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Legumes as green manure for common bean cultivated in two growing seasons at southeast Brazil

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The use of legumes in pre-cultivation on the common bean has the possibility of providing atmospheric N to the soil, making it available to this crop, and may cover part of its N demand and increase grain yield. The objective of present study was to evaluate the effect of hyacinth bean and jack bean as green manures on the production of common bean grown in two seasons. Cover crops were evaluated for fixed N_2, dry matter yield, nitrogen (N) and carbon (C) concentrations, C:N ratio and N accumulation in the shoot. The jack bean accumulated higher biomass and more total N than hyacinth bean and spontaneous vegetation (control). However, both legume species, when used as green manure, resulted in an increase in the N concentration of common bean. Compared to the spontaneous vegetation, hyacinth bean residue increased yield of common bean by 32% and jack bean residue increased the bean yield by 46%. These yields were recorded when common bean was cultivated a few weeks after residues incorporation into the soil and about seven months later, thus showing a flexibility to family farmers for making their decisions on the best cropping season.

Key words: *Phaseolus vulgaris*, *Dolichos lablab*, *Canavalia ensiformis*, family farming, symbiotic nitrogen fixation.

INTRODUCTION

In Brazil, family farmers produce a majority of the common bean (*Phaseolus vulgaris* L.) crop. Family farming is predominant in the Atlantic Forest region of Minas Gerais state, Brazil. In this region, common bean is either cropped in small mono-crop areas or intercropped with coffee. The yield of these systems is about 650–850 kg ha^{-1} (Didonet et al., 2009). While it is desirable to increase yields, the course of action followed to achieve this should be based on biological processes, be less dependent on industrial inputs, and also be consistent with the traditional practices followed by family farmers.

The common bean absorbs N at varying rates through the cropping cycle, with a higher demand between the

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beginning of flowering until pod formation (Fageria et al., 2008). Despite being a legume, common beans can benefit from biological nitrogen fixation (BNF) to achieve higher yields (Pinto et al., 2007; Cardoso et al., 2012) and, it is necessary to provide N to the crop through other sources.

Green manuring with legumes in pre-cultivation has the potential to increase soil fertility and incorporate N in production systems of family farmers (Fageria and Baligar, 2005; Nyambati et al., 2006). Their use for common bean production can prove economical as compared to that of mineral nitrogen (N) fertilizers (Teixeira et al., 2006; Pietsch et al., 2007), while improving soil properties and increasing crop productivity in a sustainable way (Teixeira et al., 2006; Whitbread et al., 2011). There are reports of the use of legumes as green manure for the common bean crop. However, its effectiveness varies considerably owing to a number of factors, such as chemical, physical, and biological soil properties; the legume species used; local growing conditions; and the residual effect of N fixed by legumes, as well as interactions of these and other factors (Roldán et al., 2007; Whitbread et al., 2011).

In the Minas Gerais Atlantic Forest Zone, family farmers grow rain-fed common beans in two seasons. In the "water season", common bean is grown at the beginning of the rainy season, from October to January. In the "dry season", common bean is grown at the end of the rainy season, from March to May. While the choice of using a green manuring approach should allow the family farmers to cultivate in these two possible planting seasons it should also allow them some flexibility in making decisions about their production systems (Coelli and Flemming, 2004). If the legumes are sown in early summer (November to January), the common bean crop can be grown either from February–March to May, immediately after the green manure is cut, or several months later, from October to January. When grown later, there could be a great time-difference between the cutting of legumes and the next common bean crop.

The hyacinth bean (*Lablab purpureus* L.) and jack bean (*Canavalia ensiformis* (L) DC.) are among the most efficient legumes in biomass production and for N supply in tropical soils (Fageria et al., 2013). Both species are already cultivated by some family farmers and can be easily grown at Minas Gerais Atlantic Forest Zone. The hyacinth bean grows well in a variety of environmental conditions and is tolerant to low soil fertility (Maass et al., 2010; Whitbread et al., 2011; Guretzki and Papenbrock, 2013). The jack bean has early maturity, is erect, and shows semi-determinate growth, in addition to being adaptable to various climatic conditions, ranging from the adverse arid and semi-arid regions to the hot and humid climate of the regions with tropical forests (Teodoro et al., 2011). Few studies have focused on the use of these legumes as green manure for common bean crop, and therefore, the objective of this study was to evaluate the effect of hyacinth bean and jack bean as green manure on the production of common bean grown in two seasons.

MATERIALS AND METHODS

The experiment was conducted from November 2012 to January 2014 at an experimental field of Fitotecnia Department at Universidade Federal de Viçosa (20° 45' S; 42° 51' W, 651 m Mean Sea Level). The climate, according to Köppen classification is Cwa, with a mean annual temperature of 19°C and a mean annual rainfall of 1300 mm. Local weather data collected during the experiment are presented in Figure 1. The experiment was set up as a randomized complete block and split-plot design. The main plot was sub-divided to represent the two growing seasons ("dry" and "water") and these were further divided to represent the three green manure (hyacinth bean, jack bean, and spontaneous vegetation), with five replicates of each. These sub-plots consisted of five rows of 6 m length, spaced at 0.50 m. The sampling area was represented by three central rows, disregarding 1 m from each end.

The soil of the area is classified as Cambisol and had the following characteristics at a depth of 0–20 cm: pH in H_2O = 5.34; available P = 23.2 mg kg^{-1}; exchangeable K = 112 mg kg^{-1}; exchangeable Ca = 2.39 $cmol_c$ kg^{-1}; exchangeable Mg = 0.25 $cmol_c$ kg^{-1}; exchangeable H+AL = 4.8 $cmol_c$ kg^{-1}; BS: base saturation = 37.9%; organic matter = 23.9 g kg^{-1}; remaining phosphorus (Rem. P) = 40 mg kg^{-1}.

Legumes as green manures

The hyacinth bean and jack bean legumes used as green manure were grown in sub-plots with ten replicates (five for each common bean cropping season). Was also grown ten replicates of a control, represented by spontaneous vegetation, comprising of marmalade grass (*Brachiaria plantaginea*), sorghum (*Sorghum arundinaceum*), and pigweed (*Amaranthus spinosus*).

The green manures were sown in November 2012, without fertilization, at a density of 9 and 12 seeds m^{-1} for jack bean and hyacinth bean, respectively. 132 days after sowing, at the beginning of the jack bean pod formation stage and early flowering for hyacinth bean, the aboveground biomass of all plots were cut and incorporated at a depth of 0–20 cm into the soil on the same day using a rotary tiller. Fresh biomass hyacinth bean was 25.68 kg ha^{-1}, fresh biomass jack bean was 47.73 kg ha^{-1} and fresh biomass spontaneous vegetation was 20.00 kg ha^{-1}. Upon cutting, the aboveground biomass green manure was sampled in an area of 2.5 m^2 per plot.

Subsequently, a sub-sample was taken, which was weighed and dried in an oven with forced air circulation at 65 °C until constant mass. These values were then converted to kg ha^{-1}.

After drying, the material was ground and samples taken to the laboratory to determine the total N concentration by the Kjeldahl method (Bremner and Mulvaney, 1982). From the multiplication of the dry biomass by their respective N concentrations, the aboveground N accumulation was estimated [N concentrations (g k-1)*dry matter yield/1000], and the transformed values in kg ha^{-1}.

The contribution of BNF to the N content of the green manure were estimated by the technique of natural abundance of ^{15}N ($\delta^{15}N$, Boddey et al., 1994), with the aid of a mass spectrometer, Finnigan MAT Delta Plus model, in the stable isotope laboratory of John M Day, Embrapa Agrobiologia). The percentage of nitrogen contributed by BNF was estimated by the equation:

$$\% \text{ N-BNF} = 100 \text{ x } \frac{\delta^{15}\text{N Spontaneous vegetation} - \delta^{15}\text{N Legumes}}{^{15}\text{N Spontaneous vegetation} - \beta}$$

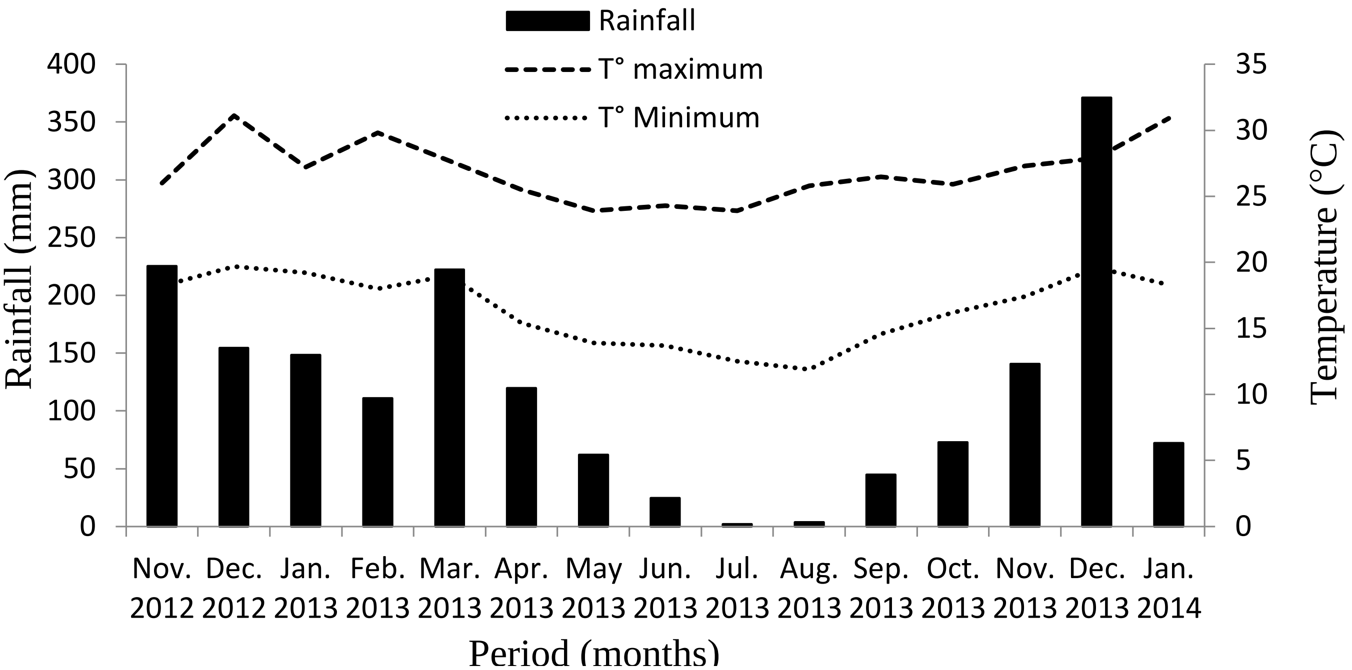

Figure 1. Monthly values of rainfall and maximum and minimum temperature (°C) during experimental period.

where; $\delta^{15}N$ spontaneous vegetation is the value of $\delta^{15}N$ obtained from plants that spontaneously grow in the region and that do not fix nitrogen and β represents the value of isotopic discrimination when $\delta^{15}N$ = 0□and is equal to -1.1□

The total carbon concentration (TC), in shoots of legumes, was also analyzed using the Finnigan MAT mass spectrometer.

Common bean cultivation

In April 2013, for "dry" season, fourteen days after green manure incorporation into the soil, one-half of amended plots were sown with common beans, cultivar Pérola. For the "water" cropping season, common beans were sown in October 2013, 6 months and 25 days after incorporation of green manure into the soil, in half size plots. Common beans were sown at a density of 15 seeds m^{-1} and a phosphorus (P) fertilizer (40 kg P_2O_5 ha^{-1}) was applied at planting time.

When more than 50% of the common bean plants had at least one open flower (that is, the R6 growth stage), 30 mature leaves from the mid portion of plants were randomly collected from each plot. These samples were then used to determine the total N content by the Kjeldahl method (Bremner and Mulvaney, 1982).

Quantification of common bean production was carried out by manually harvesting the pods of the three central rows. After drying (to 13% moisture content), the pod grains were weighed and values converted to kg ha^{-1}. The average number of pods per plant was estimated by the ratio between the total number of pods and the total number of plants. We also evaluated the average number of seeds per pod, obtained as the ratio between the total number of seeds and the total number of pods, and the average number of seeds per plant obtained as the ratio between the total seed number and the total number of plants. The weight of 100 seeds was also determined by taking four sub-samples of 100 seeds per replicate of each treatment, and the weight was measured using a 0.001 g precision balance.

Statistical analysis was undertaken using the Statistical Software SISVAR (Ferreira, 2014). Data were subjected to analysis of variance by F test and means were compared by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

Legumes as green manures

The content and amount of N-BNF provided by jack bean was higher than the hyacinth bean (Table 1). The greater supply of N-BNF by jack bean is mainly due to its higher biomass yield rather than its content of N-BNF. A high content of N-BNF in jack bean (76%) was also reported earlier (Ambrosano et al., 2013). The N-BNF content is influenced by several aspects, such as the efficiency of strains of N_2 fixing bacteria, soil fertility, water status, and phenological stage of the legume, which could explain the lower content obtained in the present study.

High yield of common bean (2436 kg ha^{-1}) was obtained with 120 kg ha^{-1} of N supplied by mineral fertilizers, in Brazil (Moreira et al., 2013); thus, the amount of N-BNF accumulated in legumes (Table 1) are able to supply significant amounts of the nutrient to the common bean crop cultivated in succession depending on mineralization rate. In this sense, the use of legumes with high potential for BNF and mass accumulation, as green manure, may reduce or even waive the use of synthetic N fertilizers for common beans.

The legume species showed different biomass accumulation, N concentration, and accumulation of N and C:N ratio after 132 days of cultivation (Table 1).

Table 1. Percentage and accumulation of N-BNF, dry matter yield, N and C concentrations, C:N ratio and N total accumulation in aboveground hyacinth bean, jack bean and spontaneous vegetation, after 132 days of cultivation.

Treatment	N-BNF	N-BNF	N	C	C:N	Dry matter	N total accumulation
	(%)	(kg ha^{-1})	g kg^{-1}			kg ha^{-1}	
Hyacinth bean	49	63.7	27^{a}	453^{a}	17^{c}	4830^{b}	130^{b}
Jack bean	58	147	22^{b}	456^{a}	21^{b}	11490^{a}	253^{a}
Spontaneous vegetation	----	----	9.1^{c}	465^{a}	51^{a}	4780^{b}	44.5^{c}

Means followed by the same letter in the column do not differ by Tukey test ($p \geq 0.05$).

Table 2. Leaf N content, yield components and grain yield of common beans cultivated in amended soil with hyacinth beans, jack bean and spontaneous vegetation. (Data are the average of two growing seasons).

Treatment	N concentration (1) (g kg^{-1})	Number pod/plant	Number seed/pod	Weight of 100 seeds (g)	Grain yield (kg ha^{-1})
Hyacinth bean	38^{a}	8.7^{a}	5.2^{ns}	23^{ns}	2128^{a}
Jack bean	39^{a}	9.3^{a}	5.2^{ns}	24^{ns}	2355^{a}
Spontaneous vegetation	29^{b}	6.1^{b}	5.1^{ns}	24^{ns}	1616^{b}

(1) N concentration in leaf common bean at flowering stage. In each column, means followed by the same letter do not differ by Tukey test ($p \geq 0.05$). ns = F value not significant for $p \geq 0.05$.

Legumes and spontaneous vegetation had similar C concentration. The jack bean accumulated more biomass than hyacinth bean and spontaneous vegetation, which were similar to each other. This result can be attributed not only to the adaptability of the jack bean to the soil and climatic conditions of the region, but also its fast vegetative growth, while hyacinth bean presented a longer life cycle and slower initial growth, resulting in less biomass accumulation in the same culture time period. This result reinforces the importance of the choice of legume species for green manuring. The biomass production of the spontaneous vegetation was similar to the hyacinth bean, but presented a higher C:N ratio and a smaller amount of accumulated N (Table 1). The spontaneous species present in the experimental field were not capable of BNF, which probably influenced the result.

The N concentrations of hyacinth bean and jack bean were higher than those of spontaneous vegetation. However, the N content of hyacinth bean was 23% higher than that of jack bean, resulting in a lower C:N ratio. It is noteworthy that at 132 days after cultivation, hyacinth bean was phenologically younger than jack bean, therefore showing a lower biomass productivity and higher N content. The jack bean accumulated more N (kg ha^{-1}) compared to hyacinth bean and spontaneous vegetation, due to its greater biomass productivity. The amount of N accumulated by the jack bean crop was nearly the double of hyacinth bean, but both species had accumulated higher amounts of N than spontaneous vegetation. Thus, these legumes are important N sources for the agroecosystem through their BNF capacity.

N transfer from crop residues to common bean

There was no significant interaction between the effects of growing season ("dry" and "water") and cover crops over any of the evaluated characteristics of the common bean crop (Table 2). Specifically, present results demonstrated no effect on the N content in leaves of common bean, number of pods per plant, number of seeds per pod, weight of 100 seeds and grain yield (Table 2). The absence of any effect due to the season can be attributed partially to the adequate climatic conditions during the “dry” and “water” cultivation periods. However, a fraction of N in the soil in both seasons ("dry" and "water") originated from hyacinth bean and jack bean residues was available for the common bean, both immediately after legume cutting, during the “dry” cultivation, as well as 6 months and 25 days after legume cutting, during the “water” cultivation.

The cover crops had significant effects on the leaf N concentration of common bean, number of pods per plant and grain yield, in the two growing seasons (Table 2). Leaf N in the common bean was high, with values just above the optimum range for the crop, ranging from 30 to 35 g kg^{-1} (Fontes, 2011). Therefore, present results indicated that apparently crop residues increased the N content in the soil, in a form readily available for uptake by the common bean, when compared to the spontaneous vegetation. The improved soil N content, either few weeks after harvesting the cover crops and after a period of almost seven months, can primarily be due to the chemical composition legume residue. This regulates the organic N mineralization rates over time

(Trinsoutrot et al., 2000). It is likely that the common bean in the "dry" season may have benefited from the mineralization of the more labile N forms, such as organic molecules of low molecular weight (Agehara and Warncke, 2005). On the other hand, common bean cultivated during the "water" season may have benefited from the slower mineralization of organic compounds of higher molecular weight and higher recalcitrance (e.g., lignin), probably resulting in N immobilization after the first weeks after soil amendment (Marschner et al., 2008), also favored by the climatic condition with mild temperature and low rainfall (Havlin et al., 2005) (Figure 1).

Although the hyacinth bean and jack bean residues did not affect differently the number of seeds per pod or the weight of 100 seeds, the number of pods per plant and grain yield were higher when compared to spontaneous vegetation residues (Table 2). The number of seeds per pod is a highly inheritable genetic characteristic and is mostly not influenced by environmental conditions (Andrade et al., 1998). On average, nine pods per plant in both cropping seasons were counted, which is similar to pod number obtained in common bean fertilized with mineral N (Crusciol et al., 2007). The increase in the number of pods per plant is justified by the possible higher N contribution from hyacinth bean and jack bean residues, which in turn causes an increase in grain yield. Some authors stated that legume green manure may provide an N input ≥ 110 kg N ha^{-1} which can result in common bean yields similar to those obtained using mineral N fertilizers (Tonitto et al., 2006).

Compared to the spontaneous vegetation, the yield of common bean in hyacinth bean amended soil increased by 32%, and under jack bean residue increased by 46%. Yields obtained in the present study in amended soils with these legumes were high, considering that the mean yield in Brazil for common bean is 650-850 kg ha^{-1} (Didonet et al., 2009). However, even in the grass amended soil the yield of common bean was higher than the national value. But the most important is that the increase in bean productivity was achieved without application of a synthetic N fertilizer. In this sense, present results contributed to improving food production under the control of family farmers using biological processes based on crop diversification and rotation.

Conclusions

i) Among the evaluated green manure, jack bean was the legume that resulted in higher common bean biomass production, accumulated more N in its tissue, by contributing to the highest amount of N-BNF to the system.

ii) Both legumes as green manure promoted increases in leaf N content of common bean and in the number of pods produced per plant, also increasing the yield of the common bean.

iii) At the Atlantic Forest Zone of Minas Gerais, southeastern Brazil, common bean can be performed well either in the "dry" season, immediately after incorporating the legume residues or later in the "water" season.

Conflict of Interests

The authors have not declared any conflict of interests.

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