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Yield and nitrogen fixation capacity by inoculated white lupin (*Lupinus albus* L.)

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Abstract. White lupin (*Lupinus albus* L.) crop can gain a renewed interest due to its higher crude protein yield than other rainfed grain legumes, and for the request as an ingredient of functional or healthy food products. It is a new crop in Sardinia (Italy) and very few studies provide information about the N fixation ability of this species which needs to be preferably evaluated at local level from direct measurements. This study was aimed at quantify the DM yield and the N fixed by white lupin, using the Isotopic Dilution method, in three Sardinian sites. Grain dry matter yield and its N proportion did not differ between sites whereas the proportion of N derived from the fixation showed a wider variation. The aerial DM of white lupin fixed from 140 to about 270 kg ha^{-1} of N. Even if results differed among sites, very high percentages of N derived from the atmosphere were found and a relationship of 53.7 kg of fixed N per tonne of grain DM produced by this legume was established.

Keywords. Legumes – % Ndfa – Fixed N – Straw – Grain yield.

Rendement et capacité de fixation d'azote par le lupin blanc inoculé **(Lupinus albus)**

Résumé. *La culture du lupin blanc (*Lupinus albus *L.) peut trouver un nouveau intérêt en raison de son rendement de protéine brut plus haut que celui d'autres légumineuses de grain et pour la requête comme un ingrédient de produits alimentaires fonctionnels ou sains. C'est une nouvelle culture en Sardaigne (Italie) et très peu d'études fournissent des informations sur la capacité de fixation de l'azote (N) de cette espèce qui doit être de préférence évaluée au niveau local avec des mesurages directs et une méthodologie appropriée. Cet étude concerne la quantification du rendement de matière sèche et de N fixé donné par le lupin blanc, en utilisant la méthode de Dilution Isotopique, dans trois localités sardes avec des caractéristiques du sol différentes. Le rendement de matière sec du grain et sa proportion de N ne diffèrent parmi les localités tandis que la proportion de l'azote provenant de l'atmosphère a montré une variation plus large. La matière sèche aérien de lupin blanc a fixé de 140 à environ 270 kg ha^{-1} de N. Même si les résultats ont différé parmi les localités, des pourcentages très élevés de N provenant de l'atmosphère ont été trouvés et une relation de 53.7 kg de N fixé par t de grain produit par cette légumineuse a été établie.*

Mots-clés. *Légumineuses – % Ndfa – N fixé – Paille – Rendement en grain.*

I – Introduction

Leguminous crops can contribute to a more sustainable agriculture in both traditional and organic farming systems. White lupin (*Lupinus albus* L.) is an interesting crop due to its low alkaloid lines, higher crude protein yield than other rainfed legumes such as pea, faba bean and narrow leafed lupin, and for the request as an ingredient of functional or healthy food products (Annicchiarico, 2008; Annicchiarico *et al.,* 2010; Chiofalo *et al.*, 2012). White lupin is an old species mainly distributed around the Mediterranean and cultivated for thousand years. There is renewed interest in it in southern Europe, where this crop is mainly grown in France, Spain and Portugal

(Huyghe, 1997). White lupin is a new crop in Sardinia (Italy), where information on its adaptation and N fixation ability is almost absent. As a general rule, legumes can fix from 1.5 to 2.5 kg of shoot N per 100 kg of shoot dry matter. However such relationship, arisen from Australian works, represents a gross generalization that cannot be considered for a widespread application. In fact, specific information concerning the effects of crop genotype and environmental conditions on legume N fixation can come only from direct measurements of N fixation, preferably at local level, using an appropriate methodology (Unkovich and Pate, 2000). This study was aimed at quantify the DM yield and the N fixed by white lupin crops in Sardinia, using the Isotopic Dilution method. The paper reports the main results obtained in the first year of the experiment.

II – Materials and methods

The field experiments were conducted during 2009-10 in Sardinia at three locations, with different soil types and rainfall amounts (Table 1). The total rainfall from September 2009 to August 2010 were 27, 9 and 44% greater than the long-term means for the Stintino, Chilivani and Sanluri sites, respectively.

Table 1. Main pedo-climatic characteristics of the three experimental locations

Location	Stintino	Chilivani	Sanluri
Latitude/longitude	40° 52' N, 8° 15' E	40° 36' N, 8° 58' E	39° 31' N, 8° 51' E
Altitude (m.a.s.l.)	6	200	60
† Avg. temp. (°C) /rainfall (mm)	15.2/590	15.2/560	17.6/430
†† Rainfall (mm)	752	612	618
Soil series (FAO, 1988)	*Haplic Nitosols*	*Lithic leptosols*	*Eutric fluvisuols*
Sand/ silt/ clay (%)	81/9/10	81/11/8	45/ 25/30
pH	6.0	6.6	7.6
Total N (g kg^{-1})	1.7	1.3	1.2
P (mg kg^{-1})	2.6	8.3	16.2

† Long-term year means.
†† From September 2009 to August 2010.

At each locations, where this crop had not been sowed before, stands of white lupin cv Multitalia were established in Autumn by sowing 60 kg ha^{-1} of viable seed. Seeds were inoculated with *Bradyrhizobium lupini* (Souche LL13). Native annual grasses and wheat, sown at a seed rate of 190 kg ha^{-1} were used as non fixing reference species. Plots were arranged in a complete randomized design with four replicates. The size of each experimental unit was 4.5 x 7.5 m. All plots were fertilized with about 100 kg ha^{-1} P_2O_5 using triple superphosphate before seeding. Seedbed was prepared using conventional tillage consisting of 30-cm depth ploughing and superficial harrowing. No irrigation, fertilizer, or herbicide was applied after sowing. A rate of 4 kg N ha^{-1} of enriched ^{15}N fertilizer (10 atom %^{15}N enriched ammonium sulfate) was applied to a 3 m^2 area of non-fixing reference species and white lupin. At maturity, dry matter production was determined by cutting the aerial biomass at 5 cm above ground level over the same 3 m^2 ^{15}N enriched area within each experimental unit, subdividing into grain and straw, and drying the material at 65°C in a forced-air oven until it reached a constant weight. Dry samples of grain and straw were ground finely enough to pass through a 1 mm mesh and subjected to elemental analyzer isotope ratio mass spectrometry at the laboratory Iso-Analytical Limited (Cheshire, United Kingdom) to determine both N and the atom%^{15}N content. The proportion of white lupin N derived from atmosphere (%Ndfa) was calculated by the ^{15}N dilution method, as described by Warembourg (1993) and the ^{15}N excess of each different legume plant portion was compared with the corresponding value of the reference non-fixing crop. The amount of N fixed was calculated by multiplying white lupin N yield (kg ha^{-1}) per %Ndfa/100. All data were analysed by ANOVA and separation of mean values by least significance difference (LSD) test at 5% probability.

III – Results and discussion

Straw dry matter yield differed significantly between sites; it ranged from 3.4 t ha^{-1} at Chilivani to 8.5 t ha^{-1} at Stintino where stems were as tall as Chilivani (Table 2). The higher straw yield was probably caused by the mild weather conditions of this site very close to the sea. Grain yield ranged from 2.2 to 2.9 t ha^{-1}. Nitrogen content of grain was from three to more than six times higher than that of the straw. Grain yields were lower to those obtained in other Italian environments (Annicchiarico, 2008).

Table 2. White lupin plant height, straw and grain dry matter yield and its N concentration at the three locations during 2009-10

	Height (cm)	Dry matter yield (kg ha^{-1})			N concentration (%)	
Location	**Plant**	**Straw**	**Grain**	**Total**	**Straw**	**Grain**
Stintino	124a	8.5a	2.2a	10.7a	1.8a	5.4b
Chilivani	80b	3.4c	2.4a	5.8c	1.1b	5.8ab
Sanluri	119a	5.5b	2.9a	8.4b	0.8b	6.1a

Means followed by the same letter within each column are not significant different at P≤0.05.

The atom%^{15}N excess in each legume biomass component was overall significantly lower than that in the corresponding component of the reference species at each locations (data not shown), due to its dilution with atmospheric N. The Ndfa of white lupin (Table 3) reached about 95% at Stintino for both biomass components, it was slightly lower at Chilivani and significantly lower at Sanluri (-40%), indicating a reduced N fixation activity at this site. The Ndfa percentages at Stintino and Chilivani sites were quite similar to those reported by Carranca *et al.* (2009) for the same legume species grown in central Portugal.

Table 3. Nitrogen yield, proportion of N derived from the atmosphere (Ndfa) and amount of fixed N in straw and grain of white lupin at the three locations during 2009-10

	Nitrogen yield (kg ha^{-1})		Ndfa (%)		Fixed N (kg ha^{-1})	
Location	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**
Stintino	162.3a	124.5a	94.6a	95.1a	153.7a	118.4a
Chilivani	40.6b	143.1a	87.2a	90.2a	35.5b	129.7a
Sanluri	48.9b	182.2a	54.2b	59.3b	27.7b	112.6a

Means followed by the same letter within each column are not significant different at P≤0.05.

The amount of fixed N in straw exceed 150 kg at Stintino whereas it was significantly lower at the remaining locations. The fixed N in the grain was similar among locations and it was on average 120 kg ha^{-1}. Nevertheless, considering the total N in grain, which is the component usually removed from the field, the proportion of grain N derived from the soil represented only 5% at Stintino and about 41% at Sanluri due to its lower Ndfa percentage. Even if below ground pool N is not considered in this work, the N balance remained positive at Stintino after grain harvesting and removal (+35 kg ha^{-1} of N), whereas it was negative at the remaining locations. The total N fixed by white lupin in its aerial biomass (straw + grain) ranged from 140 (Sanluri) to about 270 kg ha^{-1} (Stintino). It is worth noting that the lowest value reached those recorded for annual and perennial forage legumes in previous experiment carried out in Sardinia (Sulas and Sitzia, 2005; Sulas *et al.*, 2009). Considering the data from the two locations where % Ndfa for grain reached on average 92.6%, the regressions of the fixed N for white lupin grain on the corresponding DM yield (Fig. 1) showed a relationship of 53.7 kg of N per tonne of grain DM.

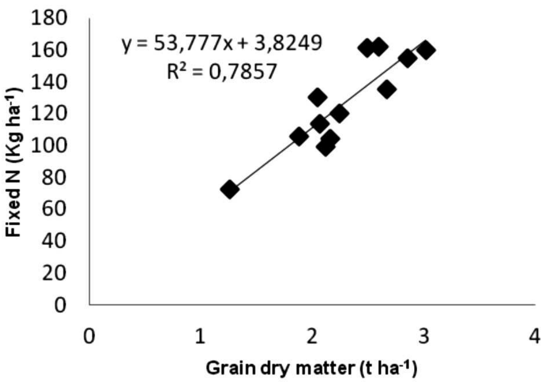

Fig. 1. The relationship between fixed N and dry matter yield in white lupin grain.

IV – Conclusions

White lupin crop showed good adaptation and performances. However, the range of Ndfa percentage was wide and a high capacity of white lupin to fix N was recorded in two of three locations under study. An interesting relationship for fixed N per tonne of grain produced by this legume was established.

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