

## Use of bio-immunization technology to manage *Fusarium* wilt (Race 1) in banana cv. Sabri growing in Tripura (India)

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### Abstract

Sabri (AAB), a traditional variety of banana is being grown commercially in North eastern region of India particularly Tripura. However, in recent years, Sabri cultivation has been severely affected by *Fusarium* wilt (Race-1). The outbreak of the disease, caused by *Fusarium oxysporum* f. sp. *ubense* Tropical Race-1 (FOC TR-1), has not only led to a significant decline in yield but has also impacted its commercial cultivation in several hotspot regions of Tripura. The virulent nature of the pathogen limits the field level success of the control initiatives through chemicals, resistance breeding and biological control, providing serious threats for banana growers in the region. This study assesses the efficacy of the bio-immunized tissue culture raised Sabri banana plantlets and the periodical spray of ICAR-FUSICONT for the community based management of banana *Fusarium* wilt. The effectiveness of this integrated approach was critically assessed by evaluating the disease management aspects along with yield restoration in hot spot regions. Banana orchards adopting this combined technology revealed significantly lower disease incidence percentage (2.98 %) compared to non-adopted plantations (28.44 %). Further, these technological interventions increased the yield from 13.33 kg/plant to 16.58 kg/plant. The findings highlighted the potential of these technologies in the management of *Fusarium* wilt and improving Sabri banana yield in Tripura.

**Key words:** Bio-immunization, ICAR-FUSICONT, *Fusarium* wilt, Sabri banana

### Introduction

Banana (*Musa* spp.) is a common economic crop grown in tropical and subtropical regions and one of the most popular exported fruits that serve as a staple diet for more than 400 million individuals globally (Abdousalami *et al.*, 2023). The banana crop economy has endured significant losses owing to an outbreak of *Fusarium* wilt since 1860, caused by *Fusarium oxysporum* f. sp. *ubense* race 1 (FOC race-1). Banana is a popular fruit of India and ranked top position in global banana production with 31.5 million tons accounting for 26.43% of global output (Hinge *et al.*, 2022). North Eastern region, offers considerable potential for banana cultivation and germplasm conservation and also serves as a geographical gateway for the rich flora and fauna of the Indian subcontinent, supporting unique banana diversity and scope for varietal improvement (Chakravarty *et al.*, 2012).

Tripura, a North Eastern state of India, possesses considerable geo-climatic variation that supports the growth of diverse plant species. Among these, banana stands out as one of the most important fruit crops in the state (Das *et al.*, 2024). Many traditional, wild, commercial cultivar/ genotypes, are being cultivated in Tripura region and 70 % area of banana cultivation is under Sabri variety followed by Samai/ Gopi/ Bangla variety (ABB) and Champa variety (AAB). Area under banana cultivation in Tripura is 13,270 ha with an annual production of 13,0085.20 MT with a productivity of 9.8 t/ha (Das *et al.*, 2020). In Tripura, under the Jhum (shifting cultivation) system, banana

is commonly used as a shade crop and is also grown along the bunds. However, banana cultivation in the region faces significant disease infestations, including *Fusarium* wilt, banana bunchy top, banana phytoplasma, and banana streak mosaic virus (Mitra *et al.*, 2022; Thangavelu *et al.*, 2024). Sabri is highly demanded by both farmers and consumers, as it is a traditional cultivar of Tripura and is believed to have originated in Tripura. This particular variety holds cultural and religious significance, with its fruits being used in various rituals. Various infections caused by fungus, virus and phytoplasma, either individually or in combination, significantly affect banana cultivation, particularly the Sabri variety. These infections have drastically altered the banana cultivation landscape in Tripura, posing a serious challenge to farmers and necessitating urgent disease management strategies (Mitra *et al.*, 2022; Uma *et al.*, 2001).

*Fusarium* wilt is the most important disease responsible to restrict banana cultivation in Tripura. Among the cultivated varieties, Sabri variety is highly susceptible to diseases, followed by Samai and Kachkela variety (Uma *et al.*, 2001). However, the prevalence of *Fusarium* wilt (Race-1) has significantly impacted Sabri banana cultivation in Tripura, altering its production and market dynamics (Uma *et al.*, 2001; Thangavelu *et al.*, 2024). The disease has caused substantial economic losses for many farmers, as infestations have significantly reduced yield and quality.

To address this challenge and revive banana cultivation in Tripura, scientists from ICAR-Central Institute for Subtropical

Horticulture, Lucknow in collaboration with College of Agriculture, Tripura have demonstrated wilt tolerant plantlets of Sabri variety developed through novel tissue culture technology. A patent-protected *in vitro* bio-immunization technology was used to develop *Fusarium* wilt resistant Sabri banana plantlets at ICAR-CISH, Lucknow. The timely application of ICAR-FUSICONT along with bio-immunized Sabri banana plants in the field further enhances disease management efforts. The current study was designed to evaluate the effectiveness of bio-immunization technology combined with ICAR FUSICONT spray in minimizing *Fusarium* wilt disease (FOC Race-1) infestation. The goal is to restore the profitability of Sabri banana cultivation by reducing disease incidence and improving plant health.

## Materials and methods

**Materials and location of the experiments:** A novel tissue culture technology in banana was developed and termed as Bio-immunization technology where, lipopolypeptide based engineered bio-molecule called ‘bio-immune’ was used and subsequently introduced into banana *in-vitro* tissue culture plantlets during *in-vitro* organogenesis (Damodaran *et al.*, 2021). The bio-molecule and the process of bio-immunization have been patented by ICAR-Central Institute for Sub Tropical Horticulture, Lucknow (patent no. 202111003761).

The technology was utilized to develop Sabri banana plantlets at Lucknow and transported to Tripura (College of Agriculture, Tripura) where primary and secondary hardening was accomplished in the shade house by following scientific and prescribed protocol for bio-immunized plant hardening. Finally secondary hardened plants were distributed among the farmer’s of Tripura for field evaluation.

This investigation was carried out at Lembucherra, Jirania region of West Tripura district. The plants were planted in the first week of June, 2022-23 & 2023-24 and all the scientific package and practices of banana cultivation were followed.



Fig. 1. Images depicting Bio-immunized sabri banana plantlets: A. Tissue culture-raised bio-immunized sabri plantlets inside culture bottles; B. Bio-immunized sabri plantlets with profused shoots and roots at ICAR-Central Institute for Subtropical Horticulture, Lucknow. C. Primary hardening of bio-immunized plantlets at the College of Agriculture, Tripura; D. Secondary hardened sabri plants used for distribution to farmers of Tripura.

Table 1. Scale for scoring different parameters for *Fusarium* wilt resistance (Viljoen *et al.*, 2017)

Disease Rating Scale	Leaf Symptoms Index	Pseudo-stem splitting	Rhizome discoloration index
1	No yellowing	No cracking	No internal symptoms
2	Yellowing of $\leq 1/3$ of the leaves	Slite cracking	Few internal spots
3	Yellowing of $1/3$ to $2/3$ of leaves	Advanced	$\leq 1/3$ discolored
4	Yellowing of $> 2/3$ of leaves		$1/3$ to $2/3$ discolored
5	Plant dead		$> 1/3$ discolored
6	---		Entire inner Rhizome

The treatments consisted of two groups representing different management practices. The adopter group included bio-immunized Sabri banana plants that received periodical spraying of ICAR-FUSICONT at critical growth stages for effective management of *Fusarium oxysporum* f. sp. *cubense* Race 1, following ICAR recommendations. Conversely, the non-adopter group comprised unimmunized tissue culture–raised Sabri plantlets grown under similar environmental conditions without the application of ICAR-FUSICONT spray.

The plant growth attributes such as plant height, plant girth, third leaf length, third leaf breadth, and yield attributes were recorded in both the adopters and non-adopter plantations. In each plot random 5 samples were drawn, from which the number of plants affected over the total number of plants were counted and expressed as % disease incidence as described in the formula given by Vernell and Hecloud (1975) (Table 1).

**Statistical analysis:** For plant height, plant girth, third leaf length and breadth, bunch weight, percent disease incidence (PDI) and disease severity index (DSI) data were obtained with five replications from systems on eight farmers’ fields in adopter and non-adopter clusters. cv. Sabri. Data were presented as mean  $\pm$  SD, and analyzed by independent two-sample t-tests. Statistical significance was accepted at  $P \leq 0.05$  and marked with asterisk (\*).

## Results and discussion

**Evaluation of bio-immunized plants in Lembucherra and Jirania region of Tripura:** Collaborative field evaluation was done by ICAR-Central Institute for Subtropical Horticulture, Lucknow and College of Agriculture, Tripura at Lembucherra and Jirania during 2022–2023 & 2023–2024 to evaluate the efficiency of bio-immunized Sabri banana plantlets along with periodical spray of ICAR-FUSICONT in managing *Fusarium* wilt.

Plant height was significantly increased in the adopter’s field with an increment of 14.86% (307.46 cm) in 2022-23 and 12.03 % (312.42 cm) in 2023-24 as compared to the non adopter 267.68 cm and 278.85 cm, respectively. Similarly, treated plants exhibited more pronounced effect in plant girth (PG) in the year 2022-2023. However bunch weight was found significantly higher in adopter’s field

throughout year (15.92 kg/plant and 16.58 kg/plant respectively), which is the clear indicator of productivity improvement with an increase of 20.51 % in 2022-23 and 24.38 % in 2023-24 (Table 2; Fig. 2). Additionally, it was also found that the bio-immunized plants sprayed with ICAR-FUSICONT had significant impact on *Fusarium* wilt management in the adopter and non-adopter orchards.

The findings of the survey data analyzed using independent ‘t’ test depicted the existence of significant differences between the adopters and non-adopters with respect to PDI (Table 1). In the year of 2022-23, PDI among the adopters was observed to be 3.35 %, while the non-adopters recorded a final PDI of 24.46 %. Similar trends were also observed in 2023-24, adopters had significantly lower disease incidence (2.98 %) than the non-adopters (28.44 %) (Table 2, Fig. 3).

Earlier, study revealed that use of consortium of *Pseudomonas aeruginosa* DRB1 and *Trichoderma harzianum* CBF2 against FOC Tropical Race 4 (FOC-TR4) reduced the disease incidence percentage significantly (Wong *et al.*, 2019). Combined application of fungicide with *Pseudomonas floescence* strain PF1 showed reduction of disease incidence up to 64 % in the green house and 75 % in field conditions against FOC Race-1 (Akila *et al.*, 2011). Further, Shen *et al.*, (2015) has documented that application of bio-fertilizer “BIO” reduced disease incidence to 20 % compared to 38 % in control in China. Based on our results and the earlier reports, it can be observed that use of bio-immunized plantlets along with the periodical application of the ICAR-FUSICONT showed significant reduction of the disease in the adopters fields (Damodaran *et al.*, 2019; 2020; 2023; Rav *et al.*, 2025). Combination of carbendazim, sodium silicate, and *Bacillus subtilis* significantly reduced the incidence and severity of *Fusarium* wilt in banana plants, which resulted in improved plant growth and yield (Zakaria *et al.*, 2023). Integrated application of *Bacillus velezensis* EB1 and Potassium sorbate reduce the *Fusarium* wilt



Fig. 2. Successful cultivation of bio-immunized banana plants of cv. Sabri in Tripura. This figure shows a banana plantation located in Tripura, India A. The images show aerial view of healthy bio-immunized plants; B & C. The image depicts banana plant bearing large bunch of bananas, with some plants visibly protective blue netting or polythene cover around the fruit bunches, used to prevent damage from pests and environmental factors. D. Fresh harvested fruits from the bio-immunized banana plants.

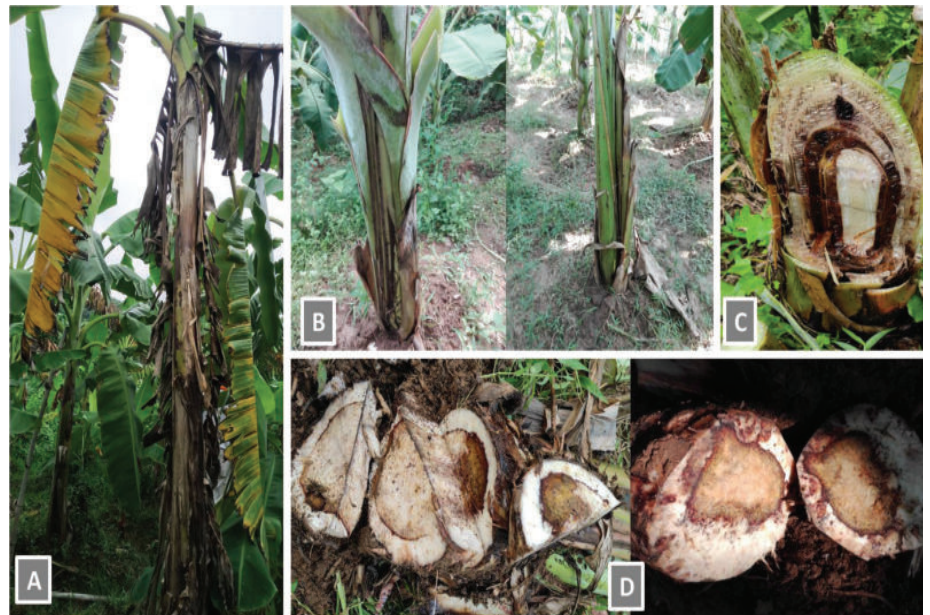


Fig. 3. External and internal symptoms of *Fusarium* wilt disease in un-immunized Sabri banana plants. A & B. banana plants affected by *Fusarium* wilt displaying characteristics like yellowing of the leaves, shading of the leaves pseudostem splitting *etc.* C. Transverse section of an un-immunized plant revealing vascular discoloration. D. Longitudinal section of the pseudostem showing necrosis of vascular strands and severe rotting.

Table 2. Effect of adoption of improved practices on plant growth, yield, and disease incidence over two years on Sabri banana in Tripura

Year	Treatments	Plant height (cm)	Plant girth (cm)	3 <sup>rd</sup> Leaf length (cm)	3 <sup>rd</sup> Leaf breadth	Bunch weight (kg)	Percent disease incidence (PDI)	Disease severity index
2022-23	Adopters	307.46 ± 4.74	97.77 ± 1.33	251.66 ± 3.61	65.28 ± 1.14	15.92 ± 0.2	3.35 ± 0.06	1.64 ± 0.02
	Non-adopters	267.68 ± 4.12	86.75 ± 1.18	235.89 ± 3.38	54.07 ± 0.94	13.21 ± 0.17	24.46 ± 0.42	3.94 ± 0.06
	<i>t</i> -value	6.34*	6.19*	3.19*	7.59*	10.32*	49.44*	36.8*
2023-24	Adopters	312.42 ± 4.78	95.35 ± 1.48	252.36 ± 4.48	62.68 ± 0.78	16.58 ± 0.27	2.98 ± 0.05	1.62 ± 0.02
	Non-adopters	278.85 ± 4.27	80.07 ± 1.25	251.00 ± 4.45	59.61 ± 0.74	13.33 ± 0.22	28.44 ± 0.48	4.05 ± 0.06
	<i>t</i> -value	5.24*	7.89*	0.22	2.86*	9.46*	52.54*	39.1*

NB: Effect of bio-immunization and periodical spraying of ICAR-FUSICONT in critical stages of development on the plant height (PH), plant girth (PG), no. of leaf (NL), 3<sup>rd</sup> leaf length (LL), 3<sup>rd</sup> leaf breadth (LB), bunch weight (BW), no. of hand per bunch (NH/B) of Banana Cv. Sabri grown in Tripura. Values are the mean ± SD of 40 replications. *t* values marked with \* are significant at alpha  $P \leq 0.05$ .

in Banana (Liu *et al.*, 2024). Sabri banana, a widely cultivated commercial cultivar in northeast India, is susceptible to *Fusarium* wilt, with field diagnosis primarily based on morphological symptoms. Typical indicators of the disease includes yellowing and progressive wilting of older leaves, starting from the margins of the leaf lamina, along with leaf blade distortion and petiole collapse, resulting in a distinctive skirt-like appearance (Fig. 3). As the infection progresses, all affected leaves eventually dry out, often accompanied by splitting of the pseudostem at the base. Internally, the disease manifests as discoloration in the rhizome and pseudostem, which was observed in both transverse and longitudinal sections. The severity of discoloration ranges from pale yellow to reddish-brown as the infection advances. These symptoms were predominantly observed in banana plants older than six months. Previous studies have also documented similar characteristic symptoms, with variations depending on banana cultivars and growing conditions (Thangavelu *et al.*, 2021).

The data (Fig. 4) revealed that adopters of bio-immunization technique in Sabri banana farming systems continuously obtained higher bunch weights and lower *Fusarium* wilt (Race 1) incidence than non-adopters in Tripura throughout 2022-23 and 2023-24. Adopters outperformed non-adopters in terms of yield and plant health in both years. The seasonal separation of adopters and non-adopters supports the efficacy of bio-immunization in reducing *Fusarium* wilt and increasing yields. This highlights the need to scale technology in Tripura emphasizing farmer training and input access. These findings should be subjected to rigorous statistical analysis in future work.

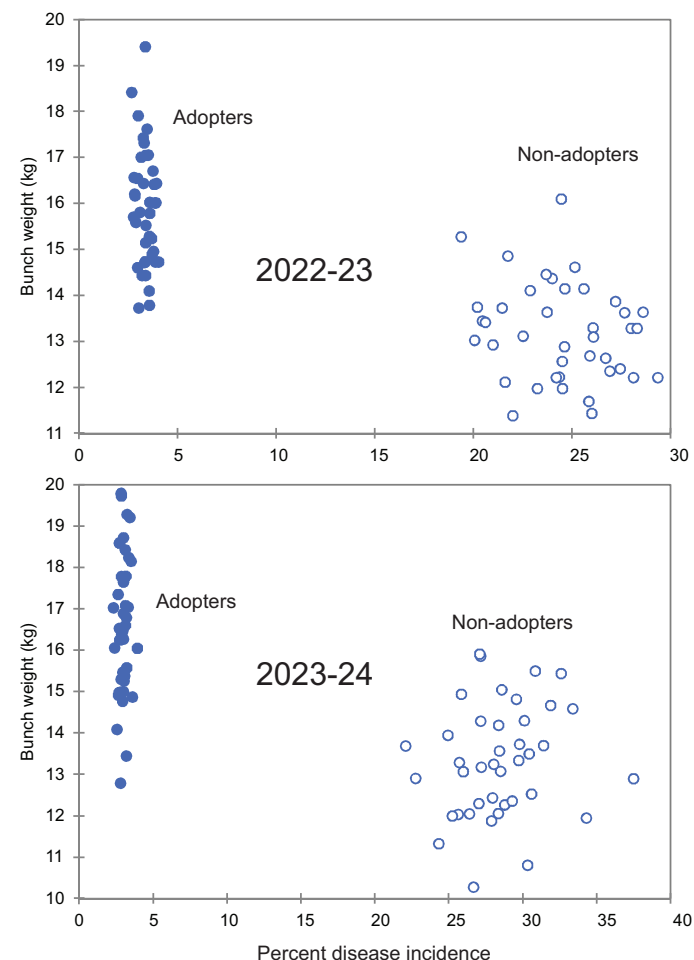


Fig. 4. Pattern of bunch weight with respect to bio-immunization technology adoption in Sabri banana cultivation in Tripura

Earlier reports were also suggested that Sabri banana from Tripura showed a disease incidence ranging from 5 to 23% and the isolated *Fusarium* species confirmed as race 1 (Uma *et al.*, 2001; Thangavelu *et al.*, 2024) and in the current study, molecular analysis of the *Fusarium* isolates collected from Tripura during the survey period were also identified as *Fusarium* race 1 (data not shown). The reason behind the better performance of the bio-immunized Sabri banana plants with periodical spraying of ICAR-FUSICONT was explained earlier by Damodaran *et al.*, 2023. The combination has the ability to reduce *Fusarium* wilt incidence significantly and probable reason was the antifungal secondary metabolites of *Trichoderma reesei* (CSR-T-3) contributing to the suppression of FOC. Further, Damodaran *et al.*, 2023 also revealed that the bio-immunization technology developed stress memory in the plant cell, which will help the plants to combat any biotic or abiotic stress efficiently. The current investigation also proven the fact that the use of bio-immunization with the periodical use of FUSICONT spray reduced the PDI and DSI significantly and also improved the plant yield attributes. Finally, it can be concluded that the technological interventions can enhanced the yield of Sabri banana variety at farmer's field in Tripura state by reducing the damages due to *Fusarium* wilt infestation.

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