The Effect of a Preparation Containing Rhizobial Nod Factors on Pea Morphological Traits and Physiology

Karolina Smytkiewicz 1, Janusz Podleśny 1, Jerzy Wielbo 2 and Anna Podleśna 1,∗

1 Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8 str., 24-100 Pulawy, Poland; ksmytkiewicz@iung.pulawy.pl (K.S.); jp@iung.pulawy.pl (J.P.)
2 Department of Genetics and Microbiology, Maria Curie-Skłodowska University (UMCS), Maria Curie-Skłodowska Sq. 5, 20-031 Lublin, Poland; jerzy.wielbo@poczta.umcs.lublin.pl
∗Correspondence: ap@iung.pulawy.pl

Abstract: The aim of the study was to determine the possibility of increasing the pea yields by improving the symbiotic nitrogen fixation through the use of a preparation containing bacterial Nod factors (NFs). Two pea cultivars were included in the experiment: Wiato (with traditional foliage) and Model (afila type). Before sowing, the seeds were soaked in distilled water (control) and in a preparation of Nod factors at a concentration of 10^{-12} M dm^{-3} H_{2}O. As a result, of the Nod factor preparation use, an acceleration of the date and uniformity of pea plant emergence was observed. The treatment had also a positive effect on the number and weight of root nodules, which resulted in a significant increase in the yield of vegetative and generative plant organs. A positive effect of seed soaking with NFs preparation was also observed in the dynamics of pea weight increase, chlorophyll content in leaves and the values of gas exchange parameters. Model cultivar of pea had generally higher values of the analysed traits than Wiato, but the response of both cultivars to Nod factors was similar. This means that application of the preparation containing NFs, may improve the growth, development, and yield of both types of pea.

Keywords: pea; Nod factors (NFs); symbiotic nitrogen fixation; yield; gas exchange parameters; chlorophyll content

1. Introduction

Legumes play an important role in crop production. Their seeds and the vegetative, above ground parts are a valuable source of good quality protein rich in essential amino acids [1,2]. These plants are used both as fodder for livestock and as food for humans [2]. One of the main reasons for the small area of legumes cultivation in Poland, is low seed yield, which varies from year to year [3]. An important factor determining the yield of plants is an adequate supply with nitrogen as an essential nutrient for their proper growth and development. For most plants, the main sources of nitrogen are soil resources and inorganic fertilisers [4]. However, nitrogen is one of the most expensive nutrients and mineral fertilisers are the highest cost in plant production [5]. Another important source of nitrogen is atmospheric air, but the gaseous form of its molecule (N_{2}) is not directly available to most plants. This form of nitrogen can be used by Fabaceae plants in a complicated process known as biological nitrogen fixation-BNF [6]. It is possible thanks to a special property possessed among others by physiologically and morphologically diverse diazotrophic bacteria [7–9]. However, a plant from legumes and the proper bacterial strain from the Rhizobium genus are necessary to the full course of this process [10]. The multistep symbiosis between rhizobia (i.e., a group of symbiotic bacteria) and legumes is species-specific, what means that individual legume species establishes symbiotic interactions with specific rhizobia species or subspecies [11,12]. The BNF process takes place in specialised nodules which are formed on the primary roots of legumes [13,14].
Pea (*Pisum sativum* L.) is an important legume cultivated in temperate climatic zones. In Poland it has been cultivated for a long time as it plays a fundamental role as a fodder and edible plant [15]. The *Rhizobium leguminosarum* bv. *viciae* strain is a symbiont of pea as well as vetch and broad bean [11,12]. The establishment of a symbiosis between a legume and bacterial strains may sometimes be unsuccessful, despite the species compatibility of bacteria strain and a host plant [16–18].

The discovery of the genetic basis of this phenomenon has allowed the identification of plant [19] and bacterial metabolites involved in the symbiosis process. The latter include specific signalling compounds excreted by rhizobia, i.e., Nod factors, which are lipochitooligosaccharide molecules (LCOs). They are produced by rhizobia in response to the presence of flavonoids in root exudates [20]. These factors participate in the exchange of information between the bacteria and a host plant. As a consequence, there is formation of root nodules [11,21,22] and improved growth of the root system [23]. However, NFs naturally present in the rhizosphere are often broken down, excessively diluted in the soil solution or degraded by soil microorganisms [24]; hence, they may be insufficient for root nodule formation. Therefore, to improve the symbiosis process a targeted treatment was undertaken consisting of the supply of rhizobial Nod factors to the soil together with the seeds [25]. As a result of breeding progress, new pea cultivars were created (as aphia type) with altered morphological habit and yield potential. Higher yield of new cultivars may result from better efficiency of symbiotic nitrogen fixation. Therefore, two pea cultivars (Wiato and Model) were included in the present study. They were registered in the Polish list of cultivars of Agricultural Crops in 1998 and 2011, respectively. The yield potential of the Wiato cultivar is 3.3 and Model 3.9 t·ha$^{-1}$ [26]. The difference in the yield of pea cultivars may result not only from their altered morphological architecture, but also from their different ability to nitrogen fixation. Therefore, there was a need to evaluate the symbiotic properties of these morphologically differentiated cultivars. The aim of this study was to evaluate the response of different pea cultivar on the application of a preparation containing NFs, which affects the process of biological nitrogen fixation and to determine its effect on the main morphological and physiological processes which influence the use of yielding potential.

2. Materials and Methods

2.1. The Conditions for Plant Growth

A three-year experiment was conducted in the greenhouse of the Institute of Soil Science and Plant Cultivation–State Research Institute in Puławy [51°24′59″ N 21°58′09″ E]. Mitscherlich pots contained a mixture of 5 kg of garden soil and 2 kg of sand. Ten pea seeds were sown into each pot. After emergence the plants were thinned and finally 5 plants were left in the pot. During plant emergence, liquid NPK fertiliser was applied which contained 0.1; 1.1 and 1.4 g·pot$^{-1}$, respectively for N, P and K. The amount of water added to the pots during watering of plants was dependent on the weight of pot with soil, plants and water. Throughout the growing season, soil moisture was maintained at 60% field water capacity and watering the plants was performed twice a day.

2.2. Experimental Factors

A two-factor experiment was set up in a completely randomised design. Two pea cultivars were included in the experiment: Wiato (normal type with traditional foliage) and Model (aphila type), which were the 1st-order factor. Seeds of both pea cultivars originated from Polish seed and breeding companies: Wiato from Poznań Plant Breeding Sp.z.o.o. and Model from Smolice Plant Breeding. The second experimental factor was the seed preparation before sowing: control-seeds were soaked in distilled water or in solution of a preparation containing Nod factors (NFs) at a concentration of 10–12 mol·dm$^{-3}$ of water. Seeds were soaked for 1 h, taking 100 mL of NFs solution or water for 1 kg of pea seeds. In our experiment, 150 seeds of both tested cultivars were soaked in water and the NF preparation.
2.3. Obtaining of a Preparation Containing NFs

Extracts containing rhizobial NFs were obtained from liquid cultures of *Rhizobium leguminosarum* bv. *viciae* GR09 (**Rlv** GR09) strain induced by a plant flavonoid extract [27]. To obtain flavonoid extract, pea seeds were surface-sterilised by immersion in 0.1% HgCl$_2$ for 3 min, then rinsed with distilled water, treated with 70% ethanol for 3 min, and rinsed again with sterile water. The whole preparation, chemical analysis and confirmation of biological activity of extract containing rhizobial NFs was performed according to the method described previously by Podlešny et al. [28].

2.4. Determination of Seedling Emergence Patterns

Plant emergence in all pots were determined in the period from 12 h after the appearance of the first cotyledons to the moment until no new plants appeared. The emergence dynamic index (Ed) was calculated from the following formula:

$$Ed = \frac{Ne}{Ns} \times 100\%$$ (1)

where Ne—number of emerged plants, Ns—number of sown seeds.

Only properly germinated and developed plants were counted.

2.5. Determination of Leaf Area and SPAD Values

Leaf area and greenness index were measured at the flowering stage. The area of stipules and tendrils as photosynthetic active organs was measured in the afila-type cultivar. Leaf area was determined with use of the AM 300 Field Portable Leaf Area Meter (ADC Bio Scientific Ltd., Hoddesdon, UK), and greenness index expressed as SPAD values were determined by use the SPAD-502 chlorophyll meter (Minolta Co., Ltd., Osaka, Japan). Stipules of an afila cultivar were used to the SPAD measurements. Every value of SPAD represented the mean of 30 measurements performed on the newest, fully developed leaves of pea from the same object.

2.6. Analysis of Chlorophyll and Carotenoid Concentrations in Leaves

Samples of harvested plant tissue were cryopreserved at $-70$ °C before the quantitative analyses of chloroplast pigments. The content of chlorophyll a, chlorophyll b and carotenoids was determined with use of spectrophotometric method proposed by Wellburn [29]. Pigments were extracted with methanol and absorbance was measured at a wavelength of 666, 653 and 470 nm.

Thawed plant tissue (200 mg per sample) was ground in a mortar on ice in 1 mL of methanol. Next 4 mL of methanol were added to the mortar, and samples were ground until a homogenous suspension was obtained. The suspension was poured into test tubes, then the mortar was rinsed with 5 mL of methanol and a new portion of the suspension was added to the previous one. The entire suspension (10 mL) was stored in darkness on ice until the spectrophotometric analysis was performed.

Prior to absorbance determinations, the suspension was shaken vigorously for more than 10 s, and 1 mL of the suspension was centrifuged at 6000 rpm for 3 min. Next, 300 μL of the supernatant was transferred to a 96-well titration plate. Absorbance was determined using the ELISA microplate reader (ASYS Hitech GmbH, UVM 340, Seekirchen am Wallersee, Austria) immediately after the sample placement on the titration plate.

The content of the analysed pigments was determined based on formulas which include sample weight, volume of extracted suspension and optical length of path during spectrophotometric analysis:

- chlorophyll a (mg/g fresh tissue weight) = \[(15.65 \times A_{666} - 7.34 \times A_{653}) \times 31.8\]/sample weight (mg),
- chlorophyll b (mg/g fresh tissue weight) = \[(27.05 \times A_{653} - 11.21 \times A_{666}) \times 31.8\]/sample weight (mg),
• carotenoids (mg/g fresh tissue weight) = \((1000 \times A_{470} - 2.86 \times \text{chlorophyll a} - 129.2 \times \text{chlorophyll b}) \times 0.144) / \text{sample weight (mg)}\).

2.7. Determination of Gas Exchange Parameters

Leaf gas exchange parameters: net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs) and water use efficiency (WUE) were determined at the flowering stage using the CIRAS-2 portable photosynthesis system (PP System Inc., Amesbury, MA, USA) equipped with a PLC(U) leaf cuvette with insert window (25 m × 7 m) and LED illumination. Samples were exposed to photosynthetically active radiation (PAR) of 500 μmol m\(^{-2}\) s\(^{-1}\) and 380 ppm of CO\(_2\). Water use efficiency was calculated with use of Yan et al. [30] formula:

\[
\text{WUE} = \frac{\text{Pn}}{\text{E}} (\mu\text{mol CO}_2 \cdot \text{mmol}^{-1} \text{H}_2\text{O})
\]

Measurements were performed on every first fully developed leaf from the top of the plant. The final result was the mean of three measurements.

2.8. Determination of the Growth Rate—RGR

To determine the dynamics of pea weight gain, plants were harvested at the three development stages: flowering (BBCH 60)—T1, green pod (BBCH 75)—T2 and full maturity (BBCH 89)—T3. The date of sowing of seeds was assumed at BBCH 00—T0. At each harvest, the fresh and dry weights of individual plant organs were determined.

The dynamics of the weight increase was determined on the basis of the relative growth rate (RGR) using a formula of Evans [31]:

\[
\text{RGR} = \left(\ln W_2 - \ln W_1\right) \left(\frac{T_2 - T_1}{T_2 - T_1}\right)^{-1} \left[g (g \cdot 24 \text{ h}^{-1})^{-1}\right]
\]

where \(W_1\)—the dry weight of the plants at the beginning of the measurement period, \(W_2\)—the dry weight of the plants at the end of the measurement period, \(T_1\)—the beginning of the measurement period, \(T_2\)—the end of the measurement period.

Root weight was determined after rinsing of whole root system from a pot (from 5 plants) on dense metal sieves and dried. After rinsing, the root nodules were peeled off the roots and their number as well as fresh and dry weight were determined.

2.9. Determination of Seed Yield and Structural Characteristics

At full pea maturity, the number of pods per plant, the number of seeds per pod and the number of seeds per plant were determined. Seed moisture was assessed using Seed Moisture Meters—SM 10 (FOSS Company, Hillerød, Denmark). The seed yield of pea was calculated at 14% of seed moisture. The weight of 1000 seeds was also calculated.

2.10. Statistical Analysis

The analytical data obtained for each harvest and measurement were the mean of the 4 pots from the same object. Statistical analysis of the obtained results was run with Statistica v.13.1. The analysis of variance and regression were performed at a significance level of \(p \leq 0.05\).

3. Results

Soaking of seeds in the preparation of NFs did not affect the data of pea plant emergence but modified its dynamics, especially between 8 and 12 days after sowing the seeds (Figure 1). Irrespective of the applied preparations, average plant emergence amounted to 97.5% for both cultivars.
The soaking of seeds with a preparation containing NFs significantly increased plant height (Figure 2) and leaf area (Figure 3); however, plants of Model cultivar were taller than those of Wiato. On the other hand, Wiato was characterised by a lower number of leaves, but they had a larger area compared to plants of the Model cultivar (Figure 3).

![Figure 1. Dynamic of emergence of pea plants in dependence on soaking of seeds. H2O—green line, NFs—red line.](image1)

![Figure 2. Selected morphological features of pea at flowering (BBCH 60). H2O—green bars, NFs—red bars; 1—height of plants, 2—number of leaves. Values that are followed by different letters differ significantly at p ≤ 0.05. Capital letter—differences between cultivars; small letters—differences between preparations.](image2)
The application of the preparation containing NFs caused a significant increase in the SPAD index and in the chlorophyll a and b content in the leaves of pea cultivars.

Although both pea cultivars were characterised by a similar value of the SPAD index (Figure 3), the Model had higher content of chlorophyll a and chlorophyll b than the Wiato (Figure 4). The application of the preparation containing NFs caused a significant increase in the SPAD index and in the chlorophyll a and b content in the leaves of pea cultivars.

![Figure 3. Values of SPAD index and leaf area of pea plants (BBCH 60). H2O—green bars, NFs—red bars; 1—SPAD, 2—leaf area. Values that are followed by different letters differ significantly at \( p \leq 0.05 \). Capital letters—differences between cultivars; small letters—differences between preparations.]

![Figure 4. Content of plant pigments in leaves of pea at flowering (BBCH 60). H2O—green bars, NFs—red bars 1—chlorophyll a, 2—chlorophyll b, 3—carotenoids. Values that are followed by different letters differ significantly at \( p \leq 0.05 \). Capital letters—the differences between cultivars; small letters—differences between preparations.]

Significant differences in the values of the selected physiological parameters were found between the two pea cultivars (Figure 5). The cultivar Model was characterised by significantly higher values of gas exchange parameters (photosynthesis intensity, transpiration and stomatal conductance) compared to the Wiato. Application of the NFs preparation increased photosynthetic intensity, stomatal conductance and transpiration intensity in leaves of both pea cultivars. However, it did not result in better water use by pea plants, as the differences in WUE values were not significant.

Figure 5. Values of gas exchange parameters at flowering (BBCH 60). H₂O—green bars, NFs—red bars. 1—Pn, 2—E, 3—Gs, 4—WUE. Values that are followed by different letters differ significantly at \( p \leq 0.05 \). Capital letters-differences between cultivars; small letters-differences between preparations.

The application of the NF preparation had a significant effect on the dynamics of aboveground part and root mass increase (Table 1). A particularly strong effect of this treatment on the weight of the aboveground parts of both pea cultivars was found in the period from emergence (T1) to flowering (T2). The use of NFs preparation caused an increase in the dynamics of root mass increment, and the aboveground part of the pea plants increased. It was found that in the period from the green pod stage (BBCH 75) to full maturity (BBCH 89), plant weight decreased. However, weight losses of plants grown from seeds soaked in the preparation containing of NFs were smaller than those soaked in water. It was also found that the root weight of pea decreased faster than the aboveground parts.

Table 1. Relative growth rate (RGR) of pea plants (aboveground part and roots) \([g (g \text{ day}^{-1})^{-1}]\).

| Harvests | Model | LSD₀.₀₅ \(H₂O\) | LSD₀.₀₅ \(LCOs\) | Wiato | LSD₀.₀₅ \(H₂O\) | LSD₀.₀₅ \(LCOs\) |
|----------|-------|-----------------|-----------------|-------|-----------------|-----------------|
| T₀–T₁    | 0.44  | 0.69            | 0.14            | 0.47  | 0.58            |
| T₁–T₂    | 1.21  | 1.48            | 0.18            | 1.16  | 1.58            |
| T₂–T₃    | -0.48 | -0.36           | 0.06            | -0.38 | -0.31           |

n.s.—differences not significant.
In both cultivars treated with the preparation of NFs, a similar increase in the number and weight of root nodules was observed (Figure 6). At the flowering stage, the increase of these features for the Model cultivar was 14.5 and 13.1%, and for the Wiato, 14.0 and 16.4%, respectively. Meanwhile, at the green pod stage, the increment for the Model cultivar amounted 15.7 and 16.4% and for Wiato 13.4 and 15.0%, respectively. Greater number and weight of root nodules was produced by the cultivar Model during flowering. Generally, the number of root nodules and their dry matter were gradually reduced with the plant growth, but the dynamics of these changes (i.e., decrease) for Wiato was higher than for Model. Although in the flowering period, the weight of 1 nodule of both cultivars was similar, in the green pod phase, a higher value of this feature was recorded in the Wiato cultivar (1.27 mg). That cultivar had less nodules, but they were characterised by greater weight.

![Figure 6](image-url)

**Figure 6.** Number and dry matter of root nodules of pea plants. **H**<sub>2</sub>O—green bars, NFs—red bars. Numbers of root nodules: 1—BBCH 60, 2—BBCH 75; dry matter of root nodules: 3—BBCH 60, 4—BBCH 75. Values that are followed by different letters differ significantly at \( p \leq 0.05 \). Capital letters—differences between cultivars; small letters—differences between preparations.

Finally, the Model cultivar treated with the NFs preparation achieved a significantly higher yield and better traits of its elements than the Wiato (Figure 7). The biggest effect on the differences in yields corresponded to pod density (Figure 7) and 1000 seed weight (data not presented).
Figure 7. Yield and yield components of pea (BBCH 89). H2O—green bars, NFs—red bars. 1—number of pods, 2—number of seeds, 3—yield of seeds. Values that are followed by different letters differ significantly at $p \leq 0.05$. Capital letters—differences between cultivars; small letters—differences between preparations.

For both cultivars, a strong relationship between root nodules mass and pea seed yield was found (Figure 8). It should be noted that the increase in the weight of root nodules in effect of application of NF preparation was largely due to their increased abundance. This was especially true in the flowering period, when the process of symbiotic nitrogen fixation is the most intensive.

Figure 8. Relationship between dry weight of root nodules and seed yield.
4. Discussion

The study showed that a higher yield with a preparation containing NFs was obtained for pea of the Model cultivar (i.e., aphila type) than for the Wiato (a type with traditional foliage). The data from the Research Centre for Cultivar Testing (COBORU) [26] show that the afila-type varieties give significantly higher yield than the varieties with traditional foliage. Thanks to intensive breeding works carried out in Poland between 2000 and 2012, the yield of peas has increased by about 45%. This was achieved largely thanks to the introduction to cultivation of narrow-leaved (i.e., aphila-type) varieties. Currently, about 90% of all pea cultivars included after 2000 in the Polish National List of Agricultural Plant Varieties belong to this type [26].

Breeders carrying out genetic work most often pay attention to the morphological features of pea plants, determining their yielding (i.e., number of pods and seeds per pod, 1000 seed weight), while to a lesser extent, they look for relations between other plant features that are more difficult to measure, such as nitrogen fixation efficiency, photosynthesis intensity and yield. Comparison of both cultivars showed that the new cv. Model had a higher yield and produced more and a greater weight of root nodules than the older cultivar Wiato. According to Kielpinski and Blixt [32], the afila gene caused a considerable reduction of leaf area, but an increase of stipule area in this type was observed. As a result, total area of foliage is comparable in normal-leaved and afila cultivars. Additionally, the high yield potential of afila cultivars is a result of light penetration into the sward several times greater than conventional cultivars. This results from the lack of normal leaves and the improved standing ability of the plants in stands.

The study showed that the treatment of pea seeds with a solution of Nod factor preparation improved the dynamics and uniformity of plant emergence, which is especially important in unfavourable environmental conditions (e.g., drought) [33]. The morphogenic effect of Nod factors accelerates seed germination and influences the growth of seedlings [23,25,34,35]. The literature reports that early events of NFs signalling lead to the production and/or activation of cytokinins (CK) and gibberellins (Gas) [20,36]. The roles of these hormones are linked with plant growth and development, because they stimulate seed germination (GAs) and increase the rate of cell division (CK).

Numerous differences between the two pea cultivars and the used preparations (water and NFs preparation) with respect to morphological and functional traits (plant height, leaf area, number of pods per plant, number of seeds per pod, number of seeds per plant and 1000 seed weight) were observed in the presented experiment. These differences also concerned physiological and biochemical traits, i.e., transpiration intensity, stomatal conductance, photosynthetic intensity and chlorophyll content in leaves. Siczek et al. [37] showed that use of NFs caused a 16.8% increase in the content of N in faba bean biomass. This can partly explain the increase of chlorophyll content (SPAD), photosynthetic intensity and final yield after the seeds were treated with a NFs preparation [4]. Moreover, application of NFs preparation improves soil biological parameters, which refers mainly to an increase in the activity of protease and acid phosphomonoesterase, i.e., enzymes involved in the N and P cycle. Enhancement of the activities of these enzymes leads to a high release of protein N and inorganic phosphorus, which are good sources of these nutrients for plants (Siczek et al. [37]).

Differences were also found in the way of interaction of cultivars with rhizobial microsymbionts. In the latter case, plants of the Model cultivar produced more nodules than plants of the Wiato, but the mass of a single nodule of both cultivars was similar. Irrespective of the intercultivar differences, the action of the rhizobial NFs preparation, in the case of many studied traits, proceeded in a similar direction for both of them.

The weight and number of root nodules was higher at the flowering (BBCH 60) than at the green pod stage (BBCH 75). This means that the death of root nodules occurs after flowering, because the symbiotically fixed nitrogen accumulated in the nodules is used for the growth of generative organs. An effect that included loss of stem, leaf and root weight
was observed in maturity. A significant part of it can be also released into the soil in the process of legume residue mineralisation (i.e., leaves, stem and nodulated roots) [38].

In the period from flowering to the green pod stage, the number of root nodules per plant and the total weight of nodules decreased. This is in contradiction with pea root nodule development, which is classified as “indeterminate”. In this type of nodule, the meristem is active throughout the whole time of development, resulting in a continuous increase in the dimensions (especially length) and weight of these organs [39,40].

According to Chaulagain and Frugoli [41], legumes balance the number of nodules formed with the plant’s need for N by integration of the outputs of least three signalling pathways. These include a pathway for nodule initiation, an inhibitory pathway for nodule number, and an inhibitory pathway based on N sufficiency. However, the presented experiment showed that the application of NFs preparation resulted in an increase in the number of nodules and in increase in pea yield. This proves that the number of “sites of molecular nitrogen reduction and symbiotic exchange”, i.e., individual root nodules, directly proportionally translates into yield. Therefore, the increase in the number of such sites on plant roots as a result of the morphogenetic action of a preparation containing Nod factors [42] can improve yield. This preparation increases the number of root nodules and intensifies the process of biological N\textsubscript{2} reduction by increasing the activity of nitrogenase, which, as a consequence, increases the amount of nitrogen taken up from the atmosphere [37,43,44]. The Nod factors preparation increased root weight dynamics to a greater extent, and to a lesser extent, the dynamics of the aboveground parts of pea. Therefore, our results confirm the large effect of NFs preparation on the formation of pea plant root weight [45].

In this experiment, a significant relationship between the weight of root nodules and the seed yield of the pea was found (Figure 1). It should be noted, however, that a limit value was found up to which an increase in mass was accompanied by an increase in yield, beyond which there was no more yield increase, and even a slight decrease. Therefore, it can be assumed that better pea yield is ensured by many nodules of smaller but sufficiently high weight than by a small number of nodules of greater weight. The explanation for this phenomenon should probably be sought in consideration of the morphology and ontogeny of the indeterminate root nodules that occur in pea [42]. According to Newcomb et al. [46], inner cortical cells contribute extensively to the infected tissue in pea nodules. Mitotic activity is restricted to a nodule meristem and occurs over a period of many weeks. NFs play a very important role in this process, by stimulation of cell division in the root and formation of the nodule meristem [20]. In the initial phase, such nodules possess only the meristem, while with progressive growth and development, further zones with different functions appear. Therefore, initially, the amount of symbiotically reduced nitrogen increases as the size and weight of individual nodules increase, while in subsequent development stages, the amount of reduced nitrogen per unit of nodule mass decreases.

These results indicate that with an increasing number of nodules, many of them reach a size that is too small for intensive nitrogen fixation. In effect, the amount of fixed nitrogen does not increase, and the achieved yield may be considerably lower.

5. Conclusions
The use of a preparation containing NFs affected the growth and development of the pea by means of favourable changes in the morphological and physiological parameters of the plants. They had better conditions for growth as a result of the greater number of nodules and the plants had increased supplies of nitrogen. The use of a preparation containing NFs is more appropriate for afila cultivars than for traditional ones because the treatment may increase their greater genetic potential for yield.

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