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The importance of biological fixation of atmospheric nitrogen by leguminous crops

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Abstract. Biological reduction of atmospheric nitrogen (BNF) is one of the most important biological processes on earth with great economic as well as ecological significance. The unique ability to convert non-reactive nitrogen into plant-available ammonia is possessed only by archaeons and bacteria belonging to the diazotrophs. Legumes are a valuable component of crop rotation and their cultivation brings many benefits. However, the most important and invaluable feature of this group of plants is their ability to fix molecular nitrogen in symbiosis with root nodule bacteria. Higher organisms remaining in symbiotic systems are able to fix approximately 200–500 kg N ha^{-1} $year^{-1}$. In contrast, non-symbiotic bacteria fix considerably less nitrogen (1–50 kg N ha^{-1} $year^{-1}$). This element, while remaining in crop residues, can be utilised by succeeding plants in the crop rotation. This is particularly important because of the possibility of reducing the use of mineral fertilisers by up to 20–25%. Thus, the importance of legumes in crop rotation is of particular importance in both organic and integrated crop production.

The aim of this study is to systematise knowledge on the importance of legumes in crop rotation, the symbiosis of legumes with symbiotic bacteria of the genus *Rhizobium*, the impact of factors interfering with this process and newly recognised methods to support it.

Keywords: biological nitrogen fixation (BNF), leguminous crops, root nodule bacteria, yield, Nod factors, Nitragin

INTRODUCTION

Nitrogen (N) is one of the most important biogenic elements conditioning high and good quality crop yields (Trawczyński, 2013). Among other things, it enters into the composition of nucleotides, proteins, vitamins and participates in numerous reactions taking place in the cells of living organisms (Adamczyk, Godlewski, 2010). Its amount in the environment is estimated at 1.6×10^{17} t, but as much as 98% of this is the non-reactive nitrogen (N_2) present in the air, which is not assimilated by plants because higher organisms take up this element in the form of ammonium and nitrate ions (Martinez-Espinosa et al., 2011). Biological fixation of atmospheric nitrogen is the process whereby soil microorganisms convert non-reactive molecular nitrogen from the air (N_2), which is not available to plants, into ammonia that is assimilated by the legumes as a result of a specific molecular dialogue (symbiosis) between these organisms. This unique ability is only possessed by bacteria classified as diazotrophs, which have the ability to fix molecular nitrogen from the air, and archaeons, i.e. small single-celled microorganisms classified as prokaryotes. Microorganisms can form different types of symbiotic systems with higher organisms, such as the symbiosis of root nodule bacteria with legumes, *Frankia radiculata* with woody plants and the symbiosis of cyanobacteria with various organisms such as corals, sponges, bryophytes, ferns and seed plants. An extremely important symbiosis for agriculture and also the best understood to date is the interaction of leguminous plants with root nodule bacteria of the genus *Rhizobium* (Peoples et al., 2009).

IMPORTANCE OF LEGUMINOUS CROPS

Leguminous plants are divided on the basis of seed weight into coarse-seeded plants – seeds weighing more than 15 g and fine-seeded plants weighing less than 15 g. The coarse-seeded legumes include peas, broad beans, vetch, soybeans and beans. On the other hand, clovers, lucerne, melilotus and sainfoin are among the small-seeded legumes. The seeds of the large-seeded legumes contain B vitamins and many valuable elements, such as potassium, phosphorus and magnesium. Due to the high content of total protein rich in lysine in the seeds (lupins, peas, broad beans, soya beans), these plants are considered an important raw material for livestock feed, as this amino acid is deficient in cereal grains. Among legumes, the highest amount of lysine is found in pea seed protein (7.2 g 100 g^{-1} protein) (Kotecki, Kozak, 2020). Legumes are also an extremely valuable component of the human diet, so their seeds are also successfully used for culinary purposes (peas, beans, soybeans, broad beans) (Matyka et al., 1985; Kotecki, Kozak, 2020).

In addition, they play an important role in the crop rotation by interrupting the frequent succession of cereals after each other, while leaving a large amount of nitrogen and other essential elements in the soil and in the crop residues. The amount of root residue dry matter including pea straw harvested from an area of 1 ha is 3 to 5 t, which contains an average of 50–80 kg of nitrogen (Kotecki, Kozak, 2020). Post-harvest residues of legumes leave potassium and humus in the soil in the amount of 35 kg ha^{-1} and in phosphorus in the amount of 25 kg ha^{-1} (Jasińska, Kotecki, 1997). Legumes are characterised by a positive balance of organic matter in the soil, which increases the amount of humus in this environment, causing an enrichment of the soil sorption complex. In addition, the legumes' strong, extensive root system makes it easier for successor plants to take up nutrients from deeper soil layers. However, the most important advantage of leguminous plants is their ability, unique in nature, to establish an efficient and effective symbiosis with root nodule bacteria of the genus *Rhizobium* and *Bradyrhizobium.* The initiation of coexistence with the microorganisms depends on a number of climatic, soil and agrotechnical factors, including mainly the plant species. Most often, this process begins at the 2–3 leaf stage, with the highest intensity of N_2 fixation occurring at the flowering stage (Kotecki, Kozak, 2020).

THE PROCESS OF BIOLOGICAL FIXATION OF ATMOSPHERIC NITROGEN

Biological reduction of atmospheric nitrogen is a highly specific process involving the conversion of unreactive molecular nitrogen N_2 into ammonia (NH_4), that is assimilable by a higher organism, i.e. the plant (Hirsch, 1992; Jensen et al., 2012). The reaction is illustrated by the following equation (Halbleib, Ludden, 2000; Martyniuk, 2019):

$$8H^+ + N_2 + 8e^- + 16MgATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16MgADP + 16Pi$$

where:
ATP – adenosine triphosphate, ADP – adenosine diphosphate, Pi – inorganic phosphate

Two biologically compatible organisms are involved in establishing symbiosis: the plant (macrosymbiont) and the plant-specific root nodule bacteria (microsymbiont) (Denison, Kiers, 2011). Soil root nodule bacteria are referred to as diazotrophs due to their ability to bind N_2. The exchange of numerous signals in molecular dialogue and the

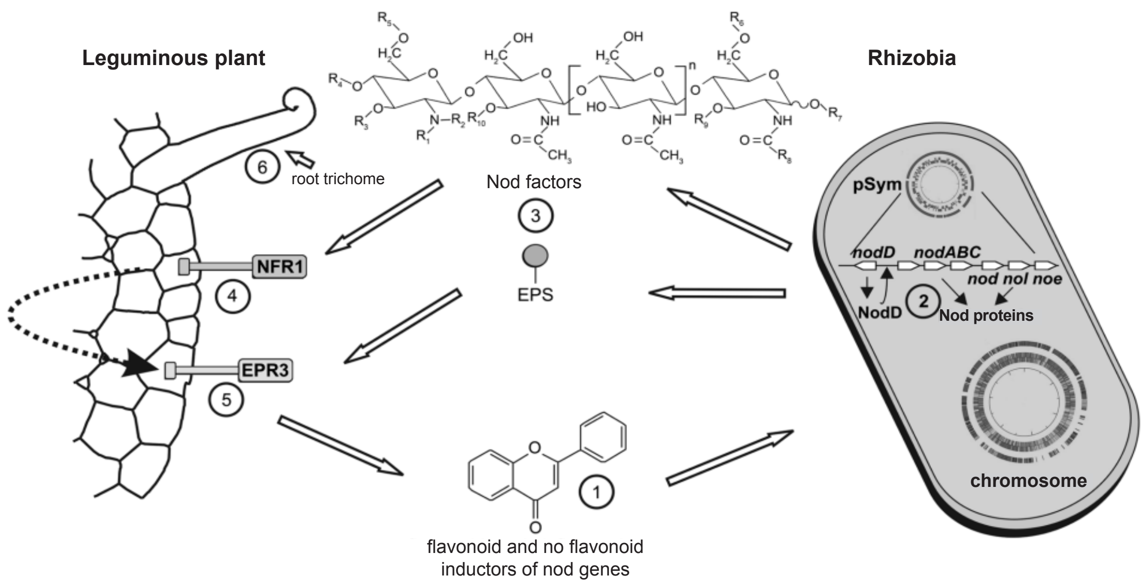

Figure 1. Initiation of rhizobia symbiosis with legumes. (1) Plant root secretions containing flavonoid compounds activate the NodD protein; (2) NodD protein induces the expression of *nod* genes responsible for the synthesis of Nod factor (NF); (3) Nod factor and EPS (an exopolysaccharide) through their NFR and EPR receptors (4 and 5) initiate a cascade of signals leading to the formation of the characteristic root trichome bend (6) and the formation of a root nodules (Stasiak et al., 2016).

mutual recognition of partners are the beginning of a highly specific interaction between macro- and microsymbiont (Mancinelli, 1996). Under the influence of stress factors (e.g. nitrogen starvation), the plant releases numerous substances into the soil environment, including flavonoids, betaines and phenolic compounds. For the initiation of the symbiotic nitrogen fixation process and its efficiency, flavonoid compounds are the most important, whose presence in the rhizosphere attracts bacteria to root trichomes and activates *nod* genes (Cooper, 2007; Skorupska et al., 2010). In response to plant metabolites, the microsymbiont releases the bacterial Nod factor into the soil. This is the most important signalling molecule in the molecular dialogue between symbiosis partners. The Nod factor is a lipochitooligosaccharide (LCO) that contributes primarily to the deformation of root hairs, thus facilitating the entry of bacteria into them, and is responsible for the formation of new meristems that are the origin of root nodule (Fig. 1). The nodules are an important organ with a complex structure in which the reduction of atmospheric nitrogen takes place. Nitrogenase, an enzyme involved in the N_2 reduction reaction, as well as leghemoglobin, a protein responsible for protecting the enzyme from excess oxygen, play an important role in this process. Root nodules, depending on the timing of meristem activity, are divided into determined (limited) and undetermined (unlimited growth) and collar-type nodules (Hadri et al., 1998).

Pea, vetch and alfalfa plants have nodules of the undetermined type, where the meristem is persistent and able to function throughout the growing season and bound nitrogen is transported to the plant organs in the form of amides. In contrast, soybean has nodules where the meristem is short-lived and nitrogen bound by reduction is incorporated into the remaining plant cells in the form of ureides (Libbenga, Harkes, 1973; Newcomb et al, 1979; Vance et al, 1987). Collar-type nodules are present in the roots of white lupin, clover and alfalfa plants. They are characterised by gradient developmental zones, a meristem subject to division throughout the growing season and nitrogen transport in the form of amides (Sujkowska, 2009; Oleńska, Małek, 2017)

EFFICIENCY OF ATMOSPHERIC NITROGEN FIXATION

Biological reduction of atmospheric nitrogen is an ecologically and economically extremely important process that allows a significant reduction in the use of mineral nitrogen fertilisers. Every year, it provides between 139 and 170 million tonnes of N to the global nitrogen cycle, of which as much as 60–80% of this amount is symbiotically bound N (Ishizuka, 1992; Martyniuk, 2012). Using yellow lupin as an example, it was shown (Wysokiński et al., 2014) that during the growing season the plant takes up about 200 kg of nitrogen, with N taken up from the atmosphere accounting for as much as 65% of this amount and nitrogen from synthetic fertilisers only 5%. It is also worth noting that more than 65 kg N ha^{-1} (30%) is taken up by the plant from the soil (Table 1).

The amount of symbiotically bound nitrogen depends, among other things, on the crop species and the efficiency of the particular rhizobial strain. Biological reduction of atmospheric nitrogen is a highly specific process in which a given rhizobial crop species can only fix nitrogen through symbiosis with the corresponding species of root nodule bacteria (Table 2).

Table 1. Symbiotic nitrogen fixation by yellow lupin (Wysokiński et al., 2014).

Sources of nitrogen	Unit	Part of the plant		Total/average in the plant
		residues post-harvest	seeds	
Total quantity of nitrogen uptake	kg N ha^{-1}	75.3	137.8	213.1
	%	35.3	64.7	100.0
of which:				
Atmosphere	kg N ha^{-1}	43.5	94.4	137.9
	%	57.8	68.5	64.7
Fertiliser	kg N ha^{-1}	3.6	6.43	10.0
	%	4.8	4.7	4.7
Soil	kg N ha^{-1}	28.2	37.0	65.2
	%	37.4	26.8	30.6

Table 2. Comparison of the efficiency of N_2 reduction by legumes and relevant symbiotic bacterial species (Mądrzak, 1995).

Plant species	Bacterial species	Bound N_2 [kg ha^{-1}year^{-1}]
Lupin (*Lupinus* sp.)	*Bradyrhizobium* sp.	20–200
Peas (*Pisum sativum* L.)	*Rhizobium leguminosarum* bv. *viciae*	55–77
Broad beans (*Vicia faba* L.)	*Rhizobium leguminosarum* bv. *viciae*	45–552
Lentils (*Lens esculenta, Lens culinaris* Medik.)	*Rhizobium leguminosarum* bv. *viciae*	88–114
Soybean (*Glycine max* (L.) Merr.)	*Bradyrhizobium japonicum*	40–200
Beans (*Phaseolus vulgaris* L.)	*Rhizobium leguminosarum* bv. *phaseoli*	40–70

FACTORS INFLUENCING THE EFFICIENCY OF THE SYMBIOTIC ATMOSPHERIC NITROGEN FIXATION PROCESS

The effectiveness and efficiency of the symbiosis process is influenced by a number of agronomic and habitat factors that can either favour the process or interfere with the exchange of signalling particles between partners. The main factors include:

- **availability of nutrients in the soil**

In the case of legumes, nitrogen fertilisation is only advisable at the beginning of the growing season before the start of symbiosis with *Rhizobium* bacteria. Its purpose is to feed the plants during the initial growth and development period before the source of N is biologically fixed nitrogen. At this stage, a so-called starter dose of 20–30 kg N ha^{-1} is recommended. Larger doses of N may interfere with the uptake of free N_2 from the atmosphere.

Phosphorus and potassium are also important in legume cultivation. These macronutrients have a number of important functions in the plant, including supporting proper water supply, leaf, root system and root nodule function, as well as influencing proper photosynthesis and the distribution of assimilates and export of bound N to intensive growing sites (Podleśna, 1999; Zahran, 1999; Bucher, 2007).

Sulphur also plays an important role in nitrogen fixation as an essential component of bacteroids. Sulphur deficiency leads to a reduction in the amount of leghemoglobin, glucose and ferredoxin in the root nodule. Also, the number and mass of nodule are reduced under conditions of sulphur deficiency, which is essential for their formation. The indispensability of sulphur in the nitrogen fixation process is evidenced by studies with the ^{34}S isotope, confirming that it can be long-distantly translocated to maintain this process (Siegl et al., 2024).

Molybdenum, which is component of the reducing protein, plays a special role in the biological reduction of atmospheric nitrogen. Despite the very low plant requirement for this nutrient (up to a few hundred grams/ha), its deficiency can significantly interfere with nitrogenase function (Rubio, Ludden, 2008; Seefeldt et al., 2009).

- **soil pH**, which affects the legume and nitrogen-fixing bacteria (Strzelec, 1988a; Paśmionka, 2017). For most legumes and their bacterial symbionts, the optimum soil pH is 6.5–7.2. In acidic soils, excessive concentrations of hydrogen ions, aluminium ions and manganese interfere with the proper growth and development of legumes. High concentrations of aluminium ions are harmful or toxic to crop plants. Too high concentration of this element in the soil restricts the uptake of water and nutrients by the plant and inhibits the development of its root system. An excessive concentration of aluminium ions also stunts the growth of *Rhizobium* bacteria, contributing to a reduction in their multiplication rate (Howieson et al., 1993). Acidic soils are deficient in calcium, phosphorus and molybdenum. These elements not only stilify the development of the two symbiosis partners, but are also involved in the process of biological reduction of molecular nitrogen, so their deficit in the soil may adversely affect the efficiency of nodulation. In Poland, as much as 83% of soils are acidic. It is therefore important to liming in order to increase soil pH and improve the efficiency of symbiosis and consequently increase the yield of legumes (Strzelec, 1988b).

- **the action of various pathogens and pests.** The greatest damage leading to disruption of nitrogen reduction due to damage to or complete degradation of root nodules is caused by larvae of the weevil *Sitona lineatus* L., which feed in the rhizosphere on the root nodules of legumes (Borowiecki, 2004).

- **temperature, salinity, and the degree of soil moisture are factors that affect the function of the nodules and their ability to fix N_2.** Excessive salt in the soil contributes to a reduction in the amount of water in the legume root nodules and thus to their premature ageing. Temperature is also an important factor in the symbiosis process. For most diazotrophs, the optimum temperature is between 15 and 30 ^{o}C. Drought stress significantly decreses the efficiency of nitrogen reduction, as soil water deficiency both stunts the growth and development of the whole plant, and interferes with the transport of symbiosis products from the nodules to other plant organs (Graham, 1992; Mengel, 1994; Sawicka, 1997).

- **crop rotation, plant species.** The efficiency of molecular nitrogen fixation depends, among other things, on the amount of symbiotic bacteria in the soil. Infrequent cultivation of a particular plant species results in low or no rhizobia strain in the soil (Martyniuk et al., 2013). The amount of symbiotically bound nitrogen also depends on the plant host species. Among leguminous plants, lupin and soybeans remaining in a symbiotic system with a bacterial strain can fix up to 200 kg N ha^{-1} $year^{-1}$ (Mądrzak, 1995). The symbiosis process can be adversely affected by the active substances of some seed dressings, as well as by pesticide residues in the soil (Streeter, 1994).

METHODS TO IMPROVE BIOLOGICAL REDUCTION OF ATMOSPHERIC NITROGEN

The efficient course of the process of biological nitrogen fixation by legumes depends mainly on ensuring favourable soil, climatic and agrotechnical conditions (i.e. plant species and cultivar, fertilisation, soil reaction) for their development and symbiosis with bacteria and the functioning of these bacteria (Szpunar-Krok, Pawlak, 2023). However, since in agricultural practice the amount and distribution of precipitation and air temperature are beyond our control, other factors are sought to maintain or increase the efficiency of the symbiotic nitrogen fixation process. To date, two main methods affecting this process are known:

- **Use of bacterial vaccines**

The prerequisite for a correct and effective biological nitrogen fixation process is the selection of the leguminous crop for the site and the presence of a suitable strain of symbiotic bacteria in the soil in sufficient numbers to guarantee effective interaction with the plants in the canopy. Unfortunately, interruptions in the cultivation of leguminous crops and unfavourable conditions for the development of atmospheric nitrogen-fixing bacteria (e.g. drought) mean that soils are often not rich in these strains. Therefore, cultivated leguminous crops cannot demonstrate their genetic yield potential and yield well below expectations.

The process of biological nitrogen reduction can be improved by using pre-sowing bacterial inoculations containing a symbiotically effective bacterial strain selected for the plant species. The inoculation process is one of the oldest agrobiotechnological methods (Bashan et al., 2014). The production of a bean plant vaccine under the name Nitragina has a long history in Poland. It was launched in 1954 at Zakład Przetwórczo-Usługowo-Handlowy „Biofood" S. C. in Wałcz (Strzelec, 1988a). Currently, vaccines of this type are also available from the Department of Agricultural Microbiology of the IUNG-PIB in Puławy, Poland. Studies conducted for years on the symbiosis of legumes with bacteria of the genus *Rhizobium* have shown that the efficiency of nodulation depends on the effectiveness of the selected strain, the abundance of bacteria, and the application technology of the preparation (Strzelec, 1988a; Martyniuk, 2012; Pudełko et al., 2017). Inoculations containing live bacterial cultures are applied in soil or as pre-sowing seed dressings (Martyniuk et al., 2013). Unfortunately, often bacteria introduced by this method face strong competition from the autochthones, resulting in lower survival and multiplication, which ultimately leads to a significant decrease in inoculation efficiency (Streeter, 1994). However, this is a method used worldwide and research by Martyniuk et al. (2013) confirm the effectiveness of the inoculations used in terms of nodulation intensity and soybean seed weight gained. The best inoculation (bacterisation) effect is achieved when the number of autochthons in the soil is low (less than 10 cells per gram) (Strzelec, 1988a; Thies et al., 1991). Studies have shown that pea, legume, and clover symbionts are common in Polish soils throughout the country. Soybean is grown infrequently hence the population of symbiotic bacteria of this plant in Polish soils is lower (Martyniuk et al., 2013; Martyniuk, 2019).

- **Use of Nod factors**

With the development of research into this complex process, work has begun to increase its efficiency at the stage of exchange of signalling molecules during the molecular dialogue between the bean plant and the relevant bacterial strain. One of the most important compounds involved in the establishment of symbiosis and the formation of root nodules is bacterial Nod factors (lipochitooligosaccharides – LCOs). This low-molecular-weight signalling compound consists of 3-6 N-acetyl-D-glucosamine residues linked by a β(1-4) glycosidic bond and a fatty acid at the C2 position at the non-reducing end (Fig. 2). These compounds are transferred between the bacterium and the plant and can be inactivated at this stage (Ovtsyna et al., 2000). A new strategy for influencing the process of growth, development and yield of legumes involves the supply of LCOs signalling compounds to sown seeds, which determines their adequate quantity already at the start of plant vegetation.

Research conducted so far has shown that Nod agents even in low, submicromolar concentrations applied as seed dressing or spray in pea (Siczek et al., 2014), vetch (Kidaj et al., 2012) or soybean (Almaraz et al., 2011) crops improved the nodulation process of these plants and increased the efficiency of biological reduction of molecular nitrogen. These compounds also have beneficial effects on the growth and development of many other crops (e.g. maize, rice, tomato, canola, cotton, barley) especially under un-

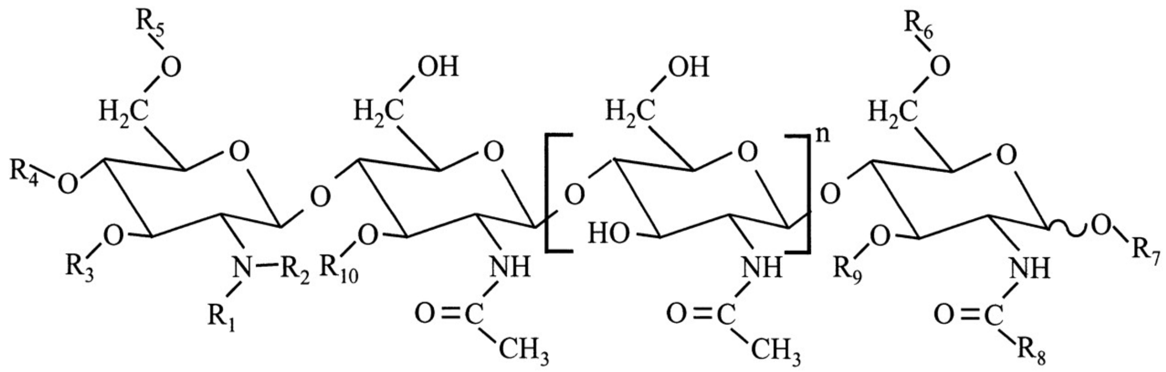

Figure 2. General structure of Nod factors produced by bacteria of the genus *Rhizobium* (D'Haeze, Holsters, 2002).

favourable environmental conditions (i.e. drought) (Souleimanov et al., 2002; Chen et al., 2006; Kidaj et al., 2012; Smith et al., 2015). In a study by Souleimanov et al. (2002), it was shown that the application of rhizobial Nod factors resulted in an increase in the leaf area and intensity of the photosynthetic process in the leaves of maize plants.

Experiment conducted by Podleśny et al. (2017) showed that Nod agents applied in the form of a spray in pea cultivation positively influenced the dynamics of growth of vegetative and generative plant organs. Spraying with the preparation containing LCO signalling particles significantly increased the number of root nodules on the pea plant at flowering and green pod stage. The preparation also stimulated nitrogenase activity at both developmental stages. The authors showed that spraying pea plants with Nod agents resulted in an improvement in the characteristics of yield structure elements and yield increase. The results of other experiments proved that equally satisfactory effects could be obtained by applying the preparation of Nod factors in the form of a seed dressing (Podleśny et al., 2013). Ongoing research has shown the beneficial effect of this treatment on the components of pea yield structure (i.e. number of pods per plant, number of seeds per pod and 1000 seed weight) and final seed yield. It was found that the morphogenetic effect of LCO signalling compounds contributed to an increase in the number of root nodules, while improving the process of symbiotic nitrogen fixation and, consequently, influenced an increase in seed yield. In a study by Siczek et al. (2020) showed that soaking legume seeds in a preparation of Nod factors did not significantly affect the number and dry weight of root nodules, but significantly increased nitrogenase activity (Table 3). It was also found that rhizobial Nod factors had a beneficial effect on protein yield of legume seeds.

Other studies have shown that the application of Nod factors in the form of a seed dressing or spray also improves photosynthesis. This compound, regardless of the form of application, significantly increased the intensity of photosynthesis, plant transpiration and plant stomatal conductance (Podleśny et al., 2014a). The results of other experiments showed that pre-sowing seed soaking in a preparation containing bacterial Nod factors favourably influenced the height of pea plants and significantly increased their leaf area at the

Table 3. Parameters of nodules and nitrogenase activity as influenced by Nod factors application with standard deviations (n = 10). Different letters in rows indicate significant differences ($p < 0.05$) (Siczek et al., 2020).

Parameter	Control	Nod factor
	Nodules	
Number [plant^{-1}]	86.8 (37.0) a	103.0 (23.2) a
Dry matter [mg plant^{-1}]	0.085 (0.026) a	0.101 (0.025) a
	Nitrogenase activity	
C_2H_4 µmol h^{-1}	4.58 (4.28) b	22.37 (7.56) a
C_2H_4 µmol h^{-1} g nodule^{-1}	51.7 (44.2) b	220.3 (48.7) a
C_2H_4 µmol h^{-1} nodule^{-1}	56.1 (50.9) b	221.1 (74.8) a

Table 4. Selected morphological characteristics of peas at flowering (BBCH 60) (Podleśny et al., 2014b).

Specification	Seed soaking	Variety		
		Milwa	Cliff	average
Plant height [cm]	water	67.4 aB*	62.8 aA	65.1 a
	LCOs	74.5 bB	66.7 bA	70.6 b
	average	70.9 B	64.7 A	
Leaf area [cm^2 plant^{-1}]	water	458 aA	614 aB	536 a
	LCOs	491 bA	652 bB	571 b
	average	474 A	633 B	
Number of leaves per plant	water	21.1 aA	19.9 aA	20.5 a
	LCOs	22.2 aA	21.9 aA	22.0 a
	average	21.6 A	20.8 A	

* LSD test range results. Values marked with the same letters are not significantly different at the 5% probability level. Upper case letters refer to data placed in rows and lower case letters refer to data placed in columns.

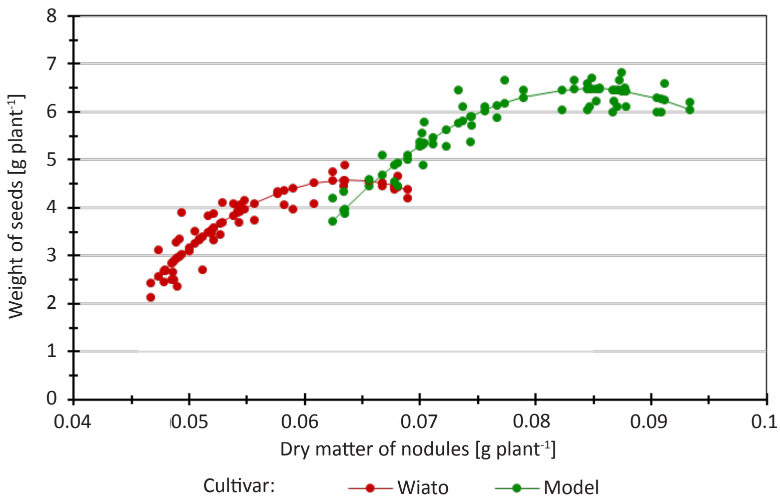

Figure 3. Relationship between root nodule dry weight and seed weight (Smytkiewicz et al., 2021).

flowering stage (BBCH 60) regardless of the cultivar tested (Table 4) (Podleśny et al., 2014b).

Using the example of pea cultivar: Wiato (traditional foliage type) and Model (afila type), it was shown that the use of selected Nod factors improves the process of molecular nitrogen reduction by increasing the mass of pea root nodules which promotes N fixation and thus increases seed yield (Smytkiewicz et al. 2021). With regard to root nodule mass, a limiting value was found up to which an increase in root nodule mass was accompanied by an increase in yield, and beyond which there was no yield increase and even a slight decrease was observed (Fig. 3) (Smytkiewicz et al., 2021).

SUMMARY

The symbiosis of legumes with root nodule bacteria of the genus *Rhizobium* is a highly specific process in which a form of molecular nitrogen, which is not available to plants, is converted into ammonia. This ability, unique in nature, of a higher organism to biologically reduce N_2 with the participation of diazotrophs allows for a significant reduction in the use of synthetic mineral fertilisers in the cultivation of legumes and successor plants. These extremely valuable properties of legumes make them an important link in crop rotation.

Unfortunately, despite the many advantages of their cultivation, the yield-forming potential of this group of plants is not fully exploited in Polish agriculture. Year-to-year variability and low seed yields are the main reasons for the still too small area under legume cultivation in Poland. For this reason, research has been conducted for years with the aim of increasing the yield of this group of plants. One such solution is to intervene in the process of biological reduction of atmospheric nitrogen increasing the efficiency of nodulation. Research by a number of authors has shown that an important agrotechnical treatment in the cultivation of leguminous crops is the use of bacterial vaccines that contain active strains of bacteria. Interfering with the exchange of signalling molecules (use of Nod factors) between symbiosis partners also appears to be a good way of improving the efficiency of biological nitrogen reduction and thus increasing legume yields. The use of LCOs signalling particles in combination with other preparations that would improve the process of biological reduction of atmospheric nitrogen and yield of legumes (e.g. with micronutrient fertilisers or bacterial vaccines) may also be important in the future.

REFERENCES

Adamczyk B., Godlewski M., 2010. Various strategies of nitrogen acquisition by plants. Kosmos. Problemy Nauk Biologicznych, 59(1-2): 211-222. (in Polish + summary in English)

Almaraz J.J., Mabood F., Zhou X., Souleimanov A., Smith D.L., 2011. Effect of Nod factor sprays on soybean growth and producitvity under field conditions. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 61: 228-234.

Bashan Y., de-Bashan L.E., Prabhu S.R., Hernandez J.P., 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). Plant and Soil, 378: 1-33.

Borowiecki J., 2004. Nowe aspekty symbiotycznego wiązania azotu. Postępy Nauk Rolniczych, 2: 9-18.

Bucher M., 2007. Functional biology of plant phosphate uptake at root and mycorhiza interfaces. New Phytologist, 173(1): 11-26.

Chen C., McIver J., Yang Y., Bai Y., Schultz B., McIver A. ,2006. Foliar application of lipochitooligosaccharides (Nod factors) to tomato (*Lycopersicon esculentum*) enhances flowering and fruit production. Canadian Journal of Plant Pathology, 87(2): 365-372.

Cooper J.E., 2007. Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. Journal Applied Microbiology, 103(5): 1355-1365.

D'Haeze W., Holsters M., 2002. Nod factors structures, responses and perception during initation of nodule development. Glycobiology, 12: 79-105.

Denison R.F., Kiers E.T., 2011. Life histories of symbiotic rhizobia and mycorrhizal fungi. Current Biology, 21: 775-785.

Graham P.H., 1992. Stress tolerance in Rhizobium and Bradyrhizobium, and nodulation under adverse soil conditions. Canadian Journal of Microbiology, 38: 475-484.

Hadri A-E., Spaink H.P., Bisseling T., Brewin N.J., 1998. Diversity of root nodulation and egizobial infection processes. pp. 347-360. In: The Rhizobiaceae; eds: H.P. Spaink, A. Kondorosi, P.J.J. Hooykaas; Kluwer Academic Publishers.

Halbleib C.M., Ludden P.W., 2000. Regulation of biological nitrogen fixation. The Journal of Nutrition, 130(5): 1081-1084.

Hirsch A.M., 1992. Development biology of legume nodulation. New Phytologist, 122: 211-237.

Howieson J.G., Robson A.D., Ewing M.A., 1993. External phosphate and calcium concentrations, and Ph, but not the products of rhizobial nodulation genes, affect the attachment of rhizobium meliloti to roots of annual medics. Soil Biology and Biochemistry, 25(5): 567-573.

Ishizuka J., 1992. Trends in biological nitrogen fixation research and application. Plant and Soil, 141(1-2): 197-209.

Jasińska Z., Kotecki A., 1997. Weight and chemical composition of post-harvest residues of some pea and faba bean cultivars. Zeszyty Problemowe Postępów Nauk Rolniczych, 446: 239-246. (in Polish + summary in English)

Jensen E.S., Peoples M.B., Boddey R.M., Gresshoff P.M., Hauggaard-Nielsen H., Alves B.J.R., Morrison M.J., 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. Agronomy for Sustainable Development, 32: 329-364.

Kidaj D., Wielbo J., Skorupska A., 2012. Nod factors stimulate seed germination and promote growth and nodulation of pea and vetch under competitive conditions. Microbiological Research, 167: 144-150.

Kotecki A., Kozak M., 2020. Część VIII. Rośliny bobowate grubonasienne (strączkowe), 95-121, Andrzej Kotecki, Uprawa roślin. Tom III, Wydawnictwo Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław.

Libbenga K.R., Harkes P.A.A., 1973. Initial proliferation of cortical cells in the formation of root nodules in *Pisum sativum* L. Planta, 114: 17-28.

Mancinelli R.L., 1996. The nature of nitrogen: an overview. Life Support & Biosphere Science, 3(1-2): 17-24.

Martinez-Espinosa R.M., Cole J.A., Richardson D.J., Wartmough N.J., 2011. Enzymology and ecology of the nitrogen cycle. Biochemical Society Transactions, 39: 175-178.

Martyniuk S., 2012. Scientific and practical aspects of legumes symbiosis with root-nodule bacteria. Polish Journal of Agronomy, 9: 17-22. (in Polish + summary in English)

Martyniuk S., 2019. Biological fixation of N_2, symbiotic bacteria of legumes in Polish soils and estimation of their numbers. Polish Journal of Agronomy, 38: 52-65. (in Polish + summary in English)

Martyniuk S., Kozieł M., Stalenga J., 2013. Effect of various strains of symbiotic bacteria on yields and nodulation of lupine and soybean. Journal of Research and Applications in Agricultural Engineering, 58(4): 67-70. (in Polish + summary in English)

Matyka S., Burczyńska-Niedziałek A., Korol W., 1985. Skład chemiczny nasion krajowych odmian roślin strączkowych grubonasiennych. Biuletyn Informacji Przemysłu Paszowego, 1: 3-10.

Mądrzak C.J., 1995. Molekularne mechanizmy symbiozy Rhizobiaceae z roślinami motylkowatymi. 232, Mądrzak C.J., Rozprawa habilitacyjna, Wydawnictwo Akademia Rolnicza w Poznaniu, Poznań.

Mengel K., 1994. Symbiotic dinitrogen fixation – its dependence on plant nutrition and its ecophysiological impact. Zeitschrift für Pflanzenernährung und Bodenkunde, 157: 233-241.

Newcomb W., Sippell D., Peterson R.L., 1979. The early morphogenesis of *Glycine max* and *Pisum sativum* root nodules. Canadian Journal of Botany, 57: 2603-2616.

Oleńska E., Małek W.G., 2017. Brodawki korzeniowe jako organy symbiozy roślin bobowatych z ich diazotroficznymi mikrosymbiontami – rozwój, budowa i funkcjonowanie w warunkach fizjologicznych oraz stresu oksydacyjnego. Polskie Towarzystwo Botaniczne, Różnorodność biologiczna – od komórki do ekosystemu. Interdyscyplinarne i aplikacyjne znaczenie badań biologicznych, pp. 87-100.

Ovtsyna A.O., Schultze M., Tikhonovich I.A., Spaink H.P., Kondorosi E., Kondorosi A., Staehelin C., 2000. Nod Factors of Rhizobium leguminosarum bv. viciae and Their Fucosylated Derivatives Stimulate a Nod Factor Cleaving Activity in Pea Roots and Are Hydrolyzed in Vitro by Plant Chitinases at Different Rates. Molecular Plant-Microbe Interactions, 13: 799-807.

Paśmionka I., 2017. Microbiological transformations of soil nitrogen. Kosmos 66(2): 185-192. (in Polish + summary in English)

Peoples M.B., Brockwell J., Herridge D.F., Rochester I.J., Alves B.J.R., Urquiaga S., Boddey R.M., Dakora F.D., Bhattarai S., Maskey S.L., Sampet C., Rerkasem B., Khan D.F., Hauggaard-Nielsen H., Jensen E.S., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis, 48: 1-17.

Podleśna A., 1999. Oddziaływanie stresu potasowego na realizację potencjału plonotwórczego bobiku. Zeszyty Problemowe Postępów Nauk Rolniczych, 469: 257-263.

Podleśny J., Wielbo J., Podleśna A., Kidaj D., 2013. Usefulness of nod preparation (LCOs) use to presowable dressing of pea seeds (*Pisum sativum* L.). Journal of Research and Applications in Agricultural Engineering, 58(4): 124-129. (in Polish + summary in English)

Podleśny J., Wielbo J., Podleśna A., Kidaj D., 2014a. The pleiotropic effects of extract containing rhizobial Nod factors on pea growth and yield. Central European Journal of Biology, 9(4): 396-409.

Podleśny J., Wielbo J., Podleśna A., Kidaj D., 2014b. The responses of two pea genotypes to Nod factors (LCOs) treatment. Journal of Food, Agriculture & Environment, 12(2): 554-558.

Podleśny J., Wielbo J., Podleśna A., Perzyński A., 2017. Effect of molybdenum and lipochitooligosaccharides on yielding of pea. Przemysł Chemiczny, 96/8: 1805-1808. (in Polish + summary in English)

Pudelko K., Narożna D., Króliczak J., Kidaj D., Wielbo J., Skorupska A., Mądrzak C., 2017. Nod Factors as potential stimulants of the lupine nodulation process. Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu, 626: 115-132. (in Polish + summary in English)

Rubio L.M., Ludden P.W., 2008. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annual Reviews of Microbiology, 62(1): 93-111.

Sawicka A., 1997. Czynniki ograniczające wiązanie azotu atmosferycznego u roślin motylkowatych i traw. Biuletyn Oceny Odmian, 29: 53-58.

Seefeldt L.C., Brain M., Hoffman B.M., Dean D.R., 2009. Mechanism of nitrogen fixation by nitrogenase: the next stage. Annual Review of Biochemistry, 78: 701-722.

Siczek A., Lipiec J., Wielbo J., Kidaj D., Szarlip P., 2014. Symbiotic activity of pea (*Pisum sativum*) after application of Nod Factors under field conditions. International Journal of Molecular Sciences, 15: 7344-7351.

Siczek A., Wielbo J., Lipiec J., Kalembasa S., Kalembasa D., Kidaj D., Szarlip P., 2020. Nod factors improve the nitrogen content and rhizobial diversity of faba bean and alter soil dehydrogenase, protease, and acid phosphomonoesterase activities. International Agrophysics, 34: 9-15.

Siegl A., Afjehi-Sadat L., Wienkoop S., 2024. Systemic long-distance sulfur transport and its role in symbiotic root nodule protein turnover. Journal of Plant Physiology, 297: 154260.

Skorupska A., Wielbo J., Kidaj D., Marek-Kozaczuk M., 2010. Enhancing Rhizobium-legume symbiosis using signaling factors, 27-54, Khan M.S, Musarrat J., Zaidi A. Microbes for Legume Improvement, Springer-Verlag, Vienna.

Smith S., Habib A., Kang Y., Leggett M., Diaz-Zorita M., 2015. LCO applications provide improved responses with legumes and nonlegumes. Biological Nitrogen Fixation, 2: 1077-1086.

Smytkiewicz K., Podleśny J., Wielbo J., Podleśna A., 2021. The Effect of a Preparation Containing Rhizobial Nod Factors on Pea Morphological Traits and Physiology. Agronomy, 11: 1457.

Souleimanov A., Prithiviraj B., Smith D.L., 2002. The major Nod factor of *Bradyrhizobium japonicum* promotes early growth of soybean and corn. Journal of Experimental Botany, 53: 1929-1934.

Stasiak G., Mazur A., Koper P., Żebracki K., Skorupska A., 2016. Symbiosis of rhizobia with legume plants (Fabaceae). Postępy Mikrobiologii, 55(3): 289-299. (in Polish + summary in English)

Streeter J.G., 1994. Failure of inoculant rhizobia to overcome the dominance of indigenous strains for nodule formation. Canadian Journal of Microbiology, 40: 513-522.

Strzelec A., 1988a. Symbiotyczne wiązanie wolnego azotu. Cz. I. Znaczenie bakterii symbiotycznych, ich występowanie w glebach i szczepionki *RHIZOBIUM* dla roślin motylkowatych. Postępy Nauk Rolniczych, 4(88): 17-30.

Strzelec A., 1988b. Symbiotyczne wiązanie wolnego azotu. Cz. II. Wpływ właściwości biotycznych i odczynu gleb na zdolność konkurencyjną szczepów *RHIZOBIUM* i ich symbiozę z roślinnym gospodarzem. Postępy Nauk Rolniczych, 5-6(35): 19-28.

Sujkowska M., 2009. The infection process during legume-Rhizobium symbiosis. Wiadomości Botaniczne 53(1/2): 35-53. (in Polish + summary in English)

Szpunar-Krok E., Pawlak R., 2023. The importance of nutrients for legumes – macroelements. Agronomy Science, 78(1): 135-151. (in Polish + summary in English)

Thies J.E., Singleton P.W., Bohlool B.B., 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Applied and Environmental Microbiology, 57: 19-28.

Trawczyński C., 2013. Assessment of mineral nitrogen content in the soil after harvest of potato tubers. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin, 267: 87-96. (in Polish + summary in English)

Vance C.P., Boylan K.L.M., Stade S., 1987. Host plant determinants of legume nodule function: similarities to plant disease situations. pp. 271-287. In: Molecular Determinants of Plant Diseases; Nishimure S.; Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin.

Wysokiński A., Faligowska A., Kalembasa D., 2014. The amount of biologically reduced nitrogen by yellow lupine (*Lupinus luteus* L.) – Preliminary results. Fragmenta Agronomica, 31(1): 121-128. (in Polish + summary in English)

Zahran H.H., 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in arid climate. Microbiology and Molecular Biology Reviews, 63(4): 968-989.

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