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Symbiotic N-fixation and its significance on crop productivity

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Abstract
Rhizobia in crop and pasture legumes' root nodules fix nitrogen symbiotically, which has significant positive effects on the environment and the economy. There are several techniques to boost nitrogen fixation, but the most of them would raise the price of photosynthate proportionately. The poor performance of crops that produce more nodules suggests that this may reduce rather than boost yields. The likelihood that past natural selection would have missed simple (i.e., commonly occurring through mutation) and fitness-enhancing trade-off-free enhancements to nitrogen fixation is one explanation for such failures. Numerous plants and rhizobial mutants that indiscriminately boost the allocation of resources to nitrogen fixation are thought to have regularly emerged but perished because the costs of survival outweighed the advantages. However, increasing nitrogen-fixation efficiency might be feasible through more intricate genetic alterations or by embracing trade-offs that natural selection has rejected. Native strains enhancing the crop productivity, nodular characteristics and maintaining better soil health.

Keywords: Biological nitrogen fixation, *Rhizobium*, nodular characteristics, symbiosis

Introduction

Biological nitrogen fixation (BNF)

Modern agriculture requires efficient, sustainable, and environmentally sound management practices for increasing crop productivity through use of appropriate N management practices (Fageria and Barbosa, 2001) [15]. Adoption of proper management strategies of N fertilizer may balance the supply of nitrogen for optimum crop production and minimizing potential losses into the environment (Fageria and Baligar, 2005; Sethi *et al*., 2021) [14, 56]. The atmosphere contains about 1015 tonnes of N_2 gas, and the nitrogen cycle involves the transformation of 3 x 109 tonnes of N_2 per year on global basis (Postgate, 1982) [47]. In the end of the nineteenth century, Hellriegel and Wilfarth discovered that microbial communities could extract nonreactive N_2 from atmosphere and convert it into a useable form known as biological nitrogen fixation (Galloway and Cowling, 2002) [18]. The fertilizer industry contributes about 25% of chemically fixed nitrogen, world production of fixed nitrogen from di-nitrogen for biological processes account for about 60% (FAO. 1990) [16] and through lightning, it accounts for about 10% of the world's fixed nitrogen supply (Sprent and Sprent, 1990) [58]. The demand for nitrogen was forecast to grow annually by 1.7% globally (FAO, 2015) [17]. Currently, approximately 2 tonnes of industrially fixed nitrogen are needed as fertilizer for crop production to equal the effects of 1 tonne of nitrogen biologically fixed by legume crops (Cheng, 2008) [11]. To produce the same amount of nitrogen as that of BNF, the Haber–Bosch process requires a temperature of 400–500 ◦C and a pressure of ~ 200–250 bars (Gilchrist and Benjamin, 2017) [20] and have the capacity to reduce the use of nitrogen fertilizers to ~0.160 billion tons per year, which corresponds to a reduction of 0.270 billion tons of coal consumed for the production process (Lesueur *et al*., 2016) [29]. As a result, compared to nitrogen that is fixed industrially, biological nitrogen has a much less impact on the world's nitrogen cycle. However, as more fertilizer-N has been utilised to produce food and cash crops in recent years, its significance as a key source of N for agriculture has decreased (Peoples *et al*., 1995) [45]. Legumes and cyanobacteria fix about 2.5 x 1011 kg of NH3 from the atmosphere each year through BNF, and the ammonia industry produces about 8 × 1010 kg of NH3 (Cheng, 2008) [11]. When symbiotic N fixers are abundant enough to sustain N pools in ecosystems and to replace N losses, biological nitrogen fixers have a significant capacity to convert N2 to organic N, frequently reaching 100 kg ha^{-1} y^{-1} (Peter *et al*., 2002) [46].

Leguminous plants and rhizobia's symbiotic relationship has the largest quantitative effect on the nitrogen cycle and has the highest capacity to contribute fixed nitrogen to soil ecosystems

(Peoples *et al.*, 2009) [44]. Rhizobia have a significant ecological function in the nitrogen cycle on earth, fixing around 65% of the nitrogen in the world's biological nitrogen fixation (Zsbrau, 1999) [68]. The first institutions in India to commercially produce rhizobia inoculants were the Indian Agricultural Research Institute in New Delhi (1956) and the Agricultural College and Research Institute in Coimbatore (now Tamil Nadu Agricultural University) (Kannaiyan *et al.*, 2001) [24]. A well-known bacterial species called *Rhizobium* sp. serves as the main symbiotic nitrogen fixer, can promote the growth of leguminous plants, and can fix nitrogen (Kiers *et al.*, 2003) [26]. Rhizobia that are effective in fixing nitrogen are necessary for increased crop productivity and healthier soil (Sethi *et al.*, 2019a; Sethi *et al.*, 2019b; Subudhi *et al.*, 2020) [54, 55, 60]. Up until 1992, there were four genera of bacteria that nodulated on roots: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Azorhizobium*. *Mesorhizobium*, *Allorhizobium*, *Methylobacterium*, and *Burkholderia* were later added as four additional genera. Rhizobia had more than 63 identified species by the year 2010, and new ones are being discovered every year. Some of the older species' names have undergone revisions (Young *et al.*, 1996) [66].

Factors influencing the efficiency of rhizobia

Mineral nitrogen content is the most significant of the soil chemical variables that affect symbiotic nitrogen fixation in pulse crops. High soil nitrogen content, whether applied or residual, generally inhibits nodulation and N_2 fixation. According to multiple studies, when nitrogen content in the root environment was between 20 and 90 mg/kg. The nitrate component in the root growth environment is specifically to blame for the reduction in symbiotic nitrogen fixation (Streeter, 1988) [59]. Rhizobia that have been inoculated face competition not only for scarce nutrients but also for interactions with local heterotrophic microorganisms and predators. As a result, injected rhizobia are less able to keep population densities high enough to ensure contact with the roots of bean plants. Rhizobia's ability to colonise the rhizosphere can be increased by manipulating this type of competition with local bacteria. Therefore, it is important to segregate native stains in order to improve N-fixation (Sethi *et al.*, 2019a; Sethi *et al.*, 2019b; Subudhi *et al.*, 2020; Verma *et al.*, 2022) [54, 55, 60, 64].

The population of R. *leguminosarum* biovar phaseoli, R. *meliloti*, *Agrobacterium tumefaciens*, *Micrococcus jlavus*, Corynebacterium sp., and Pseudomonas sp. decreased due to their vulnerability to predation, starvation, or perhaps antibiotic-producing or lytic microorganisms in a study on the survival and multiplication of introduced bacteria into soil (Acea *et al.*, 1988) [1]. It has been noted that *Rhizobium* strains that produce bacteriocin prevent the growth of strains that don't produce bacteriocin (Ahlawatt and Dadarwal, 1996). When paired with rhizobia, an antagonistic bacterium from the pigeon pea's rhizosphere increased root nodulation and crop yield (Chendrayan, 2003) [10].

Regulation of nodule development and N_2 fixation rate in legumes

Phosphorous (P) is necessary for nitrogen-fixing plants to achieve appropriate growth and nodulation (Tang *et al.*, 2001) [63]. Pea (Jakobsen, 1985) [22] and soybean (Israel, 1993) [21] nodulation was directly and positively promoted by the availability of P. It may also boost nodulation and stimulate nitrogenase activity by enhancing plant development, and it indirectly triggered nodulation by having a favorable impact on plant growth. Early research revealed that P deficit lowered root growth, limited the supply of photosynthetic carbohydrates to nodules, decreased nodule size and function, and eventually resulted in less N2 fixation (Jakobsen, 1985) [22]. (Cadisch *et al.*, 1989) [9]. The fact that P accumulates to larger concentrations in nodules relative to other organs under normal settings and especially under P-limited situations suggests that P plays a significant role in nodule functioning. Additionally, P is initially and preferentially transported into nodules during the recovery from P deficit (Pattanayak *et al.*, 2008; Pattanayak, 2016) [42, 43]. P is known to interact with a plant's need for nitrogen to limit nodule formation and to influence the symbiotic relationship between a legume and rhizobia (Wall *et al.*, 2000) [65]. Additionally, some strains that are weaker in neutral environments.

Low pH conditions also inhibited the expression of rhizobia nodulation genes and the synthesis of Nod factor (Morón *et al.*, 2005) [34]. Nod factor and genistein applied exogenously can partially reverse this decrease (Miransari and Smith, 2007) [33]. Low-pH circumstances reduce the amount of Ca^{2+} that is available in soils, which may hinder rhizobia adhesion, infection, and the development of infection threads (Sethi *et al.*, 2019b) [55]. Additionally, root hair infection-related processes, including as the development of primordia and first cell division events, are severely inhibited by low soil pH. According to Khuntia *et al.* (2022) [25], inoculation of acid tolerant plants increased pigeon pea (*Cajanus cajan* L.) growth, yield, and nutrient uptake in acidic *Alfisols*.

Crop response to different management practices:

Different management techniques, such as liming to acid soil (Sethi *et al.*, 2017; Sethi *et al.*, 2017b) [52, 53], green manuring (Kusumavathi *et al.*, 2018), and integrated nutrient management strategies, are affecting nutrient uptake, crop yield, and soil health (Sahoo *et al.*, 2022a; Sahoo *et al.*, 2022b; Garnaik *et al.* 2022; Prusty *et al.*, 2022; Swain *et al.*, 2021; Jhankar *et al.*, 2017) [36, 37, 19, 48, 61, 23]. The soil health and crop productivity are improved by recycling organic wastes into compost, vermicompost (Pandit *et al.*, 2020; Patra *et al.*, 2022; Padhan *et al.*, 2022a; Padhan *et al.*, 2022b) [39, 40, 36, 37] or in-situ decomposition (Pattanayak and Sethi, 2022) [25]. The *Rhizobium* inoculation greatly improved the French bean varieties' growth and yield parameters.

Maximum germination percentage, plant height, plant spread, number of nodules, number of branches, number of pods, pods length and yield were observed in synthetic *Rhizobium* inoculated seeds (Ahmed, *et al.*, 2008) [5]. Brar and Lal (1991) [8] also reported an increase in number of nodules per plant and yield with *Rhizobium* inoculation. Similarly, Pandher *et al*. (1991) [38] found that *Rhizobium* inoculation of moong bean significantly increases nodule per plant and yield. The results of a study by Biswas *et al.* (2020) [6] on French bean (*Phaseolus vulgaris*) in the coastal saline zone of West Bengal during the winters of 2017–18 and 2018–19 revealed that the *Rhizobium* strain V1B/bean-15 treatment combinations of 50% nitrogen and 100% molybdenum and 25% nitrogen and 50% molybdenum produced the best results in terms of growth and yield. Arka In a 2007 study, Arjun. Zaman-Allah *et al*. examined the impact of various rhizobial strains and phosphorus (P) supply in bean crops cultivated in hydroponic systems and discovered that inoculation with the right rhizobia and a supply of extra P can enhance symbiotic N_2 fixation and yield in common beans.

In a field experiment with nine different soybean rhizobia strains and one un-inoculated control in soybean cv. PK-416, the inoculation treatments showed a much greater increase in plant dry matter and pod yield than the control (Patra, *et al*., 2012) [41]. They further claimed that after soybean cropping, soil nitrogen levels decreased by 13.4–20.2% in the inoculation treatments and by 29.6–% in the control treatments that were not inoculated. When specific *Bradyrhizobium* species were used to inoculate soybean, the protein content of the seed increased (Egamberdiyeva *et al*., 2004) [12].

Field studies in northern Michigan showed 45% and 23% soybean yield increases in 2004 and 2005 where inoculant was used and field had not seen a previous soybean crop. Mehasen *et al*. (2002) [31] and Badran (2003) [4] showed that more oil yield was produced when soybean crops were inoculated and higher doses of phosphorus was applied. Inoculation of peanut with selected rhizobial strains has been demonstrated to improve crop yields (Bogino *et al*., 2006) [7]. Bhuiyan *et al* (2008) [5] found that use of high yielding chick-pea varieties along with effective rhizobial strains can enhance the yield. Inoculation with effective strains of *Rhizobium* have increased pod yield in ground nut (Sundara Rao, 1971).

Ground nut has a strong host-cultivar *Rhizobium* compatibility, as shown by Nambiar and Dart (1982) [35], who found that a specific strain of *Rhizobium* considerably boosted ground nut pod yield. Mungbean plants' height, leaf area, photosynthetic rate, and dry matter production increased after *Rhizobium* inoculation (Mehboob *et al*., 2012) [32]. Numerous researches have shown that *Rhizobium* inoculation increased mungbean yields by 10% to 37%. (Satter and Ahmed, 1992) [51]. The inoculation of mungbean resulted in an 18% rise in nitrogenase activity, according to Kothari and Saraf (1987) [27]. Mungbean productivity was dramatically boosted by *Rhizobium* inoculation and increasing the rate of micronutrients (Ahmad *et al*., 2013) [3].

Effect bioinoculation on nutrient uptake by plants and soil nutrient status

By facilitating nutrient uptake by plants, microbial inoculants like *Rhizobium* and *Bradyrhizobium* may have an impact on the chemistry of nutrients in soils. Following inoculation with effective *Rhizobium* strains, Makoi *et al*. (2013) [30] found enhanced uptake of macronutrients. According to Sethi *et al*. (2021) [56], co-inoculating Mycorrhizal and *Rhizobium* with organic and inorganic fertilisers improved *Acacia mangium* saplings' nutrient uptake and growth in acidic Soil. Under the combined usage of *Rhizobium* and FYM along with chemical fertilisers, Sharma and Verma (2011) [64] observed the highest available N, P, and K contents as well as the maximum soil organic carbon content. It was discovered that the simultaneous inoculation of chickpea with *Rhizobium* and PSB improved the uptake of N and P. (Tagore *et al*., 2014) [62]. *Rhizobium* and PSB were combined with fertiliser (P_2O_5) to dramatically boost plant growth parameters, yield characteristics, and soil N, P, and K status in cowpea. *Phaseolus vulgaris'* chlorophyll levels, shoot dry weight, nodule dry weight, and nutrient uptake were all noticeably improved by single, dual, and triple inoculations with Bacillus subtilis, *B. megaterium*, and *Rhizobium leguminosarum* bv. Phaseoli, according to Elkoca *et al*. (2010) [13]. In chickpea plants that received *Rhizobium* and Azotobacter co-inoculation, the amounts of nitrogen, phosphorus, and potassium in the shoots and roots were discovered to be much greater.

Conclusion

Such a solution is provided by legume plants' symbiotic nitrogen fixation. However, legumes are unique in that they can form a symbiotic relationship with nitrogen-fixing bacteria (collectively called rhizobia), which are housed in special root organs called nodules. Since the These approaches are still a way off, so we suggest using non-food legumes for companion planting and direct biofuel production in the interim. A biofuel feedstock must not compete with food crops for land, water, or labour in addition to being a legume. There are several such land locations in the world. However, the fact that it is a legume is more significant because it increases sustainability because it doesn't need fed nitrogen fertilisers.

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