Effect of Sulfur and Nod Factors (LCOs) on Some Physiological Features and Yield of Pea (*Pisum sativum* L.)

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Abstract The response of pea var. Medal to treatment with Nod factors (LCOs) and mineral sulfur was estimated in a pot experiment with a completely randomized design. Foliar spraying of plants was performed at the 5–6 leaf stage (BBCH 15) at concentrations of 10^{-12} M dm^{-3} and 12 g S dm^{-3} for LCOs and sulfur, respectively. The use of these factors, both individually and in combination, caused an increase in leaf area and "greenness" (SPAD), gas exchange parameters, straw and seed yields and in the root system. The number of nodules and respective nodule dry weight also increased with these treatments. A significant increase in seed yield resulted from the beneficial effects of LCOs and sulfur with an increase in the number of pods and seeds per plant compared to control plants, is clearly significant from the agricultural point of view. Although each factor improved the traits studied, the best results were achieved in the case of plants treated with both LCOs and sulfur.

Leguminous plants have a great importance in agriculture for the production of valuable seeds, which are used as food for human and fodder for animals. However, a relatively low and variable seed yield has led to a decrease in their cultivation (Graham and Vance 2003). Therefore scientists are looking at factors, which may improve the yield of legumes. Some of these studies are focused on the legumes' ability for biological nitrogen fixation (BNF) by forming a relationship with specialized nitrogen-fixing bacteria called rhizobia (van Hameren et al. 2013). The rhizobia convert atmospheric di-nitrogen into forms of nitrogen usable for the plant, whilst being housed in novel root organs – nodules. Optimizing BNF processes, such as nodulation, has the potential to increase crop yields and enhance soil fertility whilst reducing farming costs and harmful environmental impacts (van Hameren

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et al. 2013). Research into legume-rhizobia symbioses has identified numerous plant and bacterial metabolites, which are essential for the establishment of symbiosis and development of root nodules (Brewin 2004). This group of metabolites includes bacterial Nod factors (lipochitooligosaccharides-LCOs), which are bacteria-to-plant signals required for the establishment of rhizobia-legume nitrogen fixing symbioses (Cullimore et al. 2001). LCOs induce the formation of root nodules (Geurts et al. 2005; Podleśny et al. 2014a) and improve plant germination, growth and yield (Podleśny et al. 2014b) so they could be used as biofertilizers (Bhardwaj et al. 2014; Kidaj et al. 2012). On the other hand, BNF is particularly sensitive to environmental stresses such as nutrient deficiency (Divito and Sadras 2014). Varin et al. (2010) showed that sulfur has an important role in this process by demonstrating that its deficiency reduces nitrogen fixation in pea (*Pisum sativum* L.) and lucerne (*Medicago sativa* L.). Some *Rhizobium*-legume symbiotic interactions are mediated by Nod factors (LCOs), which can be sulfated (Snoeck et al. 2003). Moreover sulfur, as glutathione or ascorbate-glutathione cycle enzymes, is essential for the establishment of legume-rhizobia symbiosis, regulation of the cell cycle and growth, and for root meristem activity (Groten et al. 2005). However, the amount of sulfur in the soil profile is frequently not sufficient to fulfill the nutritional needs of legumes (Cazzato et al. 2012; Szulc et al. 2014). The aim of the present study was the evaluation of LCOs, mineral sulfur and the combined application of both factors on physiological and agricultural parameters of pea yield.

An experiment was conducted in the greenhouse, in Mitscherlich pots, which contained a mixture of soil (5 kg) and sand (2 kg) and which were planted with pea var. Medal (afila type). The plants were sprayed with: 1, control (distilled water); 2, LCOs/Nod factors (concentration: 10^{-12} M dm^{-3}); 3, sulfur (concentration: 12 g S dm^{-3}); and 4, LCOs and sulfur in the above-mentioned concentrations. Rhizobial Nod factors (LCOs) were isolated from liquid cultures of *Rhizobium leguminosarum* bv. *viciae* GR09 (*Rlv* GR09) strain induced by a plant flavonoid extract (Wielbo et al. 2007). Foliar spraying (25 ml per pot of five plants) was performed in the 5–6 leaf phase of growth (BBCH 15). Plants were harvested at three developmental phases: flowering (BBCH 60), fruit development (BBCH 75) and full maturity (BBCH 89). Dry matter of specific plant organs and seed yield were measured (Fig. 1).

Both LCOs, sulfur, and their combined use had an effect on the parameters measured. Firstly, an increase in leaf area during the flowering and green pod phases of pea growth in comparison to control plants (treated with distilled water) was observed (Table 1). Moreover, these leaves also demonstrated an increased leaf greenness index (SPAD). The application of LCOs and sulfur increased the values of the main gas exchange parameters in the pea leaves (Table 2). It is probable that these changes of photosynthesis (Pn) and transpiration (E) intensity were the result of greater leaf area and greater concentration of chlorophyll in leaves as an effect of plants treated with LCOs, sulfur or both these factors. The best results of studied traits were achieved in plants treated with both LCOs and sulfur, and were lower in plants treated with LCOs (leaf area and SPAD) and with sulfur (Pn, net photosynthesis and E, transpiration intensity). Similar responses of peas to LCOs were observed earlier (Kidaj et al. 2012; Podleśny et al. 2014a, b). The same trends in the effect of

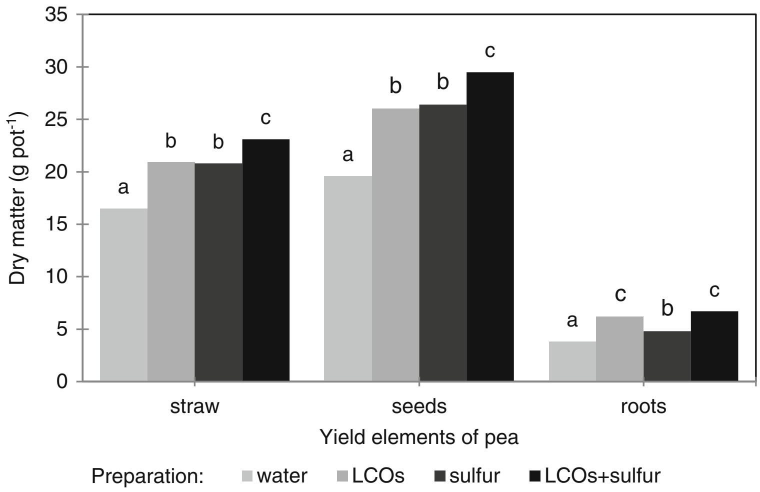

Fig. 1 Impact of LCOs (10^{-12} M dm^{-3}) and sulfur (12 g S dm^{-3}) on yield of pea plants. The weight of straw, seeds and root system was determined upon harvest at full maturity (BBCH 89). Seed yield was calculated for 14 % moisture content and expressed per pot. Roots were rinsing in dense metal sieves, dried and weighed. Different letters indicate significant differences between treatments ($p \leq 0.05$, Tukey's test)

Table 1 Impact of LCOs (10^{-12} M dm^{-3}) and sulfur (12 g S dm^{-3}) on chosen pea leaf indices during growth

Description	Preparation	Developmental phase of pea (BBCH)		
		60	75	Mean
Leaf area	Water	394 ± 8a	457 ± 7a	425 ± 7a
	LCOs	426 ± 6b	471 ± 6b	448 ± 8b
	Sulfur	431 ± 7b	480 ± 5b	455 ± 9b
	LCOs + sulfur	444 ± 6b	494 ± 6c	469 ± 9b
SPAD	Water	501 ± 12a	485 ± 8a	493 ± 6a
	LCOs	511 ± 10a	509 ± 9a	510 ± 7b
	Sulfur	514 ± 12a	508 ± 9a	511 ± 7b
	LCOs + sulfur	518 ± 12a	534 ± 10b	526 ± 9b

Leaf area (cm^2 $plant^{-1}$) was measured with using a Leaf Area Scanner AM 300 (ADC BioScientific Ltd., UK) and SPAD values were determined by using a Minolta chlorophyll meter SPAD – 502. The results were expressed in terms of mean values per four pots. Statistical analysis was performed with Statgraphic ver. 5.1 program. Results of LSD range test are shown. Values followed by similar superscript letters are not significantly different at the 5 % probability level

LCOs on soybean were found by Almaraz et al. (2007), who observed a 13 % increase in photosynthesis over controls which was accompanied by increase in stomatal conductance. Previous studies have suggested that Nod factors sprayed onto shoots stimulate carbon sink strength by increasing early cell division in meristems and this may trigger an increase in photosynthetic rate, based on photosynthetic regulation by carbon sinks. Moreover, the observed increase in stomatal

Table 2 Impact of LCOs (10^{-12} M dm^{-3} of water) and sulfur (12 g S dm^{-3}) on gas exchange parameters of pea leaves

Preparation	Parameter		
	Pn	E	Gs
Water	10.4 ± 0.3a	5.42 ± 0.14a	724 ± 14.3a
LCOs	13.0 ± 0.2c	6.37 ± 0.32b	783 ± 16.8b
Sulfur	12.1 ± 0.3b	6.14 ± 0.30b	792 ± 14.3b
LCOs + sulfur	13.8 ± 0.4d	7.08 ± 0.34c	783 ± 14.7b

Measurements of net photosynthesis intensity (Pn, μmol CO_2 m^{-2} s^{-1}), transpiration intensity (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (Gs, mmol H_2O m^{-2} s^{-1}) were performed at flowering (BBCH 60) using a CIRAS −2 device. Radiation intensity was 500 μmol m^{-2} s^{-1} and CO_2 380 ppm. Measurements were performed on the first fully developed leaf counted from the top of a plant. Different letters indicate significant differences between treatments ($p \leq 0.05$, Tukey's test)

Table 3 Impact of LCOs (10^{-12} M dm^{-3} of water) and sulfur (12 g S dm^{-3}) on number and dry matter of root nodules (mg $plant^{-1}$) and dry matter of 1 nodule (mg) during flowering (BBCH 60) and green pod (BBCH 75) stages of pea growth

Description	Preparation	Developmental phase of pea (BBCH)		
		60	75	Mean
Number of root nodules	Water	40.3 ± 2.4a	31.3 ± 2.5a	35.8 ± 3.7a
	LCOs	61.6 ± 2.6b	40.5 ± 2.1b	51.0 ± 4.4b
	Sulfur	60.4 ± 2.5b	42.4 ± 1.9b	51.4 ± 5,2b
	LCOs + sulfur	71.3 ± 2.8c	49.3 ± 2.2c	60.3 ± 4.3c
Dry matter of root nodules	Water	46.4 ± 5.3a	38.1 ± 2.7a	42.2 ± 3.1a
	LCOs	62.5 ± 5.8b	52.4 ± 3.3b	57.4 ± 3.4b
	Sulfur	61.6 ± 6.4b	53.3 ± 2.4b	57.4 ± 3.0b
	LCOs + sulfur	77.0 ± 6.9c	58.2 ± 2.1c	67.6 ± 3.2c
Dry matter of 1 nodule	Water	1.15 ± 0.03b	1.23 ± 0.04a	1.19 ± 0.05a
	LCOs	1.02 ± 0.02a	1.30 ± 0.05a	1.16 ± 0.03a
	Sulfur	1.02 ± 0.02a	1.27 ± 0.03a	1.13 ± 0.03a
	LCOs + sulfur	1.09 ± 0.03b	1.19 ± 0.03a	1.14 ± 0.04a

Nodules were removed from rinsed roots, and the number and dry weight of root nodules were determined. Presented values are the mean from an object. Different letters indicate significant differences between treatments ($p \leq 0, 05$, Tukey's test)

conductance may indicate that Nod factors improved photosynthetic rate by increasing the CO_2 supply for photosynthesis (Almaraz et al. 2007). Applied sulfur also showed a beneficial effect on gas exchange parameters, indicating that this nutrient plays an important role in these processes. According to Mazid et al. (2011) the photosynthetic apparatus is severely affected under S deficiency, mainly by the reduction of chloroplast and Rubisco content. As the largest increase in leaf area, photosynthetic activity and transpiration was observed with combined use of LCOs and sulfur, it may indicate that the use of these both factors increases their beneficial effect. Similarly, observation of the roots showed that the use of LCOs and sulfur, and particularly their use in combination, had a significant effect on the number of root nodules and their total dry matter (Table 3). The results obtained are in

Table 4 Impact of LCOs (10^{-12} M dm^{-3}) and sulfur (12 g S dm^{-3}) on pea yield structure features

Preparation	Pods plant^{-1}	Seeds pod^{-1}	Seeds plant^{-1}	Weight of 1000 seeds
Water	5.91 ± 0.21a	5.30 ± 0.24a	31.3 ± 1.2a	224 ± 12a
LCOs	6.44 ± 0.24b	5.47 ± 0.28a	35.2 ± 1.4b	220 ± 15a
Sulfur	6.53 ± 0.26b	5.38 ± 0.33a	35.1 ± 1.4b	231 ± 14a
LCOs + sulfur	7.24 ± 0.31c	5.32 ± 0.35a	38.5 ± 1.6c	230 ± 14a

The number of pods and seeds per plant and number of seeds per pod was determined upon harvest at fully ripe stage. Then weight of 1000 seeds (g) was estimated. Different letters indicate significant differences between treatments ($p \leq 0.05$, Tukey's test)

agreement with the findings of Kidaj et al. (2012) and Podleśny et al. (2014a, b) in relation to plants response to LCOs and with observations of Scherer et al. (2006) and Zhao et al. (1999) in relation to sulfur. The earlier studies of Podleśny et al. (2014a, b) found that LCOs slightly accelerated pea growth from the first developmental phases and stimulated the growth of vegetative and generative organs. Scherer et al. (2006) showed an effect of sulfur on the amount of sucrose and glucose in shoots and nodules of pea. According to these authors, when S is limiting, protein synthesis is inhibited resulting in lower yields. Moreover, pea plants fertilized with sulfur fixed more nitrogen than control plants (S0). The analysis of yield structure demonstrated the beneficial effect of LCOs and sulfur in increasing the number of pods and seeds per plant (Table 4). It can be supposed that plants sprayed with LCOs improved nitrogen fixation and additionally sprayed with sulfur more effectively used it as sulfur deficiency decrease nitrogen use efficiency (Fismes et al. 2000).

References

Almaraz JJ, Ahou X, Souleimanov A, Smith D (2007) Gas exchange characteristics and dry matter accumulation of soybean treated with Nod factors. J Plant Physiol 164:1391–1393

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13:1–10

Brewin NJ (2004) Plant cell wall remodeling in the Rhizobium-legume symbiosis. Crit Rev Plant Sci 23:293–316

Cazzato E, Laudation V, Stellacci AM, Ceci E, Tufarelli V (2012) Influence of sulphur application on protein quality, fatty acid composition and nitrogen fixation of white lupin (*Lupinus albus* L.). Eur Food Res Technol 235:963–969

Cullimore JV, Ranjeva R, Bono J-J (2001) Perception of lipo-chitooligosacchardic Nod factors in legumes. Trends Plant Sci 6:25–30

Divito GA, Sadras VO (2014) How phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? Field Crop Res 156:161–171

Fismes J, Vong PC, Guckert A, Frossard E (2000) Influence of sulfur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on calcareous soil. Eur J Agron 12:127–141

Geurts R, Fedorova E, Bisseling T (2005) Nod factor signaling genes and their function in the early stages of Rhizobium infection. Curr Opin Plant Biol 8:346–352

Graham PH, Vance CP (2003) Legumes: importance and constrains to greater use. Plant Physiol 131:872–877

Groten K, Vanacker H, Dutilleul C, Bastian F, Bernard S, Carzaniga R, Foyer CH (2005) The roles of redox processes in pea nodule development and senescence. Plant Cell Environ 28:1293–1304

Kidaj D, Wielbo J, Skorupska A (2012) Nod factors stimulate seed germination and promote growth and nodulation of pea and vetch under competitive conditions. Microbiol Res 167:144–150

Mazid M, Khan TA, Mohammad F (2011) Response of crop plants under sulphur stress tolerance: a holistic approach. J Stress Physiol Biochem 7:23–57

Podleśny J, Wielbo J, Podleśna A, Kidaj D (2014a) The responses of two pea genotypes to Nod factors (LCOs) treatment. J Food Agric Environ 12:554–558

Podleśny J, Wielbo J, Podleśna A, Kidaj D (2014b) The pleiotropic effect of extract containing rhizobial Nod factors on pea growth and yield. Cent Eur J Biol 9:396–409

Scherer HW, Pacyna S, Schultz NM (2006) Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N_2 fixation. Plant Soil Environ 52:72–77

Snoeck C, Verreth C, Hernendes-Lucas I, Martinez-Romero E, Vanderleyden J (2003) Identification of a third sulfate activation system in *Sinorhizobium* sp. strain BR816: the CysDN sulfate activation complex. Appl Environ Microbiol 69:2006–2014

Szulc W, Rutkowska B, Sosulski T, Szara E, Stępień W (2014) Assessment of sulphur demand of crops under permanent fertilization experiment. Plant Soil Environ 60:135–140

Van Hameren B, Hayashi S, Gresshoff PM, Ferguson BJ (2013) Advances in the identification of novel factors required in soybean nodulation, a process critical to sustainable agriculture and food security. J Plant Biol Soil Health 1:1–6

Varin S, Cliquet JB, Personeni E, Avice JC, Lemauviel-Levenant S (2010) How does sulfur availability modify acquisition of white clover (*Trifolium repens* L.)? J Exp Bot 61:225–234

Wielbo J, Marek-Kozaczuk M, Kubik-Komar A, Skorupska A (2007) Increased metabolic potential of Rhizobium spp. is associated with bacterial competitiveness. Can J Microbiol 53:957–967

Zhao FJ, Wood AP, McGrath SP (1999) Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil 212:209–219