of plant height

Plant height was determined by measuring from soil surface to the tip of the panicle during harvest. For this, 10 plant hills from predetermined sampling rows were randomly selected and three observations from each hill were taken for average plant height determination.

2.6.5. Number of tillers

Tillers of 10 plants (hills) were counted at 60 DAT to determine average tillers per plant.

2.6.6. Net Assimilation Rate (NAR)

The NAR represents the production efficiency of assimilatory apparatus. It is the remaining of total photosynthates after deducting the respiration. Following formula was used to compute actual net assimilation rate.

$$NAR = \frac{W2-W1}{t2-t1}\ x\ \frac{loge\ A2-loge\ A1}{A2-A1}\ \text{mg cm}^{-2}\ \text{day}^{-1}$$

where, W_2 and W_1 are dry weights, t_2 and t_1 are time points, log A_2 and log A_1 are natural logarithm of leaf area, A_2 and A_1 are leaf area without logarithm transformation.

2.6.7. Relative Growth Rate

Relative growth rate is the accumulation of dry mass on existing biomass in plants over the time period. RGR was computed by using the equation:

$$RGR = \frac{\ln W2-\ln W1}{t2-t1}\ \text{mg cm}^{-2}\ \text{day}^{-1}$$

where, W_2 and W_1 are the dry weights and t_2 and t_1 are the time points.

2.7. Statistical analysis

Collected data were entered in Excel sheet and the software "R Studio" was used for ANOVA and hypothesis testing. R Studio package 'doe bioresearch' was used for proper plot analysis and SPSS version 26 for visualization. Fisher Least Significant Difference (LSD) was used to compare the means between the treatments at a confidence level of 95% (p <0.05).

3. Results

3.1. Phenology

The rice landraces showed large contrasts in their phenological development. Simtharo was the earliest in panicle appearance and maturity; Anadi was 10 days later in first panicle appearance and 21 days later in maturity, while Tilki was further later by 21 days in panicle initiation and 34 days in maturity (Table 3).

3.2. Growth parameters

Different phenotypic variables showed differential interactions between the microbial inoculation treatments and rice landraces (Table 4; Figure 1). The number of tillers hill^{-1} at 60 DAT exhibited significant interactions between rice landraces and the microbial biopriming. The biopriming with *T. viride* and *P. fluorescens* had contrastingly positive and negative effects respectively on tiller numbers in Tilki, while the trait showed stability with least effect of biopriming on Anadi. The tillering in Simtharo exhibited intermediary responses to biopriming. However, other phenotypic variables such as root length, shoot length, plant height, leaf area index, shoot dry weight, NAR and RGR at 60 DAT, and panicle length at physiological maturity did not manifest interactions between the biopriming and rice landraces.

Root mediated biopriming of rice seedlings with beneficial microorganisms had differential effects on various phenotypic characteristics. The variables that exhibited significant biopriming effects included the number of tillers/plant (p=0.03) and dry biomass weight (p<0.01) at 60 DAT, while a derived growth variable NAR showed positive trend in response to biopriming (p=0.06) (Table 5). The plants inoculated with *P. fluorescens* had 23% less tiller numbers/plant compared to control, while other biopriming treatments were on par with the control. Similarly, the biopriming with *P. fluorescens* alone and in combination with *T. viride* also resulted in the reduction in dry shoot weight at 60 DAT by 14% and 7% respectively. These reductions in tiller number and shoot dry weight due to biopriming with *P. fluorescens* were associated with 58% decrease in NAR. The microbial biopriming did not affect the root length, shoot length, plant height, leaf area index, shoot dry weight and RGR at 60 DAT and panicle length at physiological maturity.

Three landraces Anadi, Simtharo and Tilki exhibited differences in all phenotypic variables (Table 6). The root lengths, shoot lengths and plant heights at 60 DAT showed contrasting differences in that Simtharo had the shortest roots (p = 0.04) with longest shoots (p = 0.01) and plant heights (p = 0.03), Tilki had the longest roots with shortest shoots and plant heights, while Anadi had intermediate values. Both Simtharo and Tilki had higher tillers numbers than Anadi (p < 0.01). The three landraces differed in leaf area index (p < 0.01), with Anadi, Tilki and Simtharo being in descending rank. Tilki had higher dry shoot weight and RGR at 60 DAT than Simtharo, while Anadi stood on par with both. For NAR, Tilki and Anadi stood on par differing significantly from the Simtharo (p < 0.01). Anadi had higher panicle length than Simtharo, while Tilki remained on par with the both.

Table 3. Phenology of the rice landraces

Rice landraces	First panicle appearance		Maturity	
	Date	DAS	Date	DAS
Simtharo	2 September 2021	79	11 October 2021	118
Anadi	12 September 2021	89	1 November 2021	139
Tilki	23 September 2021	100	14 November 2021	152

DAS: days after seeding of rice landraces

Table 4. Effect of interactions between rice landraces and microbial biopriming inoculants on rice growth variables in Dang valley, Nepal

Treatments	Root length (cm) at 60 DAT	Shoot length (cm) at 60 DAT	Plant height (cm) at harvest	Leaf area index (cm^2) at 60 DAT	Dry weight (g/hill) at 60 DAT	Relative growth rate (mg cm^{-2})	Net assimilation rate (mg cm^{-2} day^{-1})	Panicle length (cm)
T. viride:Anadi	25.47	107.15	132.62	55.09	41.12	6.12	43.81	24.09
T. viride:Simtharo	22.61	110.87	133.48	29.78	33.81	3.68	1.14	22.39
T. viride:Tilki	26.61	102.13	128.74	37.25	33.35	5.69	17.71	23.39
P. fluorescens:Anadi	28.07	102.82	130.89	47.67	25.87	4.80	8.35	21.85
P.fluorescens:Simtharo	21.67	117.83	139.50	31.77	23.59	4.16	0.94	23.06
P. fluorescens:Tilki	25.82	91.95	117.78	31.92	25.28	4.43	5.33	21.42
T. viride + *P.fluorescens*:Anadi	23.67	106.20	129.87	50.42	26.10	4.21	13.74	24.43
T. viride + *P. fluorescens*:Simtharo	24.48	110.10	134.58	27.02	24.40	4.91	1.31	22.69
T. viride + *P. fluorescens*:Tilki	28.13	92.54	120.68	37.82	30.65	6.42	19.67	23.11
Control:Anadi	23.40	114.94	138.34	51.40	25.33	4.28	12.85	24.20
Control:Simtharo	23.32	102.62	125.94	31.67	24.40	4.17	2.55	22.68
Control:Tilki	27.28	90.33	117.61	36.72	37.56	7.97	19.37	23.79
Mean	25.04	104.13	129.17	39.04	29.29	4.99	12.23	23.09
CV	14.01	10.07	8.81	8.66	18.25	38.30	25.70	4.40
LSD	6.07^{ns}	18.14^{ns}	19.70^{ns}	5.85^{ns}	9.25^{ns}	3.24^{ns}	19.67	1.76^{ns}
p-value (microbes*landraces)	0.57	0.39	0.63	0.15	0.12	0.44	0.11	0.15

Within the columns, means followed by the same letters are not significantly different according to LSD at 5% significance level. LSD, least significant difference; CV, coefficient of variance; DAT, days after transplanting.

Table 5. Effect of biopriming with beneficial micro-organisms on growth parameters of native rice landraces in Dang valley, Nepal

Biopriming micro-organisms	Root length (cm) at 60 DAT	Shoot length (cm) at 60 DAT	Plant height (cm)at harvest	Number of tillers per hill at 60 DAT	Leaf area index (cm^2) at 60 DAT	Dry weigh t t ha^{-1} at 60 DAT	Relative growth Rate (mg cm^{-2})	Net assimilation rate (mg cm^{-2} day^{-1})	Panicle length physiological maturity (cm)
T. viride	24.89	106.72	131.61	13.26^{a}	40.70^{a}	36.09^{a}	5.17	20.89^{a}	23.29^{a}
P. fluorescens	25.18	104.20	129.39	10.28^{b}	37.12^{a}	24.93^{c}	4.13	4.87^{b}	22.11^{a}
T. viride + *P. fluorescens*	25.423	102.95	128.37	12.99^{a}	38.42^{a}	27.05^{bc}	5.18	11.58^{ab}	23.41^{a}
Control	24.67	102.63	127.30	13.27^{a}	39.93^{a}	29.10^{a}	5.48	11.59^{ab}	23.56^{a}
Mean	25.04	104.13	129.17	12.45	39.04	29.29	4.99	12.23	23.09
CV %	13.25	5.54	5.32	14.33	14.67	8.94	38.30	25.70	5.93
LSD	3.83	6.66	7.93	2.06	6.61	3.02	1.87	11.36	1.58
p-value	0.96	0.48	0.61	0.03	0.59	0.00	0.48	0.06	0.20

Within the columns, means followed by the same letters are not significantly different according to LSD at 5% significance level. LSD, least significant difference; CV, coefficient of variance; DAT, days after transplanting

Table 6. Growth parameters of three native rice landraces in Dang valley, Nepal

Rice landraces	Root length (cm) at 60 DAT	Shoot length (cm) at 60 DAT	Plant height (cm)at harvest	Number of tillers per hill at 60 DAT	Leaf area index (cm^2) at 60 DAT	Dry weight t ha^{-1} at 60 DAT	Relative growth Rate (mg cm^{-2})	Net assimilation rate (mg cm^{-2} day^{-1})	Panicle length physiological maturity (cm)
Anadi	25.15ab	107.7a	132.93a	8.41b	51.15a	29.61ab	4.60ab	16.69a	23.64a
Simtharo	23.02b	110.3a	133.38a	14.00a	30.06c	26.55b	4.23b	14.90b	22.71b
Tilki	26.96a	94.24b	121.20b	14.93a	35.93b	31.71a	6.13a	15.52a	22.93ab
Mean	25.04	104.13	129.17	12.45	39.05	29.29	4.99	12.23	23.09
CV %	14.01	10.07	8.81	13.35	8.66	18.25	38.30	25.70	4.40
LSD	3.04	9.07	9.85	1.44	2.93	4.63	1.62	9.83	0.88
p-value	0.04	0.01	0.03	0.00	0.00	0.09	0.05	0.01	0.09

Within the columns, means followed by the same letters are not significantly different according to LSD at 5% significance level. LSD, least significant difference; CV, coefficient of variance; DAT, days after transplanting

3.3. Yield attributes

The root-mediated biopriming native rice cultivars with beneficial soil micro-organisms (*T. viride* and *P. fluorescens*) individually or in combination had significant interactive effects on most of the yield attributing characters (Figure 1). While there was no biopriming-by-landraces interaction exhibited by number of effective tillers per unit area (p = 0.20), sterility percentage (p = 0.22), and 1000-grain weight (p = 0.52), significant interactions were evident on the number of filled grains per panicle (p = 0.01), grain yield (p < 0.01), total dry biomass at physiological maturity (p < 0.01) and harvest index (p < 0.01).

The biopriming with *T. viride* increased filled grains per panicle in Anadi by 38% (94.8 vs 68.93), while having no effect on this trait of Simtharo (78.1 vs 75.43) and Tilki (8.0 vs. 6.3). None of the landraces exhibited a response to biopriming with *P. fluorescens* and combination of both *T. viride* and *P. fluorescens*.

The overall grain yield was strongly correlated with sterility (r = -0.96; p < 0.01) and filled grains per panicle (r = 0.91; p < 0.01). The biopriming with *T. viride* resulted in an increase in grain yields of Anadi by 39% (2.62 vs 1.88 t ha^{-1}) and Tilki by 111% (1.12 vs 0.53 t ha^{-1}), while causing a decrease in grain yield of Simtharo by 21% (2.20 vs 2.80 t ha^{-1}) compared to respective controls. On the other hand, the biopriming with *P. fluorescens* had neutral effects on grain yield of all three landraces – Anadi (1.98 vs 1.88 t ha^{-1}), Simtharo (2.73 vs 2.80 t ha^{-1}) and Tilki (0.59 vs 0.53 t ha^{-1}). Similarly, the biopriming with the combination of both microbes (*T. viride* and *P. fluorescens*) also resulted in a neutral effect on grain yield of the landraces – Anadi (2.09 vs 1.88 t ha^{-1}), Simtharo (3.00 vs 2.80 t ha^{-1}) and Tilki (0.81 vs 0.53 t ha^{-1}).

In contrast to the response of grain yield, Anadi and Simtharo did not show significant response to biopriming for dry biomass yield at physiological maturity (Table 6). However, Tilki underwent a 30% decrease in dry biomass yield (5.77 vs 4.02 t ha^{-1}) due to biopriming with *T. viride* compared to corresponding control. The biopriming-by-landraces interactions on grain and dry biomass yield culminated in a significant effect on the harvest index. Consequently, Anadi (0.38 vs 0.25) and Tilki (0.28 vs 0.09) responded with a significant increase in harvest index to the biopriming with *T. viride* over the respective control, while Simtharo did not exhibit significant response.

Major yield attributes such as grain yield, dry biomass and harvest index exhibited significant biopriming-by-landraces interactions. For the variables that had no significant interactions, the effects of individual factors are presented. Those variables included effective (head bearing) tillers per unit area, sterility percentage, and 1000-grain weight (Table 7). None of the three biopriming treatments affected the sterility percentage. Compared to the unprimed control, the biopriming with *P. fluorescens* alone had consistently no significant effect on the effective tillers per unit area, and 1000-grain weight. The biopriming with *T. viride* alone reduced the number of effective tillers by 12% (148.89 vs 164.89), while increasing filled grains per panicle by 20% (60.30 vs 50.22). The biopriming with the combination of both *T. viride* and *P. fluorescens* increased the number of effective tillers by 14% (187.67 vs 164.89), but had no significant effect on filled grains per panicle.

The genotype of landraces under the prevailing conditions of the experimental site mainly determined the yield attributes. With no biopriming-by-landraces interactions, the number of effective tillers per unit area, sterility percentage, and 1000-grain weight deferred significantly (p <0.01) among the three landraces of rice (Table 8). Anadi had the lowest number of effective tillers per unit area (1.4 and 1.9-fold lower than Simtharo and Tilki, respectively), highest values of 1000-grain weight (1.3 and 3.0-fold higher than Simtharo and Tilki, respectively), and intermediate level of sterility percentage (3.8-fold higher than Simtharo and 2.6-fold lower than Tilki). Contrastingly, Simtharo had the highest number of effective tillers (1.9 and 1.4-fold higher than Tilki and Anadi, respectively), lowest sterility percentage (3.8 and 10-fold lower than Anadi and Tilki, respectively), intermediate values of 1000-grain weight (1.3-fold lower than Anadi and 2.3-fold higher than Tilki). Tilki, on the contrary, exhibited the highest sterility percentage (2.6-fold higher than Anadi and 10-fold higher than Simtharo), and lowest 1000-grain weight (3-fold and 1.3-fold lower than Anadi and Simtharo, respectively), while being intermediate in the number of effective tillers per unit area (1.3-fold lower than Anadi and 1.3-fold higher than Simtharo).

Table 7. Effects of biopriming with beneficial micro-organisms on yield attribute variables of three local landraces Anadi, Simtharo and Tilki in Dang valley

Biopriming micro-organisms	Effective tillers m^{-2}	Filled grains per panicle	Sterility percentage	1000-grain weight (gm)	Grain yield (t ha^{-1})	Total dry biomass (t ha^{-1})	Harvest Index (HI)
T. viride	145.89^{c}	60.30^{a}	42.32	22.73^{b}	1.98^{a}	5.02^{b}	0.40^{ab}
P. fluorescens	158.22^{b}	51.32^{b}	44.51	23.23^{ab}	1.76^{ab}	4.06^{c}	0.43^{ab}
T. viride + P. fluorescens	187.67^{a}	55.14^{ab}	46.02	23.42^{a}	1.97^{ab}	5.20^{ab}	0.46^{a}
Control	164.89^{b}	50.22^{b}	48.88	23.11^{ab}	1.74^{b}	5.89^{a}	0.34^{b}
Mean	164.17	54.25	45.43	23.12	1.86	5.04	0.41
CV %	6.49	12.59	11.45	2.33	10.98	13.72	23.10
LSD	12.29	7.88	6.00	0.62	0.24	0.80	0.11
p-value	0.00	0.07	0.15	0.14	0.08	0.01	0.14

Within the columns, means followed by the same letters are not significantly different according to LSD at 5% significance level. LSD, least significant difference; CV, coefficient of variance; DAT, days after transplanting

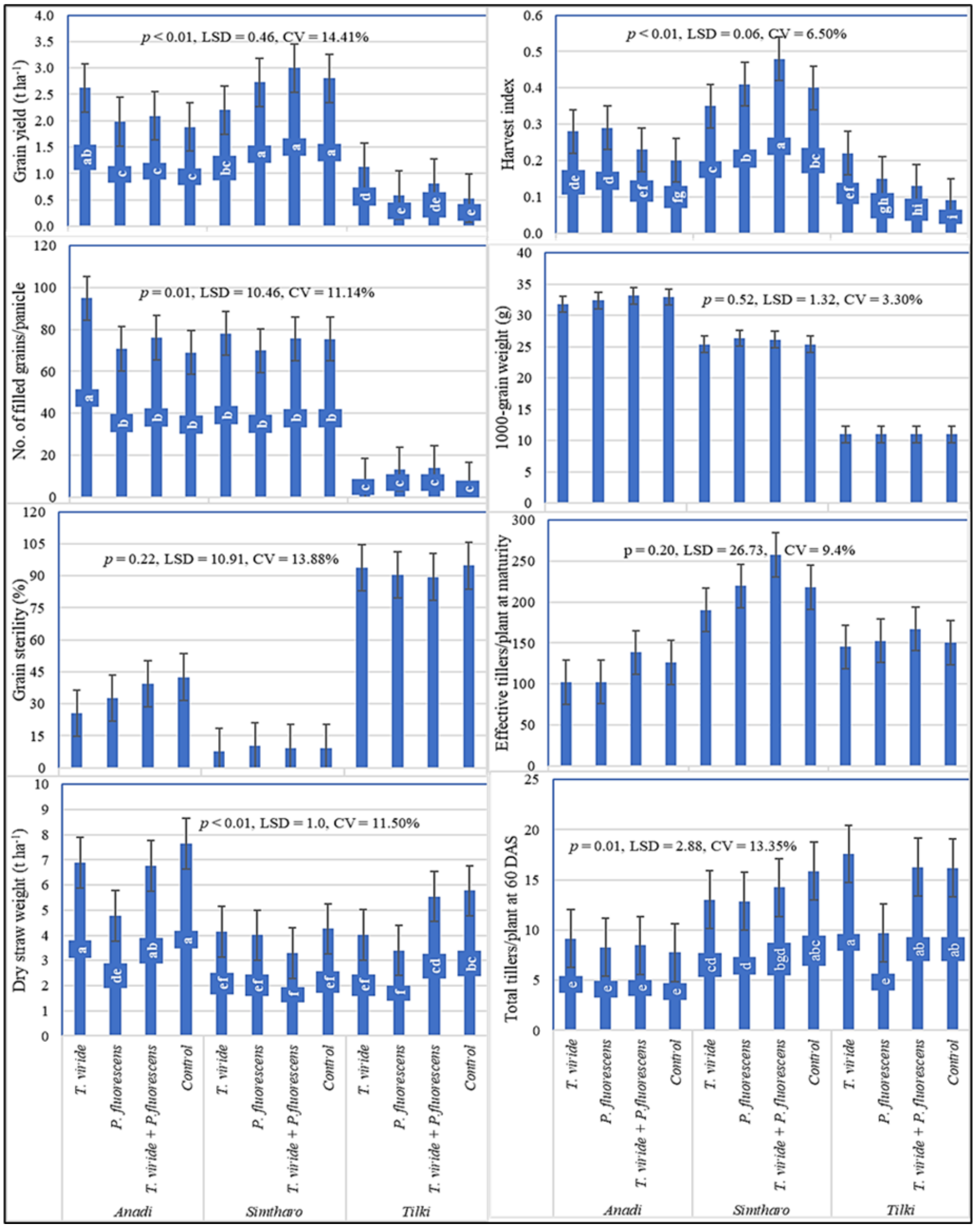

Figure 1. Growth and yield related variables showing differential landraces-by-microbial interactions. The error bars are least significant difference (LSD) values at type 1 error probability (*p*) of 0.05 across three replicates. The values depicted by individual bars having the same letters do not different at *p* = 0.05

Table 8. Yield attribute variables of three local landraces Anadi, Simtharo and Tilki in Dang valley

Rice landraces	Effective tillers m^{-2}	Filled grains per panicle	Sterility percentage	1000 grain weight (gm)	Grain yield (t ha^{-1})	Total dry biomass (t ha^{-1})	Harvest Index (HI)
Anadi	117.25^{c}	77.62^{a}	35.05^{b}	32.58^{a}	2.14^{b}	6.51^{a}	0.34^{b}
Simtharo	221.42^{a}	74.71^{a}	9.14^{c}	25.80^{b}	2.68^{a}	3.93^{c}	0.71^{a}
Tilki	153.83^{b}	10.42^{b}	92.11^{a}	10.99^{c}	0.77^{c}	4.68^{b}	0.18^{c}
Mean	164.17	54.25	45.44	23.12	1.86	5.04	0.41
CV	9.40	11.14	13.88	3.30	14.41	11.45	18.01
LSD	13.36	5.23	5.46	0.66	0.23	0.49	0.06
p value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Within the columns, means followed by the same letters are not significantly different according to LSD at 5% significance level. LSD, least significant difference; CV, coefficient of variance; DAT, days after transplanting

4. Discussion

This is the first study comparing microbial biopriming effects on three landraces Anadi, Simtharo and Tilki together with detail characterization of growth and productivity. The landraces exhibited distinctive morpho-physiological characteristics. Anadi stood out for high leaf area index, 1000 grain weight and total biomass productivity; Simtharo showed prominence for effective tiller density and low sterility, imparting high grain yield and harvest index, while Tilki was distinctive for finer grains. It should be noted that the seed yield of Tilki in our study does not reflect its potential as reported in previous studies. Tilki suffered bird damage due to being a lone crop in small-plot islands after the harvest of early maturing counterparts. It also had a high sterility percentage, possibly caused by cold temperature episodes and lodging of crop during its early reproductive stage, as an indication of its differential agroecological adaptation than other landraces. Tilki matured 34 days later than the earliest maturing Simtharo and mid-maturity type Anadi by 13 days. This exposed the small islands of Tilki plots to attract birds as well as predisposed them to lodging by wind. Application of a moderately high level of nitrogen may also be a culprit for lodging and delaying maturity, as most of the rice landraces are adapted to low-input organic production systems (Kapoor et al., 2023). Lodging of plants impairs light penetration to the underlying plants weakening the source to supply photo-assimilate to reproductive sink, leading to failure in pollination, fertilization and grain development (Fageria, 2007). It is realized that such experiments should be conducted using the crop cultivars of similar maturity class and with the consideration of their nutrient response for deciding on the types and rates of fertilizer application.

In this study, the pre-transplant biopriming of rice seedlings with *T. viride* resulted in differential interactive effects on phenotypic, yield-governing and grain quality parameters of the rice landraces. In Anadi, the biopriming increased number of filled grains per panicle and grain yield leading to an increase in the harvest index. It also increased grain yield and harvest index in Tilki, but exhibited no effects in the yield attributes of Simtharo. Anadi and Tilki showed 39% and 111% increase in seed yields, respectively due to the inoculation with *T. viride.* Our results with two rice landraces align with those of Khadka and Uphoff (2019) who realized 41% higher yield (6.29 vs 4.45 mt ha^{-1}) of Tilki with the inoculation of native isolate of *T. viride*. Though the performance of Tilki was poorer due to bird damage and other factors leading to high sterility in our study, the yield responses to the inoculant were similar to the previous study.

The inoculant effects on growth and productivity of two rice landraces in our study imply that *T. viride* enhanced the metabolic partitioning in favor of reproductive sink, leading to higher proportion of field grains, higher grain yield as well as higher harvest index. Increase in the number of tillers per plant was a common response of all land races to the inoculation with *T. viride*. Various previous studies elucidated that biopriming with *Trichoderma* spp. enhanced nutrient uptake, drought tolerance, salt tolerance, water use efficiency, photosynthesis and growth parameters of rice (Doni et al., 2014; Doni et al., 2023; Doni et al., 2018; Mishra et al., 2020; Rawat et al., 2012; Singh et al., 2023). However, because of no significant effect on net assimilation rate in our study, there is no suggestive evidence for the biopriming with *T. viride* affecting the metabolic source capacity for enhancing the yield attributes of rice. Several studies have been conducted in Nepal (Bhusal et al., 2018; Chaudhary et al., 2014) and other countries (Chinnaswami et al., 2021; Mishra et al., 2020; Seekham, Kaewsalong, & Dethoup, 2024; Seekham, Kaewsalong, Jantasorn, et al., 2024; Vijitrpanth et al., 2023) show that biopriming with *Trichoderma* spp. enhances rice plant resistance against blast, brown spot, sheath blight, stem rot, seed rot, false smut and dirty panicle diseases of rice. Suppression of various pathogen by *Trichoderma* spp. may have also contributed to the enhancement of yield in our experiment. However, no disease observation and ratings were carried out in this study to support this argument.

In this study, there was no evidence of significant yield response of any rice landraces to the rice seedling biopriming with sole culture of *P. fluorescens*. Our results contrast with earlier studies (Mathivanan et al., 2005; Nandakumar et al., 2001; Sakthivel & Gnanamanickam, 1987) which reported disease suppression and enhancement of plant growth and yield of rice due to *P. flouresens* inoculation. Less clear effect of *P. flouresens* inoculant in our study may be associated with various factors such as viability of culture over transportation and storage under prevailing high temperature conditions, immediate post-inoculation anerobic condition in the puddled rice transplanting system, and competition with native strains of microbes. The subtle effects of the seedling biopriming could have also been masked by supplemental nutrients application through chemical fertilizers (NPK at 40:60:60 kg/ha).

In this study, rice seedlings biopriming with the combined culture of *T. viride* and *P. flouresens* did not produce synergistic effects on most of the agronomic variables compared to the sole inoculation with the microbes. Previous studies deciphered pest suppression as major response mechanism of microbial biopriming contributing to enhanced crop productivity. The combined inoculum of both *T. viride* and *P. flouresens* was as effective as systemic fungicide, Carbendazim in controlling the sheath blight disease of rice (Mathivanan et al., 2005). Further study should integrate plant health aspects while evaluating the agronomic efficacy of microbial biopriming.

Our findings implicate the importance of understanding the local microbiome for the existence and abundance of respective native strains of the microbes, finetuning of microbial delivery to match cultural practices, including the types, methods and levels of supplemental nutrient application. Microbial biopriming can be more effective with organic amendment and/or supplementation with organic nutrients such as chitin, chitosan, pectin, sucrose etc. (Marian & Shimizu, 2019) Further study should examine whether microbial biopriming on local rice landraces is more effective with low-input organic nutrient management regime under direct-seeded rice culture, which provides aerobic conditions in the rhizosphere.

5. Conclusion

The three rice landraces exhibited differential interactions with seedling biopriming with the sole and mixed culture of *T. viride* and *P. flouresens*. Anadi and Tilki showed more vivid beneficial interactions with *T. viride* leading to significant enhancement of yield-governing variables including tiller growth, grain filling, grain yield and harvest index. While *P. flouresens* did not exert measurable biopriming effects, the combination of both *T. viride* and *P. flouresens* did not show any synergistic or antagonistic effects. This study identified a number of intriguing areas for further research to develop effective microbial biopriming tools and procedures in rice crop-based cropping systems, especially under low-input and organic management conditions. Use of rice landraces of similar maturity class for comparative studies, and evaluation of locally isolated microbial strains under organic, direct seeding or SRI system with the integration of pest suppression analysis in cropping systems approach are important research agenda emerged through the discussion of our results. While generating some practical agronomic solutions usable by farmers and agricultural professionals, this study has shown a promising way forward for researchers and priority reorientation by the policy makers.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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