


RESEARCH

Open Access



Impact of foliar application of phyllosphere yeast strains combined with soil fertilizer application on rice growth and yield

Gomathy Muthukrishnan^{1*}, Jeyashri Munisamy¹, Sabarinathan Kuttalingam Gopalasubramaniam^{2*}, Kizaharael Sevathapandian Subramanian³, Rajakumar Dharmaraj⁴, Dhruva Jyoti Nath⁵, Pranab Dutta⁶ and Arun Kumar Devarajan^{7*} 

Abstract

Background The application of beneficial microbes in agriculture is gaining increasing attention as a means to reduce reliance on chemical fertilizers. This approach can potentially mitigate negative impacts on soil, animal, and human health, as well as decrease climate-changing factors. Among these microbes, yeast has been the least explored, particularly within the phyllosphere compartment. This study addresses this knowledge gap by investigating the potential of phyllosphere yeast to improve rice yield while reducing fertilizer dosage.

Results From fifty-two rice yeast phyllosphere isolates, we identified three yeast strains—*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71, and *Cryptococcus* sp. Y72—that could thrive at 36 °C and possessed significant multifarious plant growth-promoting traits, enhancing rice root and shoot length upon seed inoculation. These three strains demonstrated favorable compatibility, leading to the creation of a yeast consortium. We assessed the combined effect of foliar application of this yeast consortium and individual strains with two distinct recommended doses of chemical fertilizers (RDCFs) (75 and 100%), as well as RDCFs alone (75 and 100%), in rice maintained in pot-culture and field experiments. The pot-culture experiment investigated the leaf microbial community, plant biochemicals, root and shoot length during the stem elongation, flowering, and dough phases, and yield-related parameters at harvest. The field experiment determined the actual yield. Integrated results from both experiments revealed that the yeast consortium with 75% RDCFs was more effective than the yeast consortium with 100% RDCFs, single strain applications with RDCFs (75 and 100%), and RDCFs alone (75 and 100%). Additionally, this treatment improved leaf metabolite levels compared to control rice plants.

Conclusions Overall, a 25% reduction in soil chemical fertilizers combined with yeast consortium foliar application improved rice growth, biochemicals, and yield. This study also advances the field of phyllosphere yeast research in agriculture.

Keywords Yeast, Phyllosphere, Plant growth-promoting microbes, Rice, Crop improvement

*Correspondence:

Gomathy Muthukrishnan

gomathymicro@gmail.com

Sabarinathan Kuttalingam Gopalasubramaniam

sabarinathan@tnau.ac.in; sabarimicro@hotmail.com

Arun Kumar Devarajan

aruntechnau@gmail.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

India, primarily an agricultural nation, ranks as the world's second-largest rice producer. The International Production Assessment Division's (IPAD) report from the United States Department of Agriculture (USDA) shows that in the 2022–23 season, rice production in India increased by 8.3 million tonnes from the previous year, totalling 135.7 million tonnes [1]. Annually, 30% of food is lost or wasted. Managing this loss and waste could potentially enhance food security and nutrition, although it's not a guaranteed outcome [2]. According to the report, a certain level of food loss and waste is needed as a buffer to ensure steady availability and access to food. Nonetheless, the output must be increased as agricultural land area diminishes and population size increases. Climate change, on the other hand, is known to have a negative effect on agricultural production and is expected to reduce the production of three cereal grains (rice, maize, and wheat) globally by 10 to 25%, with a 1 °C increase in mean surface temperature [3]. The use of agrochemicals has increased to improve crop yield and compensate for food loss. Annually, millions of tonnes of synthetic nutrients added to soils are not fully absorbed by plants. Excessive runoff from crop fields degrades soil fertility, harms human health, and contributes to climate change by emitting greenhouse gases, such as N₂O from nitrogen fertilizers [4, 5]. Considering all these factors, there has been a global shift to a sustainable farming approach with the primary goal of improving yield by reducing the use of agrochemicals in the field. There are many suggested approaches; among these, biofertilizers are gaining popularity, especially plant growth-promoting microorganisms (PGPM).

The microbes that exert beneficial effects on plants are termed PGPM. These microbes have the potential to alter plant performance directly through the production of specific compounds that enhance plant growth and increase the availability and uptake of nutrients in the soil (i.e., mineral solubilization, phytohormone production, nitrogen fixation, siderophore production, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production) [6]. The indirect mechanisms of plant growth promotion involves HCN production, induced systemic resistance, and suppression of pests and pathogens through metabolite production [6]. PGPM are primarily isolated from soils, rhizospheres (soil influenced by plant roots) or phyllospheres (aerial surface of the plants), and endospheres (inner tissues of plants), and it was discovered that bacteria predominate in soil and each plant compartment [7]. The soil microbiome is the primary source of plant microbiomes, which are strongly and sequentially filtered by the rhizosphere, rhizoplane (root surface), endosphere, and phyllosphere. As a result,

phyllosphere microbial diversity is lower than that of the rhizosphere, despite receiving microbial sources from air and insects [8, 9]. So, bacteria in the rhizosphere were the most extensively studied PGPM, known as plant growth-promoting rhizobacteria (PGPR). The second most widely studied microbes are from the phyllosphere compartment. The phyllosphere habitat includes all the above-ground plant parts (i.e., leaves, stems, flowers, and fruits). It is an unstable habitat where microbial life is shaped by multiple factors, like temperature, UV radiation, nutrient and water availability, and the chemical composition of the leaf surface. Bacteria dominate the leaf area, with an average of 10⁶–10⁷ bacteria per square cm of the leaf surface. However, the overall size of the phyllosphere's fungal population has not yet been estimated, but it is predicted to be smaller [10].

When it comes to the application of plant growth-promoting fungi (PGPF), the fungal origins were mostly from the soil and rhizosphere i.e., *Trichoderma* spp., *Gliocladium virens*, *Penicillium digitatum*, *Aspergillus flavus*, *Actinomucor elegans*, *Podospora bulbillosa*, and Arbuscular mycorrhizal fungi [11]. On the other hand, yeast is one of the least studied fungi, but it is gaining favor in crop improvement because it is generally recognized as safe (GRAS) for field application [12]. Since yeasts are unicellular, proliferate asexually and rapidly on simple carbohydrates, and typically utilize both fermentative and respiratory pathways, they are simpler to cultivate as a biofertilizer inoculant [13]. A diverse range of yeasts isolated from soil, rhizosphere, and, to a lesser extent, other plant parts demonstrate plant growth-promoting (PGP) characteristics, including the production of exopolysaccharide (EPS), Indole-3-acetic acid (IAA), gibberellic acid (GA), cytokinin, ACCD, siderophore [14–19], mineral solubilization [20–22], and biocontrol agents [23–25]. Given the beneficial effects of yeasts, it is critical to comprehend the role of yeast in compartments that have received little attention, such as the phyllosphere and endosphere. The significance of phyllosphere bacteria in improving rice yield and alleviating drought stress has previously been studied [26, 27], but no appropriate reports on the role of phyllosphere yeast in rice have been published. Since PGPMs alone do not achieve the same yield levels as chemical fertilizers, it is essential to explore their combined application for optimal results [28]. Nonetheless, more research is required to determine the role of yeast in crop plant improvement.

From the preceding discussion, it is clear that rice production in India must be enhanced using sustainable methods like biofertilizers. Nonetheless, to the best of the authors' knowledge, no previous publication has described the foliar application of rice phyllosphere yeast for improving rice yield. With the knowledge

gathered above, the current study is about the isolation and characterization of yeast from the rice leaf surface; the characterization of PGP properties in yeast; and understanding the individual and consortium effects of best yeast strains as well as different fertilizer dose applications on rice growth, leaf biochemicals, microbial population, and yield. Finally, the microbial and plant data will be combined using suitable integration techniques to identify the most effective beneficial yeast strains for rice [29]. Thus, this study contributes to the knowledge of the role and significance of phyllosphere yeast in crop improvement, making it a valuable asset for sustainable approaches.

Materials and methods

Collection of rice leaf samples

During the months of January, February, and March of 2019, rice leaf samples from various stages (stem elongation, flowering, and dough stages) were collected from high yielding, short-duration rice cultivar ASD 16 grown in the Killikulam region (8°40′26.616″N, 77°51′55.3392″E), Thoothukudi district, Tamil Nadu, India. The samples were collected aseptically and transported to the laboratory in an icebox for microbial analysis. During the sample collection months, the lowest and highest atmospheric temperatures were 25 °C and 36 °C. The average atmospheric temperature in the three months was 30.5 °C.

Isolation and selection of yeast

The leaf samples were washed three times with sterile water and dried with sterile tissue paper for 30 s to isolate putative phyllosphere yeast colonized on the leaf surface. The leaf imprinting technique was used to isolate phylloplane yeast [30]. Adaxial and abaxial sides of leaves were imprinted on various yeast media such as yeast extract peptone dextrose (YEPD) agar media, supplemented with chloramphenicol (50 µl ml⁻¹) and tetracycline (50 µl ml⁻¹) at pH 7. The plates were incubated for 48 h at 30 ± 1 °C. Morphologically distinct yeast colonies were purified and maintained on a YEPD slant at 4 °C, and 60% glycerol stock was preserved at -80 °C for future use. Yeast isolates were screened for growth in different temperature levels. The cell density of the purified isolates was set to OD_{600nm} 0.1, and 1 mL was added to 9 mL of YEPD broth test tubes at pH 7. Due to the variable temperature conditions observed in the phyllosphere, the test tubes were incubated at field-simulated temperatures of 25, 30, and 36 °C for five days. Yeast strains cultivated at these three temperature levels were subsequently selected for taxonomic identification.

Molecular characterization of yeast isolates and phylogenetic analysis

Genomic DNA from all the selected yeast isolates was extracted as per the described procedure [31]. The isolates were characterized by using ITS rRNA gene sequencing. Polymerase chain reaction (PCR) amplifications were performed using ITS1 as forward primer (5'-CTTGGTCATTTAGAGGAAGTAA -3') and ITS4 as reverse primer (5'-TCCTCCGCTTATTGATATGC -3'). The PCR conditions were as follows: initial denaturing at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s. Annealing at 59 °C for 30 s and extension at 70 °C for 2 min, then final extension at 72 °C for 7 min. The PCR products were resolved by electrophoresis in 1% agarose gel and purified using FavorPrep GEL/ PCR purification kit. The products were sequenced using Sanger dideoxy sequencing. Based on the obtained sequence, species were identified in the NCBI database, and the sequences of closely related species were also retrieved. The phylogenetic tree was generated using MEGA 7.0 software with a maximum likelihood algorithm, and the bootstrap analysis was based on 100 resamplings [32]. The sequence files of the yeast strains were submitted to the NCBI database via BANKIT, and accession numbers were assigned to each strain.

Assessment of direct PGP activity in yeast strain

IAA production

Indolic compound production was quantitatively measured [33]. Yeast strains were inoculated in 10 mL of YEPD broth supplemented with 1% tryptophan, incubated for 48 h at 30 ± 1 °C on a rotary shaker, while uninoculated broth served as control. Cultures were centrifuged at 10,000 rpm for 20 min, and supernatants were collected. After that two drops of concentrated orthophosphoric acid and 2 mL of Salkowski's reagent (2% 0.5 M FeCl₃ in 35% perchloric acid solution) were mixed with 1 mL of supernatant and incubated at room temperature for 25 min in the dark. The development of a reddish-pink color indicated IAA production. The absorbance was measured at 530 nm using a spectrophotometer (LAMBDA 365 UV-Vis spectrophotometer, PerkinElmer, Mumbai, India), and the quantitative estimation of IAA was performed by using a standard graph. The result is expressed as µg mL⁻¹.

GA production

GA production by yeast strains was quantified by the spectrophotometric method [34]. The yeast strains were inoculated into 10 mL of YEPD broth and incubated at room temperature for seven days, and the uninoculated broth was used as a control. Cultures were centrifuged

at 8,000 rpm for 10 min, and 2 mL of supernatant was transferred into a 15 mL reaction tube. Then, 2 mL of zinc acetate solution (21.9 g zinc acetate with 1 ml of glacial acetic acid and the volume was made up to 100 ml with distilled water) and 2 mL of 10.6% potassium ferrocyanide solution were added to the tubes, followed by centrifugation at 8,000 rpm for 10 min. A total of 5 mL of supernatant was added to 5 mL of 30% hydrochloric acid and incubated at 27 °C for 75 min. The absorbance was measured at 254 nm. The GA concentration in the samples was determined with reference to the standard GA curve. The result is expressed as $\mu\text{g mL}^{-1}$.

Qualitative assay of phosphate and potassium solubilization

Solubilization of inorganic minerals such as phosphate and potassium by yeast strains was carried out using Pikovskaya medium and Aleksandrov medium [35]. In short, spot inoculation of each yeast strain was done on these media and incubated at 28 ± 2 °C for three days. The strains that exhibited a clear halo zone around the colony on the media were identified as P and K solubilizers. Based on the area of solubilization, the solubilization index (SI) was calculated using the following formula.

$$\text{Mineral solubilization index (SI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Assessment of indirect PGP traits in yeast strain

Siderophore production

Siderophore production was evaluated for all yeast strains using Chrome Azurol S (CAS) assay [36]. Briefly, the CAS medium was prepared by adding CAS solution to the melted King's B agar medium in a 1:15 ratio. Actively grown yeast strains were spot-inoculated onto the CAS medium, and the plates were incubated in the dark at 30 ± 1 °C for ten days. An uninoculated CAS blue agar plate served as a control. Colonies with a yellow-orange halo after incubation were considered positive for siderophore production. The yeast strains were grown in King's B broth and incubated for 48 h to quantify the siderophore. After incubation, the broth was centrifuged at 10,000 rpm for 10 min. The supernatant was mixed with CAS solution in a 1:1 ratio and incubated for 20 min, with sterile king's B broth containing CAS solution as a control reference. The absorbance of the solution was then measured using a spectrophotometer at 630 nm. The siderophore production was calculated using the following formula [37]:

$$\text{Percentage of siderophore production} = \frac{A_r - A_s}{A_r} \times 100$$

whereas A_r - Absorbance of reference and A_s - Absorbance of the sample.

ACC deaminase activity

ACC deaminase activity of yeast strains was screened based on their ability to use ACC as a sole nitrogen source. Yeast strains were grown in Dworkin and Foster (DF) salts broth with ACC as a nitrogen source for 24 h. The inoculated broth was centrifuged at 10,000 rpm for 5 min at 4 °C, and the solution was washed using 1 mL of 0.1 M Tris HCl pH 7.6 and repeated several times, and the pH was maintained at 7.6 and stored the pellet at 20 °C for 30 min. The cells were suspended with 1 mL of 0.1 M Tris-HCl at pH 7.6 and centrifuged. The pellet was resuspended in 600 μL of 0.1 M Tris-HCl at pH 8.5. Toluene was added to the 30 μL and vortexed for 30 s. To this, 20 μL of 0.5 M ACC was added in one set, and blank was maintained without ACC addition. Both the tubes were vortexed and incubated at 30 °C for 15 min, and 1 mL of 0.56 M HCl was added, vortexed, and centrifuged for 5 min at 10,000 rpm at room temperature. Then 300 μL of 2,4-Dinitrophenylhydrazine (DNPH) was added, vortexed, and incubated for 30 min at 30 °C. Finally, 2 mL of 2 N NaOH was added, mixed well and absorbance was read at 540 nm [38]. The enzyme activity is expressed as $\text{nmol } \alpha\text{-ketobutyrate mg}^{-1}\text{protein h}^{-1}$.

Biocontrol activity of phyllosphere yeast strains against rice fungal pathogens

The yeast strains were screened for direct antagonism against *Helminthosporium oryzae*, *Pyricularia oryzae*, and *Sarocladium oryzae* (Standard cultures, Department of Soil Science and Agricultural Chemistry, AC & RI, Killikulam, India). In brief, a mycelial disk (5 mm diameter) of each rice pathogenic fungus was placed on the edge of potato dextrose agar (PDA) media (30 mm), and each yeast was streak inoculated close to the center of the plates. The plates were incubated for 5 to 6 days at 25 °C. Inhibition of mycelial growth was measured 5 to 6 days after the inoculation, and the percentages of inhibition by each yeast strain were calculated as follows [39]

$$\begin{aligned} \text{Inhibition percentage} &= \left[\frac{\text{(growth of pathogen in control)} - \text{(growth of pathogen with yeast strains)}}{\text{growth of pathogen in control}} \right] \times 100 \end{aligned}$$

Effect of yeast strains on the growth of rice seedlings

The effect of yeast strains on rice seedling growth was assessed in vitro using the standard roll paper towel method [40, 41]. Surface disinfection of rice seeds was performed for 1 min with 70% ethanol, followed by

20 min with a 1.65% sodium hypochlorite (NaOCl) solution. The seeds were rinsed five times with distilled water for one minute each. Yeast cells from the overnight-grown culture were pelleted after centrifugation and resuspended in the same volume of sterile water, with the final concentration adjusted to $\sim 10^8$ colony-forming units (CFU) mL^{-1} . The seeds were soaked in yeast cell suspension for 2 h before being blotted at room temperature with sterile filter paper. As wet blotters, we used sterile filter papers with a diameter of 120 mm. Five seeds were placed in a straight line at equal distances on each filter paper. Each filter paper was rolled, placed vertically in a 350 mL plastic cup with water, and incubated at 28 °C for 8 days in the growth chamber. Adequate water was regularly poured into the cups 1 cm below the seed arrangement. As a control, seeds soaked in distilled water were used. The plant phenotypic traits, such as root and shoot lengths, were measured after 10 days.

Pot-culture experiment

Selected yeast strains were tested for compatibility in terms of growth by cross-streak assay in a YEPD medium [42]. A triangular streak of three yeast colonies was made on a media plate and incubated for three days. Growth without inhibition confirmed compatibility; otherwise, the strains were considered non-compatible.

Yeast inoculums were prepared by cultivating the yeast strains in YEPD broth for 24 h. After centrifuging the yeast cells for 10 min, the pellets from the three best strains were resuspended in sterile water, resulting in a final consortium concentration of approximately 10^8 CFU mL^{-1} , with 0.2% Tween 80 as a sticking agent. The same protocol was followed for individual strain suspensions, each with a final concentration of 10^8 CFU mL^{-1} .

A pot culture experiment using a completely randomized block design (CRBD) was carried out in rice variety ASD 16 at the unit of Agricultural Microbiology, Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Killikulam, India.

Soils were sterilized in an autoclave at 121 °C for 15 min at 20 psi over three consecutive days. A 5 kg potting mixture (soil + farmyard manure in a 4:1 ratio) was placed in each mud pot. Rice seeds (ASD 16) were sown in trays, and seedlings were transplanted to the pots after 20 days, with each pot containing ten plants. Fertilizers such as urea, single superphosphate, and potassium chloride were applied at 100% and 75% recommended dose of chemical fertilizers (RDCFs) (nitrogen: phosphorus: potassium, 120:40:40 kg ha^{-1}).

Treatment details

T₁—Yeast 1 + 100% RDCFs.

T₂—Yeast 2 + 100% RDCFs.

T₃—Yeast 3 + 100% RDCFs.

T₄—Yeast consortium (Yeast 1 + Yeast 2 + Yeast 3) + 100% RDCFs.

T₅—100% RDCFs.

T₆—Yeast 1 + 75% RDCFs.

T₇—Yeast 2 + 75% RDCFs.

T₈—Yeast 3 + 75% RDCFs.

T₉—Yeast consortium (Yeast 1 + Yeast 2 + Yeast 3) + 75% RDCFs.

T₁₀—75% RDCFs.

T_C—Control.

The prepared yeast consortium and individual suspensions were foliar sprayed (five sprays per pot) on rice at the beginning of the stem elongation (55 DAS), flowering (85 DAS), and dough (100 DAS) stages. Three days after each foliar application, total chlorophyll, protein, proline, and antioxidant enzyme activity in the leaves were measured, along with the lengths of the shoots and roots. Additionally, leaf samples from each stage were stored at -4 °C for future use. Yield characteristics were recorded at the harvesting stage (115 DAS).

Estimation of the microbial population on the rice leaf surface

The microbial population on the rice leaf surface (bacteria, yeast, and actinomycetes) was assessed using the Most Probable Number (MPN) method in which the rice leaves were dipped into the sterile water for a minute, followed by serial dilution. Plating techniques involving microbial growth media viz. Luria–Bertani (LB), YEPD, and glycerol agar for bacteria, yeast, and actinomycetes, respectively. CFUs of bacteria, yeast, and actinomycetes were detected after incubating for 5–7 days. Microbial count and changes in microbe populations were studied at three different crop stages (stem elongation, flowering, and dough stage).

Estimation of total chlorophyll

To estimate the total chlorophyll content, 0.5 g of the leaf samples collected during all three stages were thoroughly ground in a pestle and mortar with 10 ml of 80% acetone. The sample mixture was then centrifuged at 10,000 rpm for 10 min in an ice-cold environment. After carefully transferring the supernatant into new tubes, 2 ml of the sample was aliquoted in a cuvette and tested for chlorophyll a, chlorophyll b, and carotenoid content by measuring multiple absorbances at 663, 646, and 470 nm, [43] and calculated using the formula as follows:

$$\text{Chlorophyll a} = 12.25 A_{663} - 27.9 A_{646}$$

$$\text{Chlorophyll b} = 21.5 A_{646} - 5.1 A_{663}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Estimation of total protein and proline content

The conventional method [44] was used to assess the total protein content ($\mu\text{g g}^{-1}$ FW) at 650 nm against various bovine serum albumin (BSA) concentrations. Proline content in rice leaves was measured at 520 nm using the acid ninhydrin and glacial acetic acid as described by Bates et al., (1973) [45]. The concentration of proline in the leaf samples was determined using the standard curve for free proline and is expressed as $\mu\text{g g}^{-1}$ FW.

Assay of antioxidant enzymes

The sample extraction for enzyme assay was done according to the protocol described by Alici and Arabaci (2016) [46]. 1 g of fresh rice leaves were taken, cleaned and homogenized in an ice-cold mortar and pestle with 50 ml of a 100 mM sodium phosphate buffer (pH 7.0) for five minutes at 4 °C. Filter paper was used to filter the homogenate, and the filtrate was centrifuged at 5,000 rpm for 15 min. The supernatant was collected, and it was used for enzyme assay of catalase, peroxidase, and polyphenol oxidase.

The catalase activity (CAT) activity was determined by monitoring the disappearance of H_2O_2 at 240 nm using the method defined by Aebi [47]. The 5 mL reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 33 mM H_2O_2 , and enzyme extract. The absorbance was recorded for 1 min once the reaction began. An extinction coefficient of 36 M cm^{-1} was used to calculate CAT activity.

The peroxidase (POD) activity was estimated [48] using guaiacol as a substrate. The 3 ml reaction mixture consisted of 100 mM potassium phosphate buffer with pH 7.0 (1.9 ml), 5 mM guaiacol (0.5 ml), 5 mM H_2O_2 (0.5 ml), and 100 μl of sample extract. The reaction mixture for blank was prepared in a similar way excluding the sample extract. The sample was added to the reaction mixture, and the absorbance was measured at 470 nm for 1 min. An extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ was used to calculate peroxidase activity.

The polyphenol oxidase (PPO) activity was assayed as per the method adopted [49]. The 3 ml reaction mixture contained 100 mM potassium phosphate buffer having pH 7.0 (2 ml), 5 mM catechol (0.5 ml), and 500 μl of sample extract (0.5 ml) in a test tube. And a blank without a sample extract. The sample was added to the reaction mixture, and the absorbance was measured at 420 nm for 1 min. The polyphenol oxidase activity was determined

by measuring the increase in absorbance resulting from the oxidation of catechol ($\epsilon = 34.5 \text{ mM}^{-1} \text{ cm}^{-1}$) at 420 nm spectrophotometrically. All three enzyme activities are expressed as variations in mmol per time unit per mg total protein ($\text{mmol min}^{-1} \text{ mg protein}^{-1}$).

Measurement of plant phenotypic and yield parameters

Plant phenotypic traits such as shoot and root length were measured during stem elongation, flowering, and dough stages. Yield parameters such as the number of productive tillers, grains per panicle, and 1000-grain weight were recorded during the harvest stage.

Gas chromatography-mass spectrometry (GC-MS) analysis of leaf extracts

Leaf samples from the control and the most effective treatment during the stem elongation phase were chosen for metabolite comparison. Leaf extracts were prepared by grinding them in 10 mL of cold water. 1N HCl was used to modify the pH to 2.0, and an equal volume of chloroform was added. These mixtures were then shaken for 24 h. Following incubation, the chloroform layer was removed, and the metabolites were concentrated using a vacuum flask evaporator before being dissolved in methanol. GC-MS analysis of methanol extracts was performed on a GCMS-TQ8040 triple quadrupole fitted with a Crossbond™ silarylene phase column (1,4-bis(dimethylsiloxy) phenylene dimethyl polysiloxane) (30 m 250 μm). The volume used for analysis was 10 μL , and the carrier gas used for GC-MS was helium at the flow rate of 1 mL min^{-1} . The injector was operated under the maximum temperature of 470 °C, and the oven temperature was 350 °C constant till the entire analysis. The extracts from control and treatment samples were prepared in triplicate, with pure methanol serving as the control. The compounds were identified by comparing the obtained spectral configurations to those in the accessible mass spectral library (NIST and WILEY libraries).

Field experiment

A field experiment was carried out at the Agricultural Microbiology Unit, Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Killikulam, India, from January to April 2020. Rice seeds (ASD 16) were planted in the nursery bed, and seedlings were transplanted to fields with plot sizes of 20 m^2 for each treatment. The treatments are listed below.

- FT₁—Yeast 1 + 100% RDCFs.
- FT₂—Yeast 2 + 100% RDCFs.
- FT₃—Yeast 3 + 100% RDCFs.
- FT₄—Yeast consortium (Yeast 1 + Yeast 2 + Yeast 3) + 100% RDCFs.
- FT₅—100% RDCFs.

FT₆—Yeast 1 + 75% RDCFs.

FT₇—Yeast 2 + 75% RDCFs.

FT₈—Yeast 3 + 75% RDCFs.

FT₉—Yeast consortium (Yeast 1 + Yeast 2 + Yeast 3) + 75% RDCFs.

FT₁₀—75% RDCFs.

FT_C—Control.

The prepared yeast inoculum ($\sim 10^8$ CFU mL⁻¹) was foliar sprayed on rice during the stem elongation, flowering, and dough stages. Finally, the rice grains were harvested from each plot, and the yield was calculated and expressed as tons per hectare (t ha⁻¹).

Statistical analysis

All experiments were conducted in triplicate, with results expressed as means with standard deviations. Statistical analyses were performed using R version 4.1.1. Tukey's post hoc test was applied using the stats package [50] to rank the yeast strains and treatments. A two-way ANOVA using the stats package was conducted to assess the effects of treatments, growth stages, and their interaction on various rice plant variables. A Venn diagram, created with the ggVennDiagram package, illustrated the percentage of unique and common rice leaf metabolites between control and yeast consortium-sprayed plants [51]. Changes in rice leaf metabolites in yeast consortium-treated plants were visualized using a volcano plot generated with ggplot2 [52]. For data integration approaches, the made4 package was used for coinertia analysis (CIA) [53], the FactoMineR package followed by the PCAtest package for Principal Component Analysis (PCA) [54, 55], the mixKernel package for Multiple Kernel Learning (MKL) [56], and the omicade4 package for Multiple Co-Inertia Analysis (MCIA) [57].

Data integration

We used unsupervised machine learning-based horizontal data integration methods, including MKL, MCIA, and PCA, to identify the optimal treatment for rice growth and yield. PCA reduces dimensionality linearly, highlighting principal components that explain the most variance. MKL combines multiple kernels to model non-linear relationships and interactions. MCIA uncovers common structures and correlations across multiple datasets, offering a comprehensive view of shared variations. These diverse techniques help select the best treatment and understand the underlying reasons for treatment groupings, as each method varies in data integration and dimension reduction approaches [29].

For the integration techniques, the parameters from the pot culture experiment were categorized into plant biochemical traits (total chlorophyll, protein, proline, and antioxidant enzyme activity: catalase, peroxidase,

and polyphenol oxidase) and plant phenotypic traits (shoot and root length) and microbial population (CFUs of bacteria, actinomycetes, and yeast), separately for each growth stage. The yield-related parameters from pot culture and actual yield from the field experiment measured during harvest were grouped into one dataset. The dataset combinations used for the integration techniques are listed in Supplementary Table 1. Unlike MCIA, which can integrate multiple datasets, CIA can only integrate two. We used CIA to combine PGP traits of yeast strains with the shoot and root lengths of rice grown in roll paper towels.

Results

Isolation and characterization of yeast isolates

Fifty-two morphologically distinct isolates were obtained from the collected leaf samples using the leaf imprinting method on different media. Among 52 isolates, 11, 16, and 25 were isolated from the stem elongation, flowering, and dough phases, respectively. The isolates were subjected to growth at different temperature levels (25, 30, and 36 °C), and all 52 isolates were grown at 25 and 30 °C, but only nine isolates (Y1, Y9, Y55, Y57, Y70, Y71, Y72, Y76, and Y78) were found to grow at 36 °C (Supplementary Table 2). DNA from the nine yeast isolates was extracted, and the ITS rRNA gene was sequenced. The sequences were compared in the NCBI database, and closely related species were obtained using BLAST. A phylogenetic tree was constructed for all the yeast isolates, and their close relatives were determined (Fig. 1). The isolated yeasts were identified as *Rhodotorula paludigena* Y1, *Dirkmeia* sp. Y9, *Pseudozyma* sp. Y55, *Pseudozyma* sp. Y57, *Dirkmeia* sp. Y70, *Pseudozyma* sp. Y71, *Cryptococcus* sp. Y72, *Dirkmeia* sp. Y76, *Dirkmeia churashimaensis* Y78. The DNA sequences of the identified yeast species were submitted to Genbank, and their accession numbers and plant host details are provided in Supplementary Table 3.

PGP of phyllosphere yeast strains

Direct PGP traits

Among the nine strains, *R. paludigena* Y1 exhibited the highest production of IAA, followed by, *Pseudozyma* sp. Y55, *Dirkmeia* sp. Y70, *Pseudozyma* sp. Y71, *Cryptococcus* sp. Y72, *Dirkmeia* sp. Y76, *D. churashimaensis* Y78 showed no significant difference in the production of IAA. The least IAA production was observed in *Dirkmeia* sp. Y9. In the case of GA production, *R. paludigena* Y1 recorded the highest GA production, followed by *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72 had the maximum GA production with a less significant difference (Table 1). The minimum amount was recorded in yeast strains *Cryptococcus* sp. Y72, *Dirkmeia* sp. Y76 and

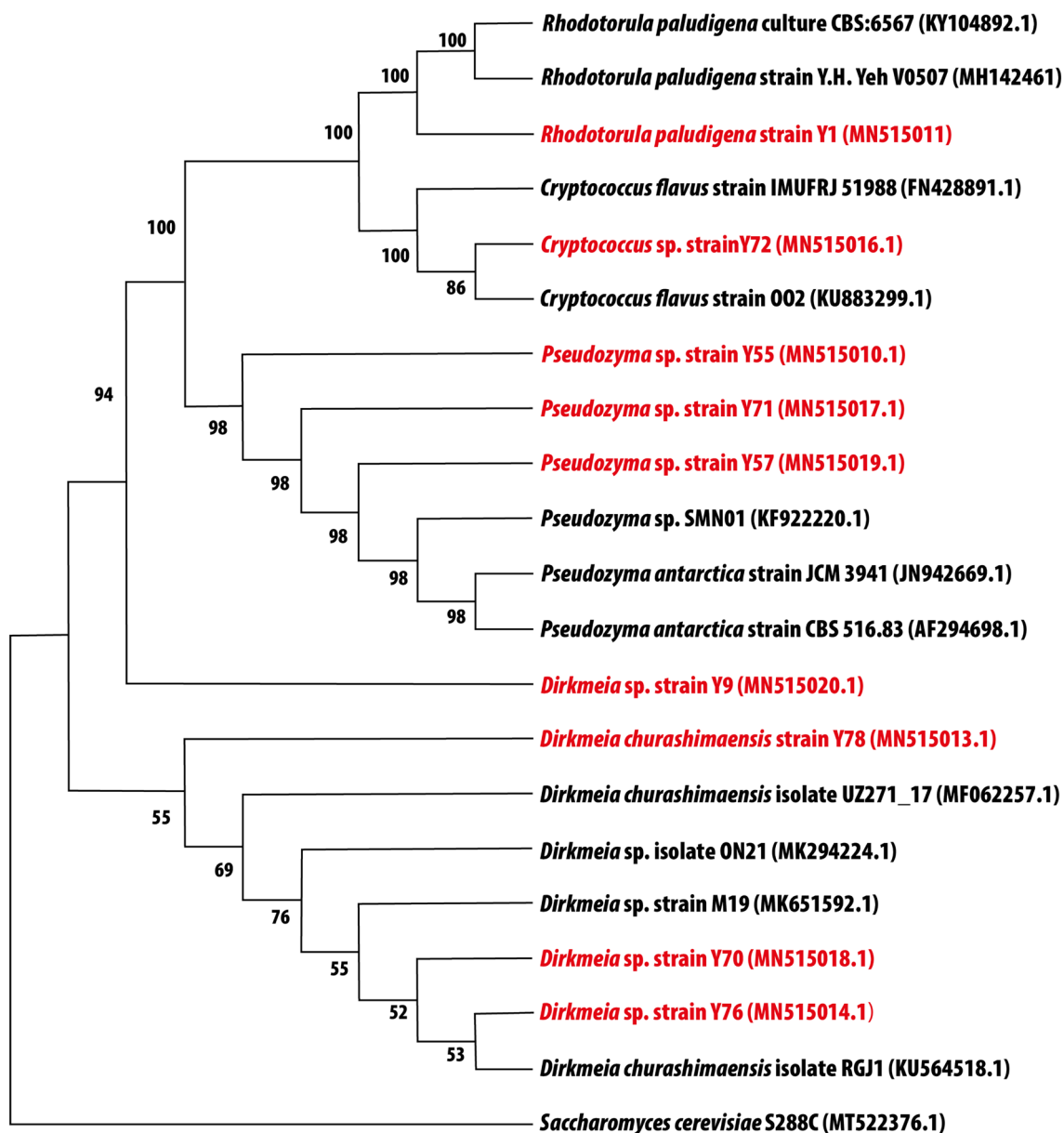


Fig. 1 The maximum likelihood phylogenetic tree based on ITS1 and ITS4 gene sequences, showing the relationships between the yeast taxa identified in this study. The bootstrap values $\geq 50\%$ (based on 100 replications) are shown at branching points. Strains in red are the yeast strains isolated from the rice phyllosphere

D. churashimaensis Y78 with no significant difference in GA production. The potential of the yeast strains for the solubilization of phosphate and potassium was determined. All nine strains solubilized the tricalcium phosphate in the plate assay method. The highest phosphate solubilization index was recorded in the strain *R. paludigena* Y1 and *Pseudozyma* sp., with the least significant difference. Whereas low phosphate solubilizing index was recorded in *Dirkmeia* sp. Y76 (Table 1). The results of the potassium solubilization study showed that all

strains had the inorganic potassium solubilization potential, but there was no considerable significant difference among them. The highest production was recorded in *R. paludigena* Y1, *Pseudozyma* sp. Y71, *Cryptococcus* sp. Y72, *Pseudozyma* sp. Y57, and *Pseudozyma* sp. Y55 and they are significantly different from the rest of the other strains, which were recorded with the least solubilization index (Table 1).

Table 1 Mean and standard deviation values (n = 3) of direct plant growth-promoting traits such as Indole acetic acid (IAA) and gibberellic acid (GA) production and mineral solubilization potential of rice phyllosphere yeast strains

Strains	IAA (µg mL ⁻¹)	GA (µg mL ⁻¹)	Solubilization index	
			Phosphate (P)	Potassium (K)
<i>Rhodotorula paludigena</i> Y1	77.2 (9.13) ^a	101 (6.60) ^a	2.84 (0.12) ^a	2.48 (0.12) ^a
<i>Dirkmeia</i> sp. Y9	5.01 (1.43) ^d	59.4 (15.6) ^{cd}	2.36 (0.17) ^{bcd}	1.33 (0.28) ^b
<i>Pseudozyma</i> sp. Y55	34.2 (3.36) ^b	6.11 (3.32) ^e	2.06 (0.05) ^{cde}	2.01 (0.01) ^a
<i>Pseudozyma</i> sp. Y57	12.6 (2.82) ^c	54.7 (7.87) ^{cd}	2.00 (0.01) ^{de}	2.13 (0.15) ^a
<i>Dirkmeia</i> sp. Y70	30.2 (11.7) ^b	44.4 (7.13) ^d	2.32 (0.12) ^{bcd}	1.21 (0.26) ^b
<i>Pseudozyma</i> sp. Y71	40.8 (2.61) ^b	84.4 (7.84) ^{ab}	2.61 (0.10) ^{ab}	2.43 (0.13) ^a
<i>Cryptococcus</i> sp. Y72	40.6 (2.52) ^b	74.2 (7.73) ^{bc}	2.51 (0.10) ^{abc}	2.35 (0.13) ^a
<i>Dirkmeia</i> sp. Y76	29.3 (2.80) ^b	7.14 (2.26) ^e	1.66 (0.28) ^e	1.33 (0.28) ^b
<i>Dirkmeia churashimaensis</i> Y78	36.0 (2.02) ^b	2.13 (1.81) ^e	2.24 (0.10) ^{bcd}	1.16 (0.28) ^b

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

Indirect PGP traits

All the yeast strains were tested for siderophore production and showed significant production (Table 2). The highest production was recorded in the strain *R. paludigena* Y1 followed by *D. churashimaensis* Y78 and *Cryptococcus* sp. Y72. While the least production was recorded in the strain *Dirkmeia* sp. Y9. The activity of ACC deaminase was assessed for all nine strains; among them, *R. paludigena* Y1 recorded the highest ACC deaminase activity, followed by *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72. The strain *Dirkmeia* sp. Y70 exhibited the lowest ACC deaminase activity (Table 2). The results of the antagonistic activity of nine yeast strains against rice fungal pathogens showed that *R. paludigena* Y1 had the highest inhibition percentage against *H. oryzae* and *P. oryzae*, while *R. paludigena* Y1 and *Pseudozyma* sp. Y71 exhibited the highest inhibition percentage against *S. oryzae* (Table 2; Supplementary Fig. 1). Similarly, the lowest

inhibition of all three fungal pathogens was recorded in *D. churashimaensis* Y78.

Relationship between yeast strains and rice growth parameters

The root and shoot length of the rice plants treated with yeast strains grown on rolled paper were measured (Fig. 2). The root length was significantly higher in the rice plants treated with *R. paludigena* Y1, followed by those treated with *Cryptococcus* sp. Y72 and *Pseudozyma* sp. Y71 recorded the maximum root length. The shortest root length was recorded in the control plants, and in the case of treated plants, the low length was noted in *Dirkmeia* sp. Y9, and *Dirkmeia* sp. Y76 treated plants. Interestingly, shoot length was observed to be higher in the rice treated with *Pseudozyma* sp. Y71. The shortest shoot length was observed in control plants as well as plants treated with *Dirkmeia* sp.

Table 2 Mean and standard deviation values (n = 3) of indirect plant growth-promoting traits such as siderophore production, ACC deaminase activity, and growth inhibition of *Helminthosporium oryzae*, *Pyricularia oryzae* and *Sarocladium oryzae* by rice phyllosphere yeast strains

Strains	Siderophore (%)	ACC deaminase activity (nmol α-ketobutyrate-mg ⁻¹ protein-h ⁻¹)	Inhibition percentage (%)		
			<i>Helminthosporium oryzae</i>	<i>Pyricularia oryzae</i>	<i>Sarocladium oryzae</i>
<i>Rhodotorula paludigena</i> Y1	26.8 (0.82) ^a	10.3 (0.65) ^a	46.6 (0.52) ^a	50.8 (2.22) ^a	40.2 (1.33) ^a
<i>Dirkmeia</i> sp. Y9	3.62 (0.51) ^a	0.60 (0.16) ^{ef}	15.2 (0.48) ^f	15.6 (0.34) ^d	15.5 (0.45) ^{de}
<i>Pseudozyma</i> sp. Y55	6.23 (0.40) ^f	0.41 (0.06) ^{ef}	28.6 (0.81) ^d	15.5 (0.25) ^{de}	20.0 (0.41) ^c
<i>Pseudozyma</i> sp. Y57	2.30 (0.65) ^{gh}	1.33 (0.07) ^{def}	18.1 (0.23) ^e	15.5 (0.41) ^{de}	17.1 (0.25) ^d
<i>Dirkmeia</i> sp. Y70	8.76 (0.76) ^e	0.15 (0.06) ^f	37.4 (1.75) ^b	18.1 (0.22) ^{cd}	15.9 (0.61) ^{de}
<i>Pseudozyma</i> sp. Y71	14.9 (0.83) ^d	5.37 (1.11) ^b	33.1 (0.21) ^c	27.4 (1.35) ^b	40.2 (1.32) ^a
<i>Cryptococcus</i> sp. Y72	17.2 (0.31) ^c	2.14 (0.24) ^c	38.6 (2.24) ^b	20.4 (1.01) ^c	38.0 (0.86) ^b
<i>Dirkmeia</i> sp. Y76	7.80 (0.71) ^{ef}	0.32 (0.06) ^{ef}	15.6 (0.32) ^{ef}	16.1 (0.62) ^d	13.8 (0.27) ^{ef}
<i>Dirkmeia churashimaensis</i> Y78	24.1 (0.31) ^b	1.55 (0.11) ^{de}	15.7 (0.33) ^{ef}	12.9 (0.15) ^{ef}	14.9 (0.44) ^{ef}

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

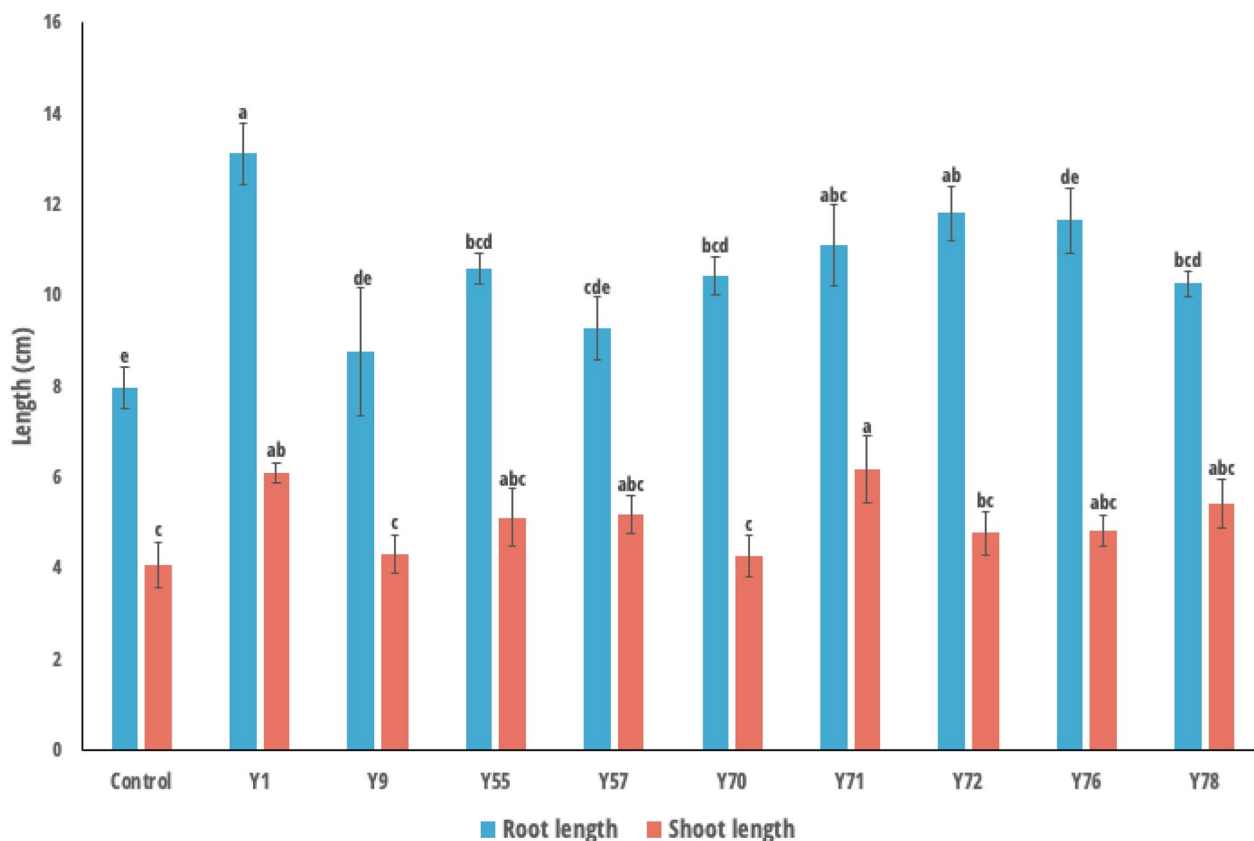


Fig. 2 Results of root and shoot length of rice obtained from the roll paper towel method treated with yeast strains. The strain codes on the plot are as follows, Y1—*Rhodotorula paludigena* Y1, Y9—*Dirkmeia* sp. Y9, Y55—*Pseudozyma* sp. Y55, Y57—*Pseudozyma* sp. Y57, Y70—*Dirkmeia* sp. Y70, Y71—*Pseudozyma* sp. Y71, Y72—*Cryptococcus* sp. Y72, Y76—*Dirkmeia* sp. Y76, and Y78—*Dirkmeia churashimaensis* Y78. Bars with different letters are significantly different according to Tukey's test. $p < 0.05$

Y9 and *Dirkmeia* sp. Y70 with no significant difference between them. Co-inertia analysis (CIA) was applied to assess the correspondence between two datasets (yeast PGP traits and rice phenotypic traits obtained from the roll paper towel method). The graphical output from this analysis is provided in Fig. 3. The CIA findings revealed a strong correlation ($RV = 0.869$, $p < 0.001$) between the two datasets with an eigenvalue of 11.8 in the first axis (Fig. 3B). The yeast PGP traits, specifically the P-solubilization index and *P. oryzae* inhibition percentage, were associated with root length, while the K-solubilization index and ACC deaminase activity were linked to shoot length (Fig. 3C, D). The first CIA axis captured the most variance (99%) in the two datasets and separates the *R. paludigena* Y1 strain from the rest of the yeast strains (Fig. 3E).

Impact of treatments on rice in pot-culture experiment

Treatments, rice growth stages, and their combined effects significantly impacted the microbial population, rice biochemical traits, and phenotypic traits (Supplementary Table 4).

Effect on leaf surface microbial population

In the case of the measured microbial population, the CFUs of bacteria, actinomycetes, and yeast in all the treatments were increased from the stem elongation to the dough stage. The highest bacterial, yeast, and actinomycetes population was recorded in plants treated with yeast consortium (*R. paludigena* Y1, *Pseudozyma* sp. Y71, and *Cryptococcus* sp. Y72) + 75% RDCFs (T_9) in all stages and that was approximately double the population measured in control plants (T_C) (Table 3). At all stages, the T_C plants had the lowest microbial population.

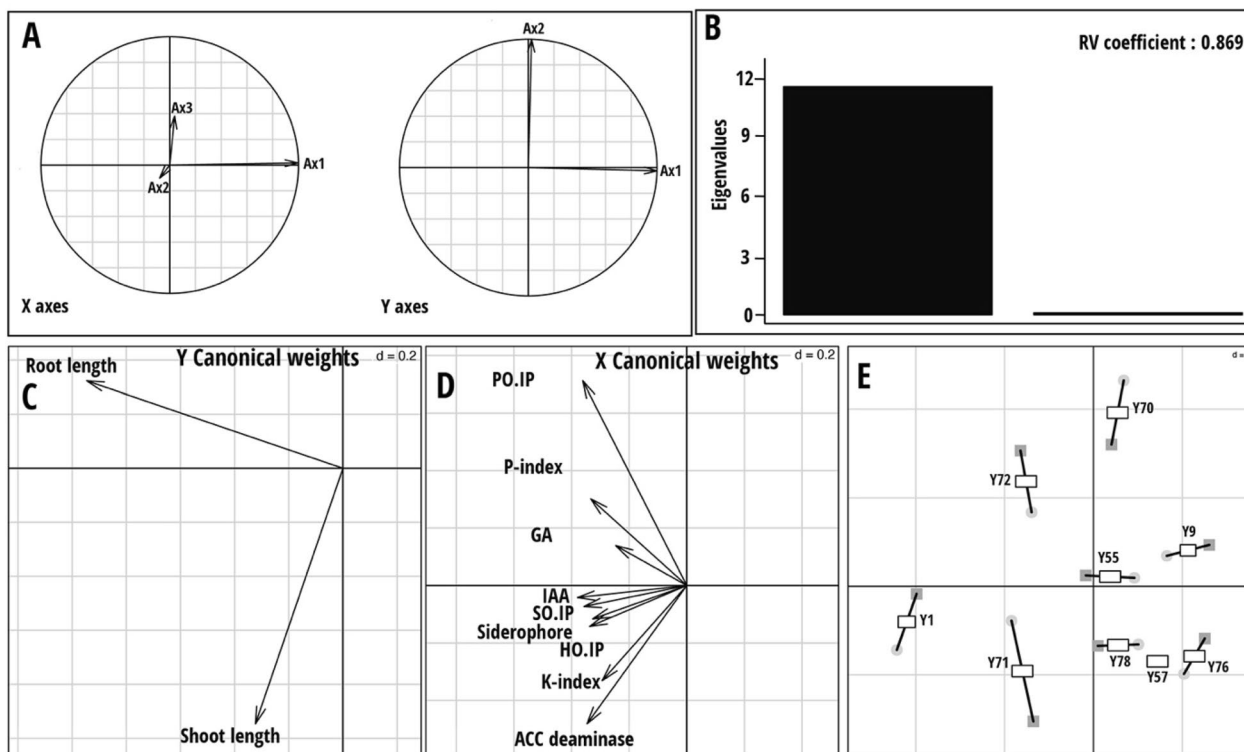


Fig. 3 Co-inertia analysis (CIA) results based on two datasets (yeast plant growth promoting traits and rice phenotypic traits). **A** Projections of the principal axes of the two datasets onto the axes of the co-inertia analysis. X axes: Yeast plant growth promoting traits; Y axes: rice phenotypic traits. **B** Scree plot of eigenvalues. **C** Correlation of plant phenotypic traits data with the first two axes of the co-inertia analysis. **D** Correlation of yeast plant growth promoting traits and plant phenotypic traits with the first two axes of the co-inertia analysis. **E** Plot of the first two components in the sample space. Each sample is represented by a square, where the two datasets for each sample are connected by lines to a center point (global score). The strain codes on the plot are as follows, Y1—*Rhodotorula paludigena* Y1, Y9—*Dirkmeia* sp. Y9, Y55—*Pseudozyma* sp. Y55, Y57—*Pseudozyma* sp. Y57, Y70—*Dirkmeia* sp. Y70, Y71—*Pseudozyma* sp. Y71, Y72—*Cryptococcus* sp. Y72, Y76—*Dirkmeia* sp. Y76, and Y78—*Dirkmeia churashimaensis* Y78

Effect on rice biochemical traits

In all treatments, the leaf chlorophyll content increased from the stem elongation stage to the flowering stage but declined slightly in the dough stage than in the stem elongation stage. The chlorophyll content was found to be higher in T_9 - treatment plants in stem elongation and flowering stages, and it was almost 3 and 2.5 times higher than the T_c plants (Table 4). *R. paludigena* Y1+75% RDCFs-treated plants (T_6) had a higher chlorophyll level during the dough stage and T_c , T_3 -, T_7 -, and T_8 - treatment plants contained comparatively lower chlorophyll content. Regarding protein and proline content in rice, T_9 - and Yeast consortium+100% RDCFs-treatment plants (T_4) had the highest levels with minimal significant variation between them (Table 4). Similarly, the T_c plants had the least protein and proline content. Protein content was higher during the flowering and dough stages compared to the stem elongation stage, but there was no substantial difference between the flowering and dough stages. The activities of antioxidant enzymes such

as catalase, polyphenol oxidase, and peroxidase revealed that T_9 - treatment plants had considerably higher activity than other treatment plants (Table 5). T_4 - treatment plants exhibited the next highest enzyme activity, with only minor differences from T_9 - treatment plants. The T_c plants had the lowest levels of enzyme activity. Catalase activity increased in all treatment plants from the stem elongation to the flowering and dough stages. In contrast, polyphenol oxidase activity rose only from the stem elongation stage to the flowering stage but dropped below the stem elongation stage in the dough stage. Peroxidase activity was higher in the flowering stage, followed by the dough stage, and with the lowest activity recorded during the stem elongation stage in all treated plants.

Effect on rice phenotypic traits

The shoot length at the stem elongation stage varied significantly among the various treatments. The plants from T_4 and T_9 sets had the longest shoots in all three stages with no significant difference. The shoot length was

Table 3 Mean and standard deviation (n = 3) values of bacterial, yeast, and actinomycetes population on rice surface at the stem elongation, flowering, and dough stages grown in pots

Treatments	Bacteria (10 ⁷ CFU cm ⁻¹)			Yeast (10 ⁵ CFU cm ⁻¹)			Actinomycetes (10 ³ CFU cm ⁻¹)		
	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage
T ₁	7.40 (0.18) ^c	14.4 (0.55) ^{ab}	17.0 (0.58) ^{ab}	11.6 (0.16) ^b	17.9 (0.15) ^c	21.7 (0.27) ^b	1.61 (0.03) ^c	1.80 (0.02) ^c	1.98 (0.02) ^c
T ₂	7.10 (0.17) ^c	13.9 (0.35) ^{bc}	16.4 (0.55) ^{abc}	10.5 (0.14) ^c	17.5 (0.14) ^{cd}	19.8 (0.25) ^d	1.57 (0.03) ^c	1.68 (0.02) ^d	1.83 (0.02) ^d
T ₃	6.90 (0.17) ^c	13.6 (0.52) ^{bc}	16.8 (0.57) ^{ab}	10.5 (0.14) ^c	17.2 (0.14) ^d	19.8 (0.25) ^d	1.50 (0.02) ^{cd}	1.55 (0.02) ^e	1.68 (0.02) ^f
T ₄	9.40 (0.23) ^b	14.6 (0.55) ^{ab}	15.6 (0.53) ^{bcd}	12.1 (0.16) ^b	19.1 (0.16) ^b	21.7 (0.27) ^b	1.98 (0.03) ^b	2.06 (0.02) ^b	2.15 (0.02) ^b
T ₅	6.10 (0.18) ^d	11.4 (0.46) ^d	16.7 (0.63) ^{ab}	9.77 (0.13) ^d	16.1 (0.13) ^e	19.6 (0.21) ^e	1.46 (0.04) ^{de}	1.54 (0.02) ^e	1.73 (0.03) ^e
T ₆	7.20 (0.18) ^c	14.1 (0.26) ^{bc}	16.8 (0.57) ^{ab}	10.8 (0.15) ^c	17.5 (0.15) ^{cd}	20.8 (0.26) ^c	1.57 (0.03) ^c	1.71 (0.02) ^d	1.83 (0.02) ^d
T ₇	6.90 (0.17) ^c	13.0 (0.50) ^c	15.1 (0.51) ^{cd}	9.85 (0.13) ^d	17.2 (0.14) ^d	19.7 (0.25) ^d	1.43 (0.02) ^{de}	1.51 (0.02) ^e	1.64 (0.02) ^{ef}
T ₈	6.10 (0.15) ^d	10.4 (0.40) ^d	14.4 (0.49) ^d	9.74 (0.13) ^d	16.2 (0.13) ^e	18.9 (0.24) ^f	1.36 (0.02) ^f	1.44 (0.01) ^f	1.59 (0.02) ^{gh}
T ₉	10.1 (0.25) ^a	15.4 (0.59) ^a	17.6 (0.60) ^a	13.4 (0.18) ^a	20.2 (0.17) ^a	23.6 (0.30) ^a	2.23 (0.04) ^a	2.89 (0.03) ^a	3.15 (0.03) ^a
T ₁₀	5.40 (0.12) ^e	11.2 (0.52) ^d	14.3 (0.53) ^d	9.56 (0.12) ^d	16.9 (0.16) ^d	18.8 (0.23) ^f	1.36 (0.02) ^f	1.39 (0.02) ^f	1.52 (0.02) ^h
T _C	4.30 (0.10) ^f	8.30 (0.32) ^e	10.3 (0.35) ^e	6.84 (0.09) ^e	8.90 (0.07) ^f	9.90 (0.12) ^g	1.12 (0.02) ^g	1.18 (0.01) ^g	1.29 (0.01) ⁱ

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

T₁, *Rhodotorula paludigena* Y1 + 100% recommended dose of chemical fertilizers (RDCFs); T₂, *Pseudozyma* sp. Y71 + 100% RDCFs; T₃, *Cryptococcus* sp. Y72 + 100% RDCFs; T₄, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs; T₅, 100% RDCFs; T₆, *Rhodotorula paludigena* Y1 + 75% RDCFs; T₇, *Pseudozyma* sp. Y71 + 75% RDCFs; T₈, *Cryptococcus* sp. Y72 + 75% RDCFs; T₉, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs; T₁₀, 75% RDCFs; T_C, Control

Table 4 Mean and standard deviation (n = 3) values of total chlorophyll, protein, and proline content in rice leaves at the stem elongation, flowering, and dough stages grown in pots

Treatments	Total chlorophyll (mg g ⁻¹ FW)			Protein (µg g ⁻¹ FW)			Proline (µg g ⁻¹ FW)		
	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage
T ₁	10.0 (0.11) ^c	13.5 (0.16) ^c	7.2 (0.16) ^d	35.4 (1.14) ^{bc}	43.9 (1.46) ^{bc}	43.0 (1.06) ^{abc}	66.7 (1.06) ^{ab}	66.8 (1.21) ^b	69.0 (1.44) ^{bc}
T ₂	8.01 (0.13) ^e	11.9 (0.29) ^d	7.3 (0.20) ^d	30.1 (2.00) ^{de}	34.5 (1.09) ^e	35.1 (1.08) ^{de}	64.7 (1.27) ^{bc}	66.4 (2.08) ^b	65.8 (1.01) ^{cd}
T ₃	5.45 (0.10) ^h	8.10 (0.06) ^f	4.2 (0.16) ^e	24.6 (1.23) ^f	27.2 (0.98) ^f	25.5 (1.17) ^{gh}	55.9 (1.02) ^{de}	60.3 (1.11) ^c	58.9 (1.03) ^{ef}
T ₄	13.2 (0.10) ^b	17.1 (0.38) ^b	9.5 (0.34) ^b	40.5 (2.00) ^{ab}	51.0 (2.30) ^a	44.4 (1.27) ^{ab}	65.5 (1.00) ^{abc}	76.2 (2.3) ^a	73.7 (2.19) ^{ab}
T ₅	7.44 (0.12) ^f	10.8 (0.23) ^e	7.7 (0.23) ^{cd}	33.8 (0.54) ^{cd}	43.5 (1.34) ^{bc}	39.7 (1.08) ^{bcd}	61.6 (1.21) ^{bc}	66.4 (1.45) ^b	66.9 (1.17) ^{cd}
T ₆	10.2 (0.14) ^c	13.4 (0.26) ^c	10.5 (0.04) ^a	37.0 (1.10) ^{abc}	47.7 (1.17) ^{ab}	41.5 (1.11) ^{abc}	64.9 (1.40) ^{bc}	67.0 (1.34) ^b	72.6 (1.26) ^{ab}
T ₇	5.53 (0.08) ^g	8.90 (0.18) ^f	4.4 (0.08) ^e	32.6 (0.78) ^{cd}	35.1 (1.16) ^{de}	33.8 (0.54) ^{ef}	60.4 (0.80) ^{cd}	65.9 (0.91) ^b	63.2 (1.22) ^{de}
T ₈	5.61 (0.13) ⁱ	8.80 (0.03) ^g	4.6 (0.13) ^e	26.4 (1.14) ^{ef}	26.6 (0.86) ^f	30.0 (1.08) ^g	55.7 (1.25) ^{de}	58.9 (1.00) ^c	55.6 (0.81) ^f
T ₉	17.4 (0.21) ^a	20.5 (0.06) ^a	9.5 (0.15) ^b	41.5 (1.40) ^a	48.0 (1.04) ^{ab}	45.1 (2.14) ^a	70.7 (1.97) ^a	78.6 (1.27) ^a	74.8 (1.00) ^a
T ₁₀	8.82 (0.15) ^d	12.5 (0.31) ^d	8.2 (0.12) ^c	34.4 (0.67) ^{cd}	40.6 (1.27) ^{cd}	38.8 (1.13) ^{cde}	63.7 (1.03) ^{bc}	66.6 (1.18) ^b	66.8 (1.32) ^{cd}
T _C	5.40 (0.10) ^j	8.70 (0.05) ^h	4.4 (0.15) ^e	18.8 (1.14) ^g	24.4 (0.90) ^f	23.7 (0.84) ^h	52.8 (0.75) ^e	51.4 (1.02) ^d	54.6 (0.94) ^f

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

T₁, *Rhodotorula paludigena* Y1 + 100% recommended dose of chemical fertilizers (RDCFs); T₂, *Pseudozyma* sp. Y71 + 100% RDCFs; T₃, *Cryptococcus* sp. Y72 + 100% RDCFs; T₄, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs; T₅, 100% RDCFs; T₆, *Rhodotorula paludigena* Y1 + 75% RDCFs; T₇, *Pseudozyma* sp. Y71 + 75% RDCFs; T₈, *Cryptococcus* sp. Y72 + 75% RDCFs; T₉, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs; T₁₀, 75% RDCFs; T_C, Control

approximately 38%, 15%, and 7.7% higher than T_C plants in stem elongation, flowering, and dough stages (Table 6). While there was less significant variation in shoot length among the treatments during the flowering stage, no significant variation was observed at the dough stage, except

in the T_C plants. The root length measured at all three stages was found to be higher in the T₉- treatment plants, and the shortest roots were recorded in the T_C plants. In the case of other treatment plants in stem elongation and

Table 5 Mean and standard deviation (n = 3) values of antioxidant enzyme activity (catalase, polyphenol oxidase, and peroxidase) in rice leaves at the stem elongation, flowering, and dough stages grown in pots

Treatments	Catalase (mmol min ⁻¹ mg protein ⁻¹)			Polyphenol oxidase (mmol min ⁻¹ mg protein ⁻¹)			Peroxidase (mmol min ⁻¹ mg protein ⁻¹)		
	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage
T ₁	0.83 (0.005) ^c	0.99 (0.006) ^d	1.34 (0.006) ^d	1.62 (0.07) ^{bc}	2.09 (0.02) ^{cd}	1.37 (0.12) ^{bcd}	1.55 (0.02) ^{bc}	1.98 (0.07) ^{bc}	1.60 (0.12) ^{bcd}
T ₂	0.46 (0.007) ^f	0.60 (0.011) ^g	0.80 (0.008) ^h	1.42 (0.08) ^{cd}	1.92 (0.02) ^{cde}	1.30 (0.08) ^{bcd}	1.43 (0.02) ^{bcd}	1.75 (0.08) ^{cde}	1.68 (0.09) ^{bc}
T ₃	0.26 (0.002) ^h	0.47 (0.003) ^h	0.74 (0.018) ⁱ	1.35 (0.16) ^{cde}	1.70 (0.04) ^e	1.12 (0.01) ^{de}	1.02 (0.04) ^f	1.56 (0.16) ^e	1.37 (0.01) ^d
T ₄	1.14 (0.005) ^a	1.34 (0.002) ^b	1.76 (0.001) ^b	1.87 (0.04) ^{ab}	2.41 (0.12) ^{ab}	1.56 (0.03) ^{bc}	1.72 (0.12) ^{ab}	2.44 (0.04) ^a	1.89 (0.03) ^b
T ₅	0.73 (0.003) ^d	0.89 (0.003) ^e	1.10 (0.002) ^f	1.58 (0.05) ^{bc}	2.02 (0.09) ^{cd}	1.21 (0.05) ^d	1.55 (0.07) ^{bc}	1.83 (0.05) ^{bcd}	1.48 (0.02) ^{cd}
T ₆	0.99 (0.009) ^b	1.09 (0.013) ^c	1.46 (0.013) ^c	1.91 (0.10) ^a	2.18 (0.01) ^{bc}	1.58 (0.07) ^b	1.65 (0.01) ^{abc}	1.95 (0.11) ^b	1.69 (0.07) ^{bc}
T ₇	0.55 (0.009) ^e	0.81 (0.013) ^e	1.19 (0.011) ^e	1.34 (0.09) ^{cd}	1.97 (0.03) ^{cde}	1.32 (0.08) ^{bcd}	1.26 (0.03) ^{def}	2.06 (0.09) ^b	1.63 (0.08) ^{cd}
T ₈	0.32 (0.001) ^g	0.53 (0.001) ^f	0.95 (0.004) ^f	1.25 (0.05) ^{de}	1.81 (0.08) ^{de}	1.12 (0.16) ^d	1.09 (0.08) ^{ef}	1.96 (0.05) ^{bcd}	1.35 (0.16) ^d
T ₉	1.16 (0.005) ^a	1.60 (0.004) ^a	1.98 (0.001) ^g	1.93 (0.02) ^a	2.68 (0.07) ^a	1.98 (0.04) ^a	1.91 (0.07) ^a	2.61 (0.02) ^a	2.21 (0.04) ^a
T ₁₀	0.74 (0.005) ^d	1.00 (0.002) ^d	1.22 (0.021) ^e	1.46 (0.03) ^{cd}	1.84 (0.07) ^{de}	1.26 (0.03) ^{cd}	1.36 (0.10) ^{cde}	1.67 (0.04) ^{de}	1.49 (0.02) ^{cd}
T _C	0.26 (0.007) ^h	0.51 (0.009) ^{hi}	0.67 (0.011) ^j	1.11 (0.10) ^e	1.27 (0.16) ^f	0.81 (0.10) ^e	0.98 (0.16) ^f	1.11 (0.12) ^f	0.71 (0.13) ^e

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

T₁, *Rhodotorula paludigena* Y1 + 100% recommended dose of chemical fertilizers (RDCFs); T₂, *Pseudozyma* sp. Y71 + 100% RDCFs; T₃, *Cryptococcus* sp. Y72 + 100% RDCFs; T₄, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs; T₅, 100% RDCFs; T₆, *Rhodotorula paludigena* Y1 + 75% RDCFs; T₇, *Pseudozyma* sp. Y71 + 75% RDCFs; T₈, *Cryptococcus* sp. Y72 + 75% RDCFs; T₉, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs; T₁₀, 75% RDCFs; T_C, Control

Table 6 Mean and standard deviation (n = 3) values of shoot and root length of rice measured at the stem elongation, flowering, and dough stages grown in pots, as well as yield parameters such as the number of productive tillers, the number of grains per panicle, and the 1000-grain weight

Treatments	Shoot length (cm)			Root length (cm)			Yield parameters		
	Stem elongation stage	Flowering stage	Dough stage	Stem elongation stage	Flowering stage	Dough stage	No. productive tillers	No. grains per panicle	1000-grain weight (g)
T ₁	39.6 (1.7) ^{abc}	59.7 (1.8) ^{ab}	68.3 (1.1) ^{ab}	22.7 (1.1) ^{ab}	31.6 (1.4) ^{abc}	43.8 (0.5) ^{ab}	11.2 (0.2) ^b	95.3 (0.6) ^c	23.9 (0.5) ^a
T ₂	35.7 (1.1) ^{bcd}	58.2 (2.0) ^{abc}	68.1 (1.5) ^{ab}	21.8 (1.2) ^{ab}	31.4 (1.1) ^{abc}	43.1 (0.7) ^{abc}	9.37 (0.6) ^{cd}	91.3 (1.2) ^d	23.8 (0.6) ^{ab}
T ₃	37.8 (0.9) ^{bcd}	58.2 (0.5) ^{abc}	67.8 (0.8) ^{ab}	21.7 (0.1) ^{ab}	30.7 (0.9) ^{abc}	42.1 (1.8) ^{bcd}	8.92 (0.3) ^{de}	89.9 (1.1) ^d	23.5 (0.6) ^{ab}
T ₄	44.6 (0.4) ^a	61.9 (0.9) ^a	70.1 (1.6) ^a	23.3 (1.1) ^{ab}	32.9 (1.0) ^{ab}	44.3 (0.6) ^{ab}	12.1 (0.3) ^{ab}	97.3 (0.4) ^b	24.1 (0.2) ^a
T ₅	41.2 (0.5) ^{abc}	56.9 (0.8) ^{bc}	67.2 (2.1) ^{ab}	19.5 (0.9) ^{bc}	30.0 (0.5) ^{bc}	42.6 (0.7) ^{bcd}	8.36 (0.4) ^{ef}	89.1 (0.7) ^d	23.4 (0.4) ^{ab}
T ₆	39.9 (0.5) ^{abc}	59.2 (1.3) ^{ab}	68.2 (0.8) ^{ab}	21.9 (1.5) ^{ab}	31.4 (1.1) ^{abc}	43.6 (1.1) ^{ab}	10.1 (0.2) ^c	95.9 (0.3) ^{bc}	23.8 (0.2) ^{ab}
T ₇	37.2 (0.4) ^{bcd}	57.2 (1.2) ^{bc}	67.1 (2.4) ^{ab}	21.7 (1.0) ^{ab}	31.3 (0.3) ^{abc}	41.0 (0.8) ^{bcd}	8.14 (0.2) ^{efg}	83.1 (0.2) ^{ef}	23.3 (0.5) ^{ab}
T ₈	33.2 (1.6) ^{de}	57.1 (1.4) ^{bc}	66.1 (2.2) ^{ab}	21.1 (1.6) ^{ab}	29.9 (1.0) ^{bc}	41.9 (1.1) ^{bcd}	7.61 (0.5) ^{fg}	81.3 (0.4) ^f	23.2 (0.3) ^{ab}
T ₉	43.9 (0.6) ^a	62.1 (2.6) ^a	70.0 (1.5) ^a	24.6 (1.3) ^a	33.5 (0.8) ^a	45.3 (1.0) ^a	12.8 (0.2) ^a	112 (0.4) ^a	24.2 (0.3) ^a
T ₁₀	36.5 (0.7) ^{cde}	57.3 (1.3) ^{bc}	66.2 (1.1) ^{ab}	19.2 (1.1) ^{bc}	29.9 (0.4) ^{bc}	41.1 (0.9) ^{cd}	7.19 (0.3) ^{gh}	84.8 (0.5) ^e	23.0 (0.5) ^{ab}
T _C	31.9 (1.6) ^e	53.9 (0.4) ^c	64.5 (0.2) ^b	18.3 (1.1) ^c	29.3 (1.2) ^c	38.1 (1.1) ^d	6.52 (0.4) ^h	75.1 (0.4) ^g	22.6 (0.6) ^b

Parameter values with different letters are significantly different according to Tukey's test. *p* < 0.05

T₁, *Rhodotorula paludigena* Y1 + 100% recommended dose of chemical fertilizers (RDCFs); T₂, *Pseudozyma* sp. Y71 + 100% RDCFs; T₃, *Cryptococcus* sp. Y72 + 100% RDCFs; T₄, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs; T₅, 100% RDCFs; T₆, *Rhodotorula paludigena* Y1 + 75% RDCFs; T₇, *Pseudozyma* sp. Y71 + 75% RDCFs; T₈, *Cryptococcus* sp. Y72 + 75% RDCFs; T₉, Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs; T₁₀, 75% RDCFs; T_C, Control

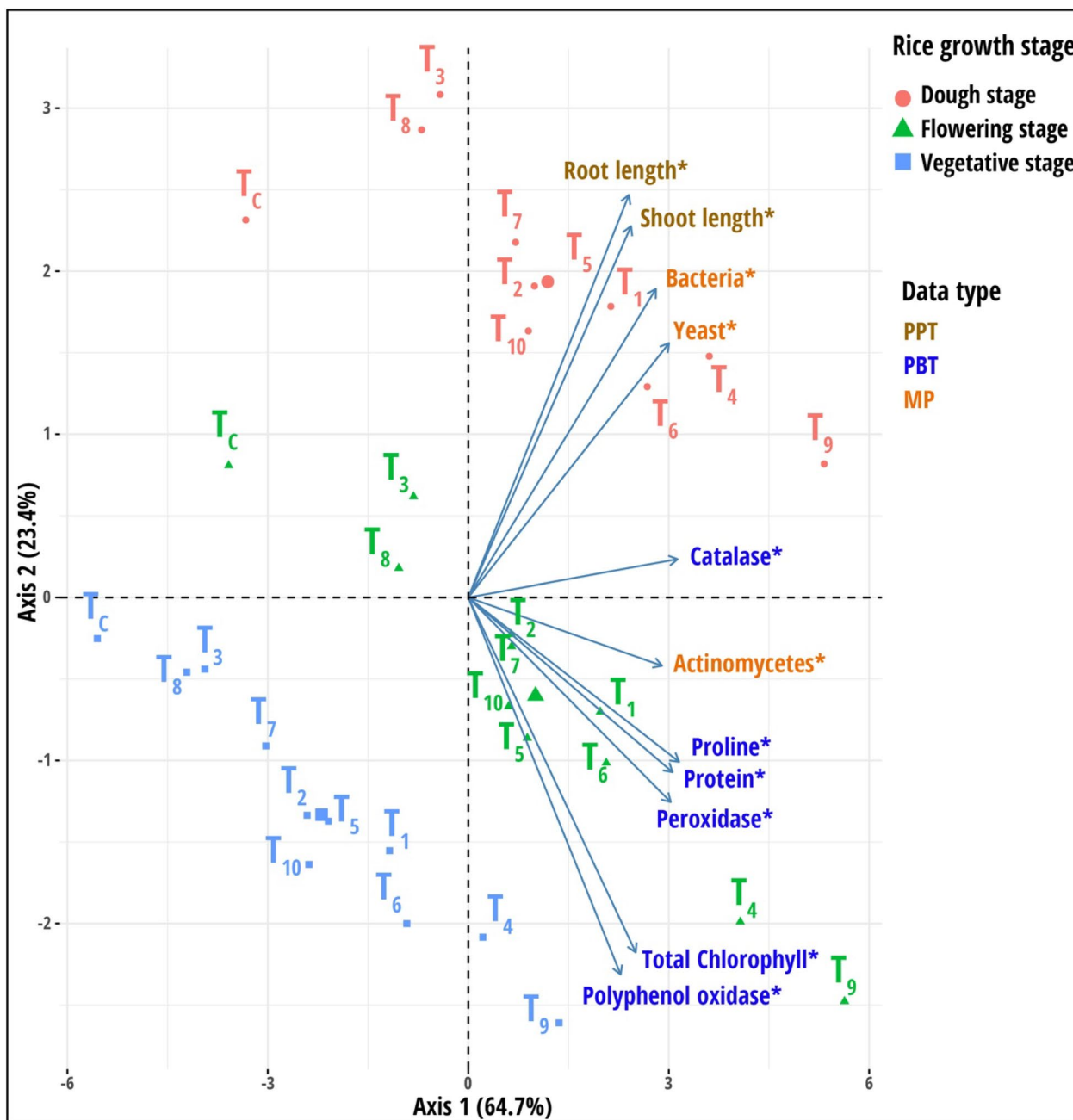


Fig. 4 Results of principal component analysis (PCA) based on the integration of all dataset categories measured in pot culture experiment. Variables marked with an asterisk are significant along the first principal component axis obtained from the PCAtest analysis. The treatments are as follows: T₁—*Rhodotorula paludigena* Y1 + 100% recommend dose of chemical fertilizers (RDCFs), T₂—*Pseudozyma* sp. Y71 + 100% RDCFs, T₃—*Cryptococcus* sp. Y72 + 100% RDCFs, T₄—Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs, T₅—100% RDCFs, T₆—*Rhodotorula paludigena* Y1 + 75% RDCFs, T₇—*Pseudozyma* sp. Y71 + 75% RDCFs, T₈—*Cryptococcus* sp. Y72 + 75% RDCFs, T₉—Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs, T₁₀—75% RDCFs, and T_C—Control. Data type abbreviations: PBT, plant biochemical traits; PPT, plant phenotypic traits; and MP, Microbial population

flowering stages, they do not have substantial differences in root length.

Relationship between the measured variables in different rice growth stages

A PCA was applied to understand the variation among plants with different treatments across three rice growth stages using measured parameters (microbial population, plant biochemicals, and plant phenotypic traits) (Fig. 4). The total variance explained by the first two PC axes was 88.1%, indicating a strong treatment impact on plants at all stages of rice development. The PC1 axis was statistically significant ($p < 0.05$), accounting for 64.7% of the overall variance. Across all rice growing phases, the PC 1 axis separated plants treated with yeast consortium with 75% RDCFs (T_9) and 100% RDCFs (T_4) from other treatments. In the PC1 axis, all variables were determined to be statistically significant. The PC axis accounted for only 23.4% of the total variance and effectively separated plant samples based on rice development stages. At the dough stage, the bacterial and yeast population variables

were dominant in plants treated with yeast consortium with 75% RDCFs (T_9) and 100% RDCFs (T_4). During the flowering period, peroxidase activity, proline, and protein content were prominent in the same treatment plants.

Comparison of metabolite profile

The PCA results based on rice leaf biochemicals, phenotypic traits, and microbial population showed that most variables were contributed by plants treated with the yeast consortium + 75% RDCFs (T_9) in the flowering stage. Hence, the leaf metabolites were extracted and profiled in T_C plants and T_9 treatment plants collected during the flowering stage (Supplementary Table 5). A total of 51 and 84 metabolites were identified in the T_C and T_9 - treatment plants, respectively, and 48 metabolites were discovered to be shared by the two treatment plants (Fig. 5A). In T_9 - treatment plants, 36 new metabolites were discovered, while 3 metabolites detected in T_C plants were absent in T_9 - treatment plants (Fig. 5A). A volcano plot was created using the 48 metabolites identified to be common in T_C and T_9 - treatment plants. T_9 - treatment plants had higher concentrations of 40 metabolites and lower concentrations of 6 metabolites than T_C plants (Fig. 5B; Supplementary Table 6). Two metabolites (Hepta-2,4-dienoic acid and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol) showed no significant changes (Fig. 5B).

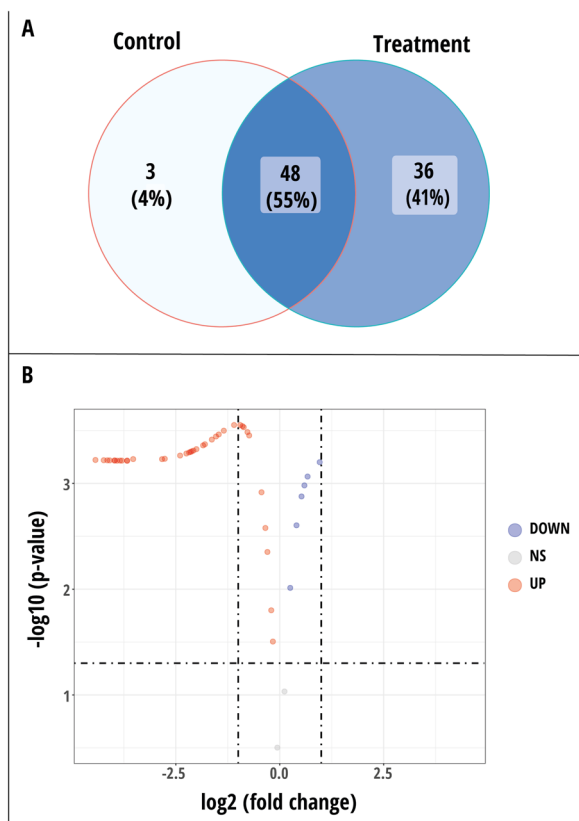


Fig. 5 **A** Comparison of the leaf metabolite profiles between control plants and those treated with the yeast consortium plus 75% RDCFs **B** Volcano plot illustrating the changes in leaf metabolites of plants treated with the yeast consortium plus 75% RDCFs compared to control plants. NS—nonsignificant

Table 7 Mean and standard deviation (n = 3) values of yield from field experiment

Treatments	Yield (t/ha)
FT ₁	3.68 (0.047) ^b
FT ₂	3.46 (0.045) ^c
FT ₃	3.42 (0.044) ^c
FT ₄	3.79 (0.048) ^{ab}
FT ₅	3.71 (0.047) ^{ab}
FT ₆	3.53 (0.045) ^c
FT ₇	3.45 (0.045) ^c
FT ₈	3.82 (0.048) ^a
FT _C	3.41 (0.044) ^c

The yield unit is mentioned as tons per hectare (t/ha). Parameter values with different letters are significantly different according to Tukey's test. $p < 0.05$

T_1 , *Rhodotorula paludigena* Y1 + 100% recommended dose of chemical fertilizers (RDCFs); T_2 , *Pseudozyma* sp. Y71 + 100% RDCFs; T_3 , *Cryptococcus* sp. Y72 + 100% RDCFs; T_4 , Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs; T_5 , 100% RDCFs; T_6 , *Rhodotorula paludigena* Y1 + 75% RDCFs; T_7 , *Pseudozyma* sp. Y71 + 75% RDCFs; T_8 , *Cryptococcus* sp. Y72 + 75% RDCFs; T_9 , Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs; T_{10} , 75% RDCFs; T_C , Control

Effect on rice yield-related parameters

The yield-related parameters were measured during the harvest stage. The number of productive tillers was significantly higher in T₉- treatment plants, which was two times greater than in T_C plants. Followed by T₄- and T₁ (*R. paludigena* Y1 + 100%)- treatment plants had maximum productive tillers (Table 6). T₉- treatment plants produced the most seeds per panicle, followed by T₄- treatment plants. The weight of 1000 grains was highest in plants that had T₉, T₄, and T₁ treatments. Other treatment plants had similar values, indicating no significant differences. In all parameters, the T_C plants recorded the least.

Treatment effect on rice yield under field condition

The yield results from the field experiment showed that FT₉- treatment plants had the highest yield, which was 15.4% higher than the FT_C plants (Table 7). Followed by, plants with FT₄, FT₆, and FT₁ treatments had the maximum yield, which was 14.5%, 12.2%, and 11.3% higher than FT_C plants, respectively (Table 7). Other treatment plants (FT₂, FT₃, FT₅, FT₇, and FT₁₀) showed no significant difference in the rice yield and the lowest yield was recorded in FT_C plants.

Relationship between the various parameter categories

MCIA was applied to analyze the ten datasets jointly. The graphical outputs of this analysis are shown in Fig. 6. The total variance captured by the first two axes was 95.3%, with the first axis capturing the highest variance (82.3%) and separating the two treatment plants, yeast consortium with 75% RDCFs (T₉) and 100% RDCFs (T₄), from other treatments. The MCIA second axis, explaining 13.1% of the data variation, emphasized the separation of T_C plants from other treatment plants (Fig. 6A). Total chlorophyll and catalase activity from all three datasets (PBT1, PBT2, and PBT3), shoot length from PPT1, root length from PPT3, bacteria from MP1, and the number of tillers from the YP dataset was well associated with T₉- and T₄- treatment plants (Supplementary Fig. 2). The RV coefficient matrix of the combined dataset showed a strong positive correlation within datasets of plant biochemical traits (PBT1, PBT2, and PBT3) and microbial population (MP2 and MP3) (Supplementary Fig. 3A). A significant positive correlation was noted between the yield parameter dataset (YP) and the plant biochemical traits dataset (PBT1, PBT2, and PBT3). However, no substantial correlation was found between other dataset categories. As a result, only all plant biochemical traits, microbial population, and yield parameters datasets were significant in separating the treatments in MCIA.

For MKL, the same datasets were used, and the combined kernel principal component analysis (KPCA)

was applied in further exploratory analysis. The results revealed that the majority of the overall variance in the data was captured by the first axis of KPCA (61%), which clearly separated the four treatments, yeast consortium with 75% RDCFs (T₉), 100% RDCFs (T₄), *R. paludigena* Y1 + 75% RDCFs (T₆) and *R. paludigena* Y1 + 100% RDCFs (T₁) from other treatments but T₉ and T₄ had the highest variables impact than T₆ and T₁ (Fig. 7A). The second axis captured the least variance (20%), which separated the fertilizer-only treatments (100% RDCFs (T₅) and 75% RDCFs (T₁₀)) from other treatments. An important variable plot was computed for each dataset to determine the impact of the variables on KPCA (Fig. 7B). In terms of the microbial community, yeast was found to be a key factor during the stem elongation stage, whereas bacteria was the critical variable during the flowering and dough stages. Catalase activity and total chlorophyll content significantly influenced plant biochemical parameters during the stem elongation stage, while the other two stages did not show a significant impact on the variables. On the other hand, shoot length was found to be an important factor during the flowering and dough stages. In the case of yield parameters data, the actual yield measured after harvest emerged as the most important variable. Correlation using the RV coefficient matrix of the combined dataset revealed a strong positive correlation within the plant biochemical traits dataset (PBT1, PBT2, and PBT3) (Supplementary Fig. 3B). A substantial positive correlation was noted between PBTs and PPT1, PPT2, and YP datasets. Microbial population datasets (MPs) also showed significant positive correlations within themselves and with PPT2, PPT3, and YP datasets. As a result, all four parameter category datasets significantly separated the treatments in KPCA.

MCIA determined that T₉ was the most effective treatment and T₄ was the next-most effective treatment. In the case of MKL, both T₉ and T₄ treatments were found to be the better treatments in KPCA, as they can separate the inseparable linear space between the samples. Additionally, PCA results support that the T₉ treatment significantly improved rice biochemical and phenotypic traits (Fig. 4). Therefore, applying the yeast consortium to rice fields with 75% RDCFs better enhances rice yield. Overall, the foliar application of yeast consortium with 75% regular fertilizer application improved rice yield.

Discussion

Yeast, a key model organism, is extensively used in the food industry, biotechnology, and medicine due to its unicellular eukaryotic nature and ease of genetic manipulation [58]. *Saccharomyces cerevisiae*, the most studied yeast, is known for its efficient homologous recombination and was the first eukaryote to have a fully sequenced

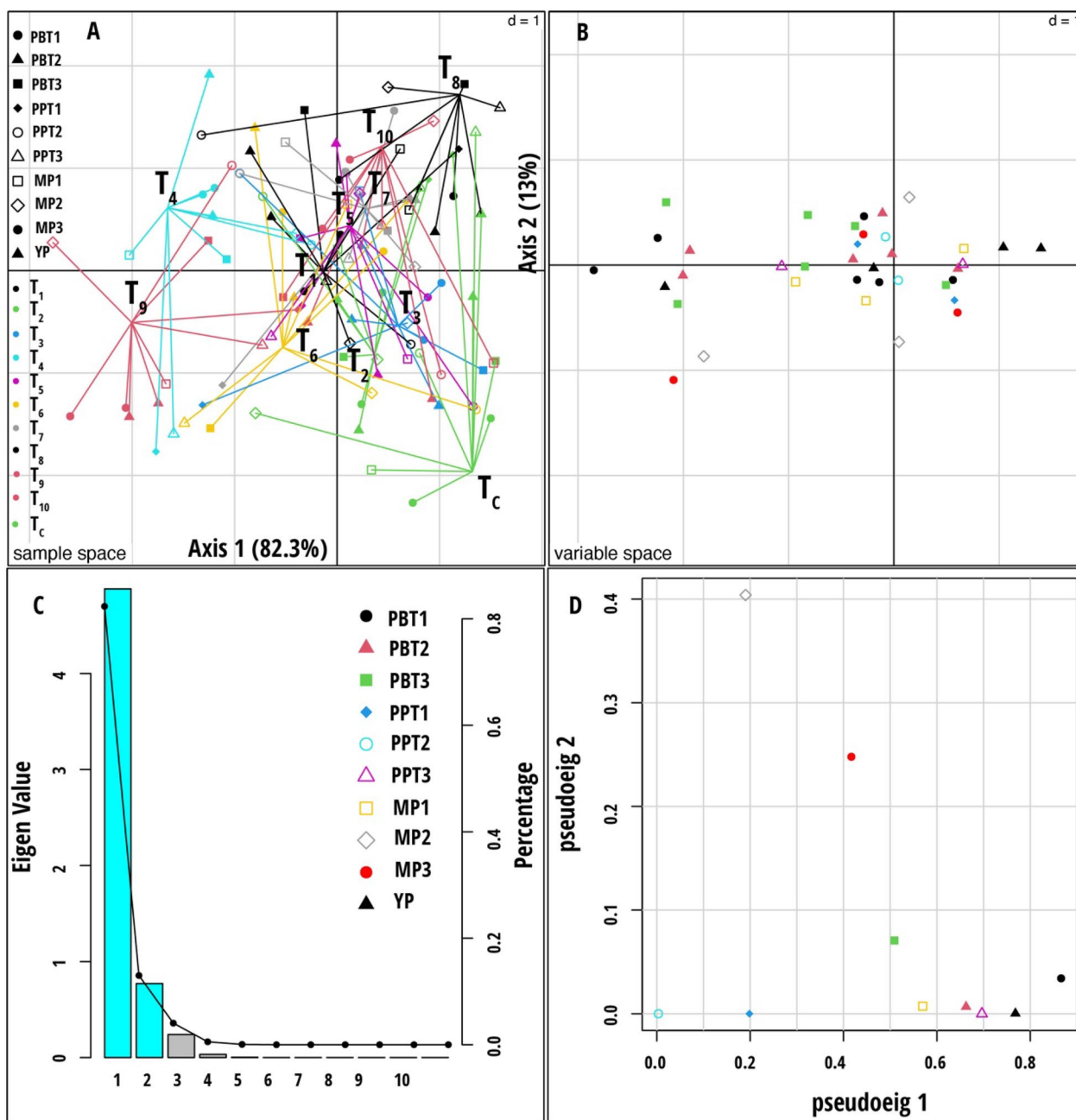


Fig. 6 Multiple co-inertia analysis (MCI) results based on nine data sets (Plant biochemical traits, plant phenotypic traits, and microbial population measured during stem elongation, flowering, and dough stages). **A** Plot of the first two components in the sample space. Each sample is represented by a colored shape, with lines connecting the nine datasets for each sample to a central point (MCI global score). **B** Variable space for each data set. **C** A scree plot of absolute eigenvalues (bars) and the proportions of variance for the eigenvectors (line). **D** A plot of data weighting space that shows the pseudo-eigenvalues space of all data sets indicating how much variance of an eigenvalue is contributed by each data set. The treatment codes used are: T₁—*Rhodotorula paludigena* Y1 + 100% recommend dose of chemical fertilizers (RDCFs), T₂—*Pseudozyma* sp. Y71 + 100% RDCFs, T₃—*Cryptococcus* sp. Y72 + 100% RDCFs, T₄—Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs, T₅—100% RDCFs, T₆—*Rhodotorula paludigena* Y1 + 75% RDCFs, T₇—*Pseudozyma* sp. Y71 + 75% RDCFs, T₈—*Cryptococcus* sp. Y72 + 75% RDCFs, T₉—Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs, T₁₀—75% RDCFs, and T_C—Control. The data set abbreviations PBT1, PBT2, and PBT3 are plant biochemical traits; PPT1, PPT2, and PPT3 are plant phenotypic traits; MP1, MP2, and MP3 are microbial population, and YP are yield parameters

genome [59]. With advancements in genome editing, non-conventional yeasts such as *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Komagataella phaffii* (formerly *Pichia pastoris*), and *Schizosaccharomyces pombe* have also become important in biotechnology and cellular biology research [60]. On the other hand, environmental yeasts have received little attention in terms of applications but have been extensively researched from an ecological standpoint. In contrast to conventional yeasts used in fermentation studies, which thrive in extremely rich culture conditions, environmental yeasts are constrained by a lack of readily available nutrients [61]. Notably, phyllosphere yeasts are subjected to environmental stresses such as high temperature, UV radiation, and dryness, which likely force them to use different metabolic routes and regulatory mechanisms for substrate utilization [10]. Thus, further efforts should be made to investigate their genetic and metabolic potential under natural conditions. They are also involved in many interactions with other microorganisms and plants, which include symbiosis, mutualism, parasitism, and competition. With these capabilities, recent reports suggest that yeast could be used for plant growth promotion and plant disease control. To fulfil the void in the search for new beneficial microbes to enhance crop production and protection, an attempt was made to isolate and develop potential yeast isolates with multiple beneficial traits for use in crop production and protection. The results obtained through various experiments have been discussed here under.

In the current study, 52 yeast isolates were obtained from the phyllosphere of rice at various phases of the rice crop. Nine of the isolates were discovered to grow at 36 °C. As previously stated, the phyllosphere is an unfavorable environment, so testing the phyllosphere microbes for specific stress levels is critical. Similarly, phyllosphere bacterial strains were tested for salinity, temperature, and osmotic stress before rice foliar treatments [26]. The ability of rice phylloplane yeast strains *Rhodotorula taiwanensis*, *Cryptococcus* aff. *laurentii*, and *Cryptococcus flavescens* to grow up to 35 °C was tested to ensure that they can withstand environmental temperatures [62]. Using ITS rRNA gene sequencing, the nine-temperature tolerant yeast genera were

identified *Rhodotorula*, *Dirkmeia*, *Pseudozyma*, and *Cryptococcus*. *Cryptococcus albidus* and *Rhodotorula mucilaginosa* were primarily found on leaves, representing more than 50% of the community [63]. The isolation of *Pseudozyma* genera from plant leaves was also reported [64, 65]. *Dirkmeia churashimaensis* was the most common basidiomycetous yeast discovered on the sugarcane phylloplane, accounting for 12% of all yeast strains identified [66]. Oleaginous red yeasts, *Rhodotorula* species, are usually a promising source of carotenoids, lipids, and exopolysaccharides (EPS), which provide essential phyllosphere microbial characteristics such as UV protection, avoidance of desiccation, and increased adhesion to the plant leaf surface [67, 68].

IAA is the most common plant hormone of the auxin class, which regulates various processes in plants from development to senescence [69]. Similarly, GA is an essential plant hormone that aids stem elongation and germination. The most common form of GA is GA₃, which is a fungal product, and plants can primarily utilize GA₁, and other GA forms are used as precursors for GA₁ synthesis [69]. The microbial origin of phytohormones and their application in plant growth and stress alleviation have been widely studied [70]. A diverse range of yeast strains isolated from plant phylloplane showed IAA production [71]. *Rhodosporidium paludigenum* isolated from rice phyllosphere was reported to produce IAA [72]. Root treatment with IAA-producing endophytic yeast has been shown to increase root and shoot length, and maximum IAA production was recorded by *Williopsis saturnus* (22.5 µg ml⁻¹). In our study, maximum IAA was produced by *R. paludigena* Y1 (77.2 ± 9.1 µg ml⁻¹), and treating rice seeds with yeast strains had minor and major improvements in shoot and root lengths, respectively [73]. The report on the production of GA by yeast is uncommon, and only production of GA in soil yeast through biochemical assay. Further confirmation of GA production needs to be studied through modern approaches. One of the most important needs of the plant is nutrients, which are provided by microbes via mechanisms such as mineralization (from organic to inorganic form), solubilization (from inorganic to soluble

(See figure on next page.)

Fig. 7 **A** Results of multiple kernel learning analysis (MKL). **A** Plot of kernel principal component analysis (KPCA) based on nine data sets (Plant biochemical traits, plant phenotypic traits and microbial population measured during stem elongation, flowering, and dough stages). **B** Plot for important variables in each dataset assessed using Crone-Crosby distance. The treatment codes used are: T₁—*Rhodotorula paludigena* Y1 + 100% recommend dose of chemical fertilizers (RDCFs), T₂—*Pseudozyma* sp. Y71 + 100% RDCFs, T₃—*Cryptococcus* sp. Y72 + 100% RDCFs, T₄—Yeast consortium (*R. paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 100% RDCFs, T₅—100% RDCFs, T₆—*R. paludigena* Y1 + 75% RDCFs, T₇—*Pseudozyma* sp. Y71 + 75% RDCFs, T₈—*Cryptococcus* sp. Y72 + 75% RDCFs, T₉—Yeast consortium (*Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72) + 75% RDCFs, T₁₀—75% RDCFs, and T_C—Control

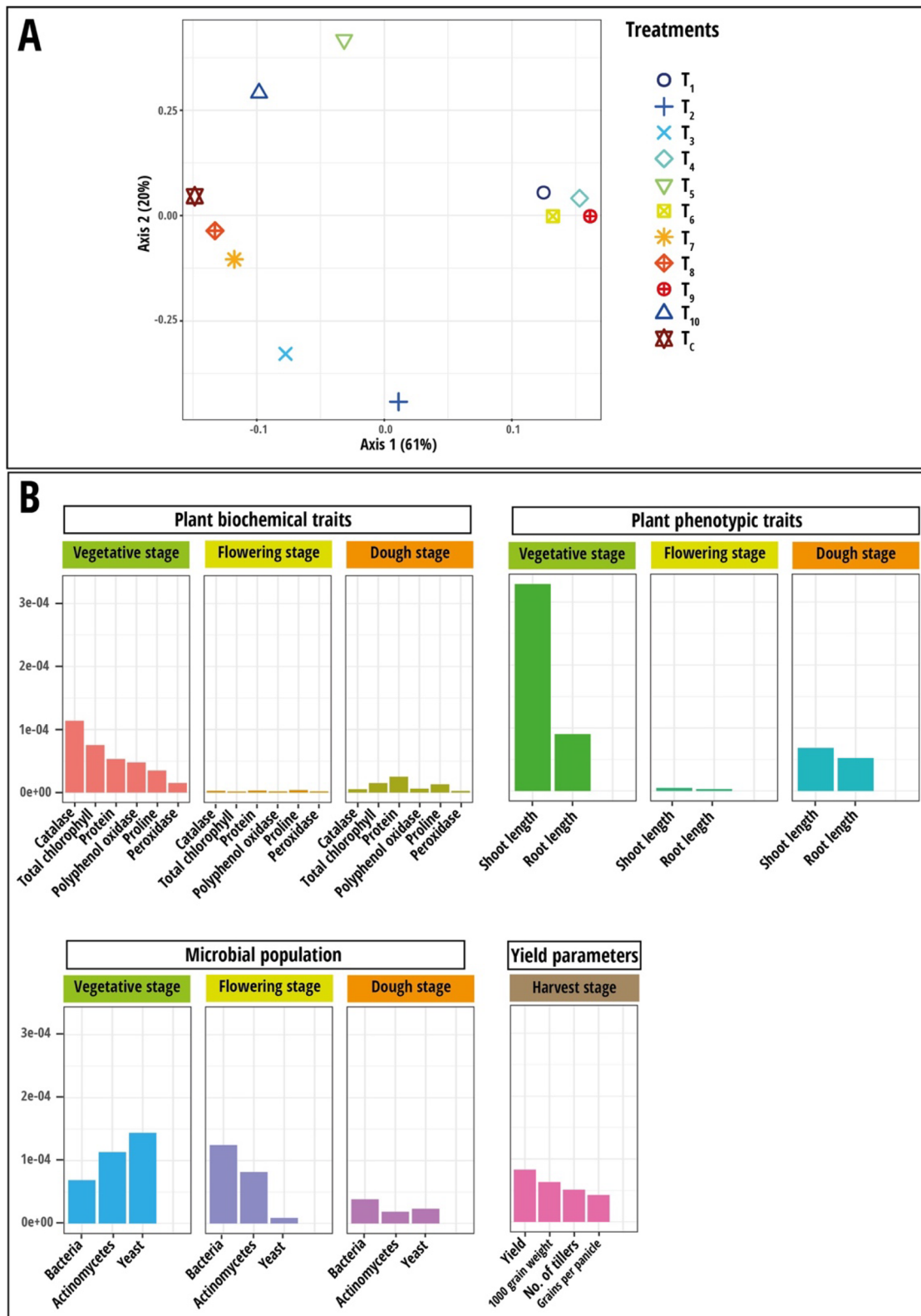


Fig. 7 (See legend on previous page.)

form), and mobilization (uptake) [74]. Plants require phosphorus and potassium as macronutrients. All nine yeast strains in this study demonstrated phosphate and potassium solubilization by forming a halo zone around the colony in the specific media. A similar study reported phosphorus solubilization by *Drosera spatulata* phyllosphere yeasts *Rhodospiridium paludigenum*, *Cryptococcus laurentii*, and *Pseudozyma* species [75]. Soil yeasts *Pichia anomala* and *Rhodotorula glutini* have been shown to solubilize potassium and enhance plant height, as well as root and stem dry weights [22]. According to our CIA results, K-index significantly increases the shoot length of plants whose seeds were treated with yeast strains.

Siderophore is a ferric iron-chelating compound with low molecular weight and strong affinity that is secreted by organisms. Siderophore-producing microbes promote plant growth by increasing iron availability and protecting plants from pathogens by increasing competition for iron sources [76]. *Pseudozyma aphidis*, a phyllosphere yeast, is known to produce the most siderophores of the 15 yeast species examined [77]. Similarly, in our study, among the nine strains, *R. paludigena* Y1 had the maximum siderophore production compared to other strains. *Trichosporon ovoides* and *Saccharomyces cerevisiae* are shown to exhibit a high production of siderophore and *sidD* gene expression under different levels of Cd²⁺ and Pb²⁺ toxicity stress [19]. Yeasts produce hydroxamate type of siderophores and are effective in controlling post-harvest diseases of apples and pears [77]. So, the results indicated that phyllosphere yeast could increase the yield by enhancing the uptake of iron and also can control the disease by making the unavailability of iron for pathogens.

During plant stress, higher ethylene levels are produced than usual, which causes leaf senescence. ACC deaminase is an enzyme that breaks the ethylene precursor ACC, thereby reducing ethylene levels. Both plants and microbes produce ACC deaminase [78]. In our case, all the yeast strains showed positive ACC deaminase activity, and high activity was recorded in *R. paludigena* Y1. High production of ACC deaminase was reported in *Pseudozyma* sp. out of the eight isolates tested [75]. Two seed-borne yeasts, *Pichia kudriavzevii* and *Issatchenkia terricola*, were found to have ACC deaminase activity as well as many other PGP functions [79]. Similarly, Liu et al., [80] discovered that rhizosphere yeast, *Cryptococcus* sp., has ACC deaminase activity and inorganic phosphate solubilization and improves the shoot biomass of *Sedum arboretum*. In our study, CIA showed a significant relationship between shoot length and ACC deaminase activity. Hence, the isolated yeast species demonstrated their potential to decrease ACC levels in rice plants,

ultimately reducing ethylene levels, enhancing plant growth, and protecting the host from various environmental stresses.

Fungal pathogens in plants cause serious crop losses with significant socioeconomic impacts. Increasingly, people recognize that fungal pathogens pose a global threat to agriculture [81]. Microbial tools such as yeast may protect plants from pathogens through various processes such as colonization, antifungal compound production, induced systemic resistance, and the mycoparasitism process [82]. Yeast isolates from the phyllosphere of rice were tested for antagonistic activity against rice pathogens. All the yeast isolates inhibited the growth of *P. oryzae*, *H. oryzae*, and *S. oryzae*. Among the nine strains *R. paludigena* Y1, *Pseudozyma* sp. Y71 and *Cryptococcus* sp. Y72 significantly controlled the rice pathogens. Pepper phyllosphere *Pseudozyma churashimaensis* conferred protection against *Xanthomonas axonopodis* and some pepper viral infections upon foliar application under field conditions [64]. Similarly, the antagonistic activity of phyllosphere yeast *Rhodotorula glutinis* against tomato *Botrytis cinerea* was reported [83]. *Cryptococcus* and some other yeast genera showed antagonistic activity against phytopathogenic fungi *Penicillium*, *Aspergillus*, and *Botrytis* of table grapes, wine grapes, and raisins [84]. The possible antagonistic mechanisms of *Rhodotorula* sp. against fungal pathogens could include siderophore production [85], chitinase production [86], and other inhibitory metabolites [87]. Hence our isolate can protect against rice foliar fungal pathogens.

The three yeast strains that were found to have prominent plant growth traits tested positive for compatibility. All three strains were found to be *Basidiomycete*. *Basidiomycota* yeasts, along with other endophytic bacteria and oomycetes, were prevalent in the *Arabidopsis thaliana* leaf microbial community [88]. Similarly, many *Basidiomycota* yeasts were reported in the rice phylloplane [62]. Hence it is possible to have good compatibility among the *Basidiomycota* yeast. According to the review by [89], applying multiple microbial species of inoculum frequently enhances nutrient availability, promotes plant growth, and yields better than applying a single microbial species. In our study, we applied individual yeast strains and the consortium of three strains along with 75% and 100% RDCFs. The results showed that consortium application, along with 75% RDCFs was the best application in rice as it significantly improved plant biochemicals and yield. In a similar study, it was discovered that the application of *Glomus mosseae* (AMF), *Bacillus subtilis* (Phosphate solubilizing bacteria), and Nitrifying microorganisms (*Nitrosomonas* + *Nitrobacter*) along with 75% of the recommended urea + superphosphate improved the potato yield and quality more than

individual microbial application and high dose fertilizers (100 and 125% RDCFs) [90]. Integrated biofertilizer consortium (*Proteobacteria* and some fungal species from *Basidiomycota* and *Ascomycota*) with 30% recommended chemical fertilizer better-improved oil palm growth and soil microbial diversity than zero and 100% dose chemical fertilizer [91]. As a result, the yeast consortium substituted 25% of the chemical fertilizers commonly used in rice cultivation while outperforming 100% chemical fertilizer applications.

Data integration approaches were invaluable in uncovering various aspects of this study. Within linear relationships, PCA and MCIA highlighted T₉ and T₄ as the best treatments, with a stronger emphasis on T₉. MKL, which captures non-linear relationships, additionally identified T₁ and T₆ as strongly related to the measured parameters. PCA loadings revealed significant variables relevant to each growth stage. MCIA demonstrated that the significance of each measured variable at each rice growth stage was closely related to specific treatments. In contrast, MKL highlighted the order of importance of each variable across different rice growth stages, rather than focusing on relationships.

We also studied the impact of yeast consortium on rice leaf metabolites. Several compounds were identified in rice leaf extract at the critical stage of rice plants from both consortium-treated plants with fertilizer and control plants. The quantity of organic acids and volatile compounds was synthesized at higher levels in the treated plants compared to the control plants. Wheat seeds exposed to two rhizosphere bacteria were found to alter leaf and rhizosphere metabolites. The metabolite profile was found to have beneficial functions such as promoting plant growth, interacting with microbes, and chemotaxis. [92]. Deproteinized leaf extract has been used as a yeast growth medium, and the metabolites produced by yeast in that medium were reported to have plant-beneficial compounds [93]. Moreover, the yeast extract was reported to act as an elicitor and induced growth and secondary metabolite production in transgenic Red sage (*Salvia miltiorrhiza*) cells in 2012 suspension culture [94]. Hence in our study, the yeast strains foliar inoculated on the rice leaf likely utilized the leaf nutrient source and induced a wide range of beneficial metabolite production in rice.

Conclusion

The primary aim of this study was to reduce the dosage of chemical fertilizers in agriculture by integrating them with underexplored microorganisms that can enhance crop improvement. Consequently, we isolated rice phyllosphere yeast and selected nine strains capable of withstanding high temperatures (36 °C), enabling survival in

the phyllosphere environment. Among these, three yeast strains, *Rhodotorula paludigena* Y1, *Pseudozyma* sp. Y71, and *Cryptococcus* sp. Y72, demonstrated better PGP traits and improved root and shoot lengths upon seed treatment. When these top three strains were combined and foliar-sprayed on rice in pot culture and field experiments with 75% of the RDCFs, the results showed significant improvements in plant biochemicals, growth, leaf microbial population, and yield compared to using 100% RDCFs alone. Thus, we found that the foliar application of the identified yeast consortium reduced fertilizer usage by 25% while achieving a better yield than regular fertilization. Therefore, this yeast consortium can be applied to rice fields with reduced fertilizer input to achieve a better yield. Reports on the role of phyllosphere yeast in crop development and biotic stress control are scarce. This study and its findings on rice phyllosphere yeast provide significant contributions to PGPM research. Future research could focus on comparing the rice yield improvement efficiency of phyllosphere yeast strains with that of bacterial strains.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40793-024-00635-9>.

Additional file 1.

Acknowledgements

The authors thank the Department of Biotechnology, Foldscope Project (E28ACN)—India.

Author contributions

GM and AKD designed the experiments. GM, SKG, and JM performed the experiments, and GM and AKD wrote the first draft of the manuscript. AKD did the data analyses. SKG, JM, KSS, RD, DJN, and PD did the proofreading of the first version. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Department of Biotechnology, Ministry of Science and Technology, grant number BT/IN/Indo-US/Foldscope/39/2015.

Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

This material is the authors' own original work, which has not been previously published elsewhere. The paper reflects the authors' own research and analysis in a truthful and complete manner.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Tuticorin 628252, India. ²Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam,

Tuticorin 628252, India. ³Department of Nanotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India. ⁴Agricultural College and Research Institute, Killikulam 628252, India. ⁵Assam Agricultural University, Jorhat, Assam 785013, India. ⁶Central Agricultural University, Umiyam, Meghalaya 793122, India. ⁷Tamil Nadu Agricultural University, Coimbatore 641003, India.

Received: 28 March 2024 Accepted: 4 November 2024

Published online: 18 December 2024

References

- IPAD. USDA. India Rice Area, Yield and Production. 2024. Retrieve from <https://ipad.fas.usda.gov/countrysummary/Default.aspx?id=IN&crop=Rice>.
- FAO. The state of food and agriculture. In: Moving forward on food loss and waste reduction. Rome: FAO; 2019.
- Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey RB, Naylor RL. Increase in crop losses to insect pests in a warming climate. *Science*. 2018;361(6405):916–9. <https://doi.org/10.1126/science.aat3466>.
- Koli P, Bhardwaj NR, Mahawer SK. Agrochemicals: harmful and beneficial effects of climate changing scenarios. In: Climate change and agricultural ecosystems. New Delhi: Woodhead Publishing; 2019. p. 65–94.
- Malhi GS, Kaur M, Kaushik P. Impact of climate change on agriculture and its mitigation strategies: a review. *Sustainability*. 2021;13(3):1318. <https://doi.org/10.3390/su13031318>.
- Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK. Plant growth promoting microbes: potential link to sustainable agriculture and environment. *Biocatal Agric Biotechnol*. 2019;21:101326. <https://doi.org/10.1016/j.bcab.2019.101326>.
- Turner TR, James EK, Poole PS. The plant microbiome. *Genome Biol*. 2013;14(6):1–10. <https://doi.org/10.1186/gb-2013-14-6-209>.
- Hassani MA, Durán P, Hacquard S. Microbial interactions within the plant holobiont. *Microbiome*. 2018;6:1–17. <https://doi.org/10.1186/s40168-018-0445-0>.
- Singh BK, Liu H, Trivedi P. Eco-holobiont: a new concept to identify drivers of host-associated microorganisms. *Environ Microbiol*. 2020;22(2):564–7. <https://doi.org/10.1111/1462-2920.14900>.
- Vorholt JA. Microbial life in the phyllosphere. *Nat Rev Microbiol*. 2012;10(12):828–40. <https://doi.org/10.1038/nrmicro2910>.
- Afeez AA, Babalola OO. Fungi that promote plant growth in the rhizosphere boost crop growth. *J Fungus*. 2023;9(2):239. <https://doi.org/10.3390/jof9020239>.
- Nimsi KA, Manjusha K, Kathiresan K, Arya H. Plant growth-promoting yeasts (PGPY), the latest entrant for use in sustainable agriculture: a review. *J Appl Microbiol*. 2023. <https://doi.org/10.1093/jambio/ixac088>.
- Botha A. The importance and ecology of yeasts in soil. *Soil Biol Biochem*. 2011;43(1):1–8. <https://doi.org/10.1016/j.soilbio.2010.10.001>.
- Nascimento FX, Rossi MJ, Soares CR, McConkey BJ, Glick BR. New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS ONE*. 2014;9(6):e99168. <https://doi.org/10.1371/journal.pone.0099168>.
- Peng X, Wang Y, Tang LJ, et al. Yeasts from Nanfeng mandarin plants: occurrence, diversity and capability to produce indole-3-acetic acid. *Biotechnol Biotechnol*. 2018;32(6):1496–506. <https://doi.org/10.1080/13102818.2018.1487337>.
- Silambarasan S, Logeswari P, Cornejo P, Kannan VR. Evaluation of the production of exopolysaccharide by plant growth promoting yeast *Rhodotorula* sp. strain CAH2 under abiotic stress conditions. *Int J Biol Macromol*. 2019;121:55–62. <https://doi.org/10.1016/j.ijbiomac.2018.10.016>.
- Ramya P, Velu G, Parimala D, Dananjeyan B. Isolation and screening of soil yeasts for plant growth promoting traits. *Madras Agric J*. 2019;106(7/9):439–43.
- Streletskii RA, Kachalkin AV, Glushakova AM, Yurkov AM, Demin VV. Yeasts producing zeatin. *PeerJ*. 2019;7:e6474. <https://doi.org/10.7717/peerj.6474>.
- El-Maraghy SS, Tohamy TA, Hussein KA. Expression of SidD gene and physiological characterization of the rhizosphere plant growth-promoting yeasts. *Heliyon*. 2020;6:e04384. <https://doi.org/10.1016/j.heliyon.2020.e04384>.
- Falih AM, Wainwright M. Nitrification, S-oxidation and P-solubilization by the soil yeast *Williopsis californica* and by *Saccharomyces cerevisiae*. *Mycol Res*. 1995;99(2):200–4. [https://doi.org/10.1016/S0953-7562\(09\)80886-1](https://doi.org/10.1016/S0953-7562(09)80886-1).
- Rosa-Magri MM, Avansini SH, Lopes-Assad ML, Tauk-Tornisiello SM, Ceccato-Antonini SR. Release of potassium from rock powder by the yeast *Torulaspora globosa*. *Braz Arch Biol Technol*. 2012;55:577–82. <https://doi.org/10.1590/S1516-89132012000400013>.
- Mohamed HM, El-Homasy RF, Abd-Elatef AEH, Salh FM, Hussein MY. Identification of yeast strains isolated from agricultural soils for releasing potassium-bearing minerals. *Geomicrobiol J*. 2017;34(3):261–6. <https://doi.org/10.1080/01490451.2016.1186762>.
- Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int J Food Microbiol*. 2013;167(2):153–60. <https://doi.org/10.1016/j.ijfoodmicro.2013.09.004>.
- Ignatova LV, Brazhnikova YV, Berzhanova RZ, Mukasheva TD. Plant growth-promoting and antifungal activity of yeasts from dark chestnut soil. *Microbiol Res*. 2015;175:78–83. <https://doi.org/10.1016/j.micres.2015.03.008>.
- Mannaizu I, Domizio P, Carboni G, Zara S, Zara G, Comitini F, Budroni M, Ciani M. Yeast killer toxins: from ecological significance to application. *Crit Rev Biotechnol*. 2019;39(5):603–17. <https://doi.org/10.1080/07388551.2019.1601679>.
- Arun KD, Sabarinathan KG, Gomathy M, Kannan R, Balachandar D. Mitigation of drought stress in rice crop with plant growth-promoting abiotic stress-tolerant rice phyllosphere bacteria. *J Basic Microbiol*. 2020;60(9):768–86. <https://doi.org/10.1002/jobm.202000011>.
- Devarajan AK, Muthukrishnan G, Truu J, Truu M, Ostonen I, Panneerselvam P, Gopalasubramanian SK. The foliar application of rice phyllosphere bacteria induces drought-stress tolerance in *Oryza sativa* (L.). *Plants*. 2021;10(2):387. <https://doi.org/10.3390/plants10020387>.
- Sedri MH, Niedbala G, Roohi E, Niazian M, Szulc P, Rahmani HA, Feiziasl V. Comparative analysis of plant growth-promoting rhizobacteria (PGPR) and chemical fertilizers on quantitative and qualitative characteristics of rainfed wheat. *Agron*. 2022;12(7):1524. <https://doi.org/10.3390/agron12071524>.
- Devarajan AK, Truu M, Gopalasubramanian SK, Muthukrishnan G, Truu J. Application of data integration for rice bacterial strain selection by combining their osmotic stress response and plant growth-promoting traits. *Front Microbiol*. 2022. <https://doi.org/10.3389/fmicb.2022.1058772>.
- Yadav RKP, Kakamanli K, Vokou D. Estimating bacterial population on the phyllosphere by serial dilution plating and leaf imprint methods. *Ecoprint Int J Ecol*. 2010;17:47–52. <https://doi.org/10.3126/eco.v17i0.4105>.
- Harju S, Fedosyuk H, Peterson KR. Rapid isolation of yeast genomic DNA: bust n'Grab. *BMC Biotechnol*. 2004;4(1):1–6. <https://doi.org/10.1186/1472-6750-4-8>.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):547. <https://doi.org/10.1093/molbev/msy096>.
- Bric JM, Bostock RM, Silverstone SE. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *AEM*. 1991;57(2):535–8. <https://doi.org/10.1128/aem.57.2.535-538.1991>.
- Holbrook AA, Edge WJW, Bailey F. Spectrophotometric method for determination of gibberellic acid. In: Gould RF, editor. *Gibberellins*. Washington: Journal of American Chemical Society; 1961. p. 159–67.
- Hu X, Chen J, Guo J. Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J Microbiol Biotechnol*. 2006;22(9):983–90. <https://doi.org/10.1007/s11274-006-9144-2>.
- Kesaulya H, Hasinu J, Tuhumury GN. Potential of *Bacillus* spp. produces siderophores in suppressing the wilt disease of banana plants. In: IOP conference series: earth and environmental science. IOP Publishing, Bristol, England. 2018.
- Ferreira CM, Vilas-Boas A, Sousa CA, Soares HM, Soares EV. Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. *AMB Express*. 2019;1:78. <https://doi.org/10.1186/s13568-019-0796-3>.
- Penrose DM, Glick BR. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol Plant*. 2003;118(1):10–5. <https://doi.org/10.1034/j.1399-3054.2003.00086.x>.
- Rabindran R, Vidhyasekaran P. Development of a formulation of *Pseudomonas fluorescens* PfALR2 for management of rice sheath blight. *Crop Prot*. 1996;15(8):715–21. [https://doi.org/10.1016/S0261-2194\(96\)00045-2](https://doi.org/10.1016/S0261-2194(96)00045-2).

40. Kandasamy S, Loganathan K, Muthuraj R, et al. Understanding the molecular basis of plant growth promotional effect of *Pseudomonas fluorescens* on rice through protein profiling. *Proteome Sci.* 2009;7:1–8. <https://doi.org/10.1186/1477-5956-7-47>.
41. Wang W, Chen LN, Wu H, Zhang H, Gao S, Yang Y, Xie S, Gao X. Comparative proteomic analysis of rice seedlings in response to inoculation with *Bacillus cereus*. *Lett Appl Microbiol.* 2013;56(3):208–15. <https://doi.org/10.1111/lam.12035>.
42. Santiago CD, Yagi S, Ijima M, et al. Bacterial compatibility in combined inoculations enhances the growth of potato seedlings. *Microbes Environ.* 2017;32(1):14–23. <https://doi.org/10.1264/jsme2.ME16127>.
43. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: *Methods in enzymology*. Cambridge: Academic Press; 1987. p. 350–82.
44. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem.* 1951;193:265–75. [https://doi.org/10.1016/S0021-9258\(19\)52451-6](https://doi.org/10.1016/S0021-9258(19)52451-6).
45. Bates LS, Waldren RA, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil.* 1973;39:205–7. <https://doi.org/10.1007/BF00018060>.
46. Alici EH, Arabaci G. Determination of SOD, POD, PPO and cat enzyme activities in *Rumex obtusifolius* L. *Annu Res Rev Biol.* 2016. <https://doi.org/10.9734/ARRB/2016/29809>.
47. Aebi H. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. New York: Academic Press Inc.; 1974. p. 673–80.
48. Fielding JL, Hall JL. A biochemical and cytochemical study of peroxidase activity in roots of *Pisum sativum*: I. a comparison of DAB-peroxidase and guaiacol-peroxidase with particular emphasis on the properties of cell wall activity. *J Exp Bot.* 1978;29(4):969–81. <https://doi.org/10.1093/jxb/29.4.969>.
49. Coseteng MY, Lee CY. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J Food Sci.* 1987;52(4):985–9.
50. Team R. Core. R: a language and environment for statistical computing. *r foundation for statistical computing*, Vienna, Austria; 2013. Online: <https://www.r-project.org>
51. Gao CH, Yu G, Cai P. ggVennDiagram: an intuitive, easy-to-use, and highly customizable R package to generate Venn diagram. *Front Genet.* 2021;12:1598. <https://doi.org/10.3389/fgene.2021.706907>.
52. Wickham H. ggplot2. *Wiley Interdiscip Rev Comput Stat.* 2011;3(2):180–5. <https://doi.org/10.1002/wics.147>.
53. Culhane AC, Thioulouse J, Perrière G, Higgins DG. MADE4: an R package for multivariate analysis of gene expression data. *Bioinformatics.* 2005;21(11):2789–90.
54. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. *J Stat Softw.* 2008;25(1):1–18.
55. Camargo A. PCAtest: testing the statistical significance of principal component analysis in R. *PeerJ.* 2022;10:e12967. <https://doi.org/10.7717/peerj.12967>.
56. Mariette J, Villa-Vialaneix N. Unsupervised multiple kernel learning for heterogeneous data integration. *Bioinform.* 2018;34(6):1009–15. <https://doi.org/10.1093/bioinformatics/btx682>.
57. Meng C, Kuster B, Culhane AC, Gholami AM. A multivariate approach to the integration of multi-omics datasets. *BMC Bioinform.* 2014;15:1–13. <https://doi.org/10.1186/1471-2105-15-162>.
58. Schindler D. Genetic engineering and synthetic genomics in yeast to understand life and boost biotechnology. *Bioeng.* 2020;4:137. <https://doi.org/10.3390/bioengineering7040137>.
59. Goffeau AEA, Aert R, Agostini-Carbone ML, et al. The yeast genome directory. *Nature.* 1997;387(6632):5–6. <https://doi.org/10.1038/38784>.
60. Stovicek V, Holkenbrink C, Borodina I. CRISPR/Cas system for yeast genome engineering: advances and applications. *FEMS Yeast Res.* 2017. <https://doi.org/10.1093/femsyr/fox030>.
61. Gouka L, Raaijmakers JM, Cordovez V. Ecology and functional potential of phyllosphere yeasts. *Trends Plant Sci.* 2022;22(11):1109–23. <https://doi.org/10.1016/j.tplants.2022.06.007>.
62. Limtong S, Kaewwichian R. The diversity of culturable yeasts in the phylloplane of rice in Thailand. *Ann Microbiol.* 2015;65:667–75. <https://doi.org/10.1007/s13213-014-0905-0>.
63. Glushakova AM, Chernov IY. Seasonal dynamics of the structure of epiphytic yeast communities. *Microbiol.* 2010;79:830–9. <https://doi.org/10.1134/S0026261710060160>.
64. Lee G, Lee SH, Kim KM, Ryu CM. Foliar application of the leaf-colonizing yeast *Pseudozyma churashimaensis* elicits systemic defense of pepper against bacterial and viral pathogens. *Sci Rep.* 2017;7(1):1–13. <https://doi.org/10.1038/srep39432>.
65. Santhanam P, Labbé C, Fietto LG, Bélanger RR. A reassessment of flocculosis-mediated biocontrol activity of *Pseudozyma flocculosa* through CRISPR/Cas9 gene editing. *Fungal Genet Biol.* 2021;153:03573. <https://doi.org/10.1016/j.fgb.2021.103573>.
66. Srisuk N, Nutaratat P, Surussawadee J, Limtong S. Yeast communities in sugarcane phylloplane. *Microbiology.* 2019;88:353–69. <https://doi.org/10.1134/S0026261719030135>.
67. Mannazzu I, Landolfo S, Da Silva TL, Buzzini P. Red yeasts and carotenoid production: outlining a future for non-conventional yeasts of biotechnological interest. *World J Microbiol Biotechnol.* 2015;31:1665–73. <https://doi.org/10.1007/s11274-015-1927-x>.
68. Beattie GA, Lindow SE. Bacterial colonization of leaves: a spectrum of strategies. *Phytopathology.* 1999;89(5):353–9. <https://doi.org/10.1094/PHYTO.1999.89.5.353>.
69. Davies PJ. The plant hormones: their nature, occurrence, and functions. In: Davies PJ, editor. *Plant hormones*. Dordrecht: Springer; 2010.
70. Jha Y. Plant microbiomes with phytohormones: attribute for plant growth and adaptation under the stress conditions. *Advances in Plant Microbiome and Sustainable Agriculture: Functional Annotation and Future Challenges.* 2020;85–103.
71. Limtong S, Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. *World J Microbiol Biotechnol.* 2012;28:3323–35. <https://doi.org/10.1007/s11274-012-1144-9>.
72. Nutaratat P, Amsri W, Srisuk N, Arunrattiyakorn P, Limtong S. Indole-3-acetic acid production by newly isolated red yeast *Rhodospiridium paludigenum*. *J Gen Appl Microbiol.* 2015;61(1):1–9. <https://doi.org/10.2323/jgam.61.1>.
73. Nassar AH, El-Tarabily KA, Sivasithamparam K. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. *Biol Fertil Soils.* 2005;42:97–108. <https://doi.org/10.1007/s00374-005-0008-y>.
74. Devi R, Kaur T, Kour D, Yadav A, Yadav AN, Suman A, Ahluwalia AS, Saxena AK. Minerals solubilizing and mobilizing microbiomes: a sustainable approach for managing minerals deficiency in agricultural soil. *J Appl Microbiol.* 2022;133(3):1245–72. <https://doi.org/10.1111/jam.15627>.
75. Fu SF, Sun PF, Lu HY, Wei JY, Xiao HS, Fang WT, Cheng BY, Chou JY. Plant growth-promoting traits of yeasts isolated from the phyllosphere and rhizosphere of *Drosera spatulata* Lab. *Fungal Biol.* 2016;120(3):433–48. <https://doi.org/10.1016/j.funbio.2015.12.006>.
76. Ahmed E, Holmström SJ. Siderophores in environmental research: roles and applications. *Microb Biotechnol.* 2014;7(3):196–208. <https://doi.org/10.1111/1751-7915.12117>.
77. Calvente V, de Orellano ME, Sansone G, Benuzzi D, de Tosetti MI. A simple agar plate assay for screening siderophore producer yeasts. *J Microbiol Meth.* 2001;47(3):273–9. [https://doi.org/10.1016/S0167-7012\(01\)00333-5](https://doi.org/10.1016/S0167-7012(01)00333-5).
78. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B. Promotion of plant growth by bacterial ACC deaminase. *Crit Rev Plant Sci.* 2007;26(5–6):227–42. <https://doi.org/10.1080/07352680701572966>.
79. Bright JP, Karunanadham K, Maheshwari HS, et al. Seed-borne probiotic yeasts foster plant growth and elicit health protection in black gram (*Vigna mungo* L.). *Sustainability.* 2022;14(8):4618. <https://doi.org/10.3390/su14084618>.
80. Liu W, Wang B, Wang Q, Hou J, Wu L, Wood JL, Luo Y, Franks AE. Characteristics of metal-tolerant plant growth-promoting yeast (*Cryptococcus* sp. NSE1) and its influence on Cd hyperaccumulator *Sedum plumbizincicola*. *Environ Sci Pollut Res.* 2016;23:18621–9. <https://doi.org/10.1007/s11356-016-7041-2>.
81. Almeida F, Rodrigues ML, Coelho C. The still underestimated problem of fungal diseases worldwide. *Front Microbiol.* 2019;10:214. <https://doi.org/10.3389/fmicb.2019.00214>.
82. Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. *Fungal Biol Rev.* 2012;26(2–3):73–83. <https://doi.org/10.1016/j.fbr.2012.07.001>.

83. Kalogiannis S, Tjamos SE, Stergiou A, Antoniou PP, Ziogas BN, Tjamos EC. Selection and evaluation of phyllosphere yeasts as biocontrol agents against grey mould of tomato. *Eur J Plant Pathol*. 2006;116:69–76. <https://doi.org/10.1007/s10658-006-9040-5>.
84. Di Canito A, Mateo-Vargas MA, Mazzieri M, Cantoral J, Foschino R, Cordero-Bueso G, Vigentini I. The role of yeasts as biocontrol agents for pathogenic fungi on postharvest grapes: a review. *Foods*. 2021;10(7):1650. <https://doi.org/10.3390/foods10071650>.
85. Calvente V, Benuzzi D, de Tosetti MS. Antagonistic action of siderophores from *Rhodotorula glutinis* upon the postharvest pathogen *Penicillium expansum*. *Int Biodeterior Biodegrad*. 1999;43(4):167–72. [https://doi.org/10.1016/S0964-8305\(99\)00046-3](https://doi.org/10.1016/S0964-8305(99)00046-3).
86. Ge L, Zhang H, Chen K, Ma L, Xu Z. Effect of chitin on the antagonistic activity of *Rhodotorula glutinis* against *Botrytis cinerea* in strawberries and the possible mechanisms involved. *Food Chem*. 2010;120(2):490–5. <https://doi.org/10.1016/j.foodchem.2009.10.042>.
87. Zhang X, Li B, Zhang Z, Chen Y, Tian S. Antagonistic yeasts: a promising alternative to chemical fungicides for controlling postharvest decay of fruit. *J Fungi*. 2020;6(3):158. <https://doi.org/10.3390/jof6030158>.
88. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol*. 2016;14:e1002352. <https://doi.org/10.1371/journal.pbio.1002352>.
89. Tabacchioni S, Passato S, Ambrosino P, et al. Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for a sustainable agriculture. *Microorganisms*. 2021;9(2):426. <https://doi.org/10.3390/microorganisms9020426>.
90. Saini I, Kaushik P, Al-Huqail AA, Khan F, Siddiqui MH. Effect of the diverse combinations of useful microbes and chemical fertilizers on important traits of potato. *Saudi J Biol Sci*. 2021;28(5):2641–8. <https://doi.org/10.1016/j.sjbs.2021.02.070>.
91. Zainuddin N, Keni MF, Ibrahim SAS, Masri MMM. Effect of integrated biofertilizers with chemical fertilizers on the oil palm growth and soil microbial diversity. *Biocatal Agric Biotechnol*. 2022;39:102237. <https://doi.org/10.1016/j.bcab.2021.102237>.
92. Mashabela MD, Tugizimana F, Steenkamp PA, Piater LA, Dubery IA, Mhlongo MI. Untargeted metabolite profiling to elucidate rhizosphere and leaf metabolome changes of wheat cultivars (*Triticum aestivum* L.) treated with the plant growth-promoting rhizobacteria *Paenibacillus alvei* (T22) and *Bacillus subtilis*. *Front Microbiol*. 2022. <https://doi.org/10.3389/fmicb.2022.971836>.
93. Jadhav RK. Yeast utilizing deproteinised leaf juice (DPJ) as a medium for growth and production of metabolites. *Plant Arch*. 2018;18(2):1716–20.
94. Chen H, Chen F. Effects of yeast elicitor on the growth and secondary metabolism of a high-tanshinone-producing line of the Ti transformed *Salvia miltiorrhiza* cells in suspension culture. *Process Biochem*. 2000;35(8):837–40. [https://doi.org/10.1016/S0032-9592\(99\)00146-6](https://doi.org/10.1016/S0032-9592(99)00146-6).

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.