AGRICULTURAL SUSTAINABILITY THROUGH NITROGEN FIXATION: APPROACHES AND TECHNIQUES

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Abstract

Agricultural sustainability balances the need for resource conservation with the needs of farmers pursuing their livelihood. Symbiotic N_2-fixing systems are used for biological nitrogen fixation it can be a major source of N in agriculture. The application of cyanobacteria in salt-affected soil remediation will reconstruct green agriculture and promote the sustainable development of human society and this approach may also serve to treat saline soils of coastal areas post tropical cyclones like Amphan and Yaas in our state.

Keywords: agricultural sustainability, cyanobacteria, symbiosis, soil microbes, salinity, eco-restoration

Production sustainability: Management of internal resources

Long-term sustainability of agricultural systems is relied on use and effective management of internal resources. Nitrogen-fixing plants reduce external inputs and improve the quality and quantity of internal resources. They also offer an economically attractive and ecologically safe system. When symbiotic N_2-fixing systems are used for biological nitrogen fixation it can be a major source of N in agriculture.

On the other hand, from non-symbiotic (associative and free-living) microorganisms nitrogen contributions are relatively minor. For this, N fertilizer supplementation is required. Nodulated legumes have been used in cropping systems for centuries among the symbiotic N_2-fixing systems. They serve multiple purposes in sustainable agriculture. They are usually used as primary sources of food, fuel, fibre and bio- fertilizer. They are also used to enrich the soil, preserve moisture and prevent soil erosion. They may be used for windbreak, ground cover, trellis, hedgerow and shade, or as a source of resins, gums, dyes and oils. Mostly ornamental flowering plants in the tropical region are legumes [1]

Some of the nodulated non-legumes, notably species of *Casuarina*, are hardy nitrogen-fixing plants. It produces high-quality fuel wood on marginal lands. It has been used for stabilizing sand dunes and eroding hillsides, as well as for reclaiming marshlands affected by fluctuating brackish/fresh water. This plant is useful for shade, windbreaks and hedges. [2]

Agricultural Sustainability

The British scholar Jules Pretty has stated several key principles associated with sustainability in agriculture. They are-

1. The incorporation of biological and ecological processes such as nutrient cycling, soil regeneration, and nitrogen fixation into agricultural and food production practices.
2. Using decreased amounts of non-renewable and unsustainable inputs, particularly environmentally harmful ones.
3. Using the expertise of farmers to both productively use the land as well as to promote the self-reliance and self-sufficiency of farmers.
4. Solving agricultural and natural resource problems through the cooperation and collaboration of people with different skills. The problems include pest management and irrigation.

Agricultural Sustainability 'considers long-term as well as short-term economics because sustainability is readily defined as forever, that is, agricultural environments that are designed to promote endless regeneration.' It balances the need for resource conservation with the needs of farmers pursuing their livelihood. It is considered to be reconciliation ecology, accommodating biodiversity within human landscapes.

N_2-Fixing Bacteria

Nitrogen-fixing bacteria are the microorganisms which are capable of transforming atmospheric nitrogen into fixed nitrogen (inorganic compounds usable by plants). More than 90 percent of all nitrogen fixation is done by these organisms, which play an important role in the nitrogen cycle.

Generally there are two kinds of nitrogen-fixing bacteria recognized. The first type, the free-living (non-symbiotic) bacteria, includes the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*. The other type consists of the mutualistic (symbiotic) bacteria; examples include *Rhizobium*, associated with leguminous plants (e.g., various members of the pea group); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses [3].

The symbiotic nitrogen-fixing bacteria multiply and stimulate formation of root nodules, enlargement of plant cells and bacteria live in intimate association by invading the root hairs of the host plants. Within the nodules of the root of plants the bacteria convert free nitrogen to ammonia, which later is used by the host plant for its development. Seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species to ensure sufficient nodule formation and optimum growth of legume crop (e.g., alfalfa, beans, clovers, peas, soybeans), especially in soil where the required bacteria is missing or lacking [4].

Nitrogen-fixing organisms can be classified into three categories: free-living N fixers, associative N fixers, and symbiotic N fixers. The last two groups can be found in the rhizosphere of legume and non-legume plants. One of the most studied mutualistic relationships of plants and nitrogen-fixing organisms is root nodule symbiosis. This is the most effective in N-fixing (20–300 Kg ha−1) . This is also more important because it involves almost all food and fodder legumes. With a molecular dialogue between the two partners, host plant and nitrogen-fixing organism through the flavonoids and isoflavonoids secreted by the host plant in its rhizosphere the mutualistic relationship is established. This molecular dialog allows recognition, infection, differentiation of root hair cells, and nodule development [5].

As several legumes are food or cash crops, some have been used to select effective strains of nitrogen-fixing bacteria for bio-fertilizer production. The legume-rhizobium association model has received most attention among the root nodule symbiosis. In this respect, many significant steps have been taken to develop inocula containing effective nitrogen-fixing bacteria (Van et al., 2018). In the last few years, significant efforts have been made to extend nitrogen fixation to crops particularly in cereals other than legumes [4].

Role of Soil Microbes in Sustainable Agriculture

In soil a vast variety of microbes like archaea, different types of bacteria, cyanobacteria, fungi are involved in ecosystem functioning. According to an estimate, 1 gm of soil contains 10^{10}-10^{11} bacteria, up to 200 m fungal hyphae and majority of them are beneficial for plants and soil. Some of them create an association with the roots of plants which cause mineral uptake from soil, few of them also decompose organic matter. Acquisition of the nutrients from the soils via the microbial association helps the plant to promote growth as well as to suppress of phytopathogens. Detrimental microbes are also present in the soil which invade plants and reduce their productivity.

PGPR (Plant Growth Promoting Rhizo-bacteria)

Plant growth is directly or indirectly facilliated by PGPR by colonising the plant root [6,7]. These soil bacteria have the ability to colonise roots and stimulate plant growth.

PGPR species belong to many different genera such as *Azoarcus, Azospirillum, Rhizobium, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Gluconoacetobacter, Pseudomonas* and *Serratia* [8]. In the recent past, to reveal the mechanisms of plant–microbe interactions many research studies has been carried out [9]. It also may affect the plant growth directly or indirectly.

Production of phytohormones, fixation of atmospheric nitrogen (N_2), synthesising iron chelators known as siderophores, solubilising inorganic minerals such as phosphorus (P), potassium (K) and zinc (Zn), making them more readily available for plant growth are involved in growth promotion [6]. PGPR also have an indirect role. They have growth inhibition activity against phytopathogens by one of the several mechanisms such as antibiotic or antifungal metabolite(s) production, depletion of iron from the rhizosphere, induced systemic resistance (ISR), production of fungal cell wall lytic enzymes and competition for binding site(s) on the roots [10]. In addition, PGPR are recognised as potential microbes. They can protect the plants from various environmental stresses [11]. A literature review shows that PGPR plays a effective role in sustainable agriculture [6, 12].

Initially it was thought that the role of PGPR was limited only to enhancing crop productivity. But, several studies confirmed that PGPR play a pivotal role in proper functioning of the agro-ecosystems [13]. Another research showed that they can be used in the restoration of degraded land, improving the quality of soil, reducing the environmental pollutants in soils and most importantly they may be able to combat climate changes [14].

Fungi

A wide variety of beneficial and pathogenic fungi that affect plants are present in soil at all stages of growth. According to an estimate, 1.5 million fungal species usually present in natural ecosystems. Only 5–10% of them have been described. Largest percentage of the biomass of soil communities is made up by soil fungi. They are the major contributors to soil nutrient cycling processes including nitrogen mineralisation, immobilisation and transformation [15].

When roots release some specific chemical compound(s) that act as nutrient sources or defence against pathogenic microbes Plant – fungal interaction initiates. As for example, compounds which are derived from cortical and epidermal cells stimulate the proliferation of fungi outside. On the other hand, release of some phenolic compounds on the surface and inside the root tissues inhibits the growth of entomopathogenic fungi [15]. The fungal species such as *Alternaria spp., Aspergillus spp., Aureobasidium spp., Candida spp., Cladosporium spp., Paecilomyces spp., Phoma spp., Penicillium spp. and Sporobolomyces spp*. are usually present in soil. They are agriculturally important and remain a focal point of many a research topic over the years. The mycorrhizal symbiotic association has been shown by 80% of all terrestrial plants [16]. In natural ecosystem where intervention activities are very less the beneficial effects of micorrhizae are great. There are two types of mycorrhiza - ectomycorrhizae and endomycorrhizae. Both are very important for sustainable agriculture.

One of the endomycorrhizas is arbuscular mycorrhiza (AM) formerly known as vesicular arbuscular mycorrhiza (VAM). It shows an obligate relationship with plants. Plants can enhance 4–20% of their photosynthate to support AM fungi. It is equivalent to the consumption of 5 billion tonnes of carbon per year by the fungi. AM fungi with the symbiotic relationship with host plants form spore in the soil with the ability to germinate under adverse environmental conditions. They also help in relieving abiotic stresses in plants [17]. By formation of a network of extra-radical hyphae AM fungi play a crucial role in soil structuring. The hypha holds soil particles together. AM fungi enhance the uptake of phosphorus in their host plant which is one of the most significant roles played by it [18]. AM fungi also interact with nitrogen-fixing bacteria and phosphate-solubilising bacteria (PSB) which help in plant growth and development. The biomass and nitrogen as well as phosphorus uptake in plant tissues has significantly increased by the dual inoculation treatment of AM fungi and bacteria [19].

Research showed that, AM fungi are found to be associated with a huge number of bacteria and fungi. As for example, some AM hyphae adhering to rhizobia and pseudomonads, appear to be the main component for root colonisation [20]. In recent times many research work on the interaction of mycorrhiza with PGPR has been done for improving crop productivity under stressful environments [21]. So, mycorrhizal fungi play an important role to benefit the host plant through acquisition of nutrients from soil and protect them from biotic and abiotic stresses.

Cyanobacteria

Cyanobacteria are photosynthetic prokaryotes. They are found commonly in lakes, ponds, springs, wetlands, streams and rivers. Cyanobacteria are also important component of soils [22]. A study shows the abundance of cyanobacteria in rice-fields and further their importance in the maintenance of rice-field fertility due to N_2 fixation. So, sometimes cyanobacteria are considered as natural bio-fertilizers [23]. The free-living cyanobacteria or symbiotic have been used for sustainable agriculture. Another research shows that free-living cyanobacteria or symbiotic cyanobacteria (like with water fern *Azolla*) fix 4–6 Tg N_2 annually. Efficient nitrogen-fixing cyanobacteria such as *Nostoc linkia*, *Anabaena variabilis*, *Aulosira fertilisima*, *Calothrix sp.*, *Tolypothrix sp.* and *Scytonema sp*. were identified from various agro-ecological regions and utilised for rice production [24].

The use of cyanobacteria and their utility in rice field has been in practice since long but recently their use with other crops has also been tested. For example, an ammonia-excreting mutant of *A. variabilis* has shown growth enhancement of wheat crop [25]. Many reports indicate that, cyanobacteria not only contribute in global N_2 supply but they are to be involved in phytohormones production in free living and symbiotic associations. For example, *Nostoc*, *Chlorogloeopsis*, *Calothrix*, *Plectonema*, *Anabaena*, *Cylindrospermum* and *Anabaenopsis* have shown indole acetic acid (IAA) production in rice and wheat rhizosphere [26]. Cyanobacteria also help in soil formation process by excreting extracellular polysaccharides, peptides and lipids during their growth in the soil [27].

Some reports are indicating that cyanobacteria can grow successfully in the saline soil where most plants (except halophytes) fail to grow and help in increasing fertility of such soils [28]. Their ability to thrive in extreme environments underlines the possibility of using cyanobacteria as an NBS for ecosystem restoration. Cyanobacteria distribute over a wide range of aquatic and terrestrial ecosystems and climatic zones and have affected major geochemical cycles (carbon, nitrogen, and oxygen) on Earth for billions of years Cyanobacteria in particular, distribute over a wide range of salt concentrations and several species can adapt to fluctuating salinity conditions [29].

The interaction between cyanobacteria and salt-tolerant plants should be considered if the cyanobacterium is utilized to improve the soil fertility in addition to performing soil remediation. It is critical to re-establish the micro-ecology in salt-affected soils and improve the salt affected soil remediation efficiency. The first challenge is the selection of suitable cyanobacterial strain. The co-culture of cyanobacteria and bacteria is also potential approach. The cultivation of cyanobacteria on a large scale should be optimized to improve productivity and decrease cost. The development of bio-remediating agents for salt-affected soil remediation also relies on other technical problems, such as harvesting and contamination control. The application of cyanobacteria in salt-affected soil remediation will reconstruct green agriculture and promote the sustainable development [30] and may serve to treat saline soils of coastal areas post tropical cyclones like Amphan [31] and Yaas in our state and thereby mediate eco-restoration of damaged coastal areas [32]

Biological Nitrogen Fixation

Di-nitrogen (N_2) is converted into plant-usable form (NH_4^+ primarily) in Biological Nitrogen Fixation (BNF) process. In this process N_2 is combined with the hydrogen ions from water. N_2-fixation is not only a biologically-mediated process. Lightning or fire can also oxidize N_2 to nitrate (NO_3^-). ~1% ammonia of the net nitrogen fixed per year is by the lightning [33]. For the N supply, organisms (eukaryotes and prokaryote both) depend directly or indirectly on BNF process as N is the main component for the synthesis of nucleic acids, proteins, and other organic nitrogenous compounds [34].

Biological nitrogen fixation is an energetically expensive process. 16 ATP molecules are required to break down a N_2 molecule. Twelve additional ATP molecules are required for NH_4^+ assimilation and transport, totalling 28 ATP molecules. The nodulating plants must provide 12 g of glucose to their bacterial partners to benefit 1 g N in part [35]. To produce the same amount of nitrogen, the Haber–Bosch process requires a temperature of 400–500 °C and a pressure of ~200–250 bars [36]. Haber- Bosch process is much more energetically expensive than BNF process. N_2 fixation is catalyzed by nitrogenase, which is quite similar in most of the nitrogen-fixing bacteria.

To meet the crop N demands Biological Nitrogen Fixation (BNF) is the one of the most sustainable approaches over N fertilizers [37]. In global N budget importance of BNF is very substantial [38].

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