Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 18, Issue 2, 2018
PRINT ISSN 2284-7995, E-ISSN 2285-3952

SYMBIOTIC FIXATION OF NITROGEN IN BELMONDO PEA VARIETY IN A SUGGESTED CROP ROTATION SYSTEM - TECHNICAL, ECOLOGICAL AND ECONOMIC EFFICIENCY

Mihai BERCA[1], Valentina-Ofelia ROBESCU[2], Gabriel CROITORU[2], Roxana HOROIAŞ[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania, prof.mihai.berca@gmail.com, unibercamihai@yahoo.com
[2]Valahia University of Targoviste, 2 Regele Carol I Blvd., 130024, Targoviste City, Romania, Emails: robescu_ofelia@yahoo.com, valentina.ofelia.robescu@gmail.com
[3]AGROVET SA, 20 Siriului Street, 014354, District 1, Bucharest, Romania, Email: roxana.horoias@gmail.com

Corresponding author: roxana.horoias@gmail.com

Abstract

In 2016 and 2017, in southern Romania, field experiences with Belmondo pea variety have been carried out. It has been found that, at about 35 days after emergence, the number of fresh nodules per plant ranged from 20 to 46 in the nitrogen-free version, with an average of about 33 nodules/plant. Since nodules were found at 80 plants/m^2, it results a number of 2,640 nodules/m^2. By weighing after oven drying, it was measured 38.24 mg/plant, which is 3.06 g/m^2 and 30.6 kg/ha. Using the balance (difference) method, the calculated amount of nitrogen extracted from peas was 262.5 kg/ha, as follows: $N_{biologically\ fixed}$ = 262.5 – 135 (control wheat) = 127.5 kg N/ha (2-year average for Belmondo peas). It results that for each kg of dry nodules a quantity of 4.17 kg N has been synthesized – a nitrogen calculation coefficient. In a crop rotation with 25% peas and a surface of 1,600 ha, an amount of 51 t N/ha is fixed, which represents 24.5% or 29.4 €/ha. Research is part of a program meant to reduce conventional inputs and increase farm profitability.

Key words: *crop rotation, efficiency, nitrogen, nodules, pea*

INTRODUCTION

Monitoring the relation management between soils and crops, many authors found that, despite the application of a large quantity of nitrogen nutrients of chemical synthesis, soil fertility is continuously reduced because the organic matter degrades itself in fast paces [2, 5]. It can be said that the Haber-Bosch synthesis models have reached their limits and for a long time are wanted natural solutions, in order to replace the bag (chemical) nitrogen with the one already having it in the air. In this respect, biosynthesis patterns are known through fixing nitrogen from air by various microorganisms (bacteria, actinomycetes). The most common and effective system is that of symbiotic fixation, which is carried out by symbiosis between legumes and bacteria. Most common are bacteria of the *Rhizobium* genus. Peas is a very important crop, which fits well in rotation with wheat, but also with rape and corn in the arid areas, including those in southern Romania.

Martins et al. (2003), studying this system on the semi-dry soils from Brazil, observed that, in addition to the nitrogen needed for the development and production of peas, the plant offers 50 kg N/ha to the following wheat crop, which corresponds to a normal nutrition in this region [10]

Studies performed in Europe by Riou (2016) [12] determined the nitrogen amount fixed by different legumes, giving the following figures: soy 60-115 kg N/ha; peas 50-100 kg N/ha; alfalfa 130-250 kg N/ha; white clover ≅200 kg N/ha [13].

Depending on the environmental conditions, the variation range of the fixed quantity is much higher. Pietsch et al., 2006 (from Boku University in Vienna) have been indicated the intervals and averages for the nitrogen fixed by different species [11]. On larger agricultural sites, such as those in India as mentioned by Marquard, (2000) and Quispel,

(1982) [9, 12] there are both wider nitrogen fixation intervals, as well as higher averages ranges for different leguminous species (as seen in Table 1).

The amount of fixed nitrogen is imposed by soil conditions, moisture, soil biology, specific technologies, and soil's nitrogenous activity. From the found literature it is worth noting that this model of the symbiosis between legumes and various bacteria brings, worldwide, over 100 million tons of nitrogen, which is a quantity comparable to that produced by chemical synthesis [13] .

Table 1. The amount of nitrogen fixed by the various annual and perennial legume crops in Austria and India

Crt.	Species	Austria		India	
		Variation (kg N/ha)	Average (kg N/ha)	Variation (kg N/ha)	Average (kg N/ha)
1.	Fava bean	100 – 450	170	100 – 300	200
2.	Peas	50 – 300	100	50 – 500	150
3.	Lentil	30 – 150	80	50 – 150	80
4.	Lupinus	50 – 400	100	140 – 200	150
5.	Soya	60 – 300	100	60 – 300	100
6.	Vetch	30 – 180	100	50 – 150	100
7.	Clover	50 – 350	250	45 – 670	250
8.	Alfalfa	100 – 400	250	90 – 340	250

Source: data from the mentioned authors.

In the agricultural area fron southern Romania, in recent years, a market for protein products has been formed, among which peas (*Pisum sativum*) gain new surfaces each year. In these circumstances, the nitrogen fertilization system suffers significant changes in both the economic, but especially in the bioeconomic (ecological) field of agricultural systems sustainability.

This research deals with the behaviour of the nitrogen biosynthesis model by symbiotic fixation on peas in a 4 year crop rotation system, in two localities in southern Romania (Alexandria and Calarasi).

MATERIALS AND METHODS

The research aim is to provide information on the amount of nitrogen fixed by Belmondo pea variety in southern Romania and the extent to which it is used by the crop rotation system. Substitution problems of industrial synthesis nitrogen with the atmospheric, symbiotically fixed one.

The proposed objectives were:

(i) determining the number, dynamics and quantity of nodules formed on the peas in the years 2016-2017;

(ii) calculating the amount of nitrogen fixed by symbiosis and its distribution in plants consumption;

(iii) establishing economic and ecological effects.

Work was carried out in two locations – Burnas Platform (Alexandria) and South Plain (Calarasi). Belmondo variety with erect port (approved in Romania and the European Union) has been used. A crop rotation system was selected, in which the peas came before wheat, the wheat before rape, the rape before corn and the corn before peas.

The soil was chernozem type, with about:

- 3.4% humus and 34% clay in Alexandria;
- 3.2% humus and 26% clay in Calarasi.

In terms of climate indicators:

- Alexandria – temperate continental climate, semiarid, with a multiannual average temperature of 10.8°C and a little over 500 mm precipitation/year;
- Calarasi – temperate continental climate, semiarid, with a multiannual average temperature of 11°C and about 500 mm precipitation/year.

In both areas, during the vegetation period, 70-72% of rainfall occurred. Research years 2016 and 2017 were favourable to pea crop, during the vegetation period falling over 300 mm precipitation/m^2.

Investigations were conducted in the field, according to the subdivision parcel method, where, alongside the location factor, phosphorus (P_0, P_{30}, P_{60}, P_{90}) and nitrogen (N_0, N_{40}, N_{80}, N_{120}) fertilizers were used.

The crop was carried out according to the technology in the area. 110 seeds/m^2 were sown, resulting in about 85 plants/m^2, out of which about 78-80 plants formed nodules and fixed nitrogen. Plants were monitored in the field, taking samples weekly to observe the formation of nodules. No inoculation was performed, the soil being rich in bacteria (*Rhizobium leguminosarum*).

It has been found that both as number and as weight, the plants completed their biosynthesis equipment at about 30-35 days after emergence. At this point, most of the samples for measurement were collected.

They were determined:

-Number of nodules/10 plants/repetition; it has been used the rehearsals average.

-Weight of nodules/m^2 by drying them in drying stove at 105°C for 3 hours, in special capsules.

-The amount of fixed nitrogen, by the Nitrogen Balance Difference method [8], using wheat as a reference plant:

$$N_{\text{fixed remaining}} = N_{\underset{\text{(harvestable)}}{\text{fixed by legume}}} - N_{\underset{\text{(harvestable)}}{\text{reference plant}}}$$

Fixed nitrogen relative to the dry nodules weight led to the calculation of a local efficiency index in nitrogen fixation of the mass of nodules. Data was statistically processed by dispersion analysis and correlations in 2D and 3D.

RESULTS AND DISCUSSIONS

Formed nodules, depending on the locality, are shown in Table 2.

Table 2. Number of nodules/plant and amount of dry nodules (g/m^2) according to research locations – average for 4 preceding plants

Crt	Location	Nodules number				Nodules weight			
		No./plant	% from M	Diff.	Sign	g/m^2	% from M	Diff.	Sign
1.	Alexandria	25.22	97.32	–0.69	–	3.06	101.26	+0.04	–
2.	Calarasi	26.61	102.68	+0.70	–	2.98	98.74	–0.03	–
Mean (M)		25.91 = 100% Interval 1 – 70			Control	3.02	100	1.49	
$DL_{5\%}$ = 1.57									

Source: results of the own experiments.

The determinations show that there are no statistically significant differences between the two locations, on average over the two analysed years (2016 and 2017). We can consider as representative and statistically assured in this area from southern Romania the number of 30 nodules/plant. We can also consider the amount of 3.02 g of dried nodules/m^2 as representative and statistically assured for the Alexandria – Calarasi region. The confidence interval is 30 ± 1.6 = 28.4 – 31.6 for the number of nodules and 3.02 ± 0.21 = 2.81 – 3.23 for weights nodules/m^2.

Please note that we found an average number of 78 plants/m^{2}in order to count the nodules. On average, 78 x 25.91 = 2,020 nodules/m^2 there were harvested. We can say that 1 g of dried nodules is actually collected from 2,020: 3.02 = 669 nodules, with small average variations from one location to another.

At the same time, remark that no inoculations have been done. Work was done in a natural inoculation system, both areas being well supplied with *Rhizobium*.

To highlight the influence of the preceding plant on nodules formation, the data are shown in Table 3, on average for the two research locations.

Table 3. Atmospheric nitrogen fixation, expressed in number and weight of nodules (g/m^2) in peas, Belmondo variety, in Southern Romania, 2016-2017

Preced. crop	Nodules number				Nodules weight			
	No/pl.	% from M	Diff.	Sign	g/m^2	% from M	Diff.	Sign
Peas	3.32	10.09	–29.53	ooo	0,29	9,65	–2,72	ooo
Wheat	55.41	168.65	+22.56	***	5,07	168,04	+2,05	***
Rape	43.19	131.46	+10.34	***	3,96	131,36	+0,95	***
Corn	29.50	89.90	–3.35	oo	2,74	90,95	–0,27	–
Mean (M)	32.55 = 100%		$DL_{5\%}$ = 2.30 $DL_{1\%}$ = 3.05 $DL_{0.1\%}$ = 3.94		3.02 = 100%		$DL_{5\%}$ = 0.33 $DL_{1\%}$ = 0.44 $DL_{0.1\%}$ = 0.56	

Source: results of the own experiments.

The number of nodules per plant is very variable in relation to the preceding plant in the crop rotation system. In the field work in which the peas comes for 4 years in a row, the natural inoculation and the number of nodules is of only 3.32 (close to 0), the loss being very significantly negative. The same thing happens with the weight of the nodules/m^2– 0.29 g/m^2 or 290 mg/m^2, significantly negative.

This phenomenon is known from literature, but also from practice. Amel (2015) finds 9 nodules/plant in a 2 years monoculture, compared to 18 nodules in a simple crop rotation system with wheat. Researches were conducted on an Indian pea. Similar results were obtained at the University of Manouba (Tunisia). In most of the studied papers, especially in warmer areas, the number of nodules/plants has hardly exceeded 30-50 pieces [6].

In our experiments, the best natural inoculation occurs in peas after wheat (55 nodules/plant and 5.07 g dry nodules/m^2)– values significantly positive compared with the average.

Another good preceding crop for peas also was the rape crop, but less good was the corn.

Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 18, Issue 2, 2018
PRINT ISSN 2284-7995, E-ISSN 2285-3952

It is to be said that after corn there was not enough time for a proper preparation of the germinative bed, as observed and confirmed by dos Santos et al. (2011) [5].
A complete picture of the natural inoculation behaviour of peas is also presented in Figure 1. In the same time, it is important to note that a single variety (Belmondo variety) has been tested, genetically adapted to good inoculation with *Rhizobium*.

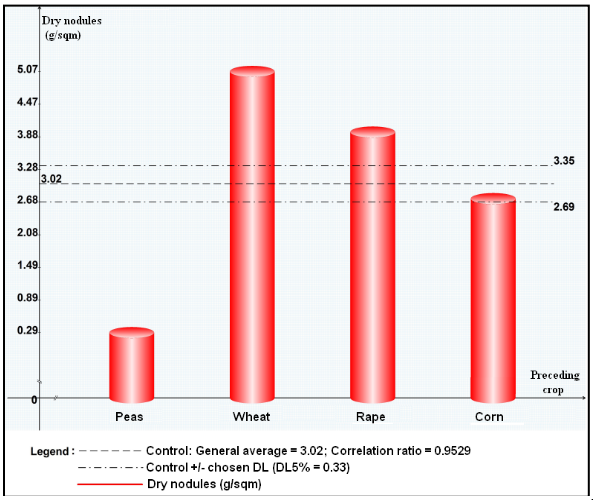

Fig. 1. Nitrogen fixation, measured in g of nodules/m^2 depending on the preceding crop– average for Alexandria and Calarasi, 2016-2017
Source: Own results.

Numerous studies and researches synthesized by Pietsch G. (Boku University, Vienna) [11] demonstrate that there are a lot of restriction factors in the natural realization of inoculation and, thus, in the reduction of fixed nitrogen. Among others, the alkalinity or the higher acidity of the soil, the lack of phosphorus, of sulphur or, on the contrary, the excess of nitrogen, too much clay content or the defective physical properties are mentioned.
Regarding the phosphorus relation with the nitrogen fixation on peas in the experiments in Alexandria and Calarasi, a small amount of phosphorus in the soil (about 6-8 mg/g soil) led to a small quantity of dry nodules, relative to the average of the two locations (2.49 g/m^2) – Figure 2. Statistically, the value is positioned very significantly negative below the average.

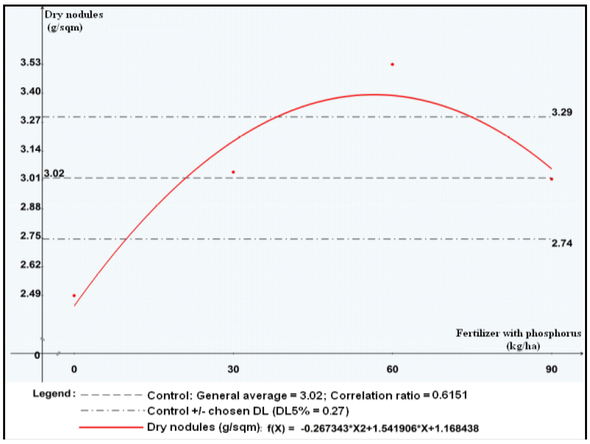

Fig. 2. The role of phosphorus in improving the symbiotic fixation of nitrogen on Belmondo peas – average for Alexandria and Calarasi, 2016-2017
Source: Own results.

Nodule formation increases significantly with doses of phosphorus, it became significant at just 10 kg P_2O_5/ha applied, but it's limited to 62 kg P_2O_5/ha, where the 1st order derivative of the function in Figure 2 indicates the maximum. The maximum production of nodules obtained in 62 kg P_2O_5/ha is about 3.55 g/m^2, i.e. 35.5 kg/ha.
Our own calculations made by using the difference method in the nitrogen balance show that every 1 kg of dry nodules are fixing 4.6 kg N ⇒35.5 x 4.6 = 163.3 kg N fixed in the version with P_{62}.In the phosphate-free version the following situation has been observed: 2.55 g/m^2 = 25.5 kg/ha = 114.75 kg fixed N.
It results that phosphorus, in its relation with *Rhizobium* in Belmondo peas, has raised an increase of 163.3 – 114.75 = 48.55 kg N/ha. This aspect improves the biological value of phosphorus applied in the autumn.
There are a lot of materials and books that inform us that, by increasing the nitrogen in the soil, the nodulation and nitrogen fixation are reduced. We quote, in this regard, Bourion et al. (2007) [3] , but also the work of L'Taief et al. (2009) [7], which claims that even at low doses of nitrogen applied to Indian pea, nitrogen fixation suffers greatly.
The results obtained are shown in Figure 3, for the average of localities, and Figure 4 separately for the two localities, for comparison.

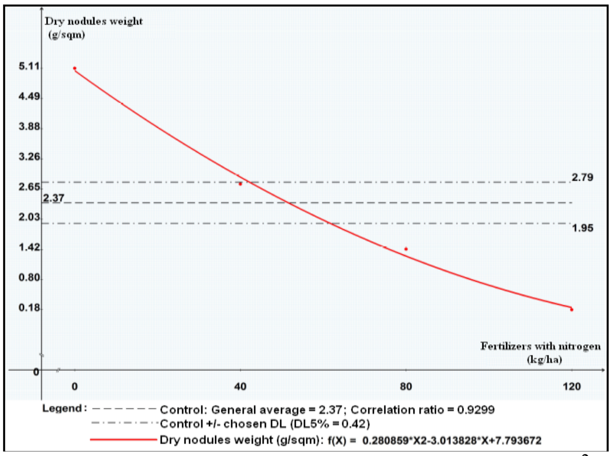

Fig. 3. Dynamics of nitrogen fixation (g of nodules/m^2) according to the applied nitrogen doses – the average for Alexandria and Calarasi, 2016-2017
Source: Own results.

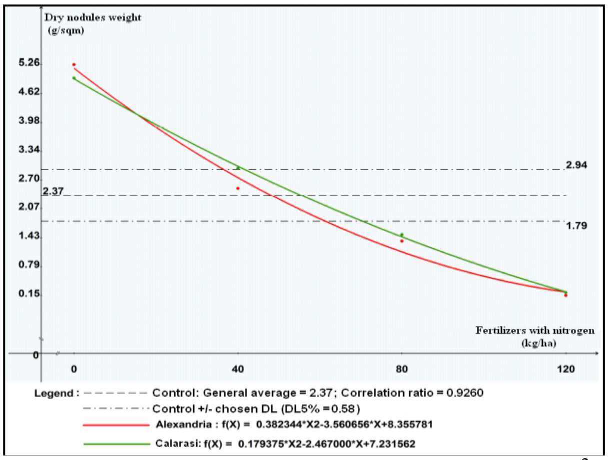

Fig. 4.Dynamics of nitrogen fixation (g of nodules/m^2) according to the applied nitrogen doses –Alexandria and Calarasi, 2016-2017
Source: Own results.

The following findings are made: cultivation of peas in poor nitrogen conditions can lead to a large amount of nitrogen fixed – 5.11 g/m^2 assumes 5.11 x 4.6 = 235 kg fixed N/ha.

This parameter is statistically assured for 92.6% of the crops that are conducted under the same soil and climate conditions. The parameter for the transformation of dry nodules into fixed nitrogen also varies depending on other agrochemical intervention factors, between 4.1 → 4.7 kg N/1 kg of nodules.

Under the research conditions, the increase with only 40 kg/ha of available nitrogen in the soil reduces by about 46% the fixed nitrogen. This means 23.6 x 4.6 = 108 kg. By applying 40 kg N/ha to the pea crop, it is lost up to 2.7 times more nitrogen by the nitrogen fixation deficit. The losses are enormous at 80 and 120 kg N/ha. At high dose, symbiotic fixation is practically cancelled. The situation is similar in the two locations, the differences between the two functions being by no means significant.

A first observation is that, under the given conditions (soil, climate, agro-technical), pea crop doesn't need nitrogen fertilizers at all and that, on the contrary, their application brings twice damages to the agricultural crop rotation system. Taking into account the negative impact of synthetic fertilizers (Haber-Bosch), the use of biological fixation models is using the symbiosis between legumes (in our case, peas) and *Rhizobium leguminosarum* species. Considering that the application of phosphorus in a dose of about 60 kg/ha leads to an increase of about 50 kg/ha of fixed nitrogen, it follows that the optimal nutritional variant with NP is N_0P_{60-70}. In our experiments, this version obtained 5.97 g dry nodules/m^2, i.e. 59.7 kg/ha x 4,6 = 275 kg fixed N/ha. That means 275 – 235 = +40 kg N/ha brought by the N_0P_{60} version compared to the N_0P_0.

The coefficient 4.6 may vary between 4.1-4.7 kg N/1 kg dried nodules for conditions such as those investigated. It depends on the fixation intensity (I), which correlates with many optimization factors

According to the calculations made by the nitrogen balance method in crop and soil, a pea crop consumption of about 127.5 kg N/ha has resulted. The difference between what is left between the self-consumption and the fixed nitrogen and which is very variable, even in small spaces in culture, can be and is going to be used by the following crops. By doing so, peas become an excellent preceding plant, primarily for wheat, but also for rape and other crops. After Chaillet and Bousquet (2009) [4] and other authors, the nitrogen supply to the soil by peas crop can be used up to 60% of wheat, by these benefit taking advantage even the third crop in rotation system.

The increase in the percentage of legumes from the crop rotation system and in the intensity of the symbiotic fixation process solves, on the one hand, the problem of the proteins of plant origin, which are increasingly wanted in Europe, and on the other hand it raises the ecological and economic sustainability of the agricultural agroecosystems.

CONCLUSIONS

On the southern Romania soils, under semiarid conditions, but with a good agrotechnics, the Belmondo pea variety obtained yields of 3.5-4.0 t/ha and symbiotically fixed between 115 and 275 kg N/ha from air.

On the analysed area, between Alexandria and Calarasi, the amount of N symbiotically fixed didn't significantly detach from one area to another.

Phosphorus fertilizers raise pea's ability to fix nitrogen with up to 40-50 kg/ha over the P_2O_5-free version.

Application of nitrogen fertilizers greatly reduces inoculation and fixation, starting with the dose of 40 kg N/ha. At 120 kg N/ha, fixation ceases. The presence of nitrogen in soil prevents the communication between bacteria and plants.

The plants with more vigorous roots form a larger number of nodules and fix more nitrogen.

Fattening option recommended to pea's crop is N_0P_{60-70}.

REFERENCES

[1]Amel Benselama, 2015. Réhabilitation de la *culture* du *Lablab* purpureus et études de son partenaire symbiotique. Ph.D. Thesis. Oran University, Algeria,http://theses.univ-oran1.dz/document/14201511t.pdf, Accessed on 11 of January 2018

[2]Berca M. *et al*., 2015, Modelul HumProd pentru evitarea colapsului agriculturii românesti, generat de distrugerea humusului, ca resursă naturală. Buletinul Institutului Politehnic din Iaşi, Tomul LXI, Fasc. 3-4, pag. 47-63

[3]Bourion Virginie *et al.,* 2007, Genetic Variability in Nodulation and Root Growth Affects Nitrogen Fixation and Accumulation in Pea. Annals of Botany, Vol. 100, N0. 3, 589-598, https://www.ncbi.nlm.nih.gov/, Accessed on 12nd of January 2018pmc/articles/PMC2533614/, Accessed on 11 of January 2018

[4]Chaillet, I., Bousquet, N., 2009, La fixation de l'azote de l'air par les plantes. Une symbiosebactérie/plante. Perspectives Agricoles, N0. 358, pp. 34-35, https://www.perspectives-agricoles.com/file/galleryelement/pj/11/78/da/2e/358_8939196083206480358.pdf, Accessed on 11 of January 2018

[5]dos Santos, J.G.D. *et al.,* 2011, Soil Management and Efficiency of Rhizobia Strains of Cowpea *Vigna unguiculata* (L.) Walp. in the Tropics. Chilean Journal of Agricultural Research, Vol. 71(4):594-600, https://scielo.conicyt.cl/pdf/chiljar/v71n4/at15.pdf, Accessed on 11th of January 2018

[6]Hmida, C., Saoudi A., 2010, Etude de la variabilité des souches de Rhizobium efficientes pour l'haricot issues par radiomutagenèse. License applied in Biotechnology, http://www.iaea.org/inis/collection/NCLCollectionStore/_Public/42/056/42056536.pdf, Accessed on 12 of January 2018

[7]L'Taief, B. *et al.,* 2009, Effets de la fertilisation azotée, de l'inoculation par *Rhizobium* sp. et du régime des pluies sur la production de la biomasse et la teneur en azote du pois chiche. BASE – Biotechnologie, Agronomie, Société et Environnement, Vol. 14(4), 537-544, http://www.pressesagro.be/base/text/v13n4/537.pdf, Accessed on 12 of January 2018

[8]Loges, R. *et al.,* 2001, Methodische Aspektezur Bestimmung der symbiontischen N2-Fixierungsleistung von Leguminosen. Arbeits gemeinschaft für Grünland und Futterbau in der Gesellschaft für Pflanzen bauwissen schaften, Wissen schaftlicher Fachverlag: Giessen, pp. 29-32, http://orgprints.org/2147/1/loges-2001-methode-stickstoff-fixierung-leguminose.pdf, Accessed on 12 of January 2018

[9]Marquard, R., 2000, Stickstoff assimilation und die Symbiose mit Knöllchen bakterien. https://geb.uni-giessen.de/geb/volltexte/2000/320/original/sticksto.html, Accessed on 12 of January 2018

[10]Martins, L.M.V. *et al*., 2003, Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biology and Fertility of Soils, Vol. 38(6): 333-339

[11]Pietsch Gabriele *et al.,* 2006, Biological Nitrogen Fixation of Different Legume Species under Water Stress – BIOfix Project. http://www.academia.edu/13923676/BIOLOGICAL_NITROGEN_FIXATION_OF_DIFFERENT_LEGUME_SPECIES_UNDER_WATER_STRESS, Accessed on 11 of January 2018

[12]Quispel, A., 1982. Frontiers of Research in symbiotic Nitrogen Fixation. Frontiers of Research in Agriculture, Indian Statistical Institute, Calcutta, pag. 206-223

[13]Riou Virginie, 2016, Legumineuses et azote: comment ça marche? SOLAG – Bulletin Sol et Agronomie des Chambres d'agriculture des Pays de la Loire, no. 6, http://www.pays-de-la-loire.chambres-agriculture.fr/fileadmin/user_upload/Pays_de_la_Loire/022_Inst-Pays-de-la-loire/Listes-affichage-FE/RetD/Vegetal/Bul-SOLAG/2016/20160905_SOLAG_legumineuses_et_azote.pdf, Accessed on 12 of January 2018.