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Articles

Improving Rice Production through Sustainable Agriculture: A study of the impact of biostimulants on plant growth

Pisit Thamvithayakorn

Rice (*Oryza sativa* L.) is an important crop consumed worldwide. It is the primary staple food of over half the world's population with Asia, South America and Sub-Saharan Africa being the largest consumers. However, rice production is facing several challenges to meet the growing demand of the rapidly increasing world population, which is expected to reach 9.26 billion by 2050. There are two main types of rice cultivated, namely *Oryza sativa*, which is commonly grown in Asia, North and South America, the European Union, the Middle East and Africa and *Oryza glaberrima*, which is solely grown in Africa. To grow, rice requires high daytime temperatures above 25°C with high humidity and cooler nights during the growing season, with annual rainfall above 100 cm and land surface which will facilitate the flooding required when seedlings are planted (**Figure 1**).

Figure 1. (A) Farmers planting rice in paddy fields in Thailand. (B) A farmer working on flooded rice terraces in Chiang Mai, Thailand. (https://www.frsthand.com/story/harvesting-asia and https://asialink.unimelb.edu.au/insights)

Based on environmental factors and water availability, there are four types of rice land ecosystems suitable for rice cultivation (**Figure 2**). Upland rice ecosystems, which rely on natural precipitation patterns in areas with limited water availability; rainfed lowland rice ecosystems, which depend on rainfall in lowland areas with adequate precipitation; irrigated rice ecosystems, where water is supplied artificially through irrigation systems for controlled management and higher yields and flood-prone rice ecosystems in areas susceptible to seasonal flooding requiring special varieties and management strategies to minimize risks

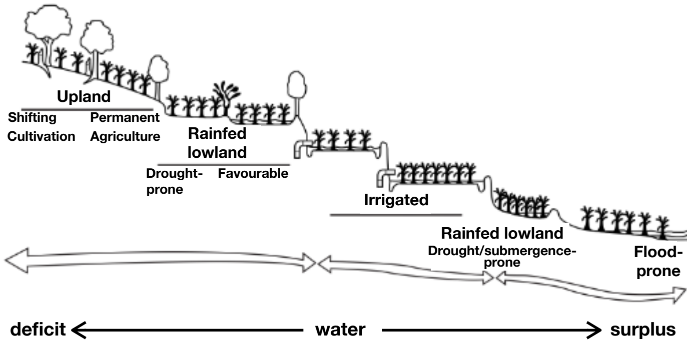

Figure 2. Rice land ecosystems including upland, rainfed lowland, irrigated and flood-prone rice ecosystems (Halwart & Gupta, 2004)

The rice production cycle usually takes about 3-4 months, but the duration can vary depending on the variety and environmental conditions (**Figure 3**). There are three phases of rice plant growth: the vegetative phase, which starts from seed germination and extends to maximum tillering (see Glossary); the reproductive phase, which includes initiation and flowering of the panicle (flowering head); and the ripening phase, during which the young grain in the panicle begins to develop starch and turns golden, signalling that it is ready for harvesting.

Climate change has a significant effect on crop production and global food security. Extreme weather events such as floods, droughts, high temperatures and salinity are common in

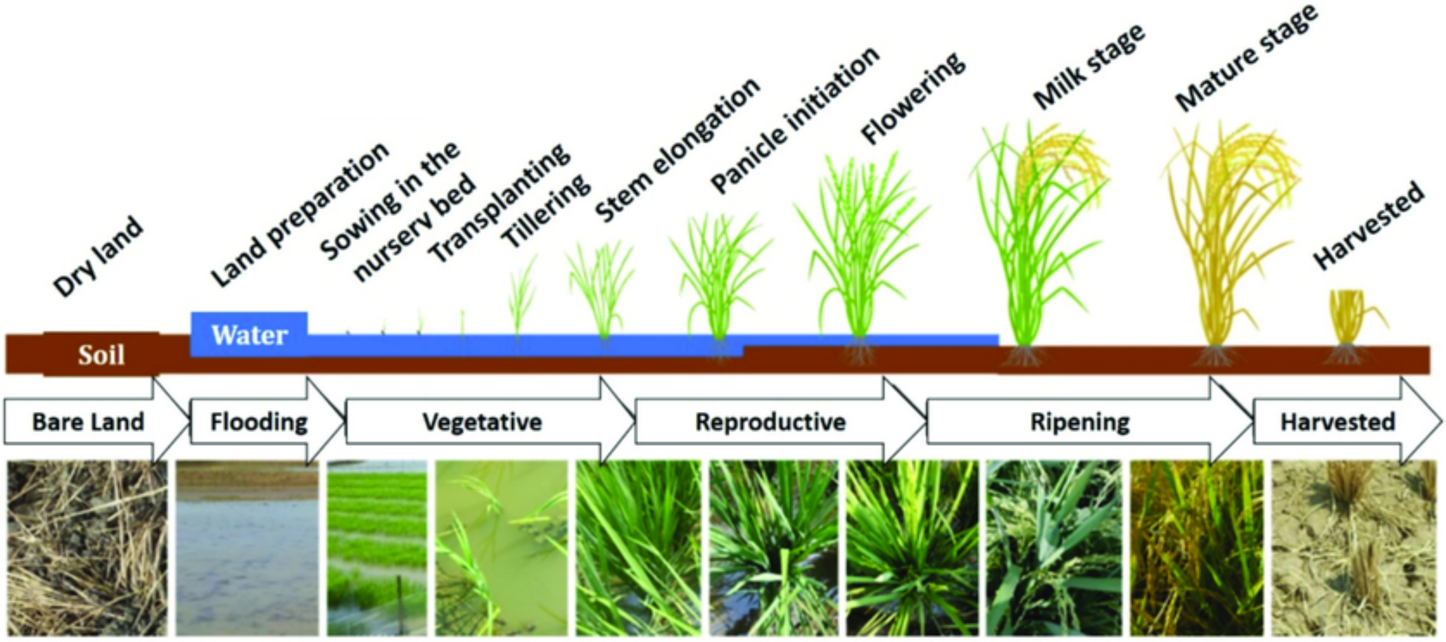

Figure 3. The rice growth phases including vegetative phase, reproductive phase and ripening phase (Sheng et al., 2022)

agriculture and significantly affect crop yields. Traditional high-input farming techniques, including chemical fertilizers, have been adopted to increase productivity. However, they have proved to be detrimental over time; they cause damage to soil structure and are expensive to use. Therefore, it is necessary to adopt cleaner and more sustainable agricultural methods that focus on improving crop productivity whilst also reducing the adverse effects of agrochemicals and climate change. One strategy identified to counteract the negative effects of agrochemicals is the use of plant biostimulants, particularly microbial biostimulants and seaweed extracts. Plant biostimulants are microorganisms and/or substances that occur naturally which stimulate natural processes in plants. When applied to plants or the rhizosphere, they enhance nutrient uptake, tolerance to biotic and abiotic stresses and crop quality (**Figure 4**).

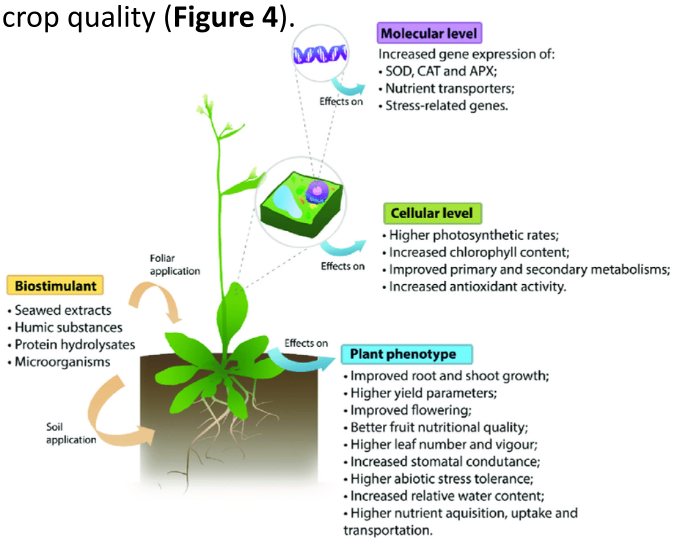

Figure 4. The beneficial effects of plant biostimulants on the soil-plant system indicating the effects at the molecular and cellular levels and the plant phenotype (Baltazar et al., 2021)

Microbial biostimulants, particularly plant growth-promoting rhizobacteria (PGPR), are essential for a plant's nutrient uptake through nitrogen fixation, phosphate solubilisation, siderophore production and disease suppression. They are also involved in various signalling cascades by means of phytohormones, secondary metabolites, amino acids, polysaccharides and antibiotics. When PGPR encounter plant roots they release signalling molecules which can trigger a cascade of biochemical reactions in the plant cells. These reactions can lead to changes in gene expression, metabolic pathways and physiological processes thereby influencing plant growth, development and response to environmental stresses (**Figure 4**). Some of the most potent PGPR that respond to various abiotic stresses which include drought, salinity and floods, are species of *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter* and *Pseudomonas*. PGPR respond to these stresses by enhancing nutrient uptake by plants, producing stress-tolerant compounds, inducing stress-responsive genes, regulating phytohormones and promoting biofilm formation. These interactions help plants tolerate stresses and enhance growth and productivity in challenging environments.

Seaweeds are macroscopic and multicellular marine algae belonging to several taxonomic groups, commonly referred to as red, green and brown seaweeds. Seaweed extracts are used to apply nutrients to plants in the form of foliar sprays and soil drenching. They contain substances that promote plant growth and yield including macronutrients, organic substances, trace elements and plant growth regulators like cytokinin, auxin and gibberellins (**Figure 4**). One of the most researched seaweeds is the brown seaweed *Ascophyllum nodosum*. Various commercial extracts from *A. nodosum* have been demonstrated to improve plant growth and mitigate abiotic and biotic stresses while improving plant defences by regulating molecular, physiological and biochemical processes.

Case Study: Investigating the use of microbial biostimulants and seaweed extracts in the promotion of rice growth and soil health

This study investigated the impact of PGPR consortium (a mixture of different strains of PGPR) and seaweed extract on rice growth and soil health. The sequence of the investigation, conducted in two stages, is illustrated in **Figure 5**. The first stage of study aimed to screen, identify and characterise the beneficial bacteria from the rhizosphere soils of paddy fields. In the second stage, the study focused on developing novel biostimulants based on PGPR consortium and seaweed extract to promote rice growth and productivity (phase 1) and investigating the bacterial communities in the rice rhizosphere soil influenced by the biostimulants using amplicon-based microbiome sequencing (phase 2).

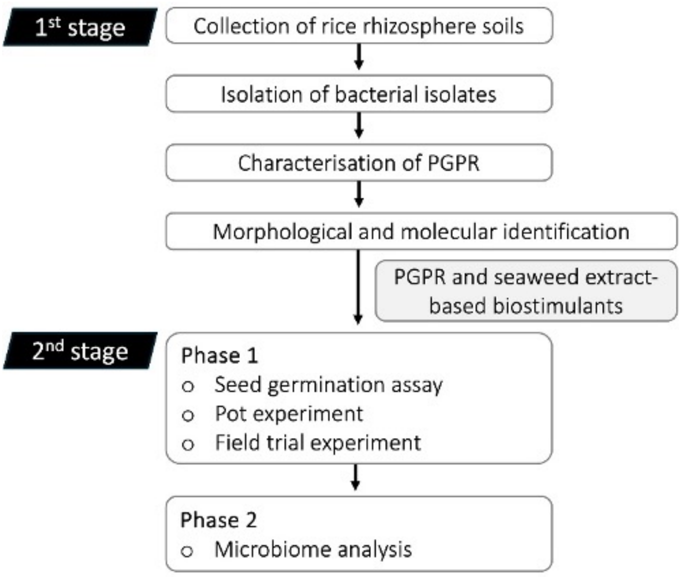

Figure 5. Schematic diagram illustrating the sequence of the investigation into the use of PGPR consortium and seaweed extract-based biostimulants on rice growth in the case study.

Method

In the first stage, in order to screen, identify and characterise the beneficial bacteria soil samples were collected from the rice rhizosphere in organic paddy fields in Northeast Thailand. Bacterial strains were isolated by diluting the soil and spreading it onto a screening agar medium to obtain pure cultures. The bacterial isolates were tested for various PGPR traits, including phosphate solubilisation, nitrogen fixation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, indole-3-acetic acid (IAA) production, siderophore production and potassium solubilisation, all of which contribute to plant growth and nutrient acquisition. The selected bacterial isolates were identified morphologically and molecularly using the Polymerase Chain Reaction (PCR) technique to determine their genus or species. The three best PGPR characteristics were chosen to produce the mixture of bacterial inoculum (PGPR consortium) for testing on the indica rice cultivar RD79 in the next stage.

The second stage involved testing the rice cultivar RD79 with four treatments: (A) *Ascophyllum nodosum* seaweed extract, (B) PGPR consortium, (C) a combination of PGPR consortium and seaweed extract and (D) a control treatment. These treatments were applied to germinating seeds (seed germination assay), pot-grown rice plants in a greenhouse (pot experiment) and rice plants in an organic paddy field (field trial experiment). In addition, microbiome analysis was conducted on the rice rhizosphere soils from the pot experiment. For the seed germination assay in phase 1, the rice seeds were surface sterilised, soaked in each treatment solution for 24 hours and incubated in petri dishes for 7 days (**Figure 6A**). The percentage of germination and seed vigour index were analysed. In both pot and field trials, the rice cultivar RD79 seeds were surface sterilised and grown in trays for 2 weeks. Afterward, the rice seedlings were soaked in each treatment using root dipping technique for 1 hour and transplanted into pots for growth under greenhouse conditions (**Figure 6B**) and into organic paddy fields for field trials in Wang Yang district, Nakhon Phanom, Thailand (**Figure 6C**). Growth parameters including plant height, number of panicles per tiller, panicle length, panicle weight and number of seeds per panicle were measured in both pot and field trial experiments.

In phase 2, rhizosphere soil samples from each treatment in the pot experiment were collected at the tillering stage (30 days after transplanting) and the harvesting stage (90 days after transplanting) for DNA extraction and sequencing. The sequencing data were analysed for bacterial diversity and to compare microbial composition across treatments.

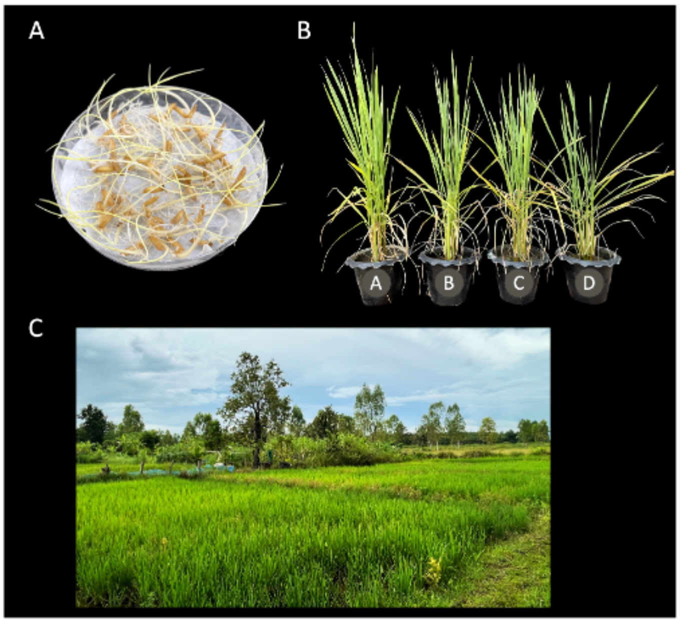

Figure 6. (A) germinated rice seeds on a petri dish, (B) a pot experiment performed under greenhouse conditions, including treatments A-D and (C) a field trial experiment in Nakhon Phanom, Thailand.

Results

In the first stage of the investigation, of the 470 bacterial strains isolated from the rhizosphere soil of the paddy fields, preliminary results showed that 57 bacterial strains exhibited at least three PGPR traits. The three best PGPR isolates from the genera *Kosakonia, Phytobacter* and *Rhizobium*, were classified in risk group 1 as safe for human health and selected for PGPR consortium production.

In the second stage, all treatments in the seed germination assay resulted in more than 90% germination with no significant differences between them after 7 days of incubation. However, the PGPR consortium (treatment B) showed the highest seed vigour index, followed by the combination of PGPR consortium and seaweed extract (treatment C), indicating effective rice growth promotion under laboratory conditions. In both pot and field trials, treatments with *A. nodosum* extract (treatment A) and the combination of PGPR consortium and *A. nodosum* extract (treatment C) enhanced plant height, panicle length, number of panicles per tiller and number of seeds per panicle compared to control treatment. These results indicated that the use of PGPR consortium combined with *A. nodosum* extract as a biostimulants on rice is effective in enhancing rice growth and yield.

Microbiome analysis from phase 2 of the experiment revealed the presence of beneficial bacteria that promote rice growth and soil health. The application of biostimulants led to significantly higher bacterial diversity compared to the control, demonstrating that biostimulants positively affect the microbial community, thereby promoting rice growth more effectively. Hence, Biostimulants introduce beneficial bacterial strains, promoting diverse bacterial colonisation, enhancing nutrient availability and improving soil structure. To our knowledge, this represents the first report on the application of a combination of PGPR consortium and *A. nodosum* seaweed extract as a novel biostimulant product on rice cultivar RD79.

Conclusion

The preliminary results of the study suggest that plant biostimulants, particularly the plant growth-promoting rhizobacteria (PGPR) and *Ascophyllum nodosum* seaweed extract, can promote the growth of indica rice and enhance the diversity of the bacterial community in rhizosphere soil of rice. The diverse bacterial community plays a role in maintaining soil ecosystem health, improving nutrient availability and disease suppression whilst enhancing rice growth and productivity. However, it is important to note that these are initial findings and further and more in-depth studies are necessary to fully exploit the benefits of these microbial and seaweed extract biostimulants and develop more efficient products in the future.

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Glossary

Agrochemicals: Chemical products used in agriculture to control pests, diseases and weeds and to enhance crop growth, such as fertilisers and pesticides.

Amplicon-based microbiome sequencing: A molecular technique that analyses the genetic makeup of microbial communities by amplifying and sequencing specific regions of their DNA to identify and measure the different microorganisms present.

Biostimulant: Substances or microorganisms applied to plants or soil to promote plant growth, improve productivity, enhance nutrient uptake and increase resistance to stress.

Microbiome: The community of microorganisms, such as bacteria and fungi that live in a specific environment, such as soil, water or within living organisms.

Organic paddy fields: Rice fields cultivated using organic farming methods that avoid synthetic chemicals and pesticides.

Panicle: A branched flower cluster found in plants like rice, where the flowers grow from a main stem.

PGPR: Plant Growth Promoting Rhizobacteria - Beneficial bacteria that colonise plant roots and enhance plant growth through mechanisms, such as nitrogen fixation, disease suppression and production of growth hormones.

PGPR consortium: A mixture of different strains of PGPR used together to boost plant growth and health.

PGPR traits: Characteristics or abilities of PGPR that promote plant growth, such as nitrogen fixation, phosphate solubilisation and production of plant hormones.

Phytohormones: Plant hormones that control various aspects of plant growth and development, including auxin, gibberellin, cytokinin, abscisic acid and ethylene.

Rhizosphere: The soil area directly surrounding plant roots, rich in microbial activity where plants interact with microorganisms.

Rhizobacteria: Bacteria that live in the rhizosphere, some of which benefit plants while others may be harmful.

Secondary metabolites: Organic compounds produced by plants, fungi or bacteria that are not essential for growth but often have roles in defence, signalling or competition.

Siderophore: Molecules produced by microorganisms and plants that bind and transport iron, which is essential for many biological processes but often limited in availability.

Signalling cascades: Complex networks of communication within cells, allowing them to respond to environmental changes by regulating processes like metabolism, gene expression and stress adaptation.

Tillering: The process by which grasses and cereals like rice produce side shoots from the base of the plant, which can increase the number of stems and potentially yield.

AUTHOR PROFILE

Pisit Thamvithayakorn is from Bangkok, Thailand and received his BSc from King Mongkut's Institute of Technology Ladkrabang. He obtained an MSc in Biotechnology and PhD in Applied Microbiology from Srinakharinwirot University. He works on plant biostimulants, focusing on plant growth-promoting rhizobacteria, soil-microbe interaction and soil microbial communities.