Measuring the BNF of Soybean Using ^{15}N-Labelled Urea with Different Atom Excess (A.E.) Content

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ABSTRACT

The soybean is a legume which has an ability to supply its major nitrogen need by the biological nitrogen fixation (BNF) process. This process is made possible by nodules formed in their roots, colonized by *Rhizobium* sp.bacteria. An accurate estimation of N gained by BNF is necessary to predict the increase or decrease of chemical fertilizer-N requirements to increase soybean production. Among several methods, the ^{15}N method was used to estimate the ability of legumes to perform BNF. The study involved soybean var. Willis (W) and a completely non-BNF soybean var. CV, which is termed as a standard crop. The standard crop is non-nodulated soybean, but it has the same main physiological traits with var. Willis. The aim of this study was to determine whether ^{15}N-labelled fertilizer with different %a.e. given to nodulated and non-nodulated soybean would not be of significant consequences for the calculation of N-BNF of W. The treatments applied were different rates of urea (20 kg N/ha and 100 kg N/ha) combined with different atom excess percentages (%a.e.)^{15}N (2% and 10%). Thus, the combination of treatments were as follows:(1) W-ll (20 kg N; 2% a.e); (2) CV-hl (100 kg N; 2% a.e); (3) W-lh (20 kg N; 10% a.e); (4) CV-hh (100 kg N; 10% a.e); (5) CV-ll (20 kg N; 2% a.e); (6) W-hl (100 kg N; 2% a.e); (7) CV-lh (20 kg N; 10% a.e); (8) W-hh (100 kg N; 10% a.e). The result of the experiment showed that a high %a.e. with a low rate of ^{15}N and a low %a.e. with a high rate of N should be used to study the %N-BNF of nodulated plants.

INTRODUCTION

Legumes, both as trees and as grain crops, are capable of supplying their nitrogen needs by the biological nitrogen fixation (BNF) process. This process is made possible by nodules formed in their roots. These nodules are colonized by the *Rhizobium* sp. bacteria. These bacteria are capable of absorbing N_2 from their environment and convert it to nitrate (NO_3). This N-source is subsequently used by the legumes, which act as the host plants.

According to Espana *et al*. [1] legumes play a vital role in sustaining agriculture. This role is manifested in providing green manure and organic material (OM). The green manure could be used as a source for several plant nutritions, especially N. As a source of OM, the green manure will thus enrich the soil organic matter (SOM) and is one of the factors which could increase soil fertility and maintain it.

Among the many grain crops used as a protein source in the form of food or cattle feed, soybean is a type of legume which has been widely studied in the past and will likely be studied in the future as well, since it is one of the globally most planted legumes.

The high protein content of soybean, which ranges from 20 to 40%, needs a high input of N. Calculations by People *et al*. [2] show that the production each ton/ha of this crop takes up 60 kg N/ha from soil. This figure is equal to the nitrogen content of 133 kg urea/ha. However, if the N source is urea, and the soybean cannot perform BNF, the figure has to be doubled to 266 kg urea/ha, since only about 40-50% of the applied urea-N can be utilized by the plant. Further according to People *et al*. [2], the production of soybean globally is around 2 t/ha. This implies that soybean has to be able to produce 120 kg N/ha by BNF. It has to be

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taken into consideration that with this figure soil fertility, especially for N-soil and OM, could be maintained.

An accurate estimation of N gained by BNF is necessary to predict the increase or decrease in the demands for nitrogen from chemical fertilizer for increasing soybean production. To carry out an accurate estimation, a method to measure BNF of legumes – in this case, soybean – is needed.

Herridge and Danso [3] have outlined several methods to estimate the ability of legumes to carry out BNF. These methods are: (a) difference method, i.e. the difference of N absorption between plants with and without BNF capability.The results are usually over- or underestimated, (b) xylem fluid method, based on the fact that the BNF process results in ureid and the non-BNF process produces nitrate (NO_3). This method is quite difficult to perform, especially in a field experiment, (c) acetylene reduction analysis (ARA) method, based on the function of nitrogenase enzyme, which plays a vital role in the BNF process. The enzyme converts acetylene (C_2H_2) to ethylene (C_2H_4). Its calculation is easy but the preparation for the calculation is difficult and there are still unanswered questions which influence the calculation, and (d) ^{15}N method, which uses ^{15}N-labelled fertilizer (urea, ZA or other N-sources fertilizer) or naturally abundant ^{15}N. This method is considered viable, but the constraints are that ^{15}N label and the equipment to count ^{15}Nare quite expensive.

According to Thomas [4], the^{15}N method is recognized as suitable and viable for use in greenhouse and field experiments. Several studies have used this method with satisfactory results [5,6,7]. In this paper, the use of the^{15}N method in researching the BNF capability of soybean is reported. In the past, the method's use in BNF research usually involved application of ^{15}N-labelled urea or ZA to the BNF and non-BNF plants at the same rate of atom excess percentages (%a.e.). However, in this study different %a.e. of the ^{15}N-labelled fertilizer wasused for BNF and non-BNF soybeans.

EXPERIMENTAL METHOD

Plant materials

In this experiment, two varieties of soybean were used: (i) the nodulated variety of var. 80 Willis (subsequently simply referred to as W), a national variety, and (ii) the non-nodulated variety of soybean var. CV, kindly donated by the IAEA. The non-nodulating soybean (CV) was used for experimental studies only and not to be used by farmers.

The ^{15}N method

In this study, the ^{15}N method was applied for the calculation of BNF in soybeans. However, a non-BNF crop was needed as a standard. The standard crops which could be used for soybeans are rice, barley, wheat or sorghum. However, the standard crop used must have the same physiological traits as the main nodulated soybean. These main traits are the same flowering time and the same maturity age. Another important trait is the same root depth to omit possible N-soil addition. Therefore, the ideal standard crop for soybean should be non-nodulated soybean. Fortunately, the standard plant can be obtained from the IAEA. The main physiological traits of soybean var. Willis and CV are all the same, except for the beginning of the pods' forming. The CV starts about 2–4 days ahead of the Willis, but this could be solved by harvesting the Willis and the CV at the same time. Two equations are usually used to calculate the BNF capability, expressed in percentage (%), of soybeans. The calculations are explained as follows:

1. If the nodulated and non-nodulated plants – namely, the Willis and the CV in this experiment – were given the same rate of ^{15}N-labelled fertilizer (for instance, both are given 20 kg N/ha or 100 kg N/ha), then the equation (1) for calculating the percentage of BNF (%BNF) is

$$\%BNF = \left(1 - \frac{\%N - diff\ in\ nodulated\ legume}{\%N - diff\ in\ non\text{-}nodulated\ legume}\right) \times 100\% \quad (1)$$

%N-diff = percentage of N derived from the ^{15}N-labelled fertilizer, whether it is N-labelled urea or ammonium sulphate. The nodulated and non-nodulated legume in this study were soybean var. Willis and CV respectively.
The %N-diff of nodulated or non nodulated legume is derived from equation (2):

$$\left[\frac{\%\ a.e.\ in\ nodulated\ or\ non\text{-}nodulated\ legumes}{\%\ a.e.\ in\ the\ N\text{-}15\ labelled\ fertilizer}\right] \times 100\% \quad (2)$$

The % atom excess (%a.e.) of the legumes is obtained by analyzing the legume samples by using ^{15}N analyzer, while the %a.e. of the ^{15}N-labelled fertilizer is provided by the label of %a.e. value on each fertilizer container.

2. Sometimes it is necessary to use different ratesof ^{15}N fertilizer for the nodulated and non-

nodulated legumes. If to both legumes are applied with, for instance, 20 kg N/ha, then this rate is sufficient for a good growth for the nodule legume but not for the non-nodulated legumes, since the roots of the nodulated legume, after it has reached an age of around one month, are already occupied by nodules which could provide N for further growth. For the non-nodulated legume, however, this is not the case. The 20 kg N/ha will be consumed by the non-nodulated legume in a short time, leavingno N from the fertilizer for further growth, resulting ina poor growth of the plant. This non-nodulated legume would be a bad standard plant for the nodulated legume, giving an overestimation of the capability of the nodulated legume. In this situation, different rates have to be used. In this case, the non-nodulated has to be applied with a higher rate of N-fertilizer, usually at a rate of 100 kg N/ha, which is equal to 217 kg Urea/ha. This high N-rate enablesthe non-nodulated legume to perform optimal growth and this would avoid the overestimated %N-BNF of the nodulated legume. The equation (1) would become equation (3) as follows:

$$\%BNF = 100 - \left[\left(1 - \frac{\%N - diff\ nodulated\ legume}{\%N - diff\ non - nodulated\ legume}\right) + \%N\% - diff\ nodulated\ legume\left(1/n - 1\right)\right] \quad (3)$$

where *n* is found from equation (4):

$$n = \frac{the\ rate\ of\ N\ applied\ to\ nodulated\ legumes}{the\ rate\ of\ N\ applied\ to\ non\text{-}nodulated\ legumes} \quad (4)$$

In which, for this example *n* is 20/100 = 0.2

For detailed information on these equations, see Hadarson and Danso [8] and Sisworo, *et al.* [9].

Treatments applied

The treatments carried out in this experiment were:

1. Soybeans Willis and CV were applied with ^{15}N-labelled Urea rates of 20 and 100 kg N/ha respectively. The ^{15}N-labelled Urea hasa low % atom excess (%a.e.) of 2.5.
2. Both soybean plants were applied with the same treatment as in treatment 1, butthe %a.e. in the ^{15}N fertilizer was high, namely 10%.
3. The rates of the ^{15}N-labelled urea appliedwere the same but the %a.e. of the ^{15}N-labelled urea for Willis and CV were 2% and 10% respectively.
4. The same treatment as in treatment 3 but the %a.e. were reversed, i.e. high %a.e. for Willis and low %a.e. for CV, which were 10% and 2% respectively.

Table 1. The treatments given in the tabulation form.

Soybean Variety	Rate of ^{15}N urea (kg N/ha)		%a.e.		Treatments code
	20	100	Low (2%)	High (10%)	
Willis	X		X		W-1
CV		X	X		CV-1
Willis	X			X	W-h
CV		X		X	CV-h
Willis	X		X		W-1
CV		X	X		W-h
Willis	X			X	W-h
CV		X		X	CV-1

In this experiment, four pairs of Willis and CV soybean were used:

W-l CV-l = Willis and CV applied with ^{15}N-labelled urea having low % a.e. (2%).

W-h CV-h = Willis and CV applied with ^{15}N-labelled urea having high %a.e. (10%).

W-l CV-h = Willis and CV given ^{15}N-labelled urea with different %a.e. Willis withlow %a.e. (2%) and CV with high %a.e. (10%).

W-h CV-l = Willis and CV given ^{15}N-labelled urea with different % a.e. Willis with high %a.e. (10%) and CV with low %a.e. (2%).

Experimental design

The randomized block design (RBD) was used in this experiment. There were four treatments where each treatment was replicated four times. There were 32 pots, 16 potsof Willis and 16 potsof CV. To test whether any difference in outcome occurred between the treatments, ANOVA was carried out as described by Little and Hills [10].

The aim of the experiment

The aim of the experiment was to determine whether the different treatments resulted in any differences in the %N-BNF of the Willis. If no differences were found, then treatment no. 4

would be highly recommended for application in further studies in determining the %N-BNF because regardless of the rate of ^{15}N-labelled urea, with the same high or low %a.e., which is applied to nodulated and non-nodulated soybeans, there will be a problem arising from the cost of the experiment, which will be explained as follows:

If the first treatment is applied (W-l and CV-l), for %N-BNF calculation, the Willis will face a problem in that by applying only 2 %a.e. at a rate of 20 kg N/ha, the % a.e. analysis in the samples could be misread. That is due to the great possibility that at 20 kg N/ha, ^{15}N-labelled urea is diluted by N-soil resulting in a small % a.e. This small %a.e. sometimes could be misread depending on the equipment used to perform the analysis. For the CV, a low %a.e. does not matter due to the high rate of ^{15}N-labelled urea applied. The decrease in the value of %a.e. diluted by N-soil is not as great as in the case of the Willis at a rate of 20 kg N/ha.

RESULTS AND DISCUSSION

The most important data to be considered in Table 2 is the %N-Fr, which is the %N-derived from fertilizer, which in this case is the ^{15}N-labelled urea. The values of %N-Fr is calculated from equation (5):

$$\left[\frac{\%\ atom\ excess\ (a.e.)\ of\ sample}{\%\ a.e.\ in\ the\ N\text{-}15\ fertilizer}\right] \times 100\% \quad (5)$$

The data shows clearly that %N-Fr of the non-nodulated soybean (CV) is higher than that of the nodulated soybean (W) (see Table 2). This is true for each treatment, as well asfor the grand total (Ro-to). The values of Ro-to for the CV (average of four plants) in %N-FrCV for roots, straw, pods (39.470, 47.246, and 46.682 respectively) are higher than the valuesof Ro-to of W in %N-FrW for the same plant parts (6.424, 8.426, and 8.121 respectively) as shown in Table 2. Had the reverse happened, i.e. %N-FrW> %N-FrCV, then it would mean that no BNF process occurred in the nodulated soybean (W). The BNF process was absent because when the ^{15}N-labelled fertilizer, in this case^{15}N urea, was absorbed by W, the absorbed ^{15}N in the plant body would be diluted by two sources of N, namely, the N derived from the BNF process and N-soil, resulting in a quite low %a.e. This would further lower the %N-Fr of W for all plant parts as shown in Table 2. As for the CV, the ^{15}N from fertilizer will be diluted only by N-soil (N-S). This is due to the CV being a non-nodulating soybean, which means the CV has no nodules in the roots resulting in a high %N-Frfor all plant parts.

Table 2. Percentage N-derived from fertilizer (%N-Fr), BNF (%N-BNF) and soil (%N-S) in roots, stover and pods of soybean Willis (W) and CV.

Plant Parts	Code of Treatment	Percentage N-derived from		
		Fertilizer (%N-Fr)	BNF (%BNF)	Soil (%N-S)
Roots	Wll	5.984	42.184	51.832
	Whh	5.895	44.826	49.279
	Wlh	8.163	46.081	45.756
	Whl	5.655	41.280	53.065
	Ro-to	6.424	42.652	49.983
	F-calculated	3.458ns	1.290ns	1.006ns
	CV (%)	19.54	12.56	12.86
	CVll	37.330	—	62.667
	CVhh	38.779	—	61.221
	CVlh	47.081	—	52.919
	CVhl	34.689	—	65.311
	Ro-to	39.470	—	60.350
	F-calculated	2.332ns	—	2.331ns
	CV (%)*	17.95	—	11.58
Stover	Wll	6.844	39.888	53.628
	Whh	9.326	42.316	48.298
	Wlh	8.232	50.170	41.598
	Whl	9.303	47.515	43.182
	Ro-to	8.426	44.987	46.586
	F-calculated	6.062**	6.460**	6.757**
	CV (%)	11.30	8.21	8.74
	CVll	39.093	—	60.097
	CVhh	49.262	—	50.738
	CVlh	49.062	—	50.398
	CVhl	51.025	—	s48.975
	Ro-to	47.246	—	54.904
	F-calculated	7.493**	—	2.670n
	CV (%)*	8.49	—	9.99
Pods	Wll	9.602	46.940	43.580
	Whh	8.203	44.627	47.176
	Wlh	8.034	45.747	46.219
	Whl	6.643	42.586	50.771
	Ro-to	8.121	44.975	46.905
	F-calculated	1.880ns	0.894ns	2.073ns
	CV (%)	21.74	8.71	8.94
	CVll	51.596	—	48.404
	CVhh	46.215	—	53.785
	CVlh	45.519	—	54.481
	CVhl	39.987	—	60.613
	Ro-to	45.682	—	54.321
	F-calculated	1.629ns	—	1.630ns
	CV (%)*	17.14	—	14.41

Remark : *CV in this table and the following tables are coefficient of variation express in percentage (%).

It is important to point out that each plant part, namely roots, stover and pods, has to be analyzed separately and not mixed in one sample, as each plant part had different N stored in them. All values of %N-Fr will be used in the following equation (6) from the section on Materials and Methods:

$$\%BNF = 100 - \left[\left(1 - \frac{\%N - diff\ nodulated\ legume}{\%N - diff\ non - nodulated\ legume}\right) + \%N\% - diff\ nodulated\ legume\left(^{1}/_{n} - 1\right)\right] \quad (6)$$

From this equation the values of %N-BNF could be calculated. Further, for the %N-S, the values of %N-BNF are only a deduction. For example, %N-S of roots, stover or pods are: 100% - %N-BNF - %N-Fr for W, while for CV it would be 100% - %N-Fr. These calculations have to be done for each plant part.

Data presented in Table 2 showed that for the W the lowest % of N is %N-Fr and the highest is %N-S, while %N-BNF is in the middle of these two values. This is true for roots, stoverand pods of the W. The values of %N-Fr, %N-BNF, and %N-Sfor roots are 6.424, 42.652, 49.983; for stover: 8.426, 44.987, 46.586; and for pods: 8.121, 44.975, 46.905 respectively. As for the CV, the highest %N is found in %N-S and the lowest is %N-Fr. The values of %N-S and %N-Fr for roots are 60.530, and 39.470; for stover are 54.904, 47.246; and for pods are 54.321, 45.682 respectively.

The data showed that for the W, the %N-BNF is able to provide N for the plant needswith nearly the same level of %N-S. This means that the nodulated soybeans are able to meet the need for N themselves through the BNF process whenever nodules are attached to their roots. Since these nodules could not occur at a location where there were no rhizobia available, this meant that the soil in the pots used in this study must already have contained at least indigenous rhizobia. It might be that those indigenous rhizobia were in the soil used because the soil was taken from a field where nodulated soybeans and cowpeas were planted frequently. Rhizobia contained in the nodules of these two species must have contaminated the soil, leaving it with the indigenous rhizobia.

Several studies reported that efficient rhizobia, developed in the lab and usually commercially available, could increase the %N-BNF up to 70–80% [1]. This shows that although only indigenous rhizobia is suspected to perform the BNF process and produce around 40% of N-BNF, it is still be able to produce the N needed by the plants studied. For the non-nodulated soybean (CV), the N-Frclearly has a significant of contribution, besides N-S, to its growth. In this study, the non-nodulated soybean was only used as a standard crop in order to calculate the %BNF of the nodulated soybean (W). Therefore, it would not be further discussed elaborately, in this paper.

The percentages of N-BNF, N-Fr, and N-S could not be expressed by the plant parts separately. It had to be done for the whole plants (roots + stover + pods). To obtain this data first the N-total uptake of each plant part had to be collected. This was done by the Kjeldahl method, where a sample of each plant part was analyzed and the values obtained were expressed in %N-total. After having obtained the %N-total, these values were multiplied by the dry weight of each plant part and were expressed in mg N/plant part.Table 2 shows these values.

Table 3. Total N-uptake in roots, stover, grain and plants by soybean var. Wilis and CV.

	N-total uptake in			
	Roots	Stover	Pods	Plants
	(mgN)			
Wll	51.530	105.594	21.550	176.924
CVll	25.384	112.012	22.419	159.807
Whh	49.679	129.053	25.666	203.031
CVhh	17.474	109.060	23.048	149.856
Wlh	43.056	132.325	19.667	194.047
CVlh	20.586	122.613	27.258	170.458
Whl	44.070	148.922	19.266	211.005
CVhl	24.072	134.953	25.533	184.578
Total mean $(\bar{x} - \rho)$				
W	47.084	128.974	21.537	196.252
CV	21.947	119.660	24.563	166.175

Table 3 shows that the N-total uptake by the W was higher than that by the CV for the roots, stover and the whole plant (roots + stover + pods) but not for the pods. As previously mentioned, the pods of the CV forms 3-4 days earlier compared to W. This might be the cause of the total-N of the CVs' pod being higher than that of W. Apparently, a large portion of N, gathered by the vegetative part of the plant, was deposited at an earlier stage in the CV and at a later stage in the W. This, then, resulted in higher total-N in pods of the CV compared to that of W. However, because the percentage of N-BNF of the W will be expressed for the whole plant this difference has no significant influence on the values of the %N-BNF of the W.

The data in Table 4 is the result of %N-BNF, %N-Fr, and %N-S multiplied by the N-total uptake of each part and expressed in mg N.

The data shows that there was no difference among the treatments for each separated plant pods This means that regardless of the %a.e. of the treatments applied, it did not influence the N-uptake by N-FNB, N-Fr and N-S. In Table 5 the data presented are for the whole plant. For example, N-FNB uptake-whole plant (mgN) = N-FNB roots + N-FNB stover + N-FNB pods whose data were

taken from Table 4. The same calculation was done for N-Fr and N-S for the whole plant.

Table 4. N-uptake derived from fixation (BNF), fertilizers (Fr), and soil (S) in the roots, stover, and pods of soybean var. Willis.

	N derived from		
	BNF	Fr	S
	(mgN)		
Roots			
Wll	19.813	2.831	27.091
Whh	21.673	2.910	25.140
Wlh	18.660	3.297	21.087
Whl	20.639	2.842	20.081
Stover			
Wll	48.819	8.083	48.693
Whh	51.775	10.382	66.898
Wlh	48.819	11.139	63.908
Whl	58.549	13.485	63.679
Pods			
Wll	8.812	2.327	10.411
Whh	11.919	2.571	11.230
Wlh	9.016	2.077	8.574
Whl	8.764	2.173	8.330
Total - X(x- total)			
Roots	20.250	2.970	23.350
Stover	56.978	10.772	60.792
Pods	9.628	2.273	9.636
F-calculated			
Roots	0.301ns	0.250ns	1.470ns
Stover	3.282ns	1.845ns	1.718ns
Pods	1.413ns	0.666ns	0.760ns
CV (%)			
Roots	22.81	29.69	23.42
Stover	19.11	17.58	20.58
Pods	26.79	20.70	33.59

The data in Table 5 shows no difference among the treatments for N-BNF and N-S but shows a differencein N-Fr.

Table 5. N-uptake derived from fixation (BNF), fertilizers (Fr) and soil (S) in plants of soybean var. Wilis.

	N derived from		
	BNF	Fr	S
	(mgN)		
Wll	77.504	13.241	86.172
Whh	85.368	15.807	102.019
Wlh	83.966	16.512	93.569
Whl	100.695	18.493	92.333
Total- x	86.883	16.013	93.523
F-calculated	1.799ns	4.237*	0.488ns
CV (%)	16.95	13.17	19.98

In this study, each treatment consisted of 4 replicates, and each replicate is represented by one individual plant. Note that N-Fr uptake depended on the roots of each individual plant, and this could mean that the differences in the outcome of the treatments were partly caused by the differing performances of the roots of each plant in the uptake of N-Fr.

Table 6 is the continuation of the data in Table 5 expressed in percentages (%). As previously mentioned, the %N-BNF, %N-Fr and %N-S for plants could not be expressed for each plant part but have to be shown for the whole plant. The data in Table 5 is obtained by using equation (7) as follows:

$$\%BNF - whole\ plant = \frac{N - BNF\ uptake\ whole\ plant}{N - total\ uptake\ whole\ plant} \times 100\% \quad (7)$$

The data in Table 6 shows that regardless of the treatments, there was no significant difference in the %N-BNF. It could be stated that whether a low %a.e. or a high %a.e. was used in combination, as conducted in this study, it would not influence the %N-BNF. Thus, the optimal combination, especially from the price of ^{15}N-labelled fertilizer, could be chosen without doubt.

Table 6. Percentage of N-derived from fixation (%N-BNF), fertilizer (%N-Fr) and soil (%N-S) in the whole plant of soybean var. Wilis.

	%N derived from		
	BNF	Fr	S
Wll	44.215	7.744	48.282
Whh	42.269	7.701	50.231
Wlh	48.817	8.622	47.652
Whl	47.535	8.756	43.710
Total -x	44.411	8.205	47.468
F-calculated	1.679ns	1.753ns	1.743ns
CV (%)	7.93	10.32	8.73

In this case, it would be a high %a.e. of the labelled fertilizer for the nodulated soybean W (20 kg N/ha) and a high %a.e. for the non nodulated soybean CV (100 kg N/ha). Consequently, the recommended treatment is Whl.

An additional observation from Table 6 is that N-BNF and N-S made nearly equal contributions to the N needed for plant growth. This is shown by the values of %N-BNF and %N-S for the Total x which are 44.411% and 47.468% respectively. On the other hand, the values for %N-Fr are quite low, less than 10%. This is expected due to the low N-fertilizer applied (20 kg N/ha) and the presence of two other sources, N–BNF and N-S,which, in this case, are apparently quite abundant.

It has to be pointed out that the capacity of nodulated plants in absorbing N from soil, fertilizersand, especially, from BNF is commonly expressed in percentages (%), which is the reason Table 6 is presented as the concluding Table in this study.

A study for determining the optimal percentage of atom excess (%a.e.) of ^{15}N-labelled

fertilizer applied to nodulated and non-nodulated soybean was carried out. The aim of this study was to determine whether ^{15}N-labelled fertilizer with different %a.e. given to nodulated and non-nodulated soybean namely the Wilis (W) and the CV would not be of significant consequences toward the calculation of N-BNF of W. The calculations of the absorption of N, whether from fertilizer (Fr), Biological Nitrogen Fixation (BNF) or soil (S) have to be done separately for each plant part, in this case, roots, stover and pods. Based on those values, the %N-BNF, %N-Fr, %N-S of W could be combined for the whole plant (roots + stover + pods). The difference atom excess method to measure N_2 fixation has previously been performed by Hardarson and Danso (1990). The results of their experiment suggested the use of a lower dose of ^{15}N (20 kg N/Na) for high atom excess content (5% a.e) and a higher dose of ^{15}N (100 kg N/ha) for low atom excess content [8]

The data showed that the four different treatments applied in this study resulted in no difference in the %N-BNF of the Wilis. It could be determined that for future studies on thenodulated soybean to calculate its %BNF, the fourth treatment is highly recommended. The fourth treatment (Wh CV) consists of using ^{15}N-labelled fertilizer with a high %a.e. at a low rate of N-fertilizer for the nodulated soybean and a low %a.e. at a high rate of N-fertilizer for the standard plant (non nodulated plant), which, in this study, was the CV.

CONCLUSION

A study for determining the optimal percentage of atom excess (%a.e) of ^{15}N fertilizer to be applied to nodulated and non-nodulated soybeans was carried out. The aim of this study was to determine whether ^{15}N-labelled fertilizer with different %a.e. given to nodulated and non nodulated soybeans, namely the Wilis (W) and the CV, would not be of significant consequences for the calculation of N-BNF of W. The calculations of the absorption of Nby the W and the CV, whether from BNF, Fror S, have to be done separately for each plant part. Based on these values the absorption of N expressed in %N-BNF could be expressed for the whole plant as is the common rule for expression of %BNF. For the purpose of further studies it is recommended that the optimal %a.e. for studying the %N-BNF of nodulated plant should be a high %a.e. with a low rate of N-fertilizer and a low %a.e. with a high rate of N-fertilizer.

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