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Review Article

A Look at Recent Advances in Nitrogen Fixation Through Common Bean Research

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Abstract

Nowadays, agriculture is in urgent need of technological changes to achieve high productivity with the least possible environmental impact. In this regard, it is crucial to examine natural biological mechanisms, such as biological nitrogen fixation (BNF), as alternatives to nitrogen fertilizers. In the case of BNF, the demand for nitrogen (N) can be fully satisfied through the symbiosis of host plants and N-fixing (or diazotrophic) bacteria. Therefore, one way for this process to be successful is the inoculation and/or co-inoculation of plants with highly efficient diazotrophic bacterial strains, a process considered by several authors as the new green revolution. Thus, plant-breeding programs should focus on increasing plants' capacity to benefit from BNF, together with enhancing other characteristics of agricultural relevance, thereby resulting in more efficient varieties that require less energy. Increasing BNF in legume plants by inoculating them with rhizobia and bacteria that stimulate plant growth increases N availability in sustainable agricultural production systems. In the case of common beans (Phaseolus vulgaris L.), despite many efforts to stimulate the adoption of BNF-related technology by producers, the agronomic efficiency of this process in field experiments is still inconsistent. In this sense, research must focus on obtaining varieties that are more responsive to N and more effective in living symbiotically with diazotrophic bacteria. The aim of this article was to discuss the current agricultural sustainability issues that involve BNF and the efficiency of inoculation and co-inoculation techniques and breeding programs in increasing the efficiency of the culture of common beans.

Keywords: Inoculation; Co-Inoculation; New Green Revolution; Environmental Impact

Introduction

The increase in the productivity of various crops, achieved after the 1960s with the green revolution, provided a high proportion of the global population with the necessary food, but also had significant impacts on socio-economic conditions and environmental sustainability. Despite this, it was a crucial step toward contemporary agriculture and the development of high-yielding varieties [1]. A key factor for this change was the genetic improvement of plants, through which many crop characteristics were improved, by focusing on the selection for greater productivity, adaptation to different environments, early cycle, resistance to abiotic (drought and flooding) and biotic (diseases and insects) stresses, and the superior quality of grains, in addition to improving nutritional quality and other commercial characteristics of added value [2]. In addition to the aforementioned features, nowadays, breeding programs for cultivable plants should focus on increasing the biological nitrogen fixation (BNF), which makes it possible to obtain more efficient varieties that require fewer inputs, with efficiency in the use of nutrients being a critical approach [3].

Despite the agricultural production improvements brought about by the green revolution and new technologies, severe environmental problems emerged, such as soil and water contamination, the release of greenhouse gases, the eutrophication of rivers, streams, lakes, and coastal marine ecosystems as well as the loss of the genetic diversity of cultures [4]. In addition to the aforementioned, agriculture is currently also responsible for other significant environmental issues, such as deforestation [5], soil and air pollution [6], the increase in greenhouse gases, climate change [7], and damage to human and animal health [8], among others [9]. warned that climate change influences seed quality, as seeds can change in size, shape, weight, and color, thereby affecting germination and initial growth. Among the negative impacts of the excessive use of synthetic nitrogen (N) fertilizers is the contamination of surface water and groundwater, caused by nitrate leaching [10].

Currently, there is a need to shift the focus of agricultural activities toward high productivity with the least possible impact. With the advancement of technological knowledge, new possibilities focused on sustainability, termed the second green revolution [11] or the new green revolution [12], have emerged. This new green revolution considers parameters related to soil microbiology, such as the microbial diversity of soil in different agro-ecosystems, the beneficial biological indicators of the soil, the improvement of plant health, through the use of biological inputs and the selection of microbial strain degraders of agricultural residues, and the capability of carrying out BNF [13].

BNF is an alternative to the use of N fertilizers, which, as already stated by [14] can promote the sustainability of agricultural systems. The N demand can be satisfied through the symbiosis of host plants and N-fixing (or diazotrophic) bacteria, which replaces the use of the problematic synthetic N and is one of the focuses of modern agriculture. Therefore, one of the paths toward the viability of BNF is the inoculation and/or co-inoculation of plants with compatible bacterial strains capable of carrying out the BNF process efficiently, which would result in the main innovation of the new green revolution mentioned by several authors [15,16].

One of the most promising breakthroughs for promoting BNF and sustainable agriculture is the co-inoculation technique, which, along with the development of cultivars with lower energy demands, can be considered one of the most promising technologies for promoting BNF and sustainable agriculture. As a result, the purpose of this study was to analyze current agricultural sustainability challenges involving BNF, as well as the efficacy of inoculation and co-inoculation procedures, as well as genetic improvement programs, in improving common bean culture (*Phaseolus vulgaris* L.).

Common Beans and N

The symbiotic association between members of the Fabaceae family (Leguminosae) and soil bacteria, mainly from the Rhizobiaceae family, allows the conversion of atmospheric N (N_2) into assimilable forms, such as ammonia (NH_3), through a process called BNF [17]. This association causes the formation of highly differentiated and specialized structures, the root nodules, in the roots where bacteria supply the plant with N, thereby protecting it from environmental stressors; in turn, bacteria obtain food, in the form of photosynthesized compounds, from the plant. As a legume, *Phaseolus vulgaris* can benefit from this type of interaction and obtain part of the N it needs for its development [17].

Phaseolus vulgaris is a widely cultivated species and one of the most important legumes in the world [18], as it is the most relevant of the 30 species belonging to the native *Phaseolus* genus of America [19]. It is the third most used legume as a food globally, only surpassed by soybeans (*Glycine max*) and peanuts (*Arachis hypogea*) [20]. Latin America, Africa, and Asia are the largest producers of the *Phaseolus* genus, with Myanmar, India, Brazil, the United States, Mexico, and Tanzania, responsible for 57% of the world's total production amounting to 15.3 million tons [21]. Myanmar is the largest producer of common beans with 6 million tons [21]. With an annual average production of 3.5 million tons of common beans and current data showing that 2019/2020 harvest yielded 3.16 million tons, which was 4.6 percent higher than the 2018/19 yield [22], Brazil is the largest consumer, with consumption estimated to have increased by 1.2 percent over one year until 2020, rising from 3.5 to 4.3 million tons, and with an annual average production of 3.5 million tons of common beans.

Despite the global importance of the culture, the financial return for small producers is low. This may be one of the reasons for the relative stagnation of the production in Latin America and Asia [23]. On the other hand, large-scale production has increased productivity in Australia, Brazil, and the United States through improved varieties and modern agricultural practices [23]. Even so, the yield of the crop is still quite below what is necessary. The average production among the world's largest common bean producers is highest in the United States, with an approximate 2,000 kg ha^{-1} yield, and lowest in India, with an approximate 400 kg ha^{-1} yield. In Brazil, which is the largest producer, the average yield is approximately 1,000 kg ha^{-1} [21]. According to [24] this figure does not reflect the yield potential of the currently recommended cultivars (> 2,000 kg ha^{1}). Among the factors that may be contributing to the low productivity of common beans is their cultivation in places with low soil fertility and the low use of inputs [25], including inoculation to increase BNF, which results in the inadequate mineral nutrition of the crops [26].

The common bean is considered a nutrient-demanding plant due to its poorly developed and superficial root system and its short life cycle, thereby making it essential that the nutrients are made available to the plant at an appropriate time and place [27]. Bean leaves are considered to have adequate macronutrients when their N, phosphorus, and potassium values are 24-52 g kg^{-1}, 4.0-6.0 g kg^{-1}, and 15-35 g kg^{-1}, respectively [27]. Besides, its cultivation generally occurs in marginal areas with little N availability. Considering the low fertility of most soils, liming and fertilization practices are indispensable to provide nutrients that are not in ideal concentrations to meet the needs of the plant [28]. The low technological level employed in the cultivation of beans also contributes to this crop's low yield in Brazil and other countries [29].

The productivity of common bean cultures should be improved substantially, as it is currently subpar. Part of this is owing to low investments in research, mainly regarding BNF. One of the practices that could be implemented widely to increase productivity in Brazil is the use of commercial inoculants already developed for common beans, based on rhizobia strains (*Rhizobium tropici*) adapted to tropical soils. The *R. tropici* inoculants supply approximately 64.2% to 75.8% of the N required by the plant through BNF [30]. In addition to inoculation with selected strains, the BNF in the common bean can be made more effective by molybdenum supplementation [31].

Substantial effort has been put toward increasing the adoption of BNF by bean producers in Brazil; however, the agronomic efficiency of BNF in field experiments is still inconsistent [32]. Besides, the production and commercialization of the only inoculant for common beans in Brazil amount to approximately only 300,000 doses, which are enough to inoculate only 5% of the cultivated area. Therefore, research must focus on improving the use of the available N and obtaining common bean varieties that are more responsive to N and more effective in BNF and in increasing the rhizobium-legume symbiotic potential, which could be crucial in terms of increasing the global bean production.

Inoculation and co-inoculation in common beans

The soils of tropical regions are generally deficient in N, which is considered as one of the limiting factors of agricultural production [28]. There are four ways in which plants can acquire the N necessary for their growth: 1) via the soil, mainly by decomposing organic matter; 2) via non-BNF (electrical discharges, combustion, and volcanism); 3) via N fertilizers; 4) via BNF [33]. BNF is the second most important biological process after photosynthesis [34]. Among systems involving plants and microorganisms, rhizobium-legume symbiosis is the most economically significant [35]. Regarding common beans specifically, the crop viability could be boosted via an increase in BNF, thereby promoting a productivity increase combined with a reduction in the environmental and economic cost of the crop. The association of beans with rhizobia is a technology capable of replacing either completely or partially N fertilization, thereby reducing the production cost and resulting in high crop yields [36]. Owing to the natural ability of common beans to establish symbiotic relationships with rhizobia, the inoculation technology in common beans is still little explored, especially in terms of co-inoculation with plant growth-promoting bacteria [32]. In Brazil, *R. tropici* SEMIA 4077 (= CIAT 899) is the type strain of its species. It is recommended as a high-performance commercial inoculant for common beans in Brazilian soils [37]. The *R. tropici* SEMIA 4080 (= PRF 81) and 4088 (= H12) strains are also recommended [38].

Despite its importance in common bean culture, BNF is still insufficient in terms of increasing productivity when compared to synthetic N fertilizers. Common beans have a lower potential for nitrogen fixation than other legumes, such as soybeans [39]. In 1930, the first successful cases of BNF in meeting 100 percent of the N requirements of soybean plants were reported [40]. Despite this, the BNF process is still understudied in modern agricultural systems, which prioritize a shorter crop cycle (with less time for the symbiotic apparatus to establish and function) and synthetic N fertilizers, primarily urea, to meet the plant's immediate N needs [24]. Furthermore, competition between the native rhizobia population and the inoculant strain can reduce the latter's efficacy in the field [41]. One of the main reasons for the low adoption of bean inoculation by producers is because of this [32]. Thus, it is necessary to improve BNF in beans by i) improving the rhizobium-legume symbiosis through the use of specific techniques and products, ii) obtaining symbiosis-responsive cultivars, and iii) developing biotechnological pathways to interfere with the symbiosis mechanism [42].

The increase of BNF in legumes through the co-inoculation with rhizobia and bacteria that promote plant growth can improve N availability in sustainable agricultural production systems [42]. Several rhizobial species can also be considered bacteria that promote plant growth, as they increase N uptake, solubilize minerals, synthesize phytohormones, and produce siderophores [43]. In addition to rhizobia, another up-and-coming group is represented by associative bacteria, capable of promoting plant growth through the production of phytohormones and the ability to carry out BNF [44]. The most used co-inoculation technique consists of combining bacteria of the *Rhizobium* genus (symbiotic) with those of the *Azospirillum* genus (associative), which produce a synergistic effect, which surpasses the productivity results obtained when each is used in isolation [45].

Co-inoculation studies on beans are recent and advancing with positive results [46]. evaluated the effects of the co-inoculation of beans with endophytic strains isolated from nodules and belonging to the genera *Bacillus*, *Paenibacillus*, *Burkholderia,* and *Pseudomonas* genera with *R. tropici* SEMIA 4077, both for biological control and for growth promotion. The results were promising, as they suggested the existence of synergy among bacteria, which supply the plants with N and protect them against rhizoctoniosis. In this respect, the use of associative bacteria can be a viable and promising alternative to stimulate the development of the culture with less environmental impact, based on the reduction or non-use of synthetic N [47]. who evaluated the effects of inoculating common bean seeds with mycorrhiza (*Glomus* spp.), *Pseudomonas fluorescens* ATCC13525, and *Bacillus subtilis* DSM108 in order to improve their nutritional status and maintain sustainability concluded that the use of bio-treatments was quite successful in reducing soil pollution through the use of fertilizers and/or pesticides. Recently, [48] conducted a study to verify the result of the co-inoculation of *R. tropici* SEMIA 4077 and *Bradyrhizobium elkanii* SEMIA 5019 in terms of the stimulation of early nodulation in common beans (cultivar BRS Esteio), which was proven by the efficiency in the development of the first stages of the cycle. [32] reported that the co-inoculation of *R. tropici* SEMIA 4077 and *A. brasilense* Ab-V5 and Ab-V6 increased significantly the number and dry mass of nodules in common beans. The root and shoot dry mass were also affected significantly by this co-inoculation, resulting in a grain yield of approximately 3,200 kg ha^{-1}, which represented increases of 11.5% and 26% compared to the yields that resulted from the use of N fertilizer and the single inoculation with *R. tropici* SEMIA 4077, respectively. The results of a study in beans co-inoculated with *R. tropici* SEMIA 4080 and *A. brasilense* Ab-V5 and Ab-V6, carried out in the southern region of Brazil, showed that the co-inoculation increased the grain yield by approximately 16%, compared to the yield that resulted from the isolated inoculation with *R. tropici* SEMIA 4080 [44]. In the work of [49] it was observed that the co-inoculation with *R. pisi* R40982 and R40983 with native *P. monteilii* R43453, increased the nodulation, growth, and productivity of different bean genotypes, compared to the isolated inoculation with *Rhizobium*, thus can be an effective bio fertilization strategy. Plants inoculated with the rhizobia lineage IITA-PAU 987 and *B. megaterium*, isolated from common beans, receive 24% of their N demand from the atmosphere, which represents an increase of 31.1% compared to the N supply resulting from rhizobia inoculation alone [50].

Co-inoculation can also help increase the tolerance of common beans to environmental stresses, thereby increasing productivity in less time than traditional plant breeding. The creation of new common bean varieties that are tolerant to environmental stress is a lengthy process, and alternative strategies can be adopted, one of which is the use of bacteria that promote plant growth [51] These bacteria improve the absorption of water and nutrients by the roots, induce the synthesis of plant hormones, such as auxins, cytokinins, and gibberellins, produce active enzymes under environmental stress conditions (water, salinity, and heavy metals), and facilitate plant resistance against pests and diseases, thereby increasing productivity, while also promoting ecological sustainability [51].

Perspectives

The use of BNF for most plants of economic importance is one of the main demands in the new green revolution, but it needs to be improved. As a result, it is critical to improving BNF's field agronomic efficiency. Plants with rhizobia and growth-promoting bacteria, such as Azospirillum strains, can be inoculated together to help with this challenge. Furthermore, obtaining genetically improved cultivars can be critical for making common bean cultivation viable by increasing N utilization, resulting in higher productivity and lower energy consumption. The economic and environmental costs of growing this legume will be significantly reduced as a result of

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