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DETERMINATION OF RESIDUAL N EFFECT OF LENTIL TYPES ON SUCCEEDING WHEAT USING N-15 ISOTOPE TRACING TECHNIQUE

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N-15 İZOTOP İZLEME TEKNİĞİ KULLANARAK MERCİMEK ÇEŞİTLERİNDEN SONRA EKİLEN BUĞDAY'A BAKİYE AZOT ETKİSİNİN BELİRLENMESİ

Abstract:

The field experiments were conducted to determine nitrogen fixing capacities of lentil (*Lens culinaris* Medic.) varieties of winter (Pul-11 and Kışlık Kırmızı-51) and summer (Sultan-1 and Pul-11) and the carry-over N effect on succeeding wheat, using N-15 isotope technique, under Central Anatolia dryland conditions. 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. In first year, biological nitrogen fixation capacities of winter and summer lentil varieties were estimated by using N-15 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 was found as 70.0 and 45.0 kgN/ha for winter and summer varieties, respectively. In 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.

Özet:

N-15 izotop tekniği kullanarak, Orta Anadolu kuru koşulları altında ekilen mercimek (*Lens culinaris* Medic.) bitkisinin kışlık (Pul-11 ve Kışlık Kırmızı-51) ve yazlık (Sultan-1 ve Pul-11) çeşitlerinin biyolojik azot fiksasyon kapasitelerini tespit etmek ve sonra üzerine ekilecek olan buğday bitkisine ne miktar azot kazandırdığını belirlemek amacıyla tarla denemeleri kurulmuştur. Etiketli azot kullanılarak gübre ve topraktan gelen azot ile beraber havadan biyolojik yolla fikse edilen azot miktarının ayrı ayrı belirlenmesi mümkündür. Birinci yıl, kışlık ve yazlık mercimek çeşitlerinin biyolojik azot fiksasyon kapasiteleri N-15 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.

Keywords: N-15 Isotope Technique, Lentil (Lens *culinaris* Medic.), Biological Nitrogen Fixation, Carry-Over Nitrogen

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

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1. 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. Nitrogen fertilizer cost is very expensive in Turkey, because of exportation. The best alternative is to grow legumes in crop rotation and increasing of their nitrogen fixation capacities could be a reliable input for sustainable agriculture at the region. The purpose of exploiting the nitrogen fixation potential of grain legumes is to save nitrogen fertilizer. The biological fixation process is especially interesting for those areas facing limited fertilizer nitrogen. In addition, in a crop rotation system, as a legume after a cereal, there are very likely fertilizer nitrogen residual effect and adequate soil moisture to the subsequent crop. Improving nitrogen fertilizer efficiency and exploitation of biologically fixed nitrogen (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, Nkonya, Mairura, Justina and Akinnifesi, 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 (Halitligil, Akın, Aydın, Yılmaz and Donmez, 1996). 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 (Akın, 1994). The objective of this study is to determine nitrogen 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 nitrogen, soil nitrogen 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°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 nitrogen fixed by the legume crop or to assess the residual nitrogen 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 nitrogen fixed by legumes, soil nitrogen and fertilizer nitrogen uptake by plants is essential for assessing the optimum nitrogen fertilizer rate for the subsequent crop (Hardarson, Danso and Zapata, 1988). Senaratne and Hardarson (1988) indicated that cultivation of legumes led to a greater exploitation of soil nitrogen by the succeeding crops. Hence, appreciable yield increases observed in the succeeding crops following legumes compared to cereal were due to nitrogen-conserving effect, carry-over of nitrogen from the legume residue and to greater uptake of soil nitrogen by the succeeding crops when previously cropped to legumes.

2. Material and Method

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

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was silty clay loam, low organic matter (1.07 %) content and alkaline soil (pH 8.1) reaction with low nitrogen (0.1 %) and phosphorus (45.0 kgP_2O_5/ha) contents. Total precipitation was around 300 mm during the vegetation period. In first year, biological nitrogen fixation capacities of winter and summer lentil varieties and in second year, the carry-over of nitrogen to wheat were determined.

First year: The field experiments were conducted to determine of biological nitrogen fixation capacities for winter and summer lentil varieties. 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_2O_5/ha as triple superphosphate before sowing. In order to determine the 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^{o}C until constant weight. Moisture contents of samples were 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 (Anonymous, 1990):

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

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

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

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

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

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

Statistical analysis of experimental data was performed.

Second year: in order to determine the carry-over N to wheat, wheat was sown on the harvested winter and summer lentil plots without any tillage and 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^2. 60.0 kgP_2O_5/ha as triplesuperphosphate fertilizer (% 42-44 P_2O_5) 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 m2 size. Harvested plants were separated into grain and straw parts. Grain and straw parts were weighted and sub-sampled. They were dried at 70^o 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 emission spectrometer) were analysed. (Faust 1981). The % 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 (Anonymous, 1990):

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

$$\% \; Ndff \; (residual) + \% \; Ndfs = 100,$$

$$\% \; Ndfs = 100 - \% \; Ndff \; (residual)$$

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

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

Soil N yield (kgN/ha) = Total N yield – Residual fertilizer N yield

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

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

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

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 data were performed

Experimental Data

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

Pul-11	Winter variety	3688 ± 170.7
Kıslık Kırmızı-51	Winter variety	4362 ± 107.7
Pul-11	Summer variety	2723 ± 66.1
Sultan-1	Summer variety	2603 ± 55.9

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

Pul-11	Winter variety	87.8 ± 4.97
Kıslık Kırmızı-51	Winter variety	99.5 ± 3.48
Pul-11	Summer variety	64.0 ± 2.05
Sultan-1	Summer variety	63.3 ± 1.51

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

Pul-11	Winter variety	74.4 ± 1.50
Kıslık Kırmızı-51	Winter variety	74.6 ± 1.43
Pul-11	Summer variety	66.7 ± 1.46
Sultan-1	Summer variety	74.3 ± 1.08

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

Pul-11	Winter variety	65.9 ± 4.26
Kıslık Kırmızı-51	Winter variety	74.2 ± 2.96
Pul-11	Summer variety	43.0 ± 1.88
Sultan-1	Summer variety	47.2 ± 1.46

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

Pul-11	Winter variety	16.7 ± 1.10
Kıslık Kırmızı-51	Winter variety	19.2 ± 1.20
Pul-11	Summer variety	6.5 ± 0.25
Sultan-1	Summer variety	5.0 ± 0.20

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.

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

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

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LSD (0.05)	0.38	0.37

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

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

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

3. Results and Discussion

First year results for lentil types: The average of total dry matter yields were found at winter varieties as 3688 and 4362 kg/ha ($p< 0.05$) for Pul-11 and Kıslık Kırmızı-51 and at summer varieties as 2723 and 2603 kg/ha for Pul-11 and Sultan-1, respectively (Table 1). The average of total N yield values was found 87.8 and 99.5 kgN/ha ($p< 0.05$) for Pul-11 and Kıslık Kırmızı-51 varieties and 64.0 and 63.3 kgN/ha for Pul-11 and Sultan-1 varieties, respectively (Table 2). The average of % Ndfa values were found 74.4 and 74.6 for Pul-11 and Kıslık

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Kırmızı-51 varieties and 66.7 and 74.3 ($p< 0.05$) for Pul-11 and Sultan-1 varieties, respectively (Table 3). The averages of fixed N values were found 65.9 and 74.2 kgN/ha ($p< 0.05$) for Pul-11 and Kıslık Kırmızı-51 varieties and 43.0 and 47.2 kgN/ha ($p< 0.05$) for Pul-11 and Sultan-1 varieties, respectively (Table 4). The average of % NUE values were found 16.7 and 19.2 ($p< 0.05$) for Pul-11 and Kıslık Kırmızı-51 varieties and of 6.5 and 5.0 ($p< 0.05$) for Pul-11 and Sultan-1 varieties, respectively (Table 5).

Second year results for wheat: In order to estimate to yield effects and carry over nitrogen from previous lentil to wheat, wheat was sown on no tillage and no nitrogen fertilizer after lentil harvesting. Harvested wheat were separated into two parts as seed and straw, dried and weighted. The average seed and straw yields of wheat were 2010 kg/ha and 3505 kg/ha when previous crop was winter lentil, and average seed and straw yields of wheat were 2258 kg/ha and 3615 kg/ha when previous crop was summer lentil, respectively. Obtained yield results were average for the region, but there were statistically no differences in average of seed and straw yields, due to no nitrogen fertilizer application to the wheat. Wheat was used only carry over nitrogen of soil. (Table 6). 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 affected ($p <0.05$) 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). The values of carried % nitrogen derived from fertilizer for seed and straw were found 1.11 and 1.11 % when the previous crop was winter lentil, and 2.82 and 2.91 % when previous crop summer lentil, respectively. Ndff % (residual) values had significantly affected ($p <0.05$) according to previous crop. Ndff % (residual) amounts were found higher at the plant parts of wheat on cultivation after s.lentil (Table 8). For calculation the amounts of fertilizer as kgN/ha carried from previous crop to wheat plant, residual fertilizer N yields (kg N/ha) were calculated from Ndff % (residual) values above given formula. Obtained results show that, the average of residual fertilizer N yield (kgN/ha) values for seed and straw were found 0.5 and 0.3 kgN/ha when previous crop was w. lentil, and 1.4 and 0.7 kgN/ha when previous crop was s. lentil, respectively. This values had significantly affected ($p <0.05$) 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). The residual % nitrogen use efficiency amounts of wheat plant parts were calculated from N rate applied to previous crop (kgN/ha) proportion to residual fertilizer N yield (kgN/ha) as given above formula. The results show that the average of residual % NUE values for seed and straw were found 5.0 and 3.0 % when the previous crop was w. lentil, and 14.0 and 7.0 % when the previous crop was s. lentil, respectively. This values had significantly affected ($p <0.05$) by the previous crop. Residual % NUE amounts were found higher at plant parts of wheat on cultivation after s.lentil (Table 10). 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. 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.

Results were summarized as; the winter types of lentil had higher total dry matter yields (kg/ha), total N yields (kgN/ha), Ndfa % and fixed N (kgN/ha) capacities than the summer varieties. Inoculation treatments had not any biological nitrogen fixing improvement effect on both winter and summer lentil. Effective nodules were observed on the root hairs, therefore

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indigenous rhizobium in the soil had been effected. In comparison to winter lentil, Kıslık-Kır.51 had higher N_2 fixing capacity than Pul-11 variety. However, Sultan-1 had higher N_2 fixation capacity than Pul-11 variety for summer treatments. 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 summer lentil.

4. Conclusion

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 affected by the previous lentil. No 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 soil nitrogen uptake of faba bean and pea were less than barley, and cultivation of these legumes had to a greater uptake of soil nitrogen by the subsequent cereal crop, comparing 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. Halitligil et al. (1996) indicated that obtained from the % Ndfu data also, make us to suggest a lentil-wheat rotation at Ankara and Eskişehir and a chickpea-wheat rotation at Konya. Kırda, Van Cleemput and Moutonnet (1996) indicated that the use of N-15 labelled fertilizers provided an advantage to separately study the behaviour of N-fertilizers as well as soil N. To determine of residual fertilizer N and to quantify the amount of biologically fixed N_2 by using of labelled N-15 fertilizer were possible. Florence et al. (2016) indicated that the importance of biological nitrogen fixation researches for reducing on nitrogen fertilizers in agriculture in the developed world and in developing countries and for increasing its importance in an agricultural fields.

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