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RESEARCH ARTICLE

Effect of fertilization with basalt powder and inoculation of *Bradyrhizobium* spp in common bean

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ABSTRACT

The common bean is a legume capable of performing biological nitrogen fixation (BNF). The objective of this work was to evaluate the effect of the application of rock powder associated with inoculation *of Bradyrhizobium* spp. in isolation or associated on the relative chlorophyll index in common bean leaves. Two experiments were conducted at the Federal Institute of Paraná Campus (IF-PR) in Ivaiporã- PR with four treatments and four completely randomized replicates. The treatments were: Inoculation of seeds with *Bradyrhizobium* spp; Inoculation *of Bradyrhizobium* spp. with basalt application; basalt application and control. The chemical analysis of the soil was performed. The seeds were inoculated with MasterFix based on *Bradyrhizobium* spp. following the manufacturer's recommendations. The relative chlorophyll index (IRC) was evaluated after the opening of the second trifoliate leaf. The IRC in experiment 1 was evaluated at 23, 38 and 53 days after sowing. In experiment 2, the IRC of the plants was evaluated at 25 and 41 days after sowing. The significant effect of treatments at 38 and 53 DAP was verified in relation to IRC, when the plants were cultivated with application of rock powder associated or not *with Bradyrhizobium* spp. There were treatment effects in relation to IRC in plants inoculated *with Bradyrhizobium* spp. and inoculated *with Bradyrhizobium* spp associated with rock powder, when compared with basalt powder and control. There was benefit of increased IRC in both treatments inoculated with *Bradyrhizobium* spp.

Keywords: *Fabaceae*, *Phaseolus vulgaris* L., biological nitrogen fixation, symbiotic process, nitrogen fixation process, inoculation process.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) belongs to the family Fabaceae, which is popularly known for its ability to perform biological nitrogen fixation (BNF) and with great nutritional requirement (Clibas, 2013; Paiter et al., 2019). The plant originates in South America and there are approximately 55 species, with the most cultivated and commercially produced group being carioca, characterized by its high levels of iron and protein in its composition. Common bean is one of the primary sources of carbohydrates, proteins, vitamins, and minerals (Ferreira et al., 2022; Paulino et al., 2022; Silva et al., 2023).

Beans and rice play a fundamental role in the diet of Brazilians. According to data from the Institute of Rural Development (IDR), seventy percent of Brazilians consume beans daily, with an average consumption per person of 15.3 kilos per year of this grain. However, the national production does not meet this need, and 150,000 tons must be imported to meet the national demand.

According to Silva (2017), about 70% of basic foods such as rice and beans are produced by family farmers. The average per capita consumption of beans in Brazil is 14 kg/inhabitant/year. However, a large part of the population is unable to access this power supply due to price fluctuations in the market (Secretaria de Estado da Agricultura e do Abastecimento [SEAB] (2018).

The limiting factors for bean production are climate, soil characteristics, pests, diseases and nutritional contents, mainly nitrogen and phosphorus (Aidar, 2002). Nitrogen can be applied in the form of nitrogen fertilizers, organic manure and through BNF performed by *symbiotic bacteria of the genus Rhizobium* present in the rhizosphere and that are capable of fixing atmospheric nitrogen. The inoculation of seeds with these bacteria and with a fertilization of 20 Kg of $^{N.ha-1}$ applied in the sowing furrow, equivalent to the application of 160 Kg $^{N.ha-1}$, can generate an increase in the production of 340 Kg.ha-1, evidencing the importance of inoculation (Pelegrin, 2009).

In The Brazilian territory, there are several rock formations that are exploited through mining, generating high amounts of products such as gravel stone used in civil construction. However, in the process of extracting this material, there is a large production of fine granulometry residue, popularly known as rock powder, so the conscious use of this material is extremely important (Theodoro et al., 2013). The Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura Pecuária e Abastecimento [MAPA] (2013) defines soil remineralizers as materialsof mineral origin that have undergone reduction and size classification by mechanical processes and that alters soil fertility rates through the addition of macro and micronutrients, such as rock powder.

In the region of the municipality of Ivaiporã, in the State of Paraná, there is a great availability of this material due to the presence of numerous quarries producing large daily volume with low cost. Thus, the use of this material presents itself as an opportunity for the integration of this residue in agroecological and organic agriculture.

The rocks have slow-dissolving minerals in their composition, which can help promote greater nutritional availability in plants. However, the use of this product in agriculture is still little discussed and evaluated, as well as its chemical properties.

The use of rock dust in agriculture provides benefits for both the physical, chemical, and biological parameters of the soil, as well as for plant development t. It also minimizes the environmental problem of tailings production mining; as well as increasing the profitability of small mining companies. However, despite the growth in the use of rock dust in agriculture, its efficiency as a source of nutrients is still discussed, as it has low solubility and requires large amounts to achieve results, as described by Bolland and Baker (2000).

The application of basalt powder to the soil, helps in the retention of water in the soil over the years, thus aiding in the maintenance of the crop in periods of drought. According to Martins (2019), in areas where rock powder is applied, an increase in root area is observed, allowing greater absorption of water and nutrients from lower layers of the soil.

The use of rock dust in agriculture can benefit two sectors: mining by marketing a tailings and agriculture by providing nutrients in the long term. The benefits of using rock dust in agriculture can be observed over time in soil balance in terms of organic matter, pH and microbiological activity is maintained.

Bacteria of the *genus Rhizobium* are symbiotic bacteria, which proliferate in the roots of legume plants. The symbiotic process between these two organisms basically consists of the availability of plant sugars and on the other hand the bacteria perform the atmospheric nitrogen fixation process and thus supplying the need for nitrogen from legumes.

In the soil, there is a community of microorganisms that is influenced by abiotic and biotic factors that can affect the efficiency of BNF. To increase the efficiency of BNF, the inoculation process is carried out, which involves adding bacteria in a solid or liquid form to the seed before sowing. However, the practice of inoculating common beans is not very common among farmers, as opposed from soybean crops, where this process is performed more frequently.

Pelegrin et al. (2009) conducted a study with the cultivation of inoculated bean and associated with 20 kg $N.ha^{-1}$ and presented a productive potential in conventional bean cultivation similar to the use of 80 Kg N.ha-1. Due to the high costs of nitrogen fertilizers, this practice has great economic viability, since the cost of the inoculant is lower when compared to nitrogen fertilizers.

Nitrogen is an important nutrient for the development of common bean because it is an essential constituent for the formation of chlorophyll, amino acids, proteins, nucleic acids among other fundamental compounds for plant metabolism. Therefore, the productivity of this crop is directly related to this nutrient, as described by Soratto (2011) in relation to the increase in the amount of grains per plant, as well as the mass of these grains, as a function of the application of nitrogen through leaf or by cover.

Nitrogen metabolism in common bean has a direct relationship with BNF, and may be benefited or impaired by the absence of it. Nitrogen is able to change plant functioning intensely compared to other nutrients. In case of nutrient insufficiency, the plant may present reduced growth, chlorotic leaves, drastic drop in production, drop in cell division rate, decrease in energy absorption by the photosynthesis process among many other problems in plant metabolism defined by (Chapin, 1980).

The SPAD index is an indestructible way to evaluate the chlorophyll content indirectly. It can be performed in a simple, practical and fast manner in the field, by measuring the green color of the leaf and the amount of chlorophyll present. The device used to measure the index is portable and easy to handle and provides a relative chlorophyll index (IRC). The relative chlorophyll index can be related to the amount of nitrogen present in the leaf area of various crops such as crops, such as beans (Soratto et al., 2004), corn (Chapman & Barreto, 1997), cotton (Malavolta et al., 2004), potato (Minotti et al., (1994), rice (Peng et al., 1993) and wheat (Follet et al., 1992).

Rock powder as a soil conditioner can influence plant metabolism and promote greater absorption and/or increase nutrient absorption by the bean plant, and result in increased chlorophyll content in leaves. However, there is still limited information available in the literature regarding the effect of rock powder on chlorophyll content in leaves.

MATERIAL AND METHODS

Two experiments were conducted at the Federal Institute of Paraná Campus (IF-PR) in the municipality of Ivaiporã- PR, delimited between 24°15'4.66"S 51°42'48.10". Each experiment consisted of four treatments and four completely randomized replications, resulting in a total of 16 plots with dimensions of 3x3 m and between each plot there was a street with a width of 1m. The treatments included: (1) Inoculation of seeds with *Bradyrhizobium* spp.; (2) Inoculation of *Bradyrhizobium* spp. with basalt application; (3) basalt application and (4) control (no treatment). The first experiment was installed on May 19, 2019 and the second on October 04, 2019.

Soil samples were collected using a probe-type trado at 25 points, with each collection at a depth of 0-10 cm. The collected samples were sent to the Ivaí Solos Soil Laboratory at IF-PR for chemical analysis, as shown in Table 1.

Table 1. Soil chemical characteristics used in experiments 1 and 2 at a depth of 0-10 cm.

pH	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Al^{3+}**	**H+Al**	**SB**	**CTC(t)**	**CTC(T)**	**V**	**m**
	--mg $^{dm-3}$--		**--------------------$cmol_c/dm^3$------------------------**							**--%--**	
5.26	9.68	3.86	3.75	0.84	0.1	3.62	4.92	8.54		57.61	

To prepare the experimental area manual weeding and rotating hoe for elimination of weed herbs. Fertilization for the bean crop followed the recommendation of Vieira Cibas (1983). To the correct soil acidity, 34 kg of calcitic limestone was used, respecting the ratio Ca:Mg of 4:1. Organic fertilization was applied at the rate of 20 kg of nitrogen for plant start-up, as described by Pelegrin (2009). The source of organic fertilizer used was chicken bedding with a Nitrogen content of 2.95% N, taking into account a 50% of nitrogen absorption rate throughout the year, as described by Fukayama (2008). A total of 32 kg of chicken bedding was used for the experiment.

The humidity and density of the chicken bed were determined to adjust in volume the amount to be applied in the area, and resulted in 166 ml of chicken bed per meter of sowing groove.

According to Vieira Cibas (1983), the ideal sowing population is 12 plants/meter. The seed germination rate was evaluated and found to be 84%. As a result, the seeds were sowed to achieve a sowing density of 15 plants/meter, with spacing of 50 cm between each plant, after fertilization in the sowing groove. The seeds were inoculated with MasterFix, a product based on *Bradyrhizobium* spp. following the manufacturer's recommendations. The relative chlorophyll index (CRF) was evaluated using the SPAD 502 Plus Konica Minolta device, after the opening of the second trifoliade leaf. IRC was measured in plants within the useful portion, disregarding a 1-meter surround. 10 points were collected in 10 sequential plants in two rows of the useful plot. The IRC in experiment 1 was evaluated at 23, 38 and 53 days after sowing. In experiment 2, the IRC of the plants was evaluated at 25 and 41 days after sowing (DAP).

The experimental design was completely randomized, with four replications with adjustment of the statistical models of growth analysis. The data were evaluated by means of variance analysis by the F test, regression or mean test ($p<0.05$).

RESULTS AND DISCUSSION

In the first experiment, at 23 DAP, it was found that there was no effect of treatments on IRC (Table 1). This result may be related to the short conduction period of the experiment, since at 23 DAP the second trifoliated leaf was opened, probably insufficient time for an efficient translocation of nitrogen to the leaves derived from the BNF process. According to Brito (2013), the beginning of nodulation in common bean plants *with Bradyrhizobium* spp. begins at 10 days after plant emergence, insufficient time to present the effect of treatments.

Table 1. Relative chlorophyll index (CRF) in common bean at 23, 38 and 53 days after sowing, with application of rock powder associated or not *with Bradyrhizobium spp*. **Experiment 1.** Means followed by the same letter vertically do not differ from each other, by the Tukey test, at the significance level of 5%.

	Days after sowing (DAP)		
Management	**23**	**38**	**53**
Control	24.21a	30.72b	32.85D
Bradyrhizobium spp	36.72a	38.65a	25.08a
Bradyrhizobium spp + basalt powder	23.90a	35.87a	37.295a
Basalt powder	24.23a	35.01a	38.55a
CV (%)	**5.86**	**9.51**	**8.67**

The significant effect of treatments at 38 and 53 DAP was verified in relation to IRC, when the plants were cultivated with application of rock powder associated or not *with Bradyrhizobium* spp. On average the treatments increased the IRC of the plants by 14.35% and 13.89% at 38 and 53 DAP respectively (Table 1).

The vegetative growth of soybean plants led to an increase in IRC, as can be seen in Figure 1. The effects of the treatments were significant for IRC according to vegetative growth. The increase in IRC is positively associated when correlated with leaf nitrogen content as demonstrated by Soratto et al. (2004), in an experiment carried out in common bean plants, which is the nutrient required on a larger scale by plants and a limiting factor of production.

The increase in nitrogen content was proportional to vegetative development and *Bradyrhizobium* inoculation and rock powder both in isolation and associated (Figure 1). This effect was possibly due to the increase in BNF over time, increasing leaf nitrogen contents.

In the control there was a positive increase in IRC, however lower when compared to treatments, as can be seen in Figure 1. Lower IRC indices may be related to lower nodulation efficiency and consequently lower available amount of nitrogen through the BNF process. Thus, evidencing the effect of the application of rock powder associated or not *to Bradyrhizobium* spp.

There was no difference between the isolated or associated applications *of*

Bradyrhizobium and rock powder. However, the application of isolated rock powder also promoted the increase in IRC, offsetting the absence of previous inoculation with Bradyrhizobium. As described by Borges (2013), the application of basalt powder can bring benefits to bean crop, due to the availability of nutrients for the crop, resulting in a better development of the plant. In the present experiment, the isolated application of rock powder presented IRCs similar to *Bradyrhizobium application* or the association of the application of rock powder + *Bradyrhizobium*. Thus, rock powder possibly influenced the plants in the greater utilization of nutrients in relation to the control, and or acted in the improvement of the BNF process by native soil bacteria.

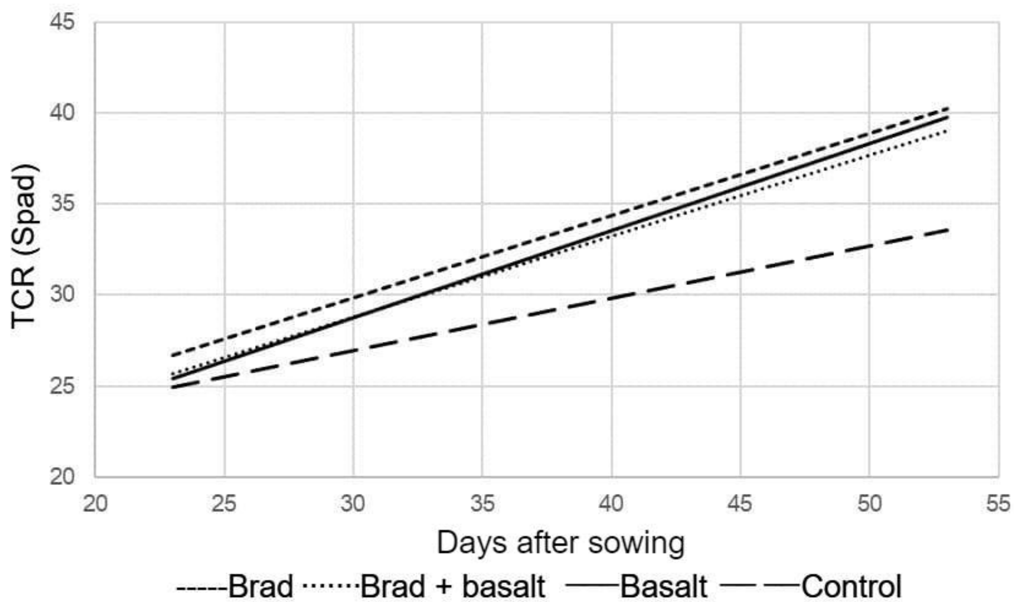

Figure 1. Relative chlorophyll index (CRF) in response to the application of rock powder associated or not *to Bradyrhizobium* spp over the days after sowing bean from Experiment 1.

In the second experiment, at 25 DAP, it was verified that there was a significant difference between the treatments, in which the highest effect of the treatments inoculated with *Bradyrhizobium spp. was* evidenced and when it was associated with rock powder, compared with the control, on average with IRC of these treatments was 26,63. The IRC of the control plants were similar to that of plants that received only rock powder. There was no difference between the isolated treatment with rock powder and the rock powder associated with *Bradyrhizobium*, evidencing the important effect of inoculation (Table 2).

At 41 days after sowing (DAP), a mean IRC of 39.77 was observed in the treatments inoculated *with Bradyrhizobium,* as well as in treatments where *Bradyrhizobium* was associated with rock powder (Table 2). This average was higher than that of control plants and those that received isolated application of rock powder.

Abdelfatta et al., 2021 conducted a study the bean crop performing fertilization as chemical fertilizers, foliars, composting with legumes and the addition of Bacillus subtilis, isolated or mixed. In the study, the highest total chlorophyll in bean leaves were observed in the treatment as equivalent percentages of chemical and foliar fertilization, composting with legume species and the addition *of Bacillus subtilis*, evidencing the role of the microorganism in biochemical processes, such as photosynthesis.

TABLE 2. Relative chlorophyll content (CRT) in common bean at 25 and 41 days after sowing (DAP), cultivated with application of rock powder associated or not *with Bradyrhizobium* spp. of **Experiment 2.** Means followed by the same letter vertically do not differ from each other, by the Tukey test, at the significance level of 5%.

	Days after sowing (DAP)	
Management	**25**	**41**
Control	23.14 C	33.46b
Bradyrhizobium spp	27.11a	40.86a
Bradyrhizobium spp + basalt powder	26.16ab	39,09a
Basalt powder	24.42bc	32.799b
CV (%)	**7.71**	**11.84**

CONCLUSIONS

There were treatment effects in relation to IRC in plants inoculated with *Bradyrhizobium* spp. and inoculated *with Bradyrhizobium* spp associated with rock powder, when compared with basalt powder and control. The benefit of increased IRC in both treatments inoculated with *Bradyrhizobium* spp was also observed.

REFERENCES

Abdelfattah, M. A., Rady, M. M., Belal, H. E. E., Belal, E. E., Al-Qthanin, R., Al-Yasi, H. M., & Ali, E. F. (2021). Revitalizing Fertility of Nutrient-Deficient Virgin Sandy Soil Using Leguminous Biocompost Boosts *Phaseolus vulgaris* Performance. *Plants,* 10, 1637. https://doi.org/10.3390/plants10081637

Aidar, H., Silva, S. C., Kluthcouski, J., & Thung, M. (2002). *Sistema de produção do feijoeiro comum em várzeas tropicais: época de plantio.* Circular técnica, 55. Santo Antônio de Goiás: Embrapa Arroz e Feijão.

Bolland, M. D. A., & Baker, M. J. (2000) Powdered granite is not an effective fertilizer for clover and wheat in sandy soils from Western Australia, *Nutrient Cycling Agroecosystems*, 56, 59–68.

Chapin, F., & Stuart, F. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics,* 11, 233–260. https://doi.org/10.1146/annurev.es.11.110180.001313

Chapman, S. C., & Barreto, H. J. (1997). Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agronomy Journal*, *89*(4), 557-562.

Ferreira, L. L. F., Carvalho, I. R., Conte, G. G., Amaral, G. C. L., Campos, J. N., Tomazele, A. A. S., Carrijo, N. S., Pereira, V. T., Souza, A. T., & Loro, M. V. (2022). Effect of biostimulant on yield characters of common bean cultivars under Southwestern Goiás conditions. *Agronomy Science and Biotechnology*, *8*, 1–13. https://doi.org/10.33158/asb.r148.v8.2022

Follett, R. H., Follett, R. F., & Halvorson, A. D. (1992). Use of a chlorophyll meter to evaluate the nitrogen status of dryland winter wheat. *Communications in Soil Science and Plant Analysis*, *23*(7-8), 687-697. https://doi.org/10.1080/00103629209368619

Malavolta, E., Nogueira, N. G. L., Heinrichs, R., Higashi, E. N., Rodrıguez, V., Guerra, E., Oliveira, S. C., & Cabral, C. P. (2004). Evaluation of nutritional status of the cotton plant with respect to nitrogen. *Communications in Soil Science and Plant Analysis*, 35, 1007–1019

MAPA - Ministério da Agricultura Pecuária e Abastecimento. (2013). Disponível em: <http://www.planalto.gov.br/ccivil_03/_ato20112014/2013/lei/l12890.htm#:~:text=%E2%80%9CDisp%C3%B5e%20sobre%20a%20inspe%C3%A7%C3%A3o%20e,%2C%20e%20d%C3%A1%20outras%20provid%C3%AAncias.%E2%80%9D>.

Minotti, P. L., Halseth, D. E., & Sieczka, J. B. (1994). Field chlorophyll measurements to assess the nitrogen status of potato varieties. *Hort Science*, *29*(12), 1497-1500. https://doi.org/10.21273/HORTSCI.29.12.1497

Paiter, A., Freitas, G., Pinto, L., Hass, L., Barreiros, M., Oliveira, A., & Grange, L. (2019). IAA production and phosphate solubilization performed by native rhizobacteria in western Paraná. *Agronomy Science and Biotechnology*, *5*(2), 70. https://doi.org/10.33158/asb.2019v5i2p70

Paulino, P. P. S., Gonçalves-Vidigal, M. C., Bisneta, M. V., Vidigal Filho, P. S., Nunes, M. P. B. A., Xavier, L. F. S., Martins, V. S. R., & Lacanallo, G. F. (2022). Occurrence of anthracnose pathogen races and resistance genes in common bean across 30 years in Brazil. *Agronomy Science and Biotechnology*, *8*, 1–21. https://doi.org/10.33158/asb.r140.v8.2022

Pelegrin, R. D., Mercante, F. M., Otsubo, I. M. N., & Otsubo, A. A. (2009). Resposta da cultura do feijoeiro à adubação nitrogenada e à inoculação com rizóbio. *Revista Brasileira de Ciência do solo*, *33*(1), 219-226.

Peng, S., Garcia, F. V., Laza, R. C., & Cassman, K. G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agronomy Journal*, *85*(5), 987-990. https://doi.org/10.2134/agronj1993.00021962008500050005x

Ruas, J. F. (2019). *Informativo técnico. Feijão Análise Mensal de Mercado CONAB*. Brasilia, DF: CONAB.

Salvador, C. A. (2018). *Informativo técnico. Feijão - Análise da Conjuntura Agropecuária*. -Curitiba, PR: CONAB.

SEAB – Secretaria de Estado da Agricultura e do Abastecimento. Departamento de Economia Rural. (2018). *Feijão – Análise e Conjuntura Disponível em:* www: agricultura.pr.gov.br

Silva, A. C., Salvador, F. V., Souza, M. H., Marçal, T. S., Dias, F. S., Mello, V. L., Carneiro, P. C. S., & Carneiro, J. E. S. (2023). Selection of common bean parents and segregating populations targeting fusarium wilt resistance and grain yield. *Agronomy Science and Biotechnology*, *9*, 1–20. https://doi.org/10.33158/asb.r173.v9.2023

Silva, R. C. (2017). *A real contribuição da agricultura familiar no Brasil*. Brasília, DF: Embrapa. https://www.embrapa.br/agropensa/busca-de-noticias/-/noticia/27405640/a-real-contribuicao-da-agricultura-familiar-no-brasil

Soratto, R. P., Carvalho, M. A. C. D., & Arf, O. (2004). Teor de clorofila e produtividade do feijoeiro em razão da adubação nitrogenada. *Pesquisa Agropecuária Brasileira*, *39*(9), 895-901.

Soratto, R. P., Fernandes, A. M., Souza, E. D. F. C. D., & Souza-Schlick, G. D. D. (2011). Produtividade e qualidade dos grãos de feijão em função da aplicação de nitrogênio em cobertura e via foliar. *Revista Brasileira de Ciência do Solo*, *35*(6), 2019-2028. https://doi.org/10.1590/S0100-06832011000600018

Theodoro, S. H., Leonardos, O. H., Rocha, E., Macedo, I., & Rego, K. G. (2013). Stonemeal of amazon soils with sediments from reservoirs: a case study of remineralization of the tucuruí degraded land for agroforest reclamation. *Anais da Academia Brasileira de Ciências*, *85*(1), 23-34. https://doi.org/10.1590/S0001-37652013000100003

Vieira, C. (1983). Cultura do feijão, MG: Editora UFV.

Vieira, R. F., & Silva, A. A. (2006). Aplicação de defensivos via água de irrigação por aspersão. In: Vieira, C., Paula Júnior, T. J., & Borém, A (Eds.). *Feijão*. (2nd ed.). p. 267-316. Viçosa, MG: Editora UFV.