Chapter 11
Biopriming is Emerging as a Supplemental Strategy for Improving Nitrogen Use Efficiency of Crop Species

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Abstract A recent review of worldwide data on nitrogen use efficiency warrants an immediate intervention of best management practices which will optimize best fertilizers option, farmer profitability and crop productivity. Nitrogen being a primary nutrient is critical for congenial plant growth. Additionally, the risk to human health due to indiscriminate use and concern due to energy intensive production of this input paves the way for use of bio-inoculants as the possible link for better utilization and use efficiency. Biopriming which involves seed priming in combination with low dosage of beneficial microbes has emerged as the most feasible eco-friendly supplement to the existing integrated plant nutrition system. In this chapter, we have presented an overview of the recent advances in biopriming with a special reference to nitrogen.

Keywords Nitrogen use efficiency · Bio-inoculants · Biopriming

11.1 Nitrogenous Fertilizer-Energy Intensive/ Non-renewable Energy

India is the second most populous country after china. India is primarily an agricultural country as 65% of its people depend on agriculture for their livelihood. Farmers of India mostly rely on organic manures for supplementing soil until middle of twentieth century. However, with the emergence of green revolution during early 1960s, demand and consumption of synthetic fertilizers increased per unit area

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Table 11.1 Various manufacturing processes of hydrogen

Process	Reaction	Approx relative energy consumption (%)
Water electrolysis	$2H_2O \rightarrow 2H_2 + O_2$	300
Coal gasification	$C + 2H_2O \rightarrow 2H_2 + CO_2$	170
Naphtha reforming	$CH_2 + 2H_2O \rightarrow 3H_2 + CO_2$	104
Nat gas reforming	$CH_4 + 2H_2O \rightarrow 4H_2 + CO_2$	100

Source: Pach (2007)

tremendously. Use of high yielding and developed irrigation facilities during the time of green revolution required higher input which was then fulfilled by fertilizers. As a consequence, Indian soils become less and less fertile. Out of many reports of nutrient deficiency, nitrogen (N) deficiency is the first one to be reported in Indian soil. As a result consumption of nitrogenous fertilizers was increased sharply from 0.06 million ton (Mt) in 1950–1951 to 10.8 Mt. in 2000–2001, about 190-fold increase in 50 years after independence (Pathak et al. 2010). Green revolution causes increase in consumption of all the fertilizers mainly phosphatic and potassic after nitrogenous fertilizers. This makes India third largest producer and consumer of fertilizers worldwide. Energy consumed in fertilizer sector per unit of output is 14.7% from 1996 to 2005. Coal, petroleum and natural gas are major sources of energy but non-renewable in nature. Production of nitrogenous fertilizers is highly energy intensive out of four nitrogen, phosphate, potash and complex type fertilizers. Ammonia (NH_3) production is a primary stage for nitrogenous fertilizers as it is basic chemical used in their production (Ray 2011). Production of NH_3 at a large scale is mainly carried out by Haber–Bosch process. Ammonia is produced using N_2 and H_2 from the atmosphere at a very high temperature and pressure. Harsh condition during the process results lower efficiency level than desirable for sustainable fertilizer production (Zhou et al. 2017). Hydrogen used in the process is chemically produced which then reacted with atmospheric N_2 and produce NH_3. Atmospheric nitrogen is used as a N_2 source which is present in adequate quantity but energy and feedstock required for the process are limited. Natural gas is one of the key input for NH_3 production which share 78.5% in total N production; external NH_3 and naphtha come after that with 16 and 5.3% share, respectively (Tewatia and Chanda 2017). Energy consumed during various process of hydrogen production was given in Table 11.1. The hydrogen produced from various processes is further reacted with atmospheric N_2 for final ammonia production.

According to Dawson and Hilton (2011), annual energy requirement for fertilizer requirement in world is approximately 1.1% of energy used globally in the year 2008. Out of the total energy input used in fertilizer production, N fertilizers alone account for more than 90% (Table 11.1).

Industries related to N fertilizers are not only energy intensive but also deals with non-renewable sources of energy which is of great concern. In developing countries like India where energy crisis is a major problem, the energy consumption of NH_3 plants is 12.48 giga calories metric ton^{-1} (Gcal MT^{-1}) and that of urea plants is 5.95 Gcal MT^{-1} in 2015 (Tewatia and Chanda 2017). This much energy consumption is

Table 11.2 World demand for fertilizer nutrient use, 2015–2020 (1000 tonnes)

Year	2015	2016	2017	2018	2019	2020
Nitrogen (N)	110,027	111,575	113,607	115,376	117,116	118,763
Phosphate ($P_2 O_5$)	41,151	41,945	43,195	44,120	45,013	45,858
Potash ($K_2 O$)	32,838	33,149	34,048	34,894	35,978	37,042
Total (N+ $P_2 O_5$ + $K_2 O$)	184,017	186,668	190,850	194,390	198,107	201,663

Source: FAO (2019)

of great concern, as most of the Indian soils are deficient in N and farmers are totally depend on nitrogenous fertilizers for crop production (Table 11.2).

11.2 Nitrogen-No Alternative Source; Supplementation and Improving Rhizosphere Efficiency Only Option

Nitrogen requirement for plant is more than any other nutrient and is a key component of chlorophyll responsible for photosynthesis, as well as amino acids, ATP and nucleic acids. Nitrogen plays major role in growth and development of plant and is a critical limiting element in soil. Though nitrogen quantity is ample in atmosphere, in Indian soils nitrogen is universally deficient element for profitable crop production and almost all soils respond to external application of nitrogen. Cost of fertilizers is still close to record highs which increase cost of cultivation and adverse effects on benefits of the farmer, also impacts environment through nitrogen losses. Also farmers may apply excess fertilizers that exceed agronomic recommendations. To get rid of this complication focus should be on management approaches and new interventions to supplement costly fertilizers, to meet crop requirement and increase efficiency especially for nitrogen. Management options are more effectual if it can target the efficiency of rhizosphere which is directly influenced by root secretions and associated microorganisms and is the seat of all complex interactions among soil, microorganisms and plant roots. To enhance nutrient availability and uptake, rhizosphere role is incredible. In soil system, 95–99% of nitrogen is in organic form which cannot be directly available to the plants, but will be mineralized into available forms by microorganisms. Rhizospheric microorganisms participate in this cycling of nitrogen and determine the plant availability. To minimize cost of cultivation and chemical fertilizer use the option in front of us is improving the rhizosphere efficiency through manipulating its microclimate which can be achieved by integration with organics and microorganisms. Bio-organic fertilizers, a combination of suitable substrate and functional microbes can effectively suppress soil-borne diseases and promote plant growth (Zhao et al. 2018). Plants shape microbiome structure through its root exudates in rhizosphere through which bio-availability of nutrients, hormonal activity and plant growth will get positively affected. Though the external application of microbes to the rhizosphere can be done through several means, but the best, effective and feasible method is seed biopriming which is the combination of both seed hydration and addition of bio-agent. Biopriming intervention is eco-friendly, low cost intensive and a potent technology for altering microclimate of the rhizosphere. This technology not only enhances the availability of nutrients especially nitrogen, but also resist the plant in abiotic and biotic stress situations.

11.3 Possible Microbes as a Suitable Agent for Biopriming

As biopriming is being emerging as an advanced form for nutrient management in agricultural crop production, the main reason underlying is the use of native and indigenous microorganisms which benefits the crop plants not only in terms of fertilization but also providing other advantageous attributes like plant growth promotion, safeguarding against pathogenic diseases and also biotic and abiotic stress (Table 11.3). The biopriming procedure was first described by Callan and co-workers in 1990 for biological control of *Pythium* preemergence damping-off of sweet corn at an optimal temperature with protection by using a biocontrol agent. Other advantages of this new priming approach are that it incite such changes in the bioprimed seeds that enhances the seed germination and emergence (Bisen et al. 2015). The indigenous strains here are emphasized because it assures of the compatibility with the crops in the region and also gets acclimatized better in the cop-soil ecosystem in comparison to the one that has been imported from outside the region (Fig. 11.1). This is also an exemplary alternative for organic production where use of chemicals for disease and nutrient management is not advised.

To get the favourable results suitability of the biopriming agent is needed to be taken under consideration which depends on many factors as aeration, quality of seed, temperature, light and duration (Kumar et al. 2020).

It improves seed viability, germination, vigour indices, plant growth and subsequent protection against diseases and finally enhances crop yield. Biopriming is the advanced technique which can be employed to improve the plant growth for which different plant growth promoting microorganisms can be used. To address the diseases and pests in crops biopriming using biocontrol agents, viz. *Trichoderma* sp., *Aspergillus* sp., Mycorrhizal fungi, etc. and antagonistic microbes can be done by suppressing various seed and soil-borne diseases as well as foliar diseases through induced systemic resistance mechanisms. Management of nutrients in the crops and soils can be accomplished with the help of biopriming agents having the ability of nutrient solubilization and improving the nutrient availability towards plants, e.g. *Rhizobium leguminosarum*, *Pseudomonas fluorescens*, vesicular-arbuscular mycorrhiza (VAM), viz. *Acaulospora* sp., *Glomus* sp. Biopriming is also a tool to aid plants during stress using such microorganisms those who elicit so-called induced systemic tolerance (IST) against biotic and abiotic stresses and can with stand high temperature, pH and salt concentrations e.g. *Colletotrichum magna*, *Piriformospora indica*, *Alternaria* sp. (Prasad et al. 2016).

Table 11.3 Different biopriming agents and various functions performed by them

	Crop	Microbial Agent	Use	Advancement	Region	Reference
Cereals	Rice (*Oryza sativa*)	*Bacillus* sp.	Dirty panicle disease (*Bipolaris* sp. and *Curvularia* sp.)	Antifungal activity (nearly 100%)	Chiang Mai, Thailand	Rangjaroen et al. (2019)
	Maize (*Zea mays*)	*Trichoderma lixii*	Salt toxicity	Proline accumulation increased by 21.87%(roots), 35.71% (leaves)	Rize, Turkey	Pehlivan et al. (2017)
	Rice (*Oryza sativa*)	*Piriformospora indica*	Cadmium stress	Root ROS decreased by 18.5%	Noida, India	Dabral et al. (2019)
Pulses and legumes	Chickpea (*Cicer arietinum*) and rajma (*Phaseolus vulgaris*)	*Pseudomonas fluorescens, Trichoderma asperellum* and *Rhizobium*	Plant growth	Germination % increased by 24–27%, plant height increased by approx. 23.6%	Varanasi, India	Yadav et al. (2013)
	Faba bean (*Vicia faba*)	*Trichoderma* spp., *Bacillus* spp., *P. fluorescens*	Root rot incidence caused by *R. solani*, *F. solani* and *S. rolfsii*	Disease incidence decreased upto 6.9–14.8% (fungal agent),6.9–23.3% (bacterial agent)	Giza, Egypt	El-Mougy and Abdel-Kader (2008)
	Soybean (*Glycine max*)	*Trichoderma* spp., *Pseudomonas fluorescent*	Nutrition	Fe uptake increased to 77%Zn uptake (90 mg/kg), N (7.5%)	Tehran, Iran	Entesari et al. (2013)
Vegetables	Carrot (*Daucus carota*)	*Clonostachys rosea*	*Alternaria* spp	Alternaria spp. incidence reduction by ≥94%	Copenhagen, Denmark	Jensen et al. (2004)
	Okra (*Abelmoschus esculentus*)	*Alcaligenes faecalis*	Against *Sclerotium rolfsii*	Mortality reduced to 20%	Varanasi, India	Ray et al. (2016)
Spices	Cumin (*Cuminum cyminum* L.)	*Pseudomonas fluorescence*, *Trichoderma harzianum*	Against drought stress	Emergence percentage (52.23%), emergence rate (0.358 seedling d^{-1})	Yasuj, Iran	Piri et al. (2019)

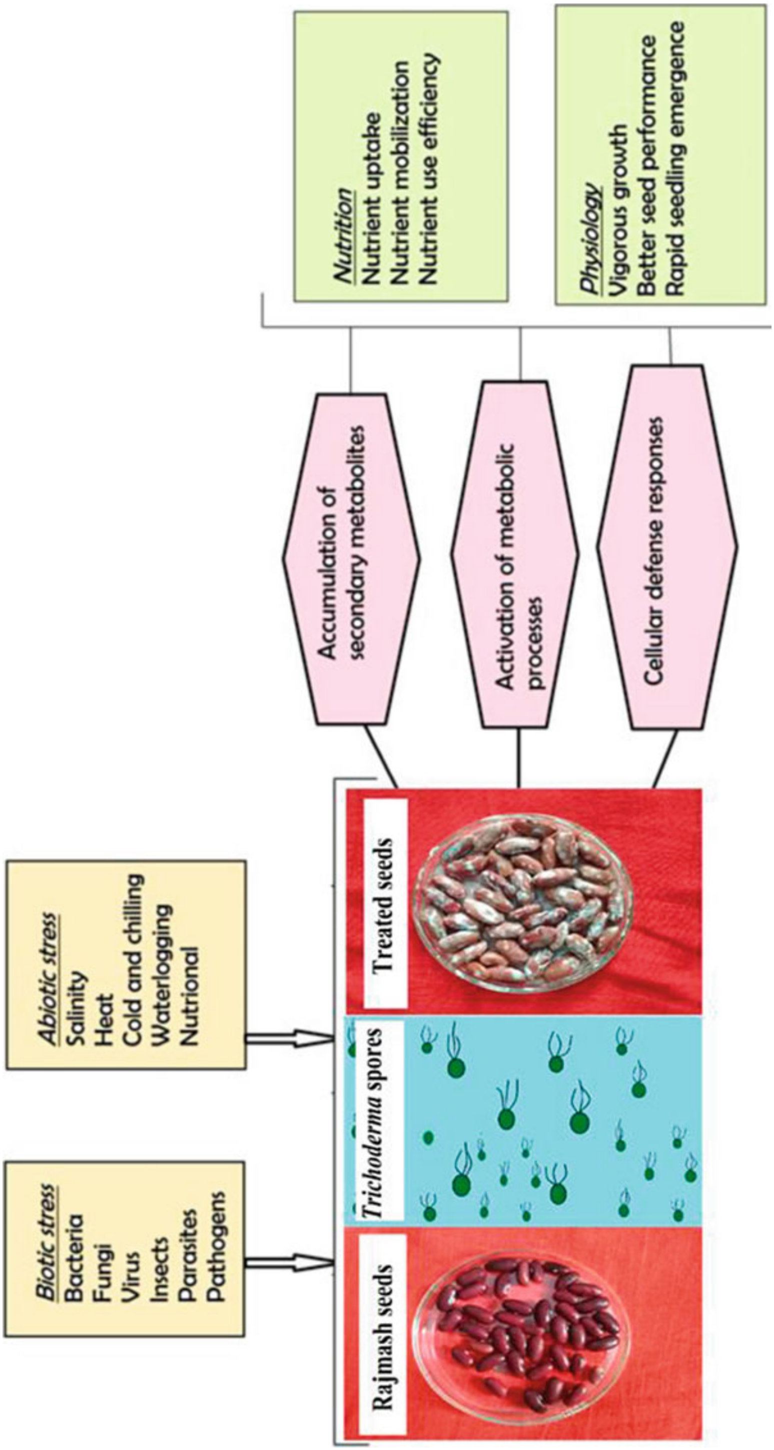

Fig. 11.1 Biopriming affecting plant growth via different mechanisms

11.4 Probable Mechanism Involved

Nitrogen is the second most limited nutrient for plants growth after water (Malik et al. 2001), despite its abundant concentration in atmosphere (78%). Nitrogen is present in the atmosphere as dinitrogen gas (N_2) and has triple bonded structure ($N \equiv N$) because of which very high energy is required to take that forms directly by plant (Shridhar 2012). Certain microbial species have the special ability to fix atmospheric N into ammonical form with the help of nitrogenase system (Halbleib and Ludden 2000). Use of synthetic nitrogenous fertilizer in agriculture is the most popular method of providing sufficient nutrient concentration to the plants. Long term and imbalance use of nitrogenous fertilizers lead to many environmental issues. Therefore, use of bio-inoculants can improve nitrogen use efficiency by various ways listed below:

1. Increase total surface area of root which improve N uptake either by increase in root growth, more branching and root hairs formation or by addition of extension in prevailing root system (e.g. mycorrhizal associations) (Saia et al. 2014).
2. Direct contribution of nutrient in soil solution pool either by biological N-fixation (BNF) or by changing the kinetics of rhizospheric processes (e.g. mineralization, nitrification inhibition, etc.) (Mohammadi and Sohrabi 2012).
3. Through microbial biomass turnover in the rhizosphere (Richardson et al. 2009).

Of all the mechanisms listed above, BNF is the most popular and efficient one. Biological nitrogen fixation is enzymatic conversion of dinitrogen into NH_3 catalysed by nitrogenase, an oxygen reactive enzyme complex (Bhat et al. 2015), by the following reaction:

$$N_2 + 8H + 8e^- + 16ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16\ ADP + 16Pi$$

Nitrogenase consists of two separate metalloprotein called larger dinitrogenase (Mo-Fe protein) and smaller dinitrogenase reductase (Fe protein) (Pathak and kumar 2017). Firstly Fe protein interacts with ATP and Mg^{2+}, accepts electron from ferredoxin or flavodoxin and becomes reduced. Finally electron flow takes place from reduced Fe protein to oxidized Mo-Fe protein and it is reduced (Fig. 11.2). It is the reduced species of Mo-Fe protein which combine with N_2 and other substrates to yield NH_3.

Nitrogen fixation by bacterial species is carried out either by symbiotic, associative or free living relationship between plants and bacteria. In symbiotic association, bacterial strains penetrate inside cortical cells of root system and form a nodule (Bhattacharjee et al. 2008). Nodules have iron containing substance known as leghaemoglobin which impart pink colour to nodule (Ampomah et al. 2012). Nodule is the primary site of nitrogen fixation. In this type of association, bacterial species provide NH_3 to plants after fixation and in return plant provides protection, energy and photosynthates to microbial species. Bacterial species like *Azotobacter* fix nitrogen in free living condition (Fig. 11.3). They are present in rhizosphere and

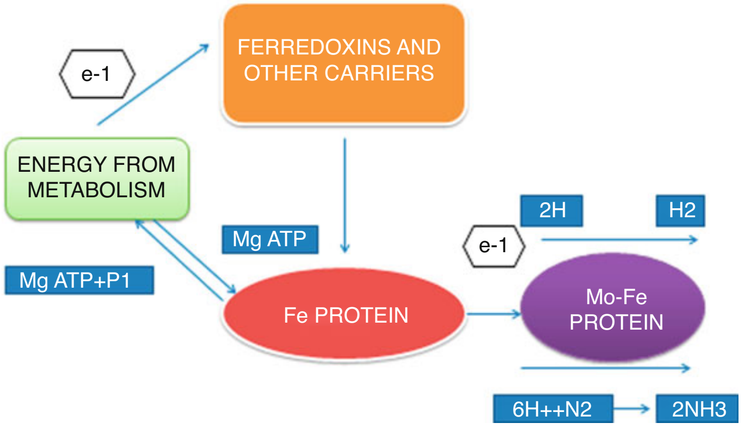

Fig. 11.2 Mechanism of N-fixation

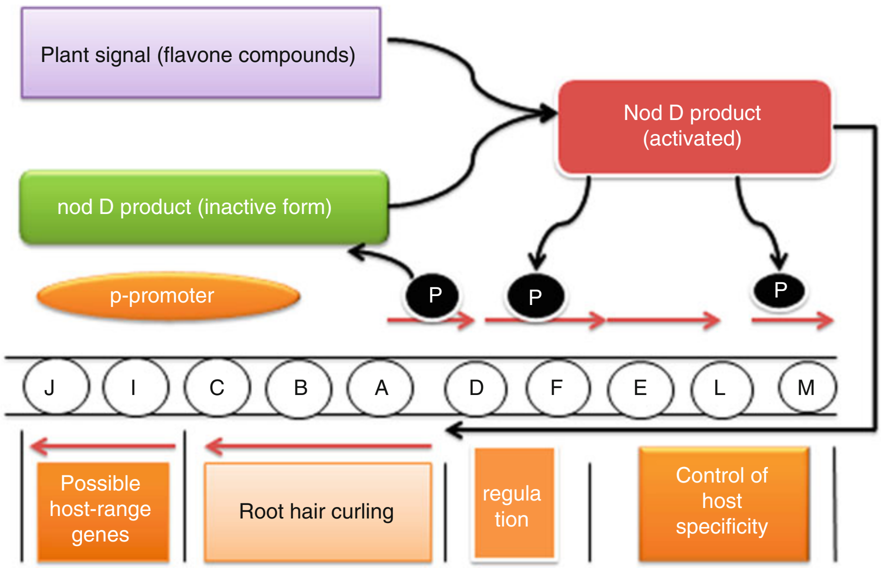

Fig. 11.3 Schematic diagram of events at molecular level in root region in Rhizobium–legume interaction of N- fixation

from there, they fix nitrogen. Plants assimilated the nitrogen from rhizosphere and in return provide energy and photosynthates through root exudates. While bacterial species like *Azospirillum* fix nitrogen by forming association with roots of Poaceae family (Duarte et al. 2020). Unlike bacteria, some fungi can also be used for improving N use efficiency through bio-priming. *Trichoderma spp.* were greatly studied for its role in augmenting N availability in soil. Some studies suggest that, its

Table 11.4 Effect of biopriming on nitrogen use efficiency

	Crops	Type of experimental	Biopriming agent	Mechanism	NUE	References
Cereals	Wheat (*Triticum aestivum* L.)	Pot experiment	*Trichoderma harzianum*	Root length and nitrogen uptake was improved	AUE of N alluvial soil (3.36%), blacksoil (0.67%), redsoil (0.18%)	Meena et al. (2016)
	Rice (*Oryza sativa*)	Pot experiment	*Trichoderma harzianum*	Growth promotion and uptake	AUE increased by 69.55%	Priya et al. (2018)
	Rice (*Oryza sativa*)	Pot experiment	*Thalassobacillus denorans* and *Oceanobacillus kapialis*	Increased uptake of nitrogen, synthesis of phytohormones and N-fixation	N content of plants increased by 20%	
	Rice (*Oryza sativa*)	Greenhouse experiment	*Azospirillum amazonense*	Nitrogen fixation and plant growth promoting activity	Nitrogen harvest index increased by 17%	Rodrigues et al. (2008)
	Maize (*Zea mays*)	Pot experiment	*T. Harzianum*	Phytohormone production, increase in photosynthetic features and root biomass	N content (root) was 8.8–9.76%	Akladious and Abbas (2012)
	Wheat (*Triticum aestivum* L.)	Greenhouse experiment	*Bacillus* spp.	Plant growth promotion and N-fixation, nitrogenase activity	N content (seeds) increased by 84.9%	Brahim et al. (2019)
	Maize (*Zea mays*)	Pot experiment	*Trichoderma* and *Bacillus*	Increased photosynthetic activity, the mobilization of photosynthates	N content (plant) increased by 25.2%	Mutetwa et al. (2019)
Pulses	Chickpea (*Cicer arietinum* L.)	Glass house pot and field experiment	*Rhizobium, Bacillus megaterium* and *Trichoderma* spp.	Growth promoting substances, N-fixation	N uptake increased by 100% in the shoots	Rudresha et al. (2004)
	Soybean (*Glycine max*)	Greenhouse conditions	*T. Harzianum, T. atroviride, pseudomonas fluorescent*	Increased root growth, enzymatic activity	Plant N content was (15.8%)	Entesari et al. (2013)
	Dry bean (*Phaseolus vulgaris*)	Shadehouse pot trial	*T. atroviride* and *Bacillus*	Increase in nodulation and dry biomass due to growth promotion activities	Plant N content was increased by 175.6%	Yobo et al. (2009)

(continued)

Table 11.4 (continued)

	Crops	Type of experimental	Biopriming agent	Mechanism	NUE	References
Vegetables and others	Amaranthus (*Amaranthus Hypochondriacus*)	Pot + field experiment	*Bacillus* spp.	PGP activity, improved uptake	ARE for N was 1.17%	Pandey et al. (2018)
	Broccoli (*Brassica oleracea* L.)	Pot experiment	*Trichoderma viride, Glomus mosseae; Acaulospora laevis* and *Pseudomonas fluorescens*	Plant growth promoting activity, production of secondary metabolites	N % increased by 142% in shoot	Tanwar et al. (2013)
	Tea (*Camellia sinensis*)	In-vivo	*Pseudomonas fluorescens, Azospirillum brasilense, Trichoderma harzianum*	Enhanced nitrogen fixation, improved plant water relations	NUE improved by 30.1%	Thomas et al. (2010)
	Native plants (*E. gamophylla, G. wickhamii* and *T. wiseana*)	Glasshouse experiment	Cyanobacteria: *Leptolyngbya* sp., *Microcoleus* sp., *Nostoc* sp. and *Scytonema* sp.	Metabolites production, N-fixation	Soil Total N increased by 14.25%	Chua et al. (2019)

potential to degrade cellulose can release huge amount of N from organic to inorganic pool of N in the rice rhizosphere (Doni et al. 2014).

11.5 Biopriming Mediated NUE in Different Crops

Several works have been done on biopriming particularly to improve nitrogen use efficiency. Increase in nitrogen use efficiency means the plant can efficiently utilize the nitrogen and is able to assimilate the nutrient into its chlorophyll, nucleic acids, protein content, etc. Different mechanisms have been explained by various investigators as the reason behind this increment in use efficiency of nitrogen. This increase is attributed to different functions of biopriming agents—N-fixation, nitrogenase activity, plant growth promoting activities, etc. (Table 11.4)

11.6 Conclusions and Way Forward

Green revolution led to the adoption of high yielding varieties whose seeds lay primarily on chemical fertilizers especially nitrogen which is produced through highly energy intensive process. Indian soils are poor in nitrogen status and almost all soils respond to the application of external nitrogen. As fossil fuels are expensive, cost of fertilizers is still at high which increase economic burden to farmer. In modern agriculture avoiding chemical fertilizers completely is not possible, but there is every need to focus on the management aspects and new interventions to supplement a part of the chemical nutrient source. The outcome of new technologies will be more effective if it is designed to increase the efficiency of rhizosphere. Rhizospheric microbes play a major role in complex interactions and nutrient cycling at root zone. The best intervention to supplement a part of energy and to manipulate rhizospheric microclimate is seed bio-priming which is an ecocentric and user friendly technique. Seed biopriming enables better plant performance even under adverse conditions, improves nutrient uptake and resists the plant against biotic and abiotic stress conditions. In current scenario, there is growing attention towards beneficial microorganisms to get higher quality and quantity of economic yields and has incredible scope in near future.

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