

## Determination to Carry-Over Nitrogen of Legume on Succeeding Crop Using <sup>15</sup>N Isotope Technique

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### Abstract

A field experiment was conducted using <sup>15</sup>N methodology to estimate the biological nitrogen fixation capacities of winter and summer lentil (*Lens culinaris* Medic.) varieties and soil nitrogen conserving effect was observed following the cultivation of wheat under dryland conditions in Central Anatolia. First year, biological nitrogen fixation capacities of winter and summer lentil varieties were estimated by using <sup>15</sup>N methodology. Second year, carry-over of N from the legume residue to wheat was determined. According to first year results, the average of fixed nitrogen were found as 70.0 and 45.0 kgN/ha for winter and summer varieties, respectively. Second year, the average of carry-over N derived from soil values were found 43.0 and 17.0 kgN/ha for succeeding wheat when the previous legumes were winter and summer lentil varieties, respectively.

**Key words:** <sup>15</sup>N Isotope Technique, Lentil (*Lens culinaris* Medic.), Biological Nitrogen Fixation, Carry-Over Nitrogen,

## Baklagil'den Sonra Ekilen Bitkiye Azotun Taşınmasının <sup>15</sup>N İzotop Tekniği Kullanılarak Tespit Edilmesi

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### Özet

<sup>15</sup>N metodu kullanarak, Orta Anadolu kuru koşulları altında kışlık ve yazlık olarak ekilen mercimek (*Lens culinaris* Medic.) bitkisinin biyolojik azot fiksasyon kapasitelerini tespit etmek ve sonar üzerine ekilecek olan buğday bitkisine ne miktar azot kazandırdığını belirlemek amacıyla bir tarla denemesi kurulmuştur. Birinci yıl, kışlık ve yazlık mercimek çeşitlerinin biyolojik azot fiksasyon kapasiteleri <sup>15</sup>N metodu kullanarak tespit edilmiştir. İkinci yıl, baklagilden sonra ekilen buğdaya taşınan bakiye azot miktarları belirlenmiştir. Birinci yıl sonuçlarına göre, ortalama fikse edilen azot miktarları kışlık ve yazlık çeşitler için sırasıyla 70.0 ve 45.0 kgN/ha olarak bulunmuştur. İkinci yıl, baklagilden sonra ekilen buğdaya ortalama topraktan taşınan azot miktarları, ön bitki kışlık ve yazlık mercimek olduğunda, sırasıyla 43.0 and 17.0 kgN/ha olarak belirlenmiştir.

**Anahtar Kelimeler:** <sup>15</sup>N İzotop Tekniği, Mercimek (*Lens culinaris* Medic.), Biyolojik Azot Fiksasyonu, Baklagil Azotunun Diğer Bitkiye Taşınması,

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## Objective

Objective of the experiments were to determine nitrogen fixing capacities of winter and summer lentil varieties and the carry-over N effect on succeeding wheat using N-15 isotope technique. The use of labelled nitrogen provides a unique tool allowing to separately study the behaviour of fertilizer N, soil N and also to quantify the amount of biologically fixed nitrogen.

## Introduction

Low precipitation, low soil organic matter, high soil pH and heavy texture are the main limiting factors in crop production under dryland Central Anatolia. Traditional farming is generally performed as fallow-cereal rotation. In addition, continuous cropping systems may cause very serious problems on the plant nutrition and plant diseases. Another point is the cost of fertilizers. N fertilizer cost is very expensive in Turkey, because of exportation. The best alternative is to grow legumes in crop rotation and increasing of their N<sub>2</sub> fixation capacities could be a reliable input for sustainable agriculture at the region. The purpose of exploiting the N<sub>2</sub> fixation potential of grain legumes is to save N fertilizer. The biological fixation process is especially interesting for those areas facing limited fertilizer N. In addition, in a crop rotation system, as a legume after a cereal, there are very likely fertilizer N residual effect and adequate soil moisture to the subsequent crop. Improving N fertilizer efficiency and exploitation of biologically fixed N (BNF) are thus of great importance for long-term sustainability of crop production in agro-ecosystems (Unkovich et al. 2008). The global high price for N fertilizer and the overall environmental impact of excessive fertilizer use (Chianu et al. 2011; Fan et al. 2006) warrant a growing interest in legume BNF, especially for farmers. Lentil, chickpea and vetch are the main legume crops which have been extensively cultivated in Central Anatolia. They are also most promising legumes in crop rotation with cereal. Lentil is generally cultivated without any nitrogen fertilizer and rhizobium inoculation by the farmers. However, some of the researchers indicated that 20-30 kgN/ha nitrogen fertilizer application as a starter dose taking for high yield. Some others advised to use of rhizobium inoculants before sowing due to the lack of rhizobium strains into the soils. The objective of this experiment is to determine N<sub>2</sub> fixation capacities of winter and spring lentil varieties which have of agronomic and economic importance for the region using by N-15 methodology. The use of labelled nitrogen provides a unique tool allowing to separately study the behaviour of fertilizer N, soil N and also to quantify the amount of biologically fixed nitrogen. According to the results, They also indicated that these results are relevant for areas with the average of annual precipitation is around 350 mm, the monthly average temperature in the dry period is below 20<sup>o</sup> C and the soil is heavy texture and alkaline reaction. Researchers were used only the conventional methods in which were not able to determine the amount of N<sub>2</sub> fixed by the legume crop or to assess the residual N contribution legumes to the subsequent crop in legume-cereal rotation studies, due to the unavailability of suitable methodology. However, the information on N-15 isotope labelling techniques involving the application of N-15 enriched fertilizer to the soil can provide reliable integrated estimates of the proportions and the amount of N<sub>2</sub> fixed by legumes, soil N and fertilizer N uptake by plants is essential for assessing the optimum N fertilizer rate for the subsequent crop (Danso 1988). Senaratne and Hardarson (1988) indicated that cultivation of legumes led to a greater exploitation of soil N by the succeeding crops. Hence, appreciable yield increases observed in the succeeding crops following legumes compared to cereal were due to N-conserving effect, carry-over of N from the legume residue and to greater uptake of soil N by the succeeding crops when previously cropped to legumes.

## Material and methods

In order to determine nitrogen fixing capacities of winter and summer lentil varieties and the carry-over N effect on succeeding wheat, the field experiments were carried out at the experimental site of the Sarayköy Nuclear Research Center in Ankara. The soil characteristic was silty clay loam, low organic matter (1.07 %) content and alkaline soil (pH 8.1) reaction with low N (0.1 %) and P (45.0 kg P<sub>2</sub>O<sub>5</sub>/ha) contents. Total precipitation was around 300 mm during the vegetation period.

First year, biological nitrogen fixation capacities of winter and summer lentil varieties were determined.

Second year, carry-over of nitrogen to wheat were determined.

### First year

In order to determine of biological nitrogen fixation capacities for winter and summer lentil varieties, the field experiments were conducted. Winter varieties were sowed in October and summer varieties in March. Barley was selected as a reference crop (non-fixing crop). Nitrogen fertilizers were applied at 10.0 % N-15 atom excess enriched ammonium sulphate for 10.0 kgN/ha rate as a starter dose to winter and summer lentils and at 2.0 %N-15 atom excess enriched ammonium sulphate for 40.0 kgN/ha rate to reference crops. Phosphorous fertilizer was applied to all parcels at 60.0 kgP<sub>2</sub>O<sub>5</sub>/ha as triple superphosphate before sowing. In order to determine of biological nitrogen fixation capacities for different growth stages, lentil and barley were harvested at maximum vegetative development, beginning of the pod formation and physiological maturity. Fresh samples were weighted, sub-sampled and dried in an oven at 70°C until constant weight. Moisture content of samples was calculated. Dried sub-samples were finely grinded to pass a 1 mm sieve. Total N (using by Kjeldahl method) and % N-15 atom excess (using by emission spectrometer) analyses were done (Faust, 1981). % Ndff (percent nitrogen derived from fertilizer), % NUE (percent nitrogen use efficiency), % Ndfa (percent nitrogen derived from atmosphere) and fixed N (kgN/ha) were calculated according to A-value concept (IAEA, 1990):

$$\% \text{Ndff} = \frac{\% \text{N-15 atom excess (plant)}}{\% \text{N-15 atom excess (fertilizer)}} \times 100$$

$$\% \text{Ndfa} = 100 \left( 1 - \frac{\% \text{Ndff}_F}{n \times \% \text{Ndff}_{NF}} \right) + \% \text{Ndff}_F \left( \frac{1}{n} - 1 \right)$$

$$\text{Where, } n = \frac{\text{Applied N fertilizer to F (kgN/ha)}}{\text{Applied N fertilizer to NF (kgN/ha)}}, \text{ F: fixing crop, NF: non-fixing crop,}$$

$$\text{Fixed N (kgN/ha)} = \frac{\% \text{Ndfa} \times \text{Total NF}}{100},$$

$$\text{Total N (kgN/ha)} = \% \text{N} \times \text{Dry matter yield (kg/ha)}$$

$$\% \text{NUE} = \frac{\% \text{Ndff} \times \text{Total N (kgN/ha)}}{\text{Applied N fertilizer (kgN/ha)}} \times 100$$

Statistical analysis of experimental datas were done.

## Second year

For determination to carry-over of N to wheat, wheat was sown on the harvested winter and summer lentil plots without tillage and any nitrogen fertilizer. A completely randomized block design was arranged with four replications. Plot size was 2.5 m x 4.2 m = 10.5 m<sup>2</sup>. 60.0 kgP<sub>2</sub>O<sub>5</sub>/ha as triplesuperphosphate fertilizer (% 42-44 P<sub>2</sub>O<sub>5</sub>) was applied to the all plots before sowing. Plants were harvested using with a parcel harvester (Hege) inside of 1.4 m x 4.0 m = 5.6 m<sup>2</sup> size. Harvested plants were separated into grain and straw parts. Grain and straw parts were weighted and sub-sampled. They were dried at 70o C until constant weight and finely grounded to pass a 1 mm sieve. Total moisture contents of grain and straw were calculated. Total N (using by Kjeldahl method) and % N-15 atom excess (using by emmission spectrometer) analyses were done (Faust 1981). % Ndff (residual), % Ndfs (percent nitrogen derived from soil), total N yield (kgN/ha), residual fertilizer N yield (kgN/ha), soil N yield (kgN/ha) and residual % NUE (percent nitrogen use efficiencies) values were calculated as follows (IAEA 1990):

$$\% \text{ Ndff (residual)} = \frac{\% \text{ N-15 atom excess (plant)}}{\% \text{ N-15 atom excess (previous fert.)}} \times 100$$

$$\% \text{ Ndff (residual)} + \% \text{ Ndfs} = 100,$$

$$\text{Than; } \% \text{ Ndfs} = 100 - \% \text{ Ndff (residual)}$$

$$\text{Total N yield (kgN/ha)} = \frac{\text{Dry matter yield (kg/ha)} \times \% \text{ N}}{100}$$

$$\text{Residual fertilizer N yield (kgN/ha)} = \frac{\% \text{ Ndff (residual)} \times \text{Total N yield (kgN/ha)}}{100}$$

$$\text{Soil N yield (kgN/ha)} = \text{Total N yield} - \text{Residual fertilizer N yield}$$

$$\% \text{ NUE (residual)} = \frac{\text{Residual fertilizer N yield (kgN/ha)}}{\text{N rate applied to previous crop (kgN/ha)}} \times 100$$

The proportion of the nitrogen derived from fixed N in the preceding crop and its N-conserving effect were calculated by the formulae as follows (Senaratne and Hardarson 1988):

$$\% \text{ Ndfu} = \left(1 - \frac{\% \text{ N-15 a.e. in the succeeding crop following the legume}}{\% \text{ N-15 a.e. in the succeeding crop following the control}}\right) \times 100$$

$$\text{Amount of N derived from the unlabelled source (kg N/ha)} =$$

$$\frac{\% \text{ Ndfu} \times \text{total N yield in the succeeding crop following the legume}}{100}$$

Statistical analysis of the experimental datas were done.

## Results and Discussion

### First Year Results for Lentil

According to the obtained results, the average of total dry matter yields were found at winter varieties as  $3688 \pm 170.7$  and  $4362 \pm 107.7$  kg/ha ( $p < 0.05$ ) for Pul-11 and Kışlık Kırmızı-51 and at summer varieties as  $2723 \pm 66.1$  and  $2603 \pm 55.9$  kg/ha for Pul-11 and Sultan-1, respectively (Table 1).

Table 1. Average of total dry matter yields (kg/ha)

Pul-11	Winter variety	$3688 \pm 170.7$
Kışlık Kırmızı-51	Winter variety	$4362 \pm 107.7$
Pul-11	Summer variety	$2723 \pm 66.1$
Sultan-1	Summer variety	$2603 \pm 55.9$

The average of total N yield values were found  $87.8 \pm 4.97$  and  $99.5 \pm 3.48$  kgN/ha ( $p < 0.05$ ) for Pul-11 and Kışlık Kırmızı-51 varieties and  $64.0 \pm 2.05$  and  $63.3 \pm 1.51$  kgN/ha for Pul-11 and Sultan-1 varieties, respectively (Table 2).

Table 2. Average of total N yields (kgN/ha)

Pul-11	Winter variety	$87.8 \pm 4.97$
Kışlık Kırmızı-51	Winter variety	$99.5 \pm 3.48$
Pul-11	Summer variety	$64.0 \pm 2.05$
Sultan-1	Summer variety	$63.3 \pm 1.51$

The average of % Ndfa values were found  $74.4 \pm 1.50$  and  $74.6 \pm 1.43$  for Pul-11 and Kışlık Kırmızı-51 varieties and  $66.7 \pm 1.46$  and  $74.3 \pm 1.08$  ( $p < 0.05$ ) for Pul-11 and Sultan-1 varieties, respectively (Table 3).

Table 3. Average of nitrogen derived from atmosphere values (%)

Pul-11	Winter variety	$74.4 \pm 1.50$
Kışlık Kırmızı-51	Winter variety	$74.6 \pm 1.43$
Pul-11	Summer variety	$66.7 \pm 1.46$
Sultan-1	Summer variety	$74.3 \pm 1.08$

The averages of fixed N values were found  $65.9 \pm 4.26$  and  $74.2 \pm 2.96$  kgN/ha ( $p < 0.05$ ) for Pul-11 and Kışlık Kırmızı-51 varieties and  $43.0 \pm 1.88$  and  $47.2 \pm 1.46$  kgN/ha ( $p < 0.05$ ) for Pul-11 and Sultan-1 varieties, respectively (Table 4).

Table 4. Average of fixed N values (kgN/ha)

Pul-11	Winter variety	$65.9 \pm 4.26$
Kışlık Kırmızı-51	Winter variety	$74.2 \pm 2.96$
Pul-11	Summer variety	$43.0 \pm 1.88$
Sultan-1	Summer variety	$47.2 \pm 1.46$

The average of % NUE values were found  $16.7 \pm 1.10$  and  $19.2 \pm 1.20$  ( $p < 0.05$ ) for Pul-11 and Kışlık Kırmızı-51 varieties and of  $6.5 \pm 0.25$  and  $5.0 \pm 0.20$  ( $p < 0.05$ ) for Pul-11 and Sultan-1 varieties, respectively (Table 5).

Table 5. Average of nitrogen use efficiency values (%)

Pul-11	Winter variety	$16.7 \pm 1.10$
Kışlık Kırmızı-51	Winter variety	$19.2 \pm 1.20$
Pul-11	Summer variety	$6.5 \pm 0.25$
Sultan-1	Summer variety	$5.0 \pm 0.20$

### Second Year Results for Wheat

The average of dry matter yields for seed and straw were found 2010 kg/ha and 3505 kg/ha when the previous crop was winter lentil; 2258 kg/ha and 3615 kg/ha when the previous crop was summer lentil, respectively. Obtained these results, there was no statistically significant differences in seed and straw yields according to previous crops (Table 6).

Table 6. The average of dry matter yields (kg/ha)

Previous crop	Plant part	
	Seed	Straw
Winter lentil	2010	3505
Summer lentil	2258	3615
LSD (0.05)	n.s.	n.s.

The average of % N-15 a.e. values for seed and straw were found 0.100 and 0.100 % when the previous crop was w.lentil; 0.230 and 0.240 % when the previous crop was s.lentil, respectively. These values had significantly effected ( $p < 0.05$ ) by the previous crop. % N-15 a.e. amounts were found higher at the plant parts of wheat on cultivation after s.lentil (Table 7).

Table 7. The average of % N-15 a.e. values

Previous crop	Plant part	
	Seed	Straw
Winter lentil	0.100	0.100
Summer lentil	0.230	0.240
LSD (0.05)	0.033	0.031

The average of % Ndff (residual) values for seed and straw were found 1.11 and 1.11 % when the previous crop was w.lentil; 2.82 and 2.91 % when the previous crop was s.lentil, respectively. % Ndff (residual) values had significantly effected ( $p < 0.05$ ) according to previous crop. % Ndff (residual) amounts were found higher at the plant parts of wheat on cultivation after s.lentil (Table 8).

Table 8. The average of % Ndff (residual) values

Previous crop	Plant part	
	Seed	Straw
Winter lentil	1.11	1.11
Summer lentil	2.82	2.91
LSD (0.05)	0.38	0.37

The average of residual fertilizer N yield (kgN/ha) values for seed and straw were 0.5 and 0.3 kgN/ha when the previous crop was w.lentil; 1.4 and 0.7 kgN/ha when the previous crop was s.lentil, respectively. This values had significantly effected ( $p < 0.05$ ) by the previous crop. Residual fertilizer N yields (kg N/ha) were found higher at the plant parts of wheat on cultivation after s.lentil (Table 9).

Table 9. The average of residual fertilizer N yield (kgN/ha) values

Previous crop	Plant part	
	Seed	Straw
Winter lentil	0.5	0.3
Summer lentil	1.4	0.7
LSD (0.05)	0.025	0.030

The average of residual % NUE values for seed and straw were 5.0 and 3.0 % when the previous crop was w.lentil; 14.0 and 7.0 % when the previous crop was s.lentil, respectively. This values had significantly effected ( $p < 0.05$ ) by the previous crop. Residual % NUE amounts were found higher at plant parts of wheat on cultivation after s.lentil (Table 10).

Table 10. The average of residual % NUE values

Previous crop	Plant part	
	Seed	Straw
Winter lentil	5.0	3.0
Summer lentil	14.0	7.0
LSD (0.05)	2.5	3.0

% Ndfu (percent nitrogen derived from unknown) and the amount of N derived from the unlabelled source (kgN/ha) values of wheat: The proportion of the nitrogen derived from fixed N in the preceding crop and its N-conserving effect were calculated with the formulas (Senaratne and Hardarson 1988) using the reference crop results given on Table 11.

Table 11. The average of % Ndfu and the amount of N derived from the unlabelled source (kgN/ha) values of wheat (seed+straw):

Rotation *	N rate KgN/ha	Total D.M Yield KgN/ha	Total N Yield KgN/ha	% N-15 weighted	N fertil. Yield KgN/ha	Soil N Yield KgN/ha	% Ndfu	Ndfu KgN/ha
B-W	40*	5310	84.3	0.305	9.2	75.1	-	-
W.L-W	0	5515	65.8	0.105	0.8	65.0	66	43
S.L-W	0	5873	75.4	0.238	2.1	73.3	22	17

\* B-W (reference): barley-wheat, W.L-W: winter lentil-wheat, S.L-W: summer lentil-wheat

According to obtained results, the average of % Ndfu (percent nitrogen derived from unknown) and the amount of N derived from the unlabelled source values were found that 66 % and 43 kgN/ha ; 22 % and 17 kgN/ha in the preceding wheat which the previous crops were winter lentil and summer lentil, respectively.

## Conclusion

The results of these experiments were summarized as follows:

The winter varieties had higher total dry matter yields (kg/ha), total N yields (kgN/ha), % Ndfa and fixed N (kgN/ha) capacities than the summer varieties of lentil. Inoculation treatments did not have any improvement effects on both of winter and summer varieties. Effective nodules were observed on the root hairs. Therefore, indigenous rhizobium was effected in our experimental soil. In comparison the winter varieties that Kışlık-Kır.51 had higher N<sub>2</sub> fixing capacity than Pul-11 variety. However, Sultan-1 had higher N<sub>2</sub> fixation capacity than Pul-11 variety for summer treatments. According to harvesting times, N<sub>2</sub> fixation capacities were found higher at the beginning of pod formation stage than the other growth stages for both experiments.

The results showed that % N-15 atom excess, residual % Ndff (percent nitrogen derived from fertilizer), residual fertilizer N yield (kgN/ha) and residual % NUE (percent nitrogen use efficiency) values were found significantly ( $p < 0.05$ ) higher in the preceding wheat when the previous crop was spring lentil. The reason of these results was related to dilution effect of N fertilizer applied to previous lentil. Senaratne and Hardarson (1988) indicated that N-15 labelled fertilizer in the soil from the previous application to the sorghum crop was again used to estimate the residual N effect of different treatments. Dry matter yields (kg/ha) and total N yields (kgN/ha) of wheat were not significantly effected by the previous lentil. No additional nitrogen fertilizer application to wheat might be the reason of these results. However, % Ndfu (percent nitrogen derived from unknown) and amount of N derived from the unlabelled source (kgN/ha) values were found higher in the preceding wheat which the previous crop was winter lentil. Senaratne and Hardarson (1988) indicated that faba bean and pea took up less soil N relative to barley, thus giving rise to an 'apparent soil N-conserving effect', their cultivation led to a greater uptake of soil N by the subsequent cereal crop than when cropped to barley. Hence, the increased yields of cereals following legumes were not due entirely to the carry-over of N from the legume residue and to the soil N-conserving effect but also due to 'other effects' which enabled the subsequent crop to exploit the soil more when preceded by legumes than by cereal.

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