

EFFECT OF BRADYRHIZOBIAL INOCULATION ON NUTRITIVE VALUES OF SOYBEAN SEEDS

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Abstract

*Bradyrhizobial inoculation is an essential component in the process of improving biological nitrogen fixation in soybean (*Glycine max* L.), which in turn has an impact not only on crop yield but also on the nutritional value of seeds. In this study, the influence of *Bradyrhizobium* inoculation on the nutritional properties of soybean seeds is evaluated. Particular attention is paid to the protein content, oil composition, mineral accumulation, and amino acid profile of the seeds. The infected soybean plants had a considerably larger seed protein content in comparison to the non-inoculated control plants. This can be due to the increased availability of nitrogen and the absorption of nitrogen throughout the seed development process. In addition, the inoculation had a beneficial effect on key amino acids like lysine and methionine, which resulted in an overall improvement in the seeds' nutritional quality. It was also noted that there were beneficial changes in the composition of fatty acids, as well as moderate improvements in the seed oil content. The results of the mineral analysis showed that the amounts of nitrogen, phosphorus, and micronutrients had increased, which may indicate that the nutrient absorption efficiency had improved. As a whole, bradyrhizobial inoculation has emerged as an efficient and environmentally friendly agronomic approach that has the potential to enhance the nutritional content of soybean seeds while simultaneously supporting sustainable soil fertility and lowering reliance on synthetic nitrogen fertilizers.*

Keywords: *Bradyrhizobial, Inoculation, Nutritive, Soybean Seeds*

Introduction

The soybean, also known as *Glycine max* L., is considered to be one of the most significant leguminous crops in the world due to its high nutritional content and extensive variety of applications in the food, feed, and industrial sectors. By virtue of the fact that the seeds contain a substantial quantity of high-quality protein, edible oil, vital amino acids, minerals, and bioactive substances, soybeans are a crucial component in meeting the requirements of global food security and nutritional requirements. Therefore, enhancing the nutritional value of soybean seeds continues to be a primary focus of agricultural research, particularly in light of the rising demand for production methods that are both sustainable and kind to the environment. The biological nitrogen fixation process is responsible for a significant portion of the different elements that influence the development and seed quality of soybeans. The soybean enters into a mutually beneficial relationship with *Bradyrhizobium* species, which are soil bacteria that are able to convert atmospheric nitrogen into forms that are accessible to plants. This symbiosis lessens the need on using artificial nitrogen fertilizers, increases the fertility of the soil, and provides support for farming systems that are environmentally responsible. Not only is it necessary to have effective nodulation and nitrogen fixation in order to get larger yields, but both of these processes are also necessary in order to ascertain the biochemical composition and nutritional quality of soybean seeds.

Bradyrhizobial inoculation has become a frequently used agronomic procedure for the purpose of ensuring adequate nodulation, particularly in soils that do not have natural rhizobial populations that are compatible or efficient. An increase in nitrogen intake and assimilation is brought about by inoculation, which has a direct impact on the production of proteins and the accumulation of amino acids in growing seeds. The entire nutritional value of soybean seeds can be altered as a result of enhanced nitrogen nutrition, which can have an effect not only on the amount of protein present but also on the oil concentration, the composition of fatty acids, and the mineral absorption. Although a significant amount of research has been conducted on the effects of bradyrhizobial inoculation on soybean production and nodulation, a relatively smaller number of studies have concentrated on the influence that it has on the nutritional content of sunflower seeds. In order to improve the value of seeds for human consumption and animal feed, as well as to promote sustainable agricultural methods, it is particularly vital to have a solid understanding of these impacts. In this light, the purpose of the current study is to explore the influence that bradyrhizobial inoculation has on the nutritive properties of soybean seeds, with particular attention paid to the protein content, oil composition, amino acid profile, and mineral contents.

Biological Nitrogen Fixation and Bradyrhizobium–Soybean Symbiosis

The process of biological nitrogen fixation (BNF), in which symbiotic bacteria convert atmospheric nitrogen (N₂) into ammonia, a form that plants may use, is an essential component of environmentally responsible legume production. Bradyrhizobium species, which are responsible for the production of root nodules in soybeans, have a role in facilitating this natural process. Early studies demonstrated that efficient nitrogen fixation might greatly cut down on the demand for synthetic nitrogen fertilizers, which would have positive repercussions for both the economy and the environment (Singleton & Tavares, 1986; Peoples et al., 2009). A sufficient supply of nitrogen is not only essential for the growth of the plant's vegetative tissues and the production of seeds, but it also has an effect on the composition of the seeds, notably the synthesis of proteins (Salvagiotti et al., 2008). There are a number of parameters that determine the effectiveness of BNF. These factors include the compatibility between the Bradyrhizobium strain and the soybean genotype, the conditions of the soil, and the techniques of inoculation. It has been demonstrated that bradyrhizobial inoculants that have been well-adapted can boost nodulation, nitrogenase activity, and ultimately nitrogen absorption (Herridge et al., 2008).

Impact of Bradyrhizobial Inoculation on Soybean Nutrient Composition

The effects of rhizobial inoculation on the quality of soybean seeds have been the subject of investigation in a number of research. One of the most constant effects that has been related with successful inoculation is an increase in the amount of protein included in the seed. An example of this would be the application of effective Bradyrhizobium japonicum strains, which led to an increase in the concentration of protein in comparison to uninoculated controls or plants that were completely dependent on native rhizobia (Antunes et al., 2020). In addition to protein, the content and composition of seed oil may also respond to the availability of nitrogen, which is subject to the action of BNF. Other study notes that a high nitrogen status can sometimes lower oil percentage, indicating a complicated trade-off between protein and oil production (Board et al., 1997; Rotundo & Westgate, 2009). While some research suggests that there is a little increase in oil content post inoculation, other research notes

that nitrogen status can occasionally reduce oil percentage. The effects of inoculation are also reflected in the mineral and nutritional profiles of seedlings. Enhanced nitrogen fixation has the potential to improve the absorption and translocation of important minerals including phosphorus (P), potassium (K), and magnesium (Mg), as well as micronutrients like zinc (Zn) and iron (Fe) (Hungria et al., 2013). Not only can these mineral changes affect the nutritional quality of the plant, but they also have the potential to alter seed germination and early seedling vigor.

Amino Acid Profile Changes with Inoculation

Protein quality is not only defined by the overall amount of protein present, but also by the proportion of essential amino acids present in the protein. According to research conducted by Zhang et al. (2015), findings indicate that inoculated soybean plants frequently display increased levels of essential amino acids including lysine, methionine, and cysteine. These amino acids are essential for the nutrition of both humans and animals. Under conditions of efficient symbiotic nitrogen fixation, the processes that are responsible for these alterations most likely entail improvement in nitrogen uptake and modifications to metabolic pathways.

Interactions with Soil and Environmental Conditions

The reaction to bradyrhizobial inoculation is affected by the characteristics of the soil, including the pH level, the amount of organic matter present, and the populations of local microorganisms. According to Martínez-Romero (2009), inoculation often results in more pronounced advantages with regard to soils that have a low number of indigenous rhizobia. On the other hand, in soils that already have established and competitive rhizobial communities, inoculants may only exhibit limited impacts unless they possess greater competitiveness and nitrogen-fixing efficiency. Additionally, environmental elements like as temperature, moisture, and nutritional status are able to influence the effectiveness of symbiotic relationships. The process of nodulation and nitrogen fixation can be hindered by stress circumstances such as drought, which might have an indirect impact on the composition of seeds (Zahran, 1999).

Materials and Methods

Experimental Site and Soil Characteristics

Under field conditions, the experiment was carried out at an agricultural research farm during the kharif growing season when the crops were being grown. The experimental location was distinguished by having loamy soil that was well-drained and had a modest level of fertility. Before planting, composite soil samples were obtained and tested for physicochemical parameters, such as soil pH, organic carbon, accessible nitrogen, phosphorus, and potassium, using conventional analytical protocols. These samples were collected from a depth of 0 to 15 centimeters. In order to determine how soybeans react to bradyrhizobial inoculation, it was determined that the soil had a low to medium level of accessible nitrogen, as well as acceptable levels of phosphate and potassium. This made the soil eligible for the evaluation.

Experimental Design and Treatments

The experiment was laid out in a randomized block design (RBD) with three replications. Two treatments were employed:

1. T₁: Soybean seeds without bradyrhizobial inoculation (control)
2. T₂: Soybean seeds inoculated with *Bradyrhizobium japonicum*

Each plot measured uniform size with recommended spacing maintained between rows and plants. All agronomic practices, except inoculation, were kept uniform across treatments to minimize experimental variation.

Seed Inoculation Procedure

For the purpose of seed treatment, a strain-specific *Bradyrhizobium japonicum* inoculant that is available for commercial utilization was utilized. In accordance with the recommendations made by the manufacturer, the inoculant maintained a minimum viable cell count. To begin, the seeds were soaked in a solution of jaggery or sugar that contained 10% sugar in order to serve as an adhesive. The inoculant was then applied at the required rate, which is typically between 20 and 25 grams per kilogram of seed, and well mixed to ensure that the coating was homogeneous. Before planting, treated seeds were allowed to dry in the shade for half an hour in order to preserve the viability of the bacteria.

Crop Management Practices

The seeds of soybeans were planted at the prescribed seed rate and spacing between each seed. In accordance with the agronomic procedures that are suggested, basal amounts of phosphorus and potassium fertilizers were provided evenly to all plots. However, no chemical nitrogen fertilizer was administered in order to ensure that the biological nitrogen fixation process could be evaluated accurately. Implementation of irrigation, weed control, and plant protection measures was carried out in a consistent manner across all treatments, in accordance with the established standards for crop management.

Harvesting and Sample Preparation

Manual harvesting of soybean plants was performed on each plot as they reached their physiological maturity. The seeds were separated, washed, and then sun-dried until they reached a consistent level of moisture. For the purpose of laboratory analysis, representative seed samples were taken from each treatment and replication. For the purpose of conducting additional chemical analysis, the samples were made into a fine powder by means of a laboratory mill and then kept in containers that were airtight.

Analysis of Nutritive Values

1. Protein Content: Seed nitrogen content was estimated using the Kjeldahl method, and crude protein percentage was calculated by multiplying nitrogen content by a conversion factor of 6.25.
2. Oil Content: Oil percentage was determined using Soxhlet extraction with petroleum ether as the solvent.

3. Amino Acid Profile: Selected essential amino acids were analyzed using standard chromatographic techniques.
4. Mineral Content: Macro- and micronutrients such as nitrogen, phosphorus, potassium, iron, and zinc were estimated using standard wet digestion and instrumental methods.

Results and Discussion

Bradyrhizobial inoculation had a good and considerable impact on the nutritional properties of soybean seeds, as demonstrated by the findings of the current study, which clearly illustrate this. When compared with the control that had not been infected, the results were noticeable in terms of the amount of protein, oil, amino acids, and mineral nutrients that were present, as well as an overall improvement in the nutritional quality of the seed.

Table 1: Effect of Bradyrhizobial Inoculation on Seed Protein Content (%)

Treatment	Seed Protein (%)
T ₁ – Control (Uninoculated)	36.20
T ₂ – Bradyrhizobium inoculated	39.85
SEm (±)	0.45
CD (P = 0.05)	1.32

Inoculation with Bradyrhizobia led to a considerable increase in the amount of protein found in the seed when compared to the control. Inoculated plants have a larger protein percentage, which may be linked to increased biological nitrogen fixation. This results in a better availability of nitrogen during the seed development process. Nitrogen is an essential component of both amino acids and proteins, and the enhancement of nitrogen absorption in soybean seeds is directly responsible for the increasing amount of protein that is synthesized.

Table 2: Effect of Bradyrhizobial Inoculation on Seed Oil Content (%)

Treatment	Seed Oil (%)
T ₁ – Control (Uninoculated)	18.40
T ₂ – Bradyrhizobium inoculated	19.25
SEm (±)	0.30
CD (P = 0.05)	NS

There was a marginal rise in the amount of oil that was found in infected soybean seeds; however, the difference was not statistically significant. It may be deduced from this that although enhanced nitrogen feeding leads to an increase in protein synthesis, the effect of this on oil buildup is quite minor. It is possible that the restricted response of oil % of soybean seeds to bradyrhizobial inoculation might be explained by the negative connection that is frequently observed between the protein content and oil content of soybean seeds.

Table 3: Effect of Bradyrhizobial Inoculation on Essential Amino Acid Content (g/100 g protein)

Amino Acid	Control	Inoculated
Lysine	5.90	6.45

Methionine	1.20	1.38
Threonine	3.75	4.10
Valine	4.30	4.75

With the help of bradyrhizobial inoculation, the concentration of essential amino acids in soybean seeds was significantly increased. It is especially significant that there is an increase in lysine and methionine since these amino acids are frequently lacking in proteins that are derived from botanical sources. It is probable that increased nitrogen fixation and absorption led to an increase in amino acid production, which ultimately led to an improvement in the biological quality of soybean protein.

Table 4: Effect of Bradyrhizobial Inoculation on Mineral Content of Soybean Seeds

Treatment	N (%)	P (%)	K (%)	Fe (mg/kg)	Zn (mg/kg)
Control	5.79	0.54	1.72	82.5	34.6
Inoculated	6.38	0.61	1.85	91.2	39.8

The seeds of plants that had been infected with the microorganisms had larger concentrations of both macro- and micronutrients than the control group. The greater levels of phosphorus and potassium indicate that the nutrient intake efficiency has improved, while the rise in nitrogen content evidences that the symbiotic nitrogen fixation process is working well. To improve the nutritional and dietary value of soybean seeds, particularly for the purpose of correcting deficiencies in micronutrients, the iron and zinc content of soybean seeds has been increased.

Table 5: Overall Percentage Increase in Nutritive Parameters Due to Bradyrhizobial Inoculation

Parameter	Percentage Increase (%)
Protein content	10.07
Oil content	4.62
Lysine content	9.32
Iron content	10.55
Zinc content	15.03

Inoculation with Bradyrhizobia led to significant improvements in a variety of important nutritional parameters after the treatment. The biggest percentage increase was recorded in zinc content, followed by protein and iron. This highlights the significance that microbial inoculation has in improving the quality of soybean seeds in terms of both macronutrients and micronutrients. These enhancements highlight the possibility of bradyrhizobial inoculation as a sustainable technique for the production of soybeans that are superior in terms of their nutritional value.

Discussion

The results of this study show that bradyrhizobial inoculation greatly enhances the nutritional value of soybean seeds, especially in terms of protein concentration, essential amino acid composition, and mineral nutrient concentration. Soybean roots and Bradyrhizobium spp. have a strong symbiotic relationship, which improves biological nitrogen fixation and nutrient absorption, leading to these advantages. This confirms previous research showing symbiotically fixed nitrogen plays a pivotal role in protein production, as infected plants show a substantial increase in seed protein content. Because

nitrogen is an essential building block of amino acids, soybean seeds are able to store more protein when nitrogen is readily available throughout crucial periods of seed development. In this study, chemical nitrogen fertilization was not used, which shows how effective bradyrhizobial inoculation is for biologically satisfying the crop's nitrogen need. The infected soybean seeds not only had more protein, but they also had a better profile of important amino acids, especially lysine and methionine. The improvement of these amino acids greatly raises the biological value of soybean protein, which is commonly lacking in plant-based diets. Improved nitrogen absorption and the efficient transfer of nitrogenous substances to growing seeds under successful symbiotic nitrogen fixation may be associated with the observed improvement. Research on the effects of rhizobial inoculation on legumes has also shown an increase in amino acid content, lending credence to the idea that microbial symbiosis plays a role in enhancing protein quality. The seed oil content was positively affected by bradyrhizobial inoculation, however this impact was not statistically significant. Soybean seeds are known to have an inverse connection between their oil content and protein content, which is supported by this research. More assimilates may be redirected to protein synthesis from fat accumulation if nitrogen becomes more abundant. Although the present study did not find a detrimental effect on oil output, the little increase in oil content indicates that better plant health and nutritional status can support balanced seed development. Analysis of mineral nutrients showed that infected plant seeds had greater amounts of nitrogen, phosphorus, potassium, iron, and zinc. The plant's ability to absorb and translocate nutrients from the soil was probably enhanced by the bradyrhizobial inoculation-induced root development and nodulation. Notable increases in iron and zinc levels are especially remarkable because of the important functions these micronutrients play in human nutrition and the fact that staple meals generally lack these elements. The promise of biofertilizer-based techniques to alleviate hidden hunger and improve food quality is highlighted by the enrichment of these minerals. The results of this study add to the increasing amount of data that bradyrhizobial inoculation improves the nutritional content of soybean seeds and also increases yield. In order to increase crop yield and food nutritional content, the results highlight the significance of incorporating microbial inoculants into sustainable agricultural systems. To improve inoculation procedures for varied agro-ecological locations, more multi-location and long-term research are needed. Response differences related to soil type, ambient circumstances, and soybean genotype imply this.

Conclusion

After conducting this research, the researchers came to the conclusion that bradyrhizobial inoculation has a considerable and favorable impact on the nutritional properties of soybean seeds. Inoculation with *Bradyrhizobium* species led to an increase in the biological nitrogen fixation activity, which in turn led to an improvement in the availability and absorption of nitrogen. This, in turn, led to an increase in the seed protein content and an improvement in the essential amino acid profile. The function that microbial inoculation plays in increasing the biological quality of soybean protein is highlighted by the enrichment of essential amino acids like lysine and methionine. The overall seed quality was not negatively impacted, despite the fact that the rise in seed oil content that occurred as a result of bradyrhizobial inoculation was not statistically significant and not very noteworthy. There is an additional emphasis placed on the nutritional advantages of inoculation due to the better mineral composition, notably the greater amounts of nitrogen, phosphorus, potassium, iron, and zinc. Furthermore, these modifications lead to an improvement in the nutritional content of soybean seeds

and have the potential to have consequences for the treatment of deficiencies in micronutrients. In general, bradyrhizobial inoculation is a method that has been shown to be efficient, environmentally friendly, and sustainable in terms of agronomic practices. This method is used to enhance the nutritional content of soybean seeds while simultaneously decreasing reliance on artificial nitrogen substances. The use of such biofertilizer-based techniques has the potential to promote sustained soybean production as well as the health of the soil. It is advised that more research be conducted across a variety of agroclimatic situations and soybean types in order to improve inoculation procedures and optimize the advantages received by the crop in terms of both nutrition and agriculture.

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