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Synergistic Effects of PGPR on Soybean Root Defense Responses and Phytochemical Exudation

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ABSTRACT:

The paper discusses the importance of soybean as a staple food and the need to increase crop production to meet the demands of a growing population. It highlights the challenges posed by abiotic and biotic stresses on soybean plants, such as drought, salinity, temperature, pests, and diseases. The paper also discusses the use of synthetic plant nutrients and chemicals to overcome these challenges, but highlights the negative impacts of prolonged usage on soil and the environment. The paper suggests that biological methods, such as plant genetic engineering and biofertilizers, offer promising solutions for promoting crop production in a sustainable and eco-friendly manner. It emphasizes the role of plant growth promoting rhizobacteria (PGPR) in enhancing plant growth and defense mechanisms through interactions with the plant's root system and secretion of beneficial compounds. The paper proposes further research on the molecular mechanisms underlying plant-PGPR interactions and the impact of root exudates on these interactions. The ultimate goal is to identify candidate genes and develop eco-friendly approaches to enhance crop production.

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1. Introduction

Soyabean (*Glycine max*) being the staple food of the majority of the world's population around 3.5 billion population depends on it for their daily calories. In order to meet the demands of the growing population, there is an urgent need to increase the crop production. Abiotic stresses such as drought, salinity, and temperature have become severe in the recent

past due to rise in global warming. In addition, crops encounter a number of biotic stresses in the form of pests and phytopathogens. For instance, loss of yield caused by diseases in soyabean plants has been estimated to be 5-30 % depending on the pathogen and climatic conditions. In order to increase the production of crop plants, synthetically manufactured plant nutrients (phosphate, urea, potassium) gained prominence during the past few decades. Further, chemicals such as endosulfan, DDT, and malathion began to be used widely to overcome the loss in crop productivity caused by the pests and weeds. But with prolonged usage, these chemicals get accumulated in the soil destroying the beneficial soil microbes and thus alter the soil ecosystem irreversibly. Consequently, water bodies surrounding the agricultural lands get contaminated with these chemicals and thus the entire food chain gets disturbed. There are several reports on the health hazards created by these chemicals on human beings, aquatic organisms and animals.

To mitigate the risks created by these chemicals, adoption of biological methods has become a requisite. Plant genetic engineering and biofertilizers have emerged as promising biological approaches to promote crop production. With the advent of genetic engineering, plants have been engineered to overcome the above-mentioned issues of biotic and abiotic stresses. Consequently, an array of transgenic plants (plants containing foreign genes introduced into its genome) were produced in the recent past with tolerance to drought, salinity, herbicides, pests and heavy metals. Transgenics developed in non-edible crops like cotton (Bt cotton - resistant to bollworms) were successfully introduced in the field conditions and is still been cultivated widely in India and other countries. But, commercialization of transgenic food crops remains a challenging task. In India, release of transgenic (Bt) brinjal to the markets remains controversial till date.

The term 'rhizosphere' (coined by Hiltner,) describes the region immediately surrounding the plant roots in the soil. The rhizosphere is the most dynamic region of the soil with several physiological and biochemical activities like respiration, uptake of water by the plants, root secretion of phytochemicals (root exudation), etc. These activities lead to continuous interaction between the soil microorganisms and the plants. The microorganism utilizes the rhizosphere as a niche for its growth and metabolism. In turn, the microbes help the plant in growth promotion in several ways such as auxin production, phosphate solubilization, siderophore production, etc. Microbes synthesize phytohormones such as auxin, gibberellins, cytokinins, and ethylene by which they improve the morphological development of the plants. Several species of *Bacillus* and *Pseudomonas* have been reported to promote plant growth through its auxin producing ability. Phosphate is the second most limiting nutrient for the plants. Most of the phosphate in the soil is in an unavailable form, either as di or tricalcium phosphates. Microbes solubilize these complex forms of phosphate through secretion of acids such as gluconic acid, malic acid, citric acid, etc. These acids lower the soil pH, dissolve the di or tricalcium phosphates into their monomers and make them available for the plants. Iron is another major nutrient required for the plant's metabolism. Microbe-secreted siderophores (iron chelating compounds) scavenge the ferric ions from the rhizosphere and make them bioavailable for the plants.

With all the above traits, soil microbes help the plant in growth promotion and hence termed as plant growth promoting rhizobacteria (PGPR). To make use of these traits, numerous studies were conducted, the consequence of which a number of bio- formulations were developed using the PGPRs (such as *Mycorrhizae*, *Azospirillum*, *Bacillus*, *Pseudomonas*, etc.), and are now available in the market as biofertilizers and biocontrol agents. However, the molecular mechanisms behind the plant-PGPR interactions - how a

bacterium is perceived by the plant; what are the changes encountered by the plant in terms of metabolism (regulation of phytochemical synthesis); and defense response are poorly understood.

Compounds secreted by the plant through roots called root exudates play a critical role in plant growth promotion through nutrient acquisition and inhibition of phytopathogens. Besides direct growth promotion, they function as a molecular dialogue to interact with the rhizospheric microbes and help the plant indirectly by recruiting beneficial microorganisms to the rhizosphere. The microbes utilize these root exudates (organic acids, sugars, amino acids, phenolics, and other secondary metabolites) as nutrients for their metabolism and growth. In turn, the microbes colonize the rhizosphere and form biofilm on the plant roots. The biofilm acts as a protective sheathing and ultimately protect the plants against invasion by the soil pathogens. Thus, root exudates with its diverse roles play a critical role in the plant-microbe interaction. Studies designed to analyze the impact of rhizobacteria on the plants with respect to root exudation would provide clues on the cross-talk between the plants and microbes. Further, comparison of the genome-wide transcriptional changes in different plants triggered by different PGPRs would give a better understanding of the interaction between the plant and the microbes. We hypothesize that an analysis of the transcriptome of crop plants like soyabean with different PGPRs will highlight the candidate genes involved in plant growth promotion. With the identified genes and its products, eco-friendly approaches can be designed to enhance crop production.

Soyabean

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops as it is one of the main sources of vegetable oil and protein [1]. Global soybean yield has continuously increased over the last century, caused by agricultural practice improvement and genetic engineering of high-yielding cultivar, but soybean yield has still not reached a plateau [2]. In order to meet the increasing food demand due to high population growth, yet facing limited area for agricultural lands, the improvement of high nutritional-yielding soybean remains a challenge. For example, multiple agricultural management techniques were implemented to achieve these aims, which include increasing nitrogen fixation, optimizing carbon utilization, adjusting soybean development process, and improving photosynthetic efficiency [3].

Plant Growth Promoting Rhizobacteria

Bacteria that colonize the rhizosphere and are able to promote plant growth are termed plant growth promoting rhizobacteria (PGPR) [4]. By enhancing the vegetative growth and yield of the plant, these bacteria promote the plant growth directly through secretion of hormones, synthesis of siderophores, fixation of nitrogen and solubilization of phosphate [5]. Indirectly, they promote the plant growth by inducing systemic resistance against pathogens, by preventing the association of pathogens with the plants through competitive colonization and by secretion of allelochemicals [6]. Several genera of bacteria such as *Rhizobium*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Agrobacterium*, and *Streptomyces* have been reported to possess plant growth promoting traits [7]. Among them, species of *Bacillus* and *Pseudomonas* are widely used as biofertilizers and biocontrol agents. Though application of these PGPRs as bio-formulations was highly successful under laboratory conditions, the success rate under field conditions remains inconsistent [8]. A better understanding of the plant-PGPR interactions would help in matching the laboratory success rates to the field levels.

As a natural engineer, Plant Growth Promoting Rhizobacteria (PGPR) play an important role in increasing plant growth, yield and enhancing plant tolerance to stressful conditions. These beneficial bacteria take up their position in the rhizosphere, around the plants' root

tissues. They may be in, or on their host tissues and help to provide nutrients to their host plants. For sustainable agriculture, PGPR transmit their extensive assistance in ecosystem management, soil structure maintenance, stress management and plant morphology and physiology modulation in an environmentally friendly manner. Plant- PGPR interactions also stimulate nutrient acquisition and accumulation, improve plant performance and enhance plants' tolerance to abiotic and biotic stresses. Beside these, PGPR are good biofertilizers and safe for our environment. Nanotechnological advances with PGPR applications are important today to increase the impact of PGPR in agriculture. Undoubtedly, PGPR concept is intimately involved with agriculture, horticulture, forestry and they are too enough to establish a vibrant environment. In this review we have focused on the versatility of PGPR-their performance and aimed to address some future prospects of PGPR as an eco-friendly tool for plant growth regulation.[9]

Bacillus Subtilis

Being gram-positive and with the ability to tolerate extreme conditions, the species of *Bacillus* (including *B. subtilis*, *B. amyloliquefaciens* and *B. methylotropicus*) have emerged as a promising PGPR in promoting the growth of crop plants and in the biocontrol of phytopathogens [10]. Mechanisms of direct plant growth promotion by the species of *Bacillus* (particularly *B. subtilis*) include phosphate solubilization, production of auxins like indole acetic acid (IAA), and emission of volatiles [11]. Besides direct growth promotion, mechanisms of indirect growth promotion by *B. subtilis* strains majorly involve the production of antibiotics and lytic enzymes. Production of antibiotics by the species of *B. subtilis* and its effect on plant protection against pathogens has been widely studied. Few among the *B. subtilis* synthesized antibiotics include iturin A, surfactin, fengycin, bacillomycin, difficidin, bacilysin, etc. These antibiotics have been demonstrated to have a protective role against several phytopathogens like *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium gramineum*, *Pythium ultimum*, *Botrytis cinerea*, *Erwinia amylovora*, and *Pectobacterium* (*Erwinia*) *carotovora* [12]. Besides direct inhibitory effect against pathogens, antibiotics like iturin A, surfactin, and fengycin induces the plant's systemic resistance and confers immunity against future pathogenic attacks [13].

Pseudomonas Putida

Species of *Pseudomonas* (gram-negative bacteria) are ubiquitously found in the soil and are well known as aggressive root colonizers [14]. Among them, species of *P. fluorescens*, *P. putida*, *P. aeruginosa* have been reported to be more effective in plant growth promotion [38,39]. *P. putida*, a versatile organism has been used as a bioinoculant for the growth promotion of several plants like canola, potato, maize, lettuce, mungbean, etc. [15]. The major mechanisms by which they promote plant growth is through the synthesis of IAA, hydrogen cyanide, antifungal metabolites, and induction of systemic resistance [16]. Species of *P. putida* possess an exclusive trait of degrading toxic metabolites in contaminated soils and hence is of great importance in the alleviation of heavy metal toxicity in plants [17]. Another exclusive trait of these genera is the production of compounds like siderophores (pyoverdine and pseudobactin) that helps the plant in iron sequestration and thereby mitigates the nutrient stress [17]. The siderophore producing ability has also been attributed to its efficiency in bioremediation and biocontrol of fungal phytopathogens like *Magnaporthe grisea*, *Dreschelaria oryzae*, and *Macrophomina phaseolina* [3].

Root Exudation

Secretion of phytochemicals through roots called root exudation play a significant role in the plant-microbe interaction. The components in the exudates serve as carbon sources and

chemoattractant for the bacteria and hence act as a driving force in bringing the plant and bacteria to form a stable association. Of the total exuded carbon, 90 % is reported to be metabolized by the rhizobacteria and hence the microbial diversity is enriched in the rhizosphere soil relative to the bulk soil [18]. The major components of root exudates include low molecular weight compounds like organic acids, sugars, amino acids, phenolics and flavonoids and high molecular weight compounds like proteins and polysaccharides [17]. Root exudation occurs either by an active process (with the expenditure of energy) or by a passive process (through diffusion). The rate of exudation and the composition of exudates are highly influenced by the biotic and abiotic factors [6].

Mechanisms Underlying Plant-PGPR Interaction

The interaction between bacteria and plants involves multifaceted mechanisms, from recruitment of bacteria towards plants (by chemotaxis); recognition of the bacteria as a PGPR or a pathogen; induction of signaling cascades; and ultimately plant biosynthesis and secretion/exudation of phytochemicals occurs to either embrace or deter the interacting bacterium. In the native environments plants are in continuous association with the microorganisms. They recognize them as beneficial or deleterious based on the chemical cues, termed microbe-associated molecular patterns (MAMPs). Pattern recognition receptors (PRRs) present on the cell surfaces of plants gets activated on binding with these MAMPs and leads to the activation of a series of signaling cascades which ultimately leads to the induction of systemic resistance in plants.

Majorly studied MAMPs include lipopolysaccharide, flagellin, elongation factor Tu (EF-Tu), cold shock proteins (CSP), and transglutaminase (TGase) GP42 from bacteria; chitin, ergosterol, and ethylene inducing xylanase (EIX) from fungi; and branched heptagluco-side from oomycetes. PRRs generally comprises of two classes namely receptor-like kinases (RLK) and receptor-like proteins (RLP). To date, very few PRRs have been identified and functionally characterized. Early physiological responses of MAMP elicited activation of PRRs in plants include generation of reactive oxygen species, nitric oxide, and ethylene; synthesis of antimicrobial compounds; and deposition of callose. However, the genes regulated during PRR activation are poorly understood. Studies conducted at the genome level would give an understanding of the genes regulated during the plant-PGPR interaction. [18]

Table 1.1 PRRs and MAMPs involved in the plant-microbe interaction.

S. No.	Name of the PRRs	Plant source of the PRRs	Corresponding Amps for the PRRs	Microbial source of the MAMPs
1.	Xa21	Soyabean	AvrXa21	Bacteria (<i>X.oryzaepv. oryzae</i> , <i>X. campestris campestris</i>) [71]
2.	RLP - CEBiP	Soyabean	Chitin	Fungus [72]
3.	LRR-RLP	Tomato	Xylanase (EIX)	Fungus (<i>Trichoderma viride</i>) [73]
4.	Glucan-binding protein (GBP)	Fabaceae	Hepta-gluco-side	Oomycete [66]

5.	A flagellin receptor FLAGELLIN SENSING 2 (FLS2)	Arabidopsis	flg22	Bacteria [74,75]
6.	EF-Tu receptor (EFR)	Arabidopsis	elf18	Bacteria [62]

Genes Involved in the Interaction Between A Plant and A PGPR

As discussed above root exudates act as a driving force in the interaction of a plant with a PGPR. The impact of root exudates on the transcriptome of the colonizing bacteria has been widely studied. Whole transcriptome analysis of the root-colonizing bacteria such as *B. amyloliquefaciens* and *B. subtilis* in response to maize and soyabean root exudates showed up- regulation of several genes involved in nutrient utilization, chemotaxis, motility and biofilm formation. In a similar study, genes involved in chemotaxis (*cheY*, *cheA*, and *pctA*), metabolism (catabolism of aromatic compounds and generation of energy) and type III secretion translocation proteins (*pscR*, *pscQ* and *pscP*) were found to be altered in the transcriptome of *P. aeruginosa* PA01 in response to root exudates of different varieties of sugarbeet. Thus, it is evident that genes involved in bacterial chemotaxis and nutrient metabolism play a critical role in the plant-PGPR interaction. In the inverse communication, PGPRs elicit a wide array of transcriptional response in the plants conferring biotic and abiotic stress tolerance. For instance, *B. subtilis* GB03 enhanced the salt tolerance in *Arabidopsis* plants by directly altering the expression of the *HKT1* gene, a Na^+ transporter in roots resulting in lower sodium accumulation. Similarly, *Paenibacillus polymyxa* enhanced the drought tolerance in *Arabidopsis* plants by up-regulating the plant *ERD15* gene. In another study, *B. subtilis* UFLA285 increased the transcription of several defense-related genes in *R. solani* infected cotton plants thereby reducing the pathogenicity of *R. solani* and the symptoms of light brown lesions in cotton stem. Similarly, *P. fluorescens* WCS417 induced systemic resistance in *Arabidopsis* and reprogrammed the plants' transcriptional machinery to increase the synthesis of defense-related compounds. [19]

Plant Defense Mechanisms

Plant defense mechanisms are complex phenomena and take place either by systemic acquired resistance (SAR) or induced systemic resistance (ISR) and are regulated by the phytohormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Figure 1.1). SA stimulate SAR, while, JA and ET stimulate ISR. Though similar in phenotype, the underlying molecular signaling cascades differ. SAR is stimulated during mechanical wounding, herbivory, and pathogenic interactions and is characterized by an increase in the synthesis of pathogenesis-related (PR) proteins and production of antimicrobials like phytoalexins. During pathogen or herbivore attack, these antimicrobials are released out through roots/shoots in the form of either exudates or volatiles to defend themselves against the attackers.

On the contrary, ISR is activated during local infection by a PGPR. Presence of a PGPR (avirulent foreign organism) enhances the accumulation of defense-related transcription factors (TFs) in the plant cells. During future infection by a pathogen, specific TFs are activated from the previously accumulated TFs, in a shorter duration with higher efficiency, thereby conferring resistance against the pathogen. ISR is mainly characterized by an enhanced expression of JA responsive genes like thionin and defensin leading to the synthesis of antimicrobial compounds.

The above studies provide clues on the key genes involved in plant stress tolerance. This knowledge has led to the generation of transgenic plants with enhanced stress tolerance. Though successful, generation of transgenics is a time-consuming process and have to be performed for each species individually. On the other hand, bio-formulations using stress tolerance imparting microorganisms can generally be used for all crop plants.

Studies undermining the molecular mechanisms behind plant-bacterial interaction were carried out mostly in Arabidopsis with a very few information on the crop plants. Moreover, there is a complete dearth of knowledge on how a specific PGPR influences plant defense mechanisms to colonize the roots and the genetic control of root exudation. Transcriptome studies focusing on the genes responsible for the synthesis and secretion of phytochemicals will provide a better understanding of the interactions between a plant and a PGPR and will help in designing new formulations for improving the agricultural productivity. The present study aims to gain insight into the impact of the PGPRs, *B. subtilis* and *P. putida*, on the expression of genes in *Oryza sativa*, the most staple food crop across the world.[20]

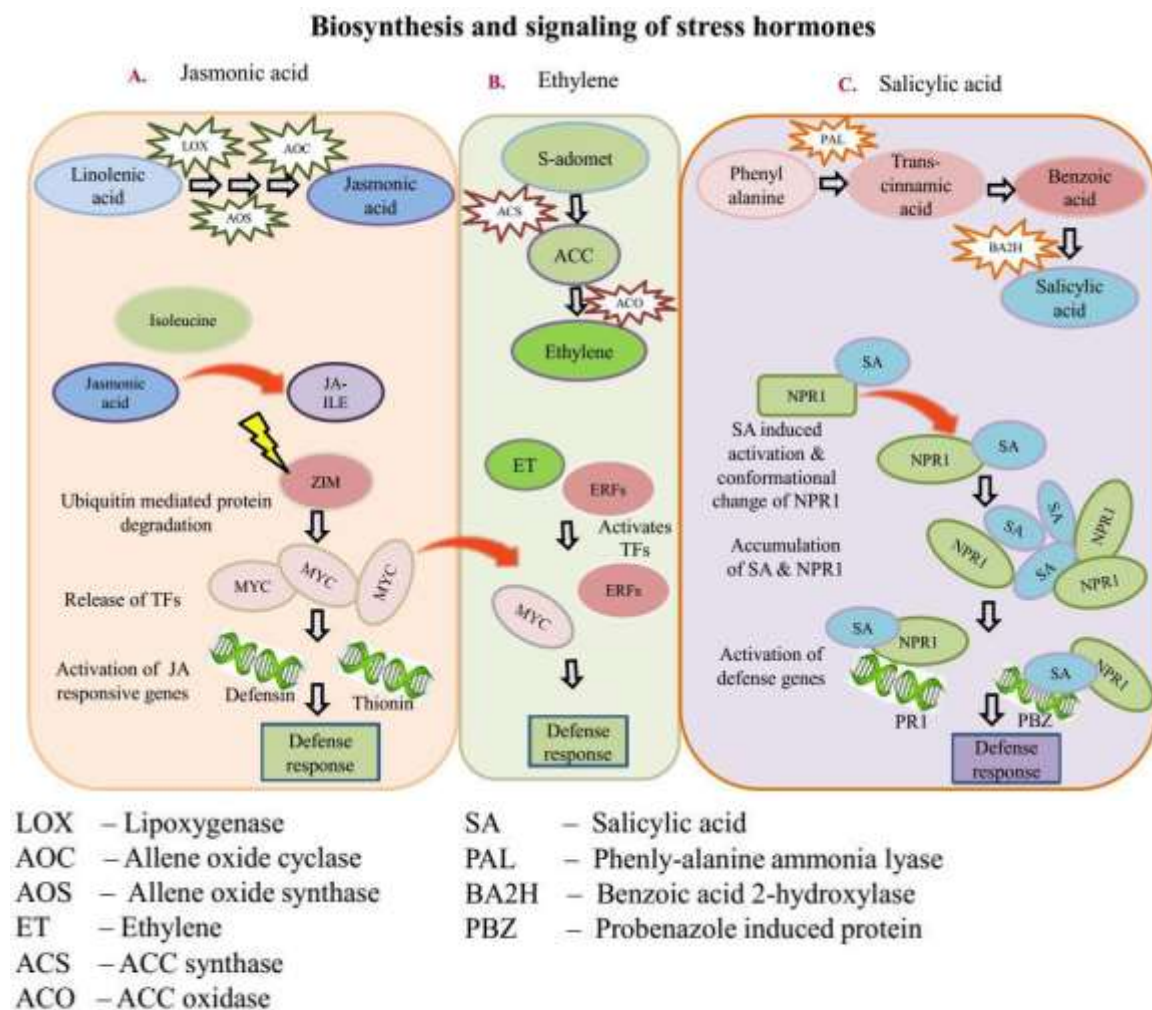


Figure 1.1 Plant defense signaling mechanisms.

2. Methodology

Isolation and Characterization of Bacterial Cultures

Ten grams of the rhizosphere soil samples (collected from three Raisen, Sehore and Hosangabad region of Madhya Pradesh India) were resuspended in 100 mL of sterile water

and serially diluted. From each of the dilutions, 100 μL was spread plated on the nutrient agar (NA) medium and King's B agar (KBA) medium for isolation of *Bacillus* and *Pseudomonas* species, respectively. After overnight incubation at 30 $^{\circ}\text{C}$, plates were observed for distinct colonies. [1]

Screening of the Bacterial Isolates for Auxin Production

The bacterial cultures were screened for the production of auxin using Salkowski reagent and orthophosphoric acid as follows. To 2 mL of the culture supernatant, 100 μL 14 of orthophosphoric acid was added followed by addition of Salkowski reagent (4 mL). After 30 min incubation at room temperature, the absorbance of the resulting mixture was measured at 530 nm using the reagent mixture as blank. The concentration of auxin was calculated using indole acetic acid (Himedia Laboratories, Mumbai, India) as the standard. [2]

Plant Growth Promotion Assay

To investigate the potential of the bacterial isolates as a PGPR, plant growth promotion test was performed using rice plants according to an earlier study with few alterations. Based on the 16S rRNA sequencing results, isolates were chosen for plant growth promotion test. [3]

Collection of Root Exudates

Rice seedlings grown for two weeks in half-strength MS media were aseptically transferred to 250 mL conical flasks containing sterile deionized water (50 mL). After transfer, the flasks were cotton plugged and kept in the plant growth room for 48 h in an orbital shaker set at 50 rpm.[3]

Chemotaxis Assay

In order to examine the chemotaxis of the PGPRs to rice root exudates (REs), chemotaxis assay was performed using the modified capillary method [100- 101]. For the assay, 150 μL of the bacterial culture with a cell density of 0.05 (OD600) was taken in a pipette tip (200 μL) and was fitted to a 2 mL syringe containing the crude RE (500 μL). [3]

Plant Growth Conditions and Preparation of Bacterial Cultures

Rice seeds were surface sterilized using Tween 80 and mercuric chloride (0.1 %) and inoculated into cultures tubes containing half-strength MS media. After 2 days of dark incubation (to facilitate germination), the culture tubes were transferred to a plant growth chamber (temperature - 25 ± 2 $^{\circ}\text{C}$; light intensity: 150-180 $\mu\text{mol m}^{-2} \text{s}^{-1}$; photoperiod - 16/8 h of light and dark) and maintained for two weeks. For treatment of rice plants, the inoculum volume of the PGPRs was optimized in Hoagland's nutrient solution. Simultaneously, the PGPRs were tagged with rifampicin resistance. [5]

Treatment of Rice Seedlings with the Pgprs

For treatment of rice plants, 250 μL and 35 μL of RR4 and RRF3, respectively, each with a cell density of 107 cells/mL were inoculated into 250 mL glass conical flasks containing 50 mL of Hoagland's nutrient solution. [6]

Sample Preparation for Microarray

Microarrays were obtained from Agilent Technologies (CA, USA) consisting of sequences from RAP-DB. [7]

Microarray Data Analysis

Gene expression and statistical analysis for the biological triplicate was done according to

manufacturer's guidelines (GeneSpring GX v12.6 software from Agilent). Percentile shift global normalization was performed using the 75th percentile shift and the differential expression patterns of genes were identified among the samples. [8]

Sample Preparation for Real-Time PCR Analysis

For validation of microarray data, 10 genes from RR4 influenced transcriptomic data and 7 genes from RRF3 influenced transcriptomic data were selected and real-time PCR analysis was performed using SYBR green reaction mix (Roche, CA, USA). [9]

Treatment of Rice Seedlings with PGPRs

Rice seeds (TKM 9) were aseptically germinated and grown in half-strength Murashige and Skoog media in a plant growth chamber (temperature - 25 ± 2 °C; light intensity: 150-180 $\mu\text{mol m}^{-2} \text{s}^{-1}$; photoperiod - 16/8 h of light and dark). [10]

Gene Expression Analysis Using Real-Time PCR

For gene expression analysis, root samples of control and treated plants were harvested at different time periods (2nd, 4th, and 10th day post-inoculation, dpi) for total RNA isolation. Treatment of rice seedlings with the PGPRs Rice seedlings. [11]

Collection of Exudates

At 48- and 96-hours post-inoculation (hpi), the root exudates that are accumulated in the deionized water were collected, filtered to remove root sheathings, and lyophilized in a freeze dryer at -37 °C under vacuum. [12]

Preparation of Root Extracts [13]

Through real-time PCR analysis, the expression of OsMS and OsPAL was found to be commonly enhanced at 96 hpi with PGPR treatment hence for analysis of MA and SA in root tissues, the root samples were 61 harvested at 96 hpi.

Investigation On the Pgpr-Regulated Phytochemicals (Malic Acid And Salicylic Acid) As Plant Growth Promoting Substances. [15]

Plant growth conditions under greenhouse [16]

Bacterial culture and treatment of rice seedlings in soilrite. [17]

Optimization of the treatment conditions for SA and MA. [18]

PGPR treatment and application of biotic and abiotic stress to rice seedlings. [19]

Estimation of chlorophyll. [20]

3. Result and Discussion

Soyabean is a staple food for billions of people worldwide, but there is a need to increase crop production to meet the growing population's demands. Abiotic and biotic stresses, such as drought, salinity, pests, and diseases, pose challenges to soyabean cultivation. Synthetic plant nutrients and chemicals have been used to overcome these challenges, but they have negative impacts on the soil and the environment. Biological methods, such as plant genetic engineering and biofertilizers, have emerged as promising alternatives. Transgenic plants with tolerance to various stresses have been developed, but the commercialization of transgenic food crops remains controversial. The rhizosphere, the region surrounding plant roots, plays a crucial role in the interaction between plants and soil microbes. Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that promote plant growth through various mechanisms, such as auxin production and phosphate solubilization. These PGPRs

can be used as biofertilizers and biocontrol agents. Root exudates, compounds secreted by plant roots, play a critical role in plant-microbe interactions and can attract beneficial microbes to the rhizosphere. Understanding the molecular mechanisms of plant-PGPR interactions and the genes involved can help in designing eco-friendly approaches to enhance crop production. *Bacillus subtilis* and *Pseudomonas putida* are examples of PGPRs that have been studied for their plant growth promoting abilities. These bacteria produce auxins, antibiotics, and siderophores that enhance plant growth and protect against pathogens. The genes and pathways involved in these interactions are still poorly understood, and further research is needed to fully understand and utilize the potential of PGPRs in agriculture.

The dynamic interactions between plant root exudates and the rhizobiome are fundamental to plant health, influencing growth, disease resistance, and overall fitness. This review synthesizes recent research on how these interactions create feedback loops that affect plant health.

Root Exudates: Composition and Functions

Root exudates consist of a diverse array of organic compounds, including sugars, amino acids, organic acids, and secondary metabolites. These exudates play crucial roles in nutrient mobilization, microbial attraction, and allelopathy [21]. For instance, sugars and amino acids serve as carbon sources that attract beneficial microbes, such as nitrogen-fixing bacteria and mycorrhizal fungi [22].

The Rhizobiome: Composition and Roles

The rhizobiome encompasses the microbial community in the rhizosphere, which includes bacteria, fungi, archaea, viruses, and protozoa. Beneficial microbes, such as Rhizobia and plant growth-promoting rhizobacteria (PGPR), enhance nutrient availability and protect plants against pathogens. Conversely, pathogenic microbes can cause diseases, undermining plant health [23].

Feedback Effects of Root Exudates and Rhizobiome Interactions

1. Promotion of Beneficial Microbes

Root exudates selectively attract beneficial microbes by providing them with nutrients. This mutualistic relationship promotes plant growth through improved nutrient uptake and enhanced microbial activity. For example, exudates can stimulate mycorrhizal fungi to solubilize phosphorus, which is then available for plant uptake [24]. In turn, healthier plants may increase exudate production, reinforcing the positive feedback loop [25].

2. Suppression of Pathogens

Certain root exudates possess antimicrobial properties or promote beneficial microbes that outcompete pathogens. For instance, flavonoids and phenolic acids can inhibit pathogenic fungi while supporting beneficial bacteria [26]. This creates a healthier rhizosphere, reducing disease incidence and enhancing plant health. Plants may further modulate exudate composition in response to pathogen attack, enhancing defense mechanisms [27].

3. Induced Systemic Resistance (ISR)

Beneficial microbes can induce systemic resistance in plants, a state of enhanced defensive capacity against a broad spectrum of pathogens. This ISR is often triggered by root exudates that signal beneficial microbes to produce ISR-inducing compounds [28]. This feedback loop improves plant immunity and may lead to changes in exudate profiles that favor ISR-associated microbes [29].

4. Microbial Community Shifts

Root exudates can cause shifts in the microbial community composition within the rhizosphere. By selectively favoring certain microbial taxa, plants can influence the structure of the rhizobiome. These changes can, in turn, affect root exudation patterns, creating a dynamic feedback system that optimizes plant-microbe interactions [30].

5. Nutrient Cycling and Soil Health

Microbial decomposition of organic matter and nutrient cycling are significantly influenced by root exudates. Beneficial microbes facilitate these processes, improving soil health and nutrient availability for plants. Enhanced soil health supports robust plant growth, which may result in increased root exudation, promoting a healthy rhizobiome [31].

4. Conclusion

In conclusion, soybean is a staple food for a large portion of the world's population, and there is a need to increase crop production to meet the growing demand. Abiotic and biotic stresses, such as drought, salinity, pests, and diseases, pose challenges to soybean production. Synthetic plant nutrients and chemicals have been used to overcome these challenges, but they can have negative effects on the environment and human health. Biological methods, such as plant genetic engineering and the use of biofertilizers, have emerged as promising approaches to promote crop production. Plant growth promoting rhizobacteria (PGPR) play a crucial role in plant growth promotion by enhancing nutrient uptake, producing hormones, and inducing systemic resistance against pathogens. Root exudates, which are compounds secreted by plants through their roots, play a critical role in the interaction between plants and PGPR. Understanding the molecular mechanisms behind plant-PGPR interactions and the genes involved in plant defense mechanisms can help in designing eco-friendly approaches to enhance crop production. The feedback interactions between root exudates and the rhizobiome are integral to maintaining plant health. These interactions create complex feedback loops that enhance nutrient availability, suppress pathogens, induce systemic resistance, and improve soil health. Understanding and leveraging these interactions can lead to improved agricultural practices, promoting sustainable plant health and productivity.

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