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Pea growth and symbiotic activity response to Nod factors (lipo-chitooligosaccharides) and soil compaction

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ABSTRACT

Growth and symbiotic activity of legumes are reduced by high soil compaction and mediated by Nod factors (LCO, lipo-chitooligosaccharides) application. Our objective was to assess the combined effects of soil compaction and Nod factors application on growth and symbiotic activity of pea. The experiment was two factorial and included soil compaction (1.30 g cm^{-3} – not compacted (control) and 1.55 g cm^{-3} – compacted soil), and Nod factors concentration (control without addition of Nod factors and use of 260 nM Nod solution) for each soil compaction. The soil (Haplic Luvisol) was packed into pots, pea (*Pisum sativum* L.) seeds were soaked with Nod factors solution or water and then plants were grown for 46 days. This study has shown that soil compaction and treatments of pea seeds with Nod factors influenced pea growth and symbiotic activity. Soil compaction significantly reduced pea growth parameters, namely plant height, dry mass, leaf area, root mass and root length and symbiotic parameters, namely mass of nodules, dry mass of an individual nodule, nitrogenase activity and total nitrogen content in plant in comparison to the non-compacted treatment. Treatment of seeds with Nod factors generally improved nearly all of the above parameters. Nitrogenase activity per pot and total plant nitrogen content were significantly reduced by soil compaction and increased by application of Nod factors in plants grown in not compacted soil. Our results demonstrate that increased symbiotic activity resulting from Nod factors addition may mitigate adverse effect of soil compaction on plant growth.

1. Introduction

Excessive soil compaction mostly results from intensive cropping and no-tillage practices, heavy vehicle and implement traffic. It harmfully influences many soil characteristics (Hamza and Anderson, 2005; Horn et al., 2003) especially shifting pore size distribution to smaller pores and, consequently, activity and diversity of soil microorganisms (Nosalewicz and Nosalewicz, 2011; Pengthamkeerati et al., 2011; Siczek and Frąc, 2012) and plant growth (Gregorich et al., 2011). Soil compaction may alter the amount of nitrogen that is supplied to agricultural system by association of nitrogen fixing bacteria with legume. Soil compaction unfavorably affects grain and protein yield of soybean (Botta et al., 2010). The effect of soil compaction on nodulation and nodule activity can be mediated by soil moisture status (Lindemann et al., 1982) and mulch applications (Siczek and Lipiec, 2011). The results of Buttery et al. (1998) showed that increasing soil moisture alleviated the effect of compaction on nodulation of soybean and common bean in sandy loam. Voorhees et al. (1976) reported that soil compaction altered the distribution of the nodules in the vertical and horizontal plane. The study of Siczek and Lipiec (2011) showed that the greatest total nodule number and weight occurred in moderately compacted soil whereas the contribution of large nodules and the dry weight of individual nodules were the greatest in the most compacted soil.

Legumes are important grain and forage crops in both temperate and tropical climates. Due to symbiotic association with gram-negative soil bacteria called rhizobia, they depend slightly on soil nitrogen status. Abi-Ghanem et al. (2011) reported that the proportion of plant N supplied by fixation varied with pea variety and ranged from 87% to 91% in a pot experiment. The field study of Hauggaard-Nielsen et al. (2010) showed that the percent of total N derived from the atmosphere at flowering in pea ranged from 65% to 92% with quantitative N_2-fixation estimates from 93 to 202 kg N ha^{-1}. At maturity these values decreased respectively to 26–81% and 48–167 kg N ha^{-1}. Considering the dry spring and a major rainfall event around flowering, it is likely that a high net mineralization of soil N took place causing the increased uptake of soil N and reduced fixation.

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Table 1
Characteristics of the soil plough layer.

Clay	Silt	Sand (g kg^{-1})	C org	Total N*	P**	K (mg kg^{-1})	Mg	pH H_2O
70	290	640	14.1	0.75	90	153	23	5.9

* As indicated by the Kjeldahl method.
** Plant available inorganic P.

Symbiotic association between legumes and rhizobia involves molecular signal exchange between both partners (Dardanelli et al., 2012; Hirsch et al., 2001). Plant roots excrete many compounds that are chemoattractans for the rhizobia. Among them flavonoids are the strongest inducers of *nod* genes. The main role of flavonoids in the initiation of symbiosis is interaction with the NodD protein. Complex flavonoids-NodD protein induces the production of key molecules that allow the plant host recognition of the bacterial partner, the Nod factors (LCO, lipo-chitooligosaccharides). Nod factors consisting of a skeleton of three to five *N*-acetyl D-glucosamine residues linked to a lipid moiety on the nonreducing end. Nod factors induce many early nodulation events that occur at the plant epidermis, cortex and pericycle. At submicromolar concentrations these molecules are responsible for membrane potential depolarization, formation of infection threads, root hair deformation, division of root cortex cells and formation of nodule primordia, and induction of early nodulin gene expression (for review see D'Haeze and Holsters, 2002). The study of Maj et al. (2009) indicated that pretreatment of red clover seeds with Nod factors significantly enhanced plant nodulation and growth. More recently, Kidaj et al. (2012) confirmed a beneficial effect of Nod factors on pea and vetch germination, nodulation and growth. The foliar application of Nod factor to soybean significantly ($P < 0.05$) increased leaf area, shoot and dry mass compared with control plants (Khan et al., 2008). Almaraz et al. (2007) also demonstrated positive response of soybean to some concentrations of Nod factors. In the above quoted study photosynthesis was increased up to 13% over the control, and this was connected with increases in plant dry weight. It has been observed that Nod factors may induce seed germination in non-legume plants such as barley (Miransari and Smith, 2009) corn and *Arabidopsis thaliana* (Prithiviraj et al., 2003). It has been recognized that addition of Nod factors affected early events of the symbiosis and growth of legume crops in stressed growth conditions including low rhizosphere temperature, low pH and water stress (Atti et al., 2005; Duzan et al., 2004, 2006; McKay and Djordjevic, 1993).

Recent evidence suggests roles for rhizobial Nod factors that go beyond the nodulation process. Duzan et al. (2005) showed that application of the Nod factor Nod Bj-V ($C_{18:1}$, MeFuc) induced soybean resistance to powdery mildew caused by *Microsphaera diffusa* by fungal growth and development hampering. On the other hand Xie et al. (1998) reported that application of Nod factors to legume roots promoted arbuscular mycorrhizal fungi (AMF) colonization.

There is currently little evidence on combined effects of soil compaction and Nod factors on the plant growth parameters, therefore our objective was to investigate whether Nod factors treatment can mediate soil compaction under the same growth conditions.

2. Materials and methods

2.1. Soil and growth conditions

The soil, a Haplic Luvisol developed from loess, was collected from the plough layer (0–20 cm) in an arable field in Lublin, Poland (51°13′ N, 22°37′ E). Soil was collected in the autumn after wheat harvest and before tillage operations, and at a soil water content corresponding to field capacity. The soil at the sampling site was under long-term (30 years) conventional tillage, with main tillage operations including pre-plough (10 cm depth) and harrowing, and mouldboard ploughing (20 cm depth). Characteristics of the soil plough layer are presented in Table 1.

After sampling, the soil was sieved through a 0.4 cm sieve. The soil was packed into PVC pots 15 cm in diameter and 40 cm high to two bulk densities: 1.30 g cm^{-3} – not compacted soil (control), with bulk density optimal for plant growth in this soil, and 1.55 g cm^{-3} – compacted soil with bulk density that is considered as the limiting for plant growth and function. A known weight of soil was compacted into successive 2 cm layers with a piston that was pressed by a hydraulic press to get bulk density 1.30 g cm^{-3} and 1.55 g cm^{-3}, respectively. Before packing into pots, soil at layers of 0–20 cm was uniformly mixed with fertilizers in amounts corresponding to P, K and Mg of 40, 50 and 35 kg ha^{-1}, respectively. The number of *Rhizobium leguminosarum* bv. *viciae* in the plough layer of the sampled soil amounted to 1.8×10^3 per g of dry soil as determined using the most probable number technique modified by Martyniuk et al. (2000) and was assessed as high (Martyniuk et al., 2005). Due to this fact, in this study pea seeds were not inoculated with *R. leguminosarum* bv. *viciae*.

The experiment was designed as a 2×2 factorial. The first factor was soil compaction (1.30 g cm^{-3} – not compacted (control) and 1.55 g cm^{-3} – compacted soil) and second factor was Nod factors concentration (control without addition of Nod factors and use of 260 nM Nod solution). Pea (*Pisum sativum* L. cv. Tarchalska) seeds were soaked for 30 min with Nod factors solution 260 nM or water. Then six seeds per pot were placed at 3 cm soil depth. After emergence seedlings were thinned to three per pot. Each of the four treatments: NC (not compacted, without Nod factors), NC + Nod (not compacted, with Nod factors), C (compacted, without Nod factors) and C + Nod (compacted, with Nod factors) were replicated four times. The pots were arranged randomly in a controlled growth-chamber environment. Day-time (16 h) and night-time (8 h) temperatures were 22 °C and 17 °C, respectively, relative humidity (RH) was 65 ± 5% and photosynthetically active radiation (PAR) amounted to 320 μmol m^{-2} s^{-1}. During pea growth soil moisture was kept on a level optimal for plant growth, corresponding to soil water potential −31 kPa (pF 2.5), by measuring the evapo-transpired water three times a week (calculated by weighing the pots) and replenishing the lost water. The experiment was started on 9th September 2011. Plants were grown for 46 days up to the flowering stage.

2.2. Preparation of exudates from sprouted seeds

Pea seeds were surface sterilized by immersion in 0.1% $HgCl_2$ for 3 min, rinsed with sterile distilled water, then treated with 70% ethanol for 3 min, followed by a sterile water wash. Then, the seeds were shaken in sterile water, in darkness, for four days at 28 °C. Sprouted seeds were separated from water suspension after decantation, and plant tissue debris were removed from the supernatant by centrifugation (4000 rpm, 10 min). Then ethyl acetate was added to the supernatant (1:10, v/v), and flavonoids were extracted with ethyl acetate from the aqueous phase in a separating dropping funnel. After that ethyl acetate was evaporated, and the