IMPACT OF NITROGEN FERTILISATION ON NODULATION CAPACITY AND NITROGEN FIXATION IN FODDER PEAS (*PISUM ARVENSE L.*)

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Abstract: *The present agricultural crop structure is dominated by grains (48%) and by technical plants (21%), detrimental to legumes that share only 11% of the cultivated arable area on Earth. If the area cultivated with legumes doubled, the necessary nitrogen fertilisers would reduce with 50% and production costs would reduce with 30%. The increase of areas cultivated with legumes would also balance the protein balance due to a larger amount of vegetal protein. The present paper points out the impact of nitrogen fertilisation on the nodulation capacity and on atmospheric nitrogen fixation in fodder peas (Pisum arvense L.). Research in the field of nitrogen fixation has pointed out the complexity of the impact of environmental nitrogen taken over by the plants on atmospheric nitrogen fixation. Legumes use nitrogen under nitric form (NO_3^-), as a result of the oxidation of ammonium nitrogen (NH_4^+) from the mineral fertilisers of from organic nitrogen. These two sources of nitrogen used by the plants (NO_3^- and fixed N_2) are strongly dependent on the plant species, on the bacterial pool and on the environmental factors. Literature presents a consolidated scientific opinion concerning the impact of mineral nitrogen on biological nitrogen fixation: an increase of the concentration of nitrate or ammonium correlates with a decrease of the legume nodulation activity (DAVIDSON and ROBSON, 1986; EAGLESHAM, 1989). Following this work resulted in the following conclusions:Research in the field of nitrogen fixation points out the complexity of the impact of the nitrogen taken over by the plants from the environment on atmospheric nitrogen fixation.Legumes use nitrogen under nitric form (NO_3^-) as a result of the oxidation of ammonium nitrogen (NH_4^+) from mineral fertilisers or from organic nitrogen. Nitrogen fertilisation also impact TN and RP plant content. Daily nitrogen fixation decreases from 0.60 mg/plant/day in the control variant to 0.05 mg/plant/day in the variant treated with N_{100}, while the nitrogen fixation rate in relation to dry matter varies between 14.57 mg/g DM and 1.79 mg/g DM in the variant fertilised with N_{100}*

Keywords: *fodder peas, nodosities, protein, fixed nitrogen.*

INTRODUCTION

Agricultural crops are nowadays dominated by grain species (48%) and by technical plants (21%), detrimental to legume crops that share only 11% of the cultivated arable area on Earth. If the area cultivated with legumes doubled, the necessary nitrogen fertilisers would reduce with 50% and production costs would reduce with 30%. The increase of areas cultivated with legumes would also balance the protein balance due to a larger amount of vegetal protein.

The economic and environmental benefits of nitrogen fixation can be summarised, according to (SERRAY, 2004), as follows: profitability, energetic efficacy, plant nutrition and human safety, environmental quality, sustainable agriculture. The importance of developing and improving biological nitrogen fixation systems has become a priority for the agricultural economy of the Earth given that, in the last 50 years, the amount of nitrogen fertilisers increased over 30 times (from 3 to 90 million tonnes), for an annual cost of over 20 million US dollars (SINGLETON et al.,1997). The present paper points out the impact of nitrogen fertilisation on the nodulation capacity and on atmospheric nitrogen fixation in fodder peas (*Pisum arvense L.*).

MATERIAL AND METHODS

Research was carried out in 2012, in plastic vegetation recipients of 600 cm^2. The soil we used had a pH (H_2O) of 6.82 and a total nitrogen content of 0.136%. The experimental setting had three variants: V_1-N_0 (control variant), V_2-N_{50} (300 mg/vase) and V_3-N_{100} (600 mg/vase). Nitrogen was applied as a solution of ammonium nitrate before sowing. In each vegetation recipient we sowed several seeds but we only kept 10 plants and all observations and measurements were summarised as an arithmetic mean of these 10 plants. From a phenological point of view, the vegetation period we studied was between sprouting and blooming. During this vegetation period, we made the following measurements and carried out the following analyses:

- Amount of dry matter (DM);
- Number of nodosities, size and colour of nodosities;
- Weight of nodosities;
- Nitrite and raw protein content in the studied legume species;
- Nitrite content of non-legume species;
- Share of fixed atmospheric nitrogen (% FaN);
- Amount of fixed N_2 (BFN);
- Nitrogen fixation rate (per vegetation day and per dry matter amount).

Observations and measurements of nodosities were made through the calculus of the nodular index according to BEN REBAH et al. (2002), using the following formula:

Nodular index = AxBxC≤18, where:
A = size of nodosities (1-small, 2-medium, 3-large)
B = colour of nodosities (1-white, 2-pink)
C = number of nodosities (1-few, 2-medium, 3-many).

The highest efficacy of nodosities is the 12-18 nodulation index interval. The total nitrogen content of the plants was measured with the Kejldhal method, and raw protein content with the formula CP = 6.25 x TN. The share of atmospheric nitrogen (% FaN) and the amount of fixed nitrogen (BFN) were measured with the nitrogen difference method suggested by ROSS et al. (2008), with the following formulas:

% FaN = [1 - (TN - kg/ha, from the reference species / TN - kg/ha, from the legume species)] x 100
BFN (kg/ha) = (% FaN x TN) / 100

The reference species for fodder peas was oats, sowed separately in a vegetation recipient.

RESULTS AND DISCUSSIONS

Research in the field of nitrogen fixation has pointed out the complexity of the impact of environmental nitrogen taken over by the plants on atmospheric nitrogen fixation. Legumes use nitrogen under nitric form (NO_3^-), as a result of the oxidation of ammonium nitrogen (NH_4^+) from the mineral fertilisers of from organic nitrogen. These two sources of nitrogen used by the plants (NO_3^- and fixed N_2) are strongly dependent on the plant species, on the bacterial pool and on the environmental factors. Literature presents a consolidated scientific opinion concerning the impact of mineral nitrogen on biological nitrogen fixation: an increase of the concentration of nitrate or ammonium correlates with a decrease of the legume nodulation activity (DAVIDSON and ROBSON, 1986; EAGLESHAM, 1989). The presence in the soil of NO_3^- ions for a longer period inhibits the development of nodosities and the activity of nitrogenase (IMSANDE, 1986; ARRESEIGOR et al. 1997).

Table 1.

Impact of nitrogen fertilisation on the nodulation capacity in fodder peas

Variants	**Dry matter**	**Nodosities**			**Weight of nodosities (DM)**		**Efficacy of nodosities[1)]**			
	g/pl	%	no/ plant	%	g/pl	%	A	B	C	Nodular index AxBxC
Control variant (N_0)	1.64	100	22	100	0.33	100	3	2	3	18
N_{50}	1.28	78	16	73	0.19	58	2	2	2	8
N_{100}	1.06	65	4	18	0.04	12	1	1	1	1

[1)] After BEN REBAH et al. (2002), Nodular index = AxBxC≤18

Data presented in (Table 1) show that applying nitrogen fertilisers in peas has a negative impact on the nodulation capacity of fodder peas. Thus, for a rate of N_{100}, the amount of dry matter diminishes with 35%, the number of nodosities decreases from 22 to 4 per plant, and the weight of nodosities decreases with about 80%. The efficacy of nodosities, determined by calculating the nodular index, shows critical decreases: from 18 (in the control variant) to 1 (in the variant fertilised with N_{100}). This latter index shows that the nodosities are very small in size and white in colour, which shows their lack of efficacy in the biological nitrogen fixation process.

Table 2.

Impact of nitrogen fertilisation on total nitrogen (TN) and raw protein (PB) content

Variants	**Legume TN**		**PB**		**Non-legume TN[1)]**	
	%	**mg/pl**	**%**	**mg/pl**	**%**	**mg/pl**
Control variant (N_0)	2.78	45	17.37	285	2.06	21
N_{50}	2.89	37	18.06	231	2.13	22
N_{100}	2.66	28	16.62	176	2.30	26

[1)] Reference plant: oats

Nitrogen fertilisation also impacts TN and RP content in plants. Thus, compared to the control variant, applying a moderate rate of N_{50} results in a slight increase of the amount of nitrogen in the plants (from 2.78% to 2.89%) and of the raw protein content (from 17.37% to 18.06%), but both TN and RP amounts decrease when applying a rate of N_{100}. For a rate of N_{100}, the amount of TN decreases to 28 mg/pl compared to the control variant (45 mg/pl), and the amount of RP decreases to 176 mg/pl compared to 285 mg/pl in the control variant (Table 2).

Table 3.

Impact of nitrogen fertilisation on fixed nitrogen amount (BFN) and on nitrogen fixation rate in fodder peas

Variants	**% FaN**[1)]	**Fixed N_2 (mg/pl)**[1)]	**Nitrogen fixation rate**	
			Fixed N_2/plant/day (mg)[2)]	**Fixed N_2/g DM (mg)**
Control variant (N_0)	53.3	23.9	0.60	14.57
N_{50}	40.5	15.0	0.38	11.72
N_{100}	7.1	1.9	0.05	1.79

1) The nitrogen difference method, after ROSS et al. (2008)
2) Vegetation duration (sprouting- blooming): 40 days

The nitrogen applied has a strong negative impact on the atmospheric nitrogen fixation capacity (Table 3). Thus, the share of atmospheric nitrogen (% FaN) decreases to 53.3% (in the control variant) to 7.1% in the variant treated with N_{100}. In the variant treated with N_{50}, % FaN is 40.5%. The amount of fixed nitrogen (BFN) is 23.9 mg/pl in the control variant and of only 1.9 mg/pl in the variant treated with N_{100}, i.e. a decrease of over 90%.

The daily nitrogen fixation rate decreases from 0.60 mg/plant/day in the control variant to 0.05 mg/plant/day in the variant treated with N_{100}, and the nitrogen fixation rate in relation to the dry matter varies between 14.57 mg/g DM and 1.79 mg/g DM in the variant treated with N_{100}.

CONCLUSIONS

Research in the field of nitrogen fixation points out the complexity of the impact of the nitrogen taken over by the plants from the environment on atmospheric nitrogen fixation.

Legumes use nitrogen under nitric form (NO_3^-) as a result of the oxidation of ammonium nitrogen (NH_4^+) from mineral fertilisers or from organic nitrogen.

Nitrogen fertilisation also impact TN and RP plant content.

Daily nitrogen fixation decreases from 0.60 mg/plant/day in the control variant to 0.05 mg/plant/day in the variant treated with N_{100}, while the nitrogen fixation rate in relation to dry matter varies between 14.57 mg/g DM and 1.79 mg/g DM in the variant fertilised with N_{100}.

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