#### Article

# Potential of five legume species as green manure in the highland area of San Luis Potosí, Mexico

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#### Abstract

The green fertilizers (AV) are an alternative to improve the fertility and conservation of agricultural soils in semi-arid zones. For this reason, the potential of five legume species as AV in the highlands of San Luis Potosi, Mexico was evaluated. The study species were: 1) black dolichos beans (*Lablab purpureos*); 2) beans brown dolichos (*Lablab purpureos*); 3) red dolichos beans (*Lablab purpureos*); 4) yorimon bean (*Vigna unguiculata*); and 5) chickpea (*Cicer arietinum*). The trial was carried out under a randomized block design with three repetitions. In the stage of flowering of each species the evaluation was carried out. The variables that were measured were: biomass, carbon capture, carbon footprint, nitrogen in plant and C/N ratio. The results indicate that Dolichos red was the most outstanding in biomass production (6.5 t ha<sup>-1</sup>), carbon capture (2.97 t ha<sup>-1</sup>) and carbon footprint (-4.3 t ha<sup>-1</sup>). *Cicer arietinum* excelled in concentrating N in its tissues (2.03, 1.47 and 1.87% in aerial part, root and whole plant) and could contribute to the soil up to 83 kg ha<sup>-1</sup> N. *Cicer* sp., Had the lowest C/N ratio with 24.12, which would have a faster mineralization than the other species. In conclusion, the five species have the potential to be used as AV in the study area and are an important alternative to improve agricultural soils and the environment.

Keywords: Lablab, Cicer, Vigna, carbon, nitrogen.

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### Introduction

In Mexico, as in the world, agricultural soils are degraded as a result of various human activities. An analysis carried out estimates that a global loss of 0.3% of annual crop yields occurs due to erosion. If these rates continue, there could be a total reduction of 10% in the yield of food production by the year 2050, equivalent to 150 million ha of crop production (FAO, 2016). In the state of San Luis Potosi, Mexico, the misuse of soils has caused different damage to their properties, which has caused problems in crop yields, in addition to losing fertility in each cycle (Martínez, 2014).

The high energy required for the production of fertilizers, the high costs of fertilizers, the lack of subsidies for agricultural products and credit limitations, in some cases, have aroused interest in the search for more efficient technological alternatives for the supply of fertilizers of organic matter (MO) and nitrogen in crops (De la Cruz, 2015). In the case of the energy required in the synthesis of fertilizers, a requirement of 64.65 MJ kg<sup>-1</sup> N for urea and 52.62 MJ kg<sup>-1</sup> N from other sources of N is estimated (IDEA, 2007).

One of these alternatives, to improve the fertility and conservation of soils, consists of the use of legumes in association and rotation as green fertilizers (AV), in order to exploit their potential for the fixation of atmospheric N<sub>2</sub> and at the same time the contribution of organic matter of rapid decomposition to improve soil fertility (Prager *et al.*, 2012). This natural input has been recognized for contributing to the recovery of fertility and physical characteristics of the soil, as well as for its ability to control pests, weeds and nematodes, but especially for its ability to increase the concentration of MO in the soil (Beltrán *et al.*, 2009). García *et al.* (2010) adds that AV contribute to carbon sequestration, which is of great environmental benefit. It is estimated that this element has been lost in soils at a rate of 66 ±12 billion tons worldwide since 1850 (FAO, 2016).

A first step, for the development and adoption of an agricultural technology, is the evaluation of the performance of varieties in a given environment, since it is known that the physiological and agronomic behavior of a plant material is defined by the genetic part, the environment and the interaction between both. Several studies on the evaluation of legume varieties for use as green fertilizers in arid zones were made in the state of Baja California Sur with the species of the genus *Lablab* (Beltrán *et al.*, 2005 and 2009). The authors conclude that the soils improved significantly in terms of their physical and chemical properties, and that the contributions of N when the AV are incorporated into the soil can reach 240 kg ha<sup>-1</sup>. However, in the semiarid zone of the Potosino highlands, where there are more than 20 thousand hectares under intensive horticultural systems, the use of AV has not been reported. In this regard, García *et al.* (2010) point out the need to continue researching with species with the potential to tolerate and be productive under adverse conditions (arid and semi-arid zones).

The objective of the study was to evaluate the potential of five legume species as green manures in the potosino highlands (San Luis Potosí, Mexico) through the generated biomass, carbon capture, carbon footprint, nitrogen and the C/N ratio.

The present work was carried out in the campus of the Faculty of Agronomy and Veterinary, of the UASLP, located in common Palma de la Cruz, municipality of Soledad de Graciano Sánchez, state of San Luis Potosí, Mexico. 22° 13' 36''north latitude, 100° 51' 22'' west longitude, altitude: 1 837 meters above sea level. The climate is semiarid with average temperature of 17.9 °C and precipitation of 341 mm year<sup>-1</sup>. The soil presented the following characteristics: texture, sandy loam; pH, 7.6, electrical conductivity, 2 dS m<sup>-1</sup>, organic matter, 1.1% (low) and inorganic nitrogen, 1.7 mg kg<sup>-1</sup> (very low).

The materials evaluated as green fertilizers were: 1) black dolichos beans (*Lablab purpureus*); 2) brown bean dolichos (*Lablab purpureus*); 3) red dolichos beans (*Lablab purpureus*); 4) yorimon bean (*Vigna unguiculata*); and 5) chickpea for fodder use (*Cicer arietinum*). The dolichos and yorimon species were chosen for their adaptation and good performance as AV in arid environments, similar to the study area (Beltrán *et al.*, 2005 and 2009). On the other hand, *Cicer arietinum* was chosen for being a species that has presented good growth in the central and middle zones of the state of San Luis Potosi, and due to its legume condition. The search for species with potential as AV is widely recommended (García *et al.*, 2010).

The AV were planted on June 13, 2013. The population density was 5.5 plants per m<sup>2</sup>. The experimental plots were of 3 rows of 5 m long x 0.90 m wide each (13.5 m<sup>2</sup>). The distribution of the treatments was through a random block design with 3 repetitions. In total, an area of 202.5 m<sup>2</sup> was available. The cultivation practices consisted in two weeding and manual weeding during the first 30 days, from the sowing of the AV. Irrigations were performed to maintain a soil moisture available between 70% and 90%.

At 115 days after sowing, plant sampling was carried out, which was the moment of beginning of the phenological stage of flowering. Six complete plants representative of the population were collected by treatment and repetition. In the laboratory, the samples were washed with distilled water and the root of the aerial part of each plant was separated. They were then placed in perforated paper bags and placed in a forced air drying oven at 65 °C until constant weight was obtained.

The variables that were measured as indicators of the potential of the legumes as green manure in the highland area of San Luis Potosí, Mexico, were:

1) Biomass, is an important variable in the evaluation of the growth and production of the plant, indicate the productive potential of a species in a certain area and integrate its genetic capacity with the interaction with the environment (Ávila *et al.*, 2010). This variable was measured according to the dry weight of each sample, weighing independently the root of the aerial part. To obtain the biomass per hectare, it multiplied in individual result by the population density that was 55 000 plants ha<sup>-1</sup>.

2) Carbon in biomass, this variable represents a value of the photosynthetic efficiency of the plants and is important for the soil since it is related to the sustainability of the agricultural systems affecting the properties of the soil related to the sustained yield of the crops. It was estimated considering that 45% of dry weight is composed of carbon in plant species (Azcon and Talon, 2008). In this way, the biomass obtained per hectare multiplied by 0.45.

3) Carbon footprint means the amount of  $CO_2$  captured in the plant biomass plus the  $CO_2$  equivalent that would be released in the manufacture of nitrogen fertilizer (in this case, ammonium nitrate) in the amounts of N that were fixed by the AV. The captured  $CO_2$  was estimated by multiplying the carbon in biomass (kg ha<sup>-1</sup>) by 3.67 (conversion factor from C to  $CO_2$ , quotient resulting from the molecular weights of carbon dioxide 44 and carbon 12). The  $CO_2$  equivalent emitted into the atmosphere per kg of nitrogen fixed in the form of fertilizers and their transport, was estimated with the value 3.7 kg of  $CO_2$  kg N<sup>-1</sup> (Yara, 2015).

4) Concentration of nitrogen in the plant, this is the most limiting element in world agriculture, it is fixed by soil microorganisms in association with legumes, such as the AV under study. Nitrogen in vegetable tissue was made through the Kjeldahl method (Rodríguez and Rodríguez, 2002), and represents the amount of nitrogen present per biomass obtained. This value considers proportionally the nitrogen in the plant.

5) Nitrogen per ha<sup>-1</sup> (kg N ha<sup>-1</sup>), the nitrogen content per plant was multiplied by the density of plants per hectare. This value considers the total absolute nitrogen fixed that would be incorporated into the soil.

6) C/N ratio, it is useful to know the rate of decomposition of an AV and integrate into the soil (Martínez *et al.*, 2008). It was determined with the quotient of the division between the total carbon and estimated total nitrogen.

Analysis of variance was made for all the variables under study. In the case of the percentage variable of N in the plant, prior to the ANOVA, the values were transformed with the function arc  $\sin \sqrt{\text{porcentaje}}$ , with the intention of normalizing the percentage values (Reyes, 1992). In the variables where significant differences were found, we made a comparison of means with the Tukey test ( $p \le 0.05$ ). The experimental design software of FAUANL was used to analyze the data obtained (Olivares, 2012).

The results obtained in biomass indicate a good growth of the five species in the experimental plot of study (Figure 1a). A study done in arid zones reports results similar to those of this trial (Beltrán *et al.*, 2009). In this study, dry weights were reported per plant of 90 and 80 g per plant of the genus *Lablab* and *Vigna*, respectively. In the present trial, the most outstanding species was *Lablab* (red dolichos beans), with weights greater than 109 g per plant ( $p \le 0.05$ ), while *Vigna* (yorimon bean) obtained average dry weight per plant of 67.4 g.

Beltran *et al.* (2009) report having had, with a population density of 62 500 plants ha<sup>-1</sup>, total dry weight of 5.6 t ha<sup>-1</sup> of biomass produced by Lablab. This trial had a density of 55 000 plants, which gives a total dry weight of 5.9 t ha<sup>-1</sup> and 2.7 t ha<sup>-1</sup> of fixed carbon (Figure 1b). It is also relevant to have obtained dry weights greater than those found in tropical areas as reported by Sosa *et al.* (2014), who obtained yields of aerial part of 4 t ha<sup>-1</sup> with an association of *Mucuna pruriensis* L. var. Utilis with *Zea mays* var. ICA 305. The carbon fixed by the plant species, which will be incorporated into the soil, will be indispensable for the growth of plants and the establishment of biota, since it intervenes in all the structural and metabolic processes of living beings (Lehmann and Kleber, 2015).

The environmental gain of the AV is observed in Figure 1c, where red dolichos beans managed to have the best environmental value when estimating a carbon footprint of -4.3 t ha<sup>-1</sup> CO<sub>2</sub> equivalent emitted into the atmosphere (the negative sign represents CO<sub>2</sub> captured per kg of N in the biomass) (Bentrup *et al.*, 2004). This value includes the CO<sub>2</sub> savings of the manufacture and transportation of synthetic chemical nitrogen in the units in which the N was set by the AV.

Regarding the results obtained in nitrogen, stands out of the species *Cicer arientnum* (chickpea), which managed to concentrate 2.03%, 1.47% and 1.87% of N in aerial part, root and total. In the case of the root, it was statistically superior to the rest of the species ( $p \le 0.05$ ) (Figure 1d). In a study conducted in Oaxaca by Ruiz and Loaeza (2003), where they used the wheat, soybean and chickpea species as AV, they obtained N concentrations in vegetable tissue of 1.73, 1.29 and 0.92, respectively. These values are similar to the species studied in this test and, in the case of chickpea, the difference is double. However, the results obtained by the *Lablab* and *Vigna* species are lower than those reported by Beltrán *et al.* (2009) in these same genres. The concentrations of nitrogen found in the plant tissue were, on average, more than 4%.

Garate and Bonilla (2008), mention that mineral contents among plant species vary, even when comparisons are established using the same organs and the same physiological age. They indicate several determining factors: a) the genetic endowment of the plant; b) the availability of nutrients in the soil or in the culture medium; and c) the phenological moment or age of the plant and the plant organ or tissue that is considered. In this regard, we consider that the differences found in the contents of N in the plant, of this trial, are merely genetic, rather than the availability of nutrients, since the five species grew in a soil with the same chemical and nutrient characteristics, as well as having been harvested in the same phenological stage.

The results in total nitrogen, which can be incorporated into the soil, which depends on the concentration of N in the tissues and biomass, the species *Cicer* sp. it stands out again It obtained the highest value in the whole plant with 83 kg N ha<sup>-1</sup> of incorporating the entire plant to the soil and 14.86 kg N ha<sup>-1</sup> in root, in this case it was greater than the rest of the species statistically (p < 0.05) (Figure 1e). Such values mean up to 50% of the nitrogen needed for a subsequent crop, such as: lettuce, chili, squash, cilantro, garlic, onion and others, which are economically important crops in the altiplano area. However, the 83 kg of N ha<sup>-1</sup>, are much less than the 240 kg of N ha<sup>-1</sup> reported to have obtained Beltrán *et al.* (2009) with the Lablab genre in Baja California Sur. The differences may be due to the high concentrations of N in plant tissue they obtained, as mentioned above (>4%), as well as the high population densities (62 500 plants ha<sup>-1</sup>).

The results in the C/N ratio indicate that the chickpea (*Cicer arietinum*) is the species that would have more facility to incorporate the nitrogen of its tissues into the soil and be arpovigated by the subsequent crops. The values obtained are the lowest of the group of species under study (ratio < 25 in the whole plant) (Figure 1f). According to Martin and Rivera (2000) plants with a C/N ratio of less than 25, N mineralization is rapid, while plants with C/N ratio greater than 25 form a stable coverage, which contributes to the increase of the content of matter organic and therefore to

improve the structure of the soil and to protect it from the impact of rain and solar radiation; in addition, it favors the development of the radical system, the formation of nodules and the symbiotic fixation of nitrogen.



Figure 1. a) Dry weight of the five AV. b) Carbon in the five AV. c) Carbon footprint in the five AV (CO<sub>2</sub> captured in the AV). d) Nitrogen in vegetal tissue in the five AV (%). e) Nitrogen (N) in vegetal tissue that would be contributed to the soil of the five AV. f) C/N ratio of the five AV. Means followed with the same letter in each bar indicate that they are statistically.

# Conclusions

The five species of the study: black dolichos beans (*Lablab purpureos*), brown dolichos beans (*Lablab purpureos*), red dolichos beans (*Lablab purpureos*), yorimon beans (*Vigna unguiculata*) and chickpeas (*Cicer arietinum*), have potential to be used as green manures in the highland area of San Luis Potosí, Mexico, and are an important alternative to improve agricultural soils and the environment.

The red dolichos bean species was the most outstanding in biomass production (6.5 t ha<sup>-1</sup>), carbon capture (2.97 t ha<sup>-1</sup>) and carbon footprint (-4.3 t ha<sup>-1).</sup> The species *Cicer arietinum* excelled by concentrating N in its tissues (2.03, 1.47 and 1.87% in aerial part, root and total, respectively), which could contribute to the soil up to 83 kg N ha<sup>-1</sup>. It also obtained the lowest C/N ratio (24.14), which would have a faster mineralization than the other species in the study.

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