Bul. Agron. (31) (1) 31 – 36 (2003)

Nitrogen Transfer of Two Cultivar Faba Bean (*Vicia faba* L.) to Oat (*Avena sativa* L.)

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ABSTRACT

Nitrogen fixed by the legume could be used by other plants, such as through rhizodeposition and direct transfer between roots. The possibility of N transfer in intercropping legum-cereal have long been observed, especially legum with short and dense root. This experiment had been carried out to determine whether there was N transfer from faba bean (Vicia faba L.) growing in association with oat (Avena sativa L.) and whether there was difference between two cultivars of faba bean in the amount of N transfer. Methods used were complete-mixed-root (CMR) between faba bean and oat, and mixed half the root of faba bean with oat (SR). As a tracer isotope ^{15}N *was used in form of* $K^{15}NO_3$. *The experiment was carried out from May 2000 through January 2001 in Institute of Agronomy and Plant Breeding, University of Goettingen Germany. The data of CMR method had great variance. Using SR method a positive value of* ^{15}N *enrichment was found in oat indicated that transfer N from faba bean cv. Minica and Scirocco occurred. There was no difference found in the amount of N transferred between the two tested cultivars.*

Key words: N transfer, ^{15}N*, Faba bean, Oat*

INTRODUCTION

Because of the ability of legumes to fix N_2 from atmosphere through their symbiosis with *Rhizobium* bacteria, legume crops are often included in intercropping systems. Giller and Wilson (1991) described mechanisms by which legume nitrogen can be made available to other plants, included rhizodeposition, root and nodule senescence, and direct transfer of N between roots. Nitrogen transfer from legume to cereal might have the potential for further manipulation to increase cereal yields (Vandermeer, 1989).

Addition yield from mixtures of non legumes and faba beans compared to mean yield of sole crops are often to be found (Jensen, 1986; Bulson *et al.*, 1997; Li *et al.*, 1999). Li *et al.* (1999) suggested that the beneficial effect of faba bean/maize mixtures was the results of transfer of substances from faba bean to maize via roots. In the field studies neither Danso *et al.* (1987), who used the ^{15}N dilution method, nor Cochran and Schlentner (1995), who using N difference method, found an evidence of N transfer from faba bean to cereals. The roots of faba bean are relatively large and sparse so that sloughing of legume roots is not provided. On the other hand, N transfer to non-legume could be proved for numerous grain legumes such as pea (Jensen, 1996) and soybean (Van Kessel *et al.*, 1985; Martin *et al.*, 1991; Hamel *et al.*, 1991).

The proportion of N, which transferred from legume to non-legume plants, depends on the ability of N_2 fixation by the legume (Ta and Faris, 1987) and the growing condition of the legume (Giller and Wilson. 1991). Since species or cultivar of legume plants, which have different growth habit, may have a different capacity to fix N, it is likely that the proportion of N transfer could be also different between species and cultivars.

Faba bean is a wildly cultivated grain legume, it occupies nearly 2.3 x 10^6ha world-wide (FAO, 2000). There is a lack of information on N transfer and the difference of N transfer from different cultivars of faba bean. Therefore, the following experiment had been carried out with the aim: to determine whether there was N transfer from faba bean, to measure the proportion of N transferred from faba bean to companion plant oat (*Avena sativa* L) and to examine whether there are differences between cultivars of faba bean in the amount of N transfer.

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MATERIAL AND METHODS

Plants, Nutrients and Growth Conditions

The experiment has been carried out at the beginning of summer, in the glasshouse of the Institute of Agronomy and Plant Breeding, University of Goettingen, from May 2000 through January 2001. At that time temperature in the glasshouse was at 15.9 - 29.0 °C and relative humidity of 34.8 - 62.7 %. Two cultivars of faba bean (*cv. Minica* and *Scirocco*) were used as donor plants and onm(..Uplants and on..Uoat (*cv. Lut* was used as receiver plant. Faba bean *cv. Minica* had big seed and *cv. Scirocco* had smaller seed. A nutrient solution (Vincent, 1970) containing basic nutrient, but without N, was given every two days together with pouring. In experiment 1 plants were planted in vermiculite, an N-free media, and in experiment 2 a clay loam soil from the university experimental field was used only in receiver pot. The soil contained for about 0.15 % total N and 1.64 % total C with pH_{H2O} 6.30.

Experimental Designs

The contribution of faba bean-N to the N nutrition of companion plant was studied using two technique named as the complete-mixture root (CMR) and split root (SR). All treatments in these experiments have been arranged in a randomised complete block design with six replications.

Complete-Mixture Root

Plants were grown in PVC-pot holding 12 litre N-free vermiculite. One day after the root emergence in moist filter paper, seedlings of faba bean and oat were transplanted to the experiment units. Before planting, seedlings of faba bean were inoculated with Rhizobium. Twelve seedlings of faba bean *cv. Minica* were planted in a circle and one seedling of oat was planted in the centre (treatment M). The same treatment was done to faba bean *cv Scirocco* (treatment S). To see whether there was N transported through air, oat was planted alone in 12-litre-PVC pot and twelve plants of legume were placed nearby in six 5-litre-PVC pots (treatment m and s). Control of unlabelled fertilized (C1) and labelled- fertilized-oat growing alone (C2) were also included.

Three weeks after transplanting, all pots, except treatment C1 and pots of faba bean in treatment s, were fertilised with 10 mg N, 5 atom % ^{15}N as $K^{15}NO_3$. Pots C1 was fertilized with the same amount of N using unlabelled KNO_3.

Split Root

The split root technique used is based on Jensen (1996), who worked with pea and barley. Faba bean was grown with the root divided between two pots. The experiment unit was a 2-litre pot which contained only moist-N-free vermiculite ('Donor' pot) and a 5-litre pot as 'Receiver' pot which contained a mixture of soil and vermiculite (1:1 $^{v}/_{v}$).

Seeds were germinated in N-free vermiculite, which have been washed with deionized water before transplanted into the experimental units. Four days after sowing, when the radicle root had ± 5 cm long, the major part of the radicle was cut to promote the lateral roots to grow. The upper 2 cm of the out coming root was left intact. Then, seedlings were replanted and grown in N-free-vermiculite for further 12 days to allow the plants to produce the lateral roots before being transplanted into the experimental units. Half part of faba bean root was placed in 'Donor' pot and the other part in 'Receiver' pot. One seedling of oat (one week old) was planted in 'Receiver' pot, 6 cm apart from faba bean. As control, one seedling of oat was planted alone in a 5-litre pot. Ten days later, half of pots 'Donor' received 20 mg N, 5 atom % ^{15}N (as KNO_3). The amount of 5 atom % ^{15}N was added every 3 days to the 'Donor' pot for continuous labelling. The other half of pots 'Donor' and oat growing alone as were control plants were fertilised with non ^{15}N-enrichced KNO_3 with the same amount of N.

Harvest and Analytical Methods

Plants harvested 90 days after transplanting. In experiment 1 estimation of N transfer will be calculated based on the shoot-N because the difficulty to separate roots of faba bean growing together with oat. In experiment 2, plants were separated into shoot and root. This was possible because the roots of faba bean and oat have different colour. Faba bean roots have darker colour then the oat roots. The shoots and roots of the plants were washed with deionized water, dried at 60°C for 24 hours, weighted, grounded to ≤ 2 mm and analyzed for total N and $^{15}N/^{14}N$ ratio (Finnigan MAT 251) at Isotope Laboratory for Biological and Medical Research of the University of Göttingen. Before calculating N transfer, the ^{15}N excess of the plants was calculated with the ^{15}N enrichment of oat growing alone as control plant.

Calculation of N Transfer

Estimation of faba bean N contribution to total N of oat in experiment 1 was calculated through comparing the δ ^{15}N of labelled N fertilizer and δ ^{15}N units uptake found in oat in treatment M, m, S and s. If the value of δ ^{15}N units uptake of oat in treatment M, m, S and s were found to be lower than δ ^{15}N of labelled N fertilizer, it indicates that there was some amount of nitrogen transferred from legume to oat.

The of value of δ ^{15}N units uptake was calculated using equation :

$$\delta\ ^{15}N \text{ units uptake} = \frac{|\delta\ ^{15}N \text{ unit receiver} - \delta\ ^{15}N \text{ unit control (C1)}|}{|\text{total N receiver} - \text{total N (C1)}|} \quad (1)$$

whereas δ ^{15}N unit (receiver or control) was :

$$\delta\ ^{15}N \text{ unit} = \delta\ ^{15}N \times \text{total N} \quad (2)$$

In the experiment 2, the transfer of N was estimated using a method known as 'Donor root enrichment' (Giller *et al.*, 1991). The proportion of N in the receiver plant derived from donor plant root (% Ndft root) was calculated under assumption that N from donor plant deposited in the rhizosphere and taken up by the receiver plant had the same ^{15}N enrichment during the labelling period as the donor root at the time of harvest.

$$\%\ \text{Ndft root} = \frac{\text{atom } \%\ ^{15}N \text{ excess}_{\text{receiver plant}}}{\text{atom } \%\ ^{15}N \text{ excess}_{\text{donor root}}} \times 100 \quad (3)$$

The amount of N transferred (mg plant $^{-1}$) is calculated as :

$$\text{N transferred} = \%\ \text{Ndft root} \times \text{total N receiver} \quad (4)$$

$$\%\ \text{N transfer} = \frac{\text{N transferred} \times 100}{(\text{total N donor} + \text{N transferred})} \quad (5)$$

RESULTS AND DISCUSSION

The data of experiment 1 was found to have greatly variance within replications, therefore only the mean values were showed in Table 1.

The oat growing together with faba beans showed the lowest δ ^{15}N value, but it presumably as a result of competition, since total N uptake was lower than that from oat growing alone surrounded by faba (treatment m and s). If the intercropped non legume had less ^{15}N as well as more total N than the sole crop non legume it would indicate that the ^{15}N had been diluted from the legume (Martin *et al.,* 1991). The δ ^{15}N uptake of oat growing together with cv. *Minica* was much lower than δ ^{15}N value of labelled N fertilizer. The lower value of δ ^{15}N uptake could be caused by uptake N from mineralization (Giller and Wilson, 1991). During the first weeks of this experiment, faba beans were transplanted twice because the microclimate inside the glasshouse was not favorable. This situation could lead to mineralization of decayed-seed.

Table 1. Means value of shoot dry matter, N uptake, N content and δ ^{15}N uptake of oats in experiment 1.

Treatment	Dry Matter (mg)	Total N uptake (mg)	δ ^{15}N (‰)	N content (%)	δ ^{15}N uptake (‰)
C1 (control $^{-15}$N)	170	1.01	68.208	0.59	
C2 (control $^{+15}$N)	220	1.51	4366.095	0.65	
OM	170	2.68	2278.638	1.12	9040.974
OS	210	1.42	3939.530	0.65	19630.479
Om	370	1.91	5585.948	0.72	15817.665
Os	200	1.37	4348.230	0.71	16133.405

Note : OM = oat growing together with faba bean *cv. Minica*
OS = oat growing together with faba bean *cv. Scirocco*
Om = oat growing surrounded by faba bean *cv. Minica*
Os = oat growing surrounded by faba bean *cv. Scirocco*

In experiment 2 faba bean was 16 days older than oat. Although the oat grew poorly, oat in the 'Receiver' pot did not get any additional N fertilizer. The source for N for oat, solely, came from soil and N, which deposited by faba bean. The half root of faba bean growing in 'Donor' pot was not included for calculating dry matter weight, total N uptake and ^{15}N enrichment. Since this half root was directly contacted to N fertilizer, it would add a considerable amount of N and ^{15}N to the whole plant basis.

There were no different in plant dry weight and total N uptake of oat growing with ^{15}N-fertilised faba beans or unlabelled-fertilized faba beans, although the root of oat growing together with unlabelled-fertilized *Minica* contained lower N compared to oats root growing together with ^{15}N-fertilized *Minica.* Oat growing with ^{15}N-fertilized *Scirocco* showed higher ^{15}N enrichment compared to oat growing with unlabelled-fertilized *Scirocco,* whereas in oat growing with ^{15}N-fertilized *Minica* only the root had higher ^{15}N enrichment (Table 2).

Table 2. Plants dry matter, total N uptake and weighted % ^{15}N excess of oat growing together with faba bean fertilized with or without ^{15}N.

	Dry matter (mg plant^{-1})			Total N uptake (mg plant^{-1})			Weighted % ^{15}N excess		
	Shoot	Root	plant	Shoot	Root	plant	Shoot	Root	Plant
Oat(+*Minica* ^{-15}N)	1875	695	2552	37.757	6.415	44.173	0.038	0.001	0.0009
Oat(+*Minica* ^{+15}N)	1870	1028	2898	40.405	10.369	50.774	0.064	0.025	0.0017
HSD(0,05)	ns	ns	ns	ns	0.047	ns	Ns	0.018	ns
Oat (+*Scirocco* ^{-15}N)	2152	810	2962	45.768	7.068	52.835	0.023	0.00002	0.0001
Oat (+*Scirocco* ^{+15}N)	2177	1215	3392	47.086	10.027	57.112	0.037	0.0096	0.0008
HSD(0,05)	ns	ns	ns	ns	ns	ns	Ns	0.028	0.029

Note: ns = no significant

Oat(+*Minica* ^{-15}N) = Oat growing together with *cv. Minica* fertilized by KNO_3

Oat(+*Minica* ^{+15}N) = Oat growing together with *cv. Minica* fertilized by $K^{15}NO_3$

Oat(+*Scirocco* ^{-15}N) = Oat growing together with *cv. Scirocco* fertilized by KNO_3

Oat(+*Scirocco* ^{+15}N)= Oat growing together with *cv. Scirocco* fertilized by $K^{15}NO_3$

% ^{15}N excess was based on the difference between ^{15}N enrichment of oat growing together with faba beans and oat control

Growing together with faba bean did not influence oat growth, but reduced total N uptake of oat and shoot-N was much more affected than root-N (Table 3). Between the two cultivars of faba bean, *Minica* had larger shoot dry weight and tended to accumulate more shoot-N (P = 0,090) than *Scirocco*. There was a tendency that oat growing together with *Minica* had a lower N uptake compared to the oat growing with *Scirocco*.

Since oat did not directly fertilized with ^{15}N, the positive value of ^{15}N enrichment found in shoot and root of oat was an indication that N had been transferred from faba beans to oat. Oat growing together with *Minica* had significantly more ^{15}N enrichment than oat growing together with *Scirocco* (Table 3). The higher ^{15}N excess in oat growing with Minica appeared, more likely, because of oat growing together with *Minica* took up less N compared to the oat growing with *Scirocco*, rather than because of *Minica* transferred more N than *Scirocco*. Table 4 showed that the proportion of N in oat that came from *Minica* was not significantly higher than the proportion of N came from *Scirocco*.

Table 3. Plants dry matter, N uptake and weighted ^{15}N excess of oat and faba bean.

	Dry matter (mg plant^{-1})			Total N uptake (mg plant^{-1})			Weighted %^{15}N excess		
	Shoot	Root	plant	Shoot	Root	Plant	Shoot	Root	Plant
oat control	2425	1080	3505	60,349 a	10.377	70.816 a			
oat (+ Minica)	1870	1028	2898	40,405 b	10.369	50.774 b	0.064	0.025	0.002 a
oat (+ Scirocco)	2177	1215	3392	47,086 ab	10.027	57.112 ab	0.037	0.010	0.001 b
HSD(0,05)	ns	ns	ns	0.014	ns	0.038	Ns	ns	0,020
Minica (+ oat)	6815 a	3790	10 605	151.042	36.355	187.397	36.418	3.002	0.215
Scirocco (+ oat)	5562 b	3747	9 308	126.535	40.114	166.649	18.437	2.037	0.125
HSD(0,05)	0.036	ns	ns	Ns	ns	ns	Ns	ns	ns

Note: ns = no significant.

Numbers with different letters indicates significant difference.

% ^{15}N excess was based on the difference between ^{15}N enrichment of oat growing together with faba bean and oat control.

Table 4. Estimated N-transfer[1)] from faba bean cv *Minica* and *Scirocco* to oat.

Treatments	Donor root enrichment method		
	N transfer[2)] (%)	N transferred (mg N plant^{-1})	Ndft root[3)] (%)
Oat growing With *Minica*	0.68	1.280	2.51
Oat growing With *Scirocco*	0.58	0.927	1.62
HSD(0,05)	ns	ns	ns

Note : 1) = calculation for plant excluded the half root in pot 'donor'
2) = N transferred as percentage of faba bean-N
3) = N transferred as percentage of oat-N
ns = no significant.

Despite the low N nutrition condition created in 'Receiver' pot to enhance faba bean-N-fixation, there seemed no immediate benefit of N to oat. At the time of harvest, *Minica* contained in average 1.98% N and *Scirocco* 1.99% N. This N uptake of faba beans was roughly three times of total N in oat but only less than 0.68% of faba bean's N was transferred to oat. Giller *et al.* (1991) and Jensen (1996) found that the amounts of N transferred from bean to companion plant in intercropping might improve under a severe limitation on the growth of the beans such as an insect attack or shading.

We found no correlation between the amount of N, which transferred to oat, neither to the amount of N taken up by faba beans, nor the amount of N left in the media of 'Receiver' pot after harvest.

Jensen (1996) explained that the donor root enrichment method may give the most reliable estimation of N transfer in continuous split root labelling because the ^{15}N enrichment of donor root is probably similar than the ^{15}N enrichment of N deposited. But, we must also consider that at 90 days after sowing, faba bean was at the end of pod filling stage. The ^{15}N enrichment of faba beans roots might be different with the ^{15}N enrichment of N deposited and taken up by oat since decomposition and senescence of nodules and root had been occurred.

We concluded that transfer of N from faba bean to associated plant does occur, although the amount of N transferred was very small (less than 0.68% of faba bean's N). The result of experiment also showed that there was no difference in the amount of N transfer between the two tested cultivars, *Minica* and *Scirocco.*

ACKNOWLEDGEMENTS

We would like to thank R. Langell for helping in N analyses, Gaby and Mrs Hofmann for helping during preparation. The work was funded by Prof. Werner Schulze-Stiftung im Stifterverband für die Deutsche Wissenschaft.

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