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# Relationship between nitrogen form and the development and yield of *Lupinus albus* L. from different countries

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## Abstract

This paper discusses the influence of form of nitrogen (N) used as fertilizer, such as $N_2$, $NH_4^+$, $NO_3^-$, [$NH_4^+$+$NO_3^-$] and $-NH_2$, on the development and yield and the protein content and yield of low-alkaloid cultivars of *Lupinus albus* L. from Poland, Spain, and Chile. The experiments were carried out in a greenhouse and plants were grown in perlite. The different forms of N used significantly influenced lupin development and yield. Plants only developed normally in treatments where N was delivered in the molecular form $N_2$ or as [$NH_4^+$+$NO_3^-$]. For the other forms of N anomalies like necrosis, chlorosis, and small leaves were present. In contrast to cv. Butan, the N used as $NH_4^+$ disturbed flowering in cvs. Multolupa and Marta, which produced no seed. Moreover, N form also influenced protein seed protein content and yield.

**Additional key words**: *Bradyrhizobium lupini*, legume proteins, lupin, nitrogen-forms.

## Resumen

**Relación entre diferentes formas de nitrógeno y el desarrollo y rendimiento de *Lupinus albus* L. originario de diferentes países**

El objetivo de este trabajo fue estudiar la influencia de diferentes formas de nitrógeno ($N_2$, $NH_4^+$, $NO_3^-$, [$NH_4^+$+$NO_3^-$] y $-NH_2$), utilizadas como fertilizante, sobre el desarrollo, el rendimiento vegetativo y el contenido y rendimiento proteico de diferentes variedades dulces de *Lupinus albus* L. originarias de Polonia, España y Chile. Los experimentos se llevaron a cabo en invernadero, utilizando perlita como sustrato. Las plantas se desarrollaron adecuadamente sólo cuando el aporte de nitrógeno fue en forma de $N_2$ o como [$NH_4^+$ + $NO_3^-$]. Cuando se utilizaron las otras formas de nitrógeno se observaron anomalías como necrosis, clorosis u hojas de tamaño reducido. En contraste con el cultivar Butan, cuando se utilizó $NH_4^+$, los cultivares Multolupa y Marta presentaron alteraciones en la floración, que dieron lugar a una carencia de semillas. Además, la forma del nitrógeno utilizada tuvo una importancia crucial en el contenido proteico de las semillas, así como en el rendimiento proteico.

**Palabras clave adicionales:** altramuz, *Bradyrhizobium lupini,* formas nitrogenadas, proteínas de leguminosas.

## Introduction

Among all grain legumes, lupin (*Lupinus* spp.) appears to have the highest seed protein content. Seed of some lupin species e.g. *Lupinus luteus* L. contain about 50% protein (Jasińska and Kotecki, 1993). The plant is therefore considered to be a potential protein source and seems to be important nutritionally. Nitrogen (N) is an indispensable element in protein and other important N compounds. It can be taken up by plants in the form of $NH_4^+$ or $NO_3^-$ and under specific condition in amine form $-NH_2$ (urea). Plant preference to a particular form depends on plant species, pH and soil temperature.

All legumes, including lupins, use the atmospheric N ($N_2$) for biological fixation and protein biosynthesis, and synthesis of other N containing compounds e.g. amino acids, nucleic acids, vitamins, polyamines, alkaloids, etc, which are necessary for plant life. In N fixation, atmospheric N is converted into ammonia, which is subsequently available for biosynthesis of N containing molecules. Fixed N is not free. Plants contribute signi-

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ficant amounts of energy, in the form of photosynthates and other nutritional factors to the bacteria (Hardy and Havelka, 1975; Havelka *et al*., 1984; Zachariassen and Power, 1987; Loomis and Connor, 1992). Legume symbiotic N fixation can be inhibited by the presence of both, nitrate and ammonium in the soil (Latimore *et al*., 1977; Evans, 1982; Evans *et al*., 1987; Malik *et al*., 1987; Carroll and Mathews, 1990; Daimon *et al*., 1999; Daimon and Yoshioka, 2000).

The present work is a continuation of earlier studies (Ciesiołka *et al*., 2005). The aim of this work was to answer the following question: does replacement of atmospheric N, with other forms, such as $NH_4^+$, $NO_3^-$, $[NH_4^+ + NO_3^-]$ and $-NH_2$ influence the development and yield of different cultivars of white lupin of the same species and cultivated in countries with different soil and climatic conditions?

## Material and Methods

Three low alkaloid cultivars of *Lupinus albus* L. grown under different climatic conditions (Poland, Spain and Chile) were used. Seed of cv. Butan was supplied by Dr. Stanisław Stawiński, Plant Breeding and Acclimatization Station Przebędowo, near Poznań, Poland. Seed of cv. Marta was supplied by Servicio de Investigaciones Agrarias (Badajoz, Spain) and seed of cv. Multolupa from Dr. E. Von Baer, Campex-Semillas Baer and AVELUP (Temuco-Chile). Grade 2 perlite was purchased from RHP Agro-Perlite, The Netherlands. Cultures of *Bradyrhizobium lupini* were supplied by the Institute of Soil Science and Plant Cultivation (Puławy, Poland). The different N forms used as a fertilizer $(NH_4)_2SO_4$, $NaNO_3$, $NH_4NO_3$, $NH_2CONH_2$ were supplied by POCh, Gliwice, Poland. The fertilizer Azofoska containing K and P was purchased from Fosfory-Gdańsk, Poland. Micronutrients (B, Zn, Mn, Cu, Mo, Fe) and Mg were supplied by Organica-Łódź, Poland.

The experiments were carried out in sterilized perlite in controlled greenhouse during April-August, 2005. Lupin seed was disinfected with 1.5% sodium hypochlorite, and sown in 5.0 L perlite filled pots (20 cm diameter × 18 cm high) on 12 April.

The different N forms were applied in seven treatments: $T_1$, $N_d$ (control); $T_2$, plants inoculated with *B. lupini* (atmospheric N); $T_3$, plants inoculated with *B. lupini* plus a small amout of N as $[NH_4^+ + NO_3^-]$; $T_4$, N as $NO_3^-$ applied as $NaNO_3$; $T_5$, N as $NH_4^+$ applied as $[(NH_4)_2SO_4]$; $T_6$, N as $NH_4^+ + NO_3^-$ applied as $(NH_4NO_3)$; $T_7$, N as $-NH_2$ applied as $[NH_2CONH_2]$.

Each of the seven treatments was applied to all three cultivars and there were six replicates. Each pot contained five seeds. The total N dose and other components (mg in 60 ml of water pot$^{-1}$) used in the experiment are given in Table 1. The N dose used in $T_4$, $T_5$, $T_6$ and $T_7$ was applied four times, two weekly. During vegetative growth plants were irrigated with sufficient water to avoid separate N from the perlite.

The greenhouse experiments ended on 17 July for 'Butan' and on 23 July for 'Marta' and 'Multolupa'.

During vegetative growth, plant development was observed and recorded as follows: i) plant growth, ii) leaf colour and fall, iii) time of finish and start of flowering and iv) maturation time. First detailed observations of the lupins were made after one month, and the last 3 weeks prior to harvest. In all treatments the harvest time for 'Butan' was 7 July and for 'Multolupa' and 'Marta' 28 July.

Seed crude protein was determined by the Kjedahl method with a Kjeltec Auto Distillation 2200 apparatus (FOSS TECATOR).

**Table 1.** Total nitrogen dose (mg in 60 ml of water pot$^{-1}$) used in the experiment

| Treatment | Nitrogen form | N | $P_2O_5$ | $K_2O$ | MgO | Micronutrients (B, Zn, Mn, Cu, Mo, Fe) |
|---|---|---|---|---|---|---|
| $T_1$ | Nd | 0.0 | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_2$ | $N_2$ | Bact susp[1] | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_3$ | $N_2+[NH_4^+ + NO_3^-]$ | Bact susp + 29.0 | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_4$ | $NO_3^-$ | 116.0[2] | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_5$ | $NH_4^+$ | 116.0 | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_6$ | $[NH_4^+ + NO_3^-]$ | 116.0 | 22.0 | 34.0 | 8.0 | 9.0 |
| $T_7$ | $-NH_2$ | 116.0 | 22.0 | 34.0 | 8.0 | 9.0 |

[1] Bacterial suspension of *Bradyrhizobium lupini.* [2] The total dose of N (116 mg) used in $T_4$, $T_5$, $T_6$ and $T_7$ was four applications of 29 mg two weekly.

**Table 2.** Observations of the development of white lupin cvs. Butan, Multolupa and Marta after 1 month of vegetative growth

| Treatment | Nitrogen form | Symptoms |
|---|---|---|
| $T_1$ | Nd | Light-green leaves, weak with small leaf area. After two months lower leaves abscised |
| $T_2$ | $N_2$ | Normal plant development, leaves dark-green |
| $T_3$ | $N_2$ +[$NH_4^+$+$NO_3^-$] | Normal plant development, leaves dark-green |
| $T_4$ | $NO_3^-$ | Chlorosis of middle leaves |
| $T_5$ | $NH_4^+$ | Lower leaves dry, upper leaves dark-green. In cvs. Marta and Multolupa there was disturbed flowering and pod set. Many leaves abscised in these cultivars. After 2 months, plant growth was distinctly disturbed |
| $T_6$ | [$NH_4^+$+$NO_3^-$] | Healthy dark green leaves |
| $T_7$ | –$NH_2$ | Many leaves chlorotic and yellow |

A one-factor analysis of variance was conducted using Statgraphics plus version 4.1 programme. Means were compared using Duncan's test. Significance level was set at $p < 0.05$.

## Results

The three white lupin cultivars developed properly in treatments $T_2$, $T_3$ and $T_6$, where N was in the form $N_2$, $N_2$ +[$NH_4^+$+$NO_3^-$] and [$NH_4^+$+$NO_3^-$] respectively. Plants in the other treatments were mal-developed and showed leaf yellowing and chlorosis (Table 2). In 'Multolupa' and 'Marta' N applied as $NH_4^+$ disturbed flowering and there was a lack of pods and seeds. The first flower appeared on 'Butan' on 20 May. Flowering of 'Multolupa' and 'Marta' began one week later. At the start of July, pods became dry; leaves started to fall and shoots started to lignify.

The average of seed yield pot$^{-1}$, average yield of vegetative part (leaves, stalks, pods and roots) on a dry matter basis and single grain weights (SGW) for the treatments are shown in Table 3. The effect of the different N forms on these parameters depended on lupin cultivar. In 'Butan', the highest seed yields were observed in $T_2$, $T_3$ and $T_5$, where N was applied as $N_2$, $N_2$+[$NH_4^+$+$NO_3^-$] and $NH_4^+$ respectively. The lowest yield was in the control treatment ($T_1$). The seed yield in $T_4$ and $T_7$ (N as $NO_3^-$ and –$NH_2$ respectively), was 1.82 and 2.30 g respectively. For 'Multolupa' and 'Marta', the highest seed yield was in $T_2$ and $T_3$ ($N_2$ and $N_2$+[$NH_4^+$+$NO_3^-$], respectively), and also when N was used as [$NH_4^+$+$NO_3^-$] ($T_6$). Again the lowest seed yield was from the control ($T_1$). When N was applied as $NH_4^+$ ($T_5$), no seed was harvested from either of these cultivars. Although the dry mass of the vegetative plant parts among cultivars was different (Table 3), the yield for all three cultivars was the highest for the $N_2$ and $N_2$+[$NH_4^+$+$NO_3^-$] treatments ($T_2$ and $T_3$). The effect of other N forms depended on cultivar. For example, in 'Butan', except for $T_2$ and $T_3$, the highest dry mass yield was in for $T_5$ ($NH_4^+$). In 'Multolupa' and 'Marta' it was from $T_2$ and $T_3$. As with seed the lowest dry mass yield, in all three cultivars was from the control ($T_1$). Nitrogen form also affected single grain weight (SGW) (Table 3). Generally, the highest SGW for all cultivars was observed in the both $N_2$ forms. The SGW did not always correlate with the amount of seeds. Particularly, in the case of the [$NH_4^+$+$NO_3^-$] form ($T_6$) for 'Butan' and 'Multolupa' and from $T_1$ for 'Marta' (data not presented).

The effect of various N forms on seed protein content and yield is given in Table 4. The different N forms significantly affected seed protein content. In 'Butan', the protein content ranged from 25% ($T_1$) to 35% ($T_6$); for 'Multolupa' from 34% ($T_1$) to 37% ($T_2$); and for 'Marta' from 32% ($T_1$) to 38% ($T_3$). In all cultivars seed from $T_1$ (control) had the lowest protein content and yield. Moreover, N form had a variable effect on seed protein yield. Treatments $T_2$ and $T_3$ had the greatest positive effect on protein yield. This is most evident in 'Multolupa' and 'Marta'. This is due to the high protein content and seed yield of these treatments (Table 3).

## Discussion

As a plant model, *Lupinus albus* was chosen by its economic and ecological importance. The results showed that the three lupin cultivars responded differently to N form irrespective of their origin. Generally, in 'Butan', except in $T_1$, the difference among treatments were not as significant as in 'Multolupa' and 'Marta'.

**Table 3.** The effect of different nitrogen forms on average seed and dry vegetative plant yield (leaves, stalks, pods and roots) and single seed weight of three white lupin cultivars

| Treatment | Average seed yield (g pot$^{-1}$) | Total vegetative yield (g pot$^{-1}$) | Single seed weight (g pot$^{-1}$) |
|---|---|---|---|
| *cv. Butan* | | | |
| $T_1$ | 0.84 ± 0.21[1]a[2] | 5.90 ± 1.20 a | 128.50 ± 19.30 a |
| $T_2$ | 2.65 ± 0.27 c | 8.40 ± 0.90 c | 214.70 ± 32.20 b |
| $T_3$ | 2.68 ± 0.29 c | 8.20 ± 0.90 c | 226.80 ± 34.00 b |
| $T_4$ | 1.82 ± 0.36 c | 7.50 ± 0.70 b | 158.40 ± 23.80 ab |
| $T_5$ | 2.71 ± 0.45 c | 8.20 ± 0.50 c | 210.90 ± 31.60 b |
| $T_6$ | 2.46 ± 0.75 c | 7.80 ± 0.70 b | 184.20 ± 27.60 b |
| $T_7$ | 2.30 ± 0.50 b | 7.10 ± 0.80 b | 189.00 ± 28.40 b |
| *cv. Multolupa* | | | |
| $T_1$ | 0.97 ± 0.59 b | 6.70 ± 1.20 b | 210.00 ± 31.50 ab |
| $T_2$ | 4.24 ± 0.40 d | 14.00 ± 1.70 e | 273.50 ± 41.00 b |
| $T_3$ | 3.84 ± 0.41 d | 13.50 ± 0.80 e | 267.90 ± 40.20 b |
| $T_4$ | 1.30 ± 0.53 b | 10.60 ± 0.70 c | 177.70 ± 26.70 a |
| $T_5$ | — | 2.60 ± 0.30 a | — |
| $T_6$ | 2.57 ± 0.68 c | 11.90 ± 0.90 d | 256.80 ± 38.50 b |
| $T_7$ | 1.76 ± 0.88 b | 12.20 ± 0.70 d | 229.80 ± 34.50 ab |
| *cv. Marta* | | | |
| $T_1$ | 1.04 ± 0.62 b | 6.40 ± 0.70 b | 214.80 ± 32.20 ab |
| $T_2$ | 2.82 ± 0.77 c | 12.10 ± 1.40 d | 241.30 ± 36.20 b |
| $T_3$ | 3.47 ± 0.60 c | 13.00 ± 0.80 de | 266.70 ± 40.00 b |
| $T_4$ | 1.14 ± 0.41 b | 8.80 ± 1.23 c | 166.80 ± 25.00 a |
| $T_5$ | — | 3.10 ± 0.60 a | — |
| $T_6$ | 1.34 ± 0.72 b | 10.90 ± 1.70 d | 190.70 ± 28.60 ab |
| $T_7$ | 0.93 ± 0.83 b | 11.20 ± 3.10 d | 206.70 ± 31.00 ab |

[1] Standard deviation (SD). [2] Letters a-e indicate statistical significance at $p < 0.05$ within the same cultivar.

The latter two cultivars were much more sensitive to the form of N used, particularly for the $NH_4^+$ form ($T_5$), where there was no yield. Although the lupin cultivars differed in their seed yield, a common feature was that highest seed yields were collected from the treatment where N was used in the form of $N_2$ ($T_2$ and $T_3$). Normal development was also obtained when the N supplied was in cation and anion form ($T_6$). Disturbed plant development was seen where N was applied in the other forms. Important factors that could trigger disturbed plant development are pH and salt concentration. According to Górecki and Grzesiuk (2002) the recommended dose of salt, in plant nutrient media should be in the range of 0.3-0.5%. To eliminate this factor, the total N used in treatments $T_4$, $T_5$, $T_6$ and $T_7$ was divided and applied four times, two weekly. It is concluded that the observed disturbed growth, i.e. early leaf yellowing and fall and chlorosis, was associated to the N form used. In lupin, pH was not as significant due to the lupin's adaptation to acid soils (Gulewicz *et al.*, 1993). Cultivars Marta and Multolupa, from Spain and Chile, respectively, were most sensitive to the chemical form of N applied than the Polish 'Butan' (Table 3). In contrast to 'Butan', there was a negative effect on 'Multolupa' and 'Marta' when N was supplied as cation ($T_5$). This negative effect appeared during flowering and resulted in no pod or seed formation. It is very interesting as $NH_4^+$ in a complex with $NO_3^-$ ($T_3$ and $T_6$) appeared to have beneficial effect on the parameters measured. The high sensitivity of both cultivars to [$NH_4^+$] ($T_5$) may have been due to environmental factors such as temperature, humidity, water supply, soil type and light, which affect the development and yield of *L. albus* (Rodrigues *et al.*, 1993; Talhinas *et al.*, 1996; Mullins *et al.*, 1999). In $T_3$, where seeds were inoculated with *Bradyrhizobium lupini*, the addition of a small initial

**Table 4.** Content and yield of crude protein in white lupin seed harvested from different nitrogen treatments

| Treatment | cv. Butan | | cv. Multolupa | | cv. Marta | |
|---|---|---|---|---|---|---|
| | Protein % (N × 6.25) | Protein yield (mg pot$^{-1}$) | Protein % (N × 6.25) | Protein yield (mg pot$^{-1}$) | Protein % (N × 6.25) | Protein yield (mg pot$^{-1}$) |
| T1 | 25.2 ± 0.2[1]a[2] | 212.0 ± 1.4a | 33.6 ± 0.1a | 325.7 ± 0.6a | 31.6 ± 0.0a | 316.3 ± 0.0a |
| T2 | 30.1 ± 0.3b | 798.2 ± 8.2b | 37.4 ± 0.2b | 1,584.9 ± 10.2b | 37.8 ± 0.0b | 1,066.8 ± 0.1b |
| T3 | 32.5 ± 0.0c | 872.1 ± 0.8c | 35.2 ± 0.0c | 1,351.3 ± 1.0c | 38.1 ± 0.4c | 1,323.8 ± 12.8c |
| T4 | 34.2 ± 0.1d | 621.9 ± 2.7d | 35.3 ± 0.1c | 458.5 ± 1.0d | 36.3 ± 0.3d | 413.8 ± 3.0d |
| T5 | 31.8 ± 0.0e | 863.1 ± 1.2e | — | — | — | — |
| T6 | 35.1 ± 0.1f | 864.1 ± 3.7e | 34.8 ± 0.2d | 894.4 ± 6.0e | 35.3 ± 0.2e | 473.2 ± 2.6e |
| T7 | 34.2 ± 0.3d | 787.3 ± 3.8b | 35.9 ± 0.4e | 632.5 ± 5.0f | 37.4 ± 0.0f | 348.2 ± 0.2f |
| Sown seed | 33.7 ± 0.2d | — | 38.3 ± 0.3b | — | 36.6 ± 0.2d | — |

[1] Standard deviation (SD). [2] Letters a-e indicate statistical significance at $p < 0.05$ within the same cultivar.

dose of N in the form of [$NH_4^+$+$NO_3^-$], did not have a negative effect on plant development in any cultivars. Further, in 'Butan' and 'Marta', N addition increased seed protein content and protein yield (Table 4).

Normal lupin plant development observed in treatments $T_2$, $T_3$ and $T_6$ correlates with yield structure parameter. Hence, these N forms seem to positively influence assimilation and photosynthetic processes responsible for the production of vegetative and reproductive structures. The results showed distinct influence on production of seed yield and plant vegetative structure related to genetic and ecological conditions. This work showed that $N_2$ uptake by plants via *B. lupini* gave the best plant development and protein yield in 'Multolupa' and 'Marta'. This is not so clear in 'Butan'. Among other forms of N used, N in the form [$NH_4^+$+$NO_3^-$] ($T_3$ and $T_6$) gave similar results to $N_2$ ($T_2$). The presence of N in both the cation and anion form, in the development and yield of white lupin is interesting in the light of the results obtained for these ions separately. In particular, the negative effect of the cation form ($T_5$) on 'Multolupa' and 'Marta'. However, the «negative effects» of $NH_4^+$ are not apparent when comparing seed (Table 3) and protein yield (Table 4) for $T_4$ and $T_6$. The latter treatment with nitrate and ammonium gave higher yields than $T_4$ with only nitrate for all three cultivars.

In all cases, harvested seeds had a significantly lower SGW compared with the seeds that were sown. The SGW determined by breeders was 230-320 mg for 'Butan', 330-420 mg for 'Multolupa', and 300-400 mg for 'Marta'. This could be due to conditions in the greenhouse experiment, compared with natural conditions: the use of perlite instead of soil, high temperatures, shading, low humidity or water stress during the summer. Another possible reason might be related to the common negatively correlation between seed weight and seed protein content (cereals, legumes, oil crops). As determined by the conditions of the experiment, fixation of atmospheric N by white lupins gave the best conditions for SGW. This statement is supported by Ciesiołka *et al.* (2005).

Various N forms had different effects on the seed protein content and protein yield. However, the most beneficial effect on these parameters included both $N_2$ treatments. This was particularly evident in the case of 'Multolupa' and 'Marta'. In 'Butan', a beneficial effect of N as [$NH_4^+$+$NO_3^-$] form was also observed (Table 4). These results agree with the data of Ciesiołka *et al.* (2005).

In conclusion, the form of N used as a fertilizer had major significance on the development and yield of all three low-alkaloid cultivars of *L. albus*. Generally, in treatments $T_2$, $T_3$ and $T_6$ normal plant development was observed. This is correlated with the yield results. The effect of N form depended on the origin of the lupin cultivar, thus 'Marta' and 'Multolupa' coming from similar climatic conditions, contrasted with 'Butan', which responded with a different behaviour, e.g. no yield, when the $NH_4^+$ form of N was used.

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