

The influence of effective soybean seed treatment on root biomass formation and seed production

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Abstract: The soya seed was treated before sowing with the following biological active substances: Lignohumate B, Lexin, Lexenzym, brassinosteroid, and "Complex treatment" (a mixture of saturated sugar solution, Lexin, fungicide treatment Maxim XL 035 FS and remedial pinolen substance Agrovital). During growing, the influence of biological active substances on root biomass formation and the activity of bacteria for nitrogen fixation was observed. Evaluated parameters were shoot biomass formation and dry mass formation of plants. Harvest values were considered an important output of the whole year soya growth process. As can be observed from the results, the most effective seed treatments were Lexenzym, Lexin, and "Complex treatment", where the yields were high. Moreover, the "Complex treatment" in comparison with the control variant (not treated) improved statistically conclusively not only the final yield but was helpful also for bacteria nodulation and nitrogen fixation (N₂). All biologically active compounds supported the root and shoot biomass formation and the whole plant growth.

Keywords: *Glycine max* L.; seed dressing; biological fixation of nitrogen; germination; root system

Healthy seeds with high vigour are the basis for the successful growing of all plants in agriculture. Such seeds are a primary precondition of effective and high production, high yields at all stages from germination, and especially later during the formation of quality and vitality root biomass. Strong roots are necessary for basic life processes in plants (Henshaw et al. 2007). Seed vigour influences not only the seeding quality but also root and shoot biomass formation (Finch-Savage et al. 2010). To support the health and vitality of seeds not only fungicides are used, but they are also treated with biologically active compounds, too (Procházka et al. 2017). Seed treatment is mainly a biological process; however, it can also be a chemical or physicommechanical process or a combination of the mentioned methods. It should lead to the reduction of a negative influence of the environment and support germination, vitality, and growth of healthy plants with high yield potential (Khanzada et al. 2002). In the case of leguminose,

an effect of quick decline could be observed in vitality and possible germination of seeds (Maity et al. 2000, Murthy et al. 2003). For example, Maity et al. (2000) and Goel et al. (2003) mentioned many possibilities to reduce the decrease in germination and vitality. Important are treatments with biologically active compounds, supporting the metabolism of seeds; it makes them convenient for stimulation of germination.

Biologically active compounds are variable grow regulators, enzymes, substances related to bioenergetics in plants, or photosynthetic pigments that are a part of the protein complex. They can change the energy from sunshine to chemical energy (Dřimalová 2005). Many biologically active compounds showed a direct positive influence on seed germination and growth of soya. Some of the researchers reported a positive effect of biologically active substances based on the combination of synthetic auxins, humin acids, and fulvic acids. Quite the same

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effect could be observed using the synthetic variant of some brassinosteroids, as there was a positive correlation with auxins (Procházka et al. 1998, 2017).

The group of most quality humic substances comprises Lignohumate and Lignohumate B, which is a solution of humic acids. Acids arise from the organic transformation of residues during wood processing. It contains active humus acids that are 50% of humic acids and 50% of fulvic acids. Humus acids have a positive influence on the plant growing. They help the plant during intake, distribution, and incorporation of nutrient substances. Among the biologically active compounds, it is not only humate substances but also phytohormones, and such combinations have become popular in agriculture (Lee and Bartlett 1976, Veselá et al. 2005, Domingos et al. 2009). This group of solutions with active substances used in field experiments and practice comprises, for example, Lexin – concentrated solution of humic and fulvic acids in combination with auxins. A positive effect of Lexin is on cellular growth and division, root growth, lignification and other plant characteristics in anatomy and morphology. As a result, high productivity in quantity and quality was observed (Adamčík et al. 2016). Other biologically active compounds with a positive impact on the plants' growth are brassinosteroids (phytohormones). Such hormones help fight against stress conditions, especially drought, low or too high temperatures or soil salinity. They can also support the development of roots (Kohout 2001, Nováková et al. 2014, McGuinness et al. 2019).

The experiment was established to determine the effect of soybean seed treatment on biologically active compounds, on the formation of root biomass and its dry matter, number of nodules, shoot mass and dry matter, and yield elements – the yield and the qualitative composition of the produced seeds (oiliness, protein content and amount of fibre).

MATERIAL AND METHODS

Field trials were carried out during the growing seasons from 2016 to 2018 with a very early soybean cv. Merlin (000+). To maintain the uniformity of the methodology, the variants were treated each time immediately before sowing, according to the scheme shown in Table 1. The sowing rate was determined based on the recommendations of a seed company; it was 68 seeds per square meter for cv. Merlin. In all cases (in all variants), the seeds were inoculated with Nitrazon+.

The following biologically active compounds were used in the experiment:

- Lignohumate B is a mixture of humic acids produced in the process of organic transformation from waste wood with the ratio of humic and fulvic acids 1:1 (Procházka et al. 2018);
 - Lexin is a concentrated solution of humic acids, fulvic acids, and auxins supporting plant cell division and elongation. Improving influence on root formation and growth and increase of yield was observed (Adamčík et al. 2016);
 - Brassinosteroids are a relatively new group of steroid phytohormones from the terpenic family. They were found in oilseed rape (*Brassica napus* L.) pollen in the USA in 1970 (Nováková et al. 2014). Substance No. 4154 (brassinosteroid), a synthetic analogue of natural 24 – epibrassinolide (2 α ,3 α ,17 β -trihydroxy-5 α -androstan-6-on), was used in the experiment (McGuinness et al. 2019);
 - Lexenzym is a concentrate of humic acids and fulvic acids, enriched with phytohormones, vitamins, and enzymes (Procházka et al. 2018);
 - Complex treatment – a mixture of a saturated solution of saccharose, Lexin, fungicide Maxim XL 035 FS, and surfactant agent pinolene (Agrovital). The experiment was designed as long plots, with three replications (1000 m² each) in the area of Studeněves, Czech Republic. Weather details of the experimental years and locality are presented in Table 2.
- The pre-crops of soybean were spring barley, winter wheat, spring barley, and winter wheat in order from 2016–2018. For all experimental variants, the same growing technology was used:
- stubble breaking with disc harrow directly after pre-crop harvest;

Table 1. Scheme of pre-sowing seed treatment

Treatment	Dose per 20 kg of seed
Lignohumate B (LIG)	25.7 mL, water
Lexin (LEX)	6.5 mL, water
Brassinosteroid (BRS)	2.2 mL substance 4154, water saturated solution of saccharose
"Complex treatment" (COM)	6.5 mL Lexin 10 mL Agrovital 20 mL Maxim XL 035 FS
Lexenzym (LEXZ)	6.5 mL, water
Untreated control (UTC)	200 mL water

Total volume of all solutions was 200 mL

Table 2. Characterization of experimental location

Year	Sowing date	Harvest day	Altitude (m a.s.l.)	Average annual temperature (°C)	Annual sum of precipitation (mm)	pH	P	K	Mg	Ca
							(ppm (Mehlich 3))			
2016	23. 4.	10. 10.	306	8.2	684	7.1	107	295	165	3487
2017	21. 4.	21. 10.	314	9.8	587	7.3	103	260	174	4250
2018	22. 4.	13. 10.	325	9.8	491	6.8	68	344	213	4250

All soil blocks are loam, arenic cambisol

- chisel ploughing into 30 cm;
- NPK 15 fertilising (15.0% N, 6.6% P, 12.5% K) dose 200 kg/ha applied before sowing in spring;
- pre-sowing tillage – 2× cultivator in 6 cm depth;
- seed treatment and sowing;
- a pre-emergent herbicide treatment (Plateen 41.5 WG – flufenacet, metribuzin dose 2.0 kg/ha);
- Harvest Class Lexion 760 with adapter S750.

The results of the field trial were processed by a general linear model (GLM ANOVA) using the SAS statistical program, version 9.4 (Carry, USA). Differences between the mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test at the level of significance $P = 0.05$.

The influence of biologically active compounds on root biomass growth was examined in phase BBCH 73, i.e., in the time of growing, when the root biomass should be strong, robust, and in full activity with a lot of nodules for nitrogen fixation. From each experimental variant and each experimental parcel, 15 plants were taken. Every plant was hoed around and pulled gently out (depth 30 cm), which was followed with soil preparation with water to preserve the roots not damaged. The number of root nodules was counted. Then, the root biomass was cut away from shoot mass and weighed in fresh mass and dry mass (after 24 h drying at 105°C). This experiment indicated the weight of root mass and its dry matter. At the end of cultivation, the yield of seeds from each of the experimental variant was evaluated. After the soybean harvest, the seeds were analysed for oil, protein, and fibre content using an NIR spectrophotometer (OmegAnalyzer G Bruins Instruments; Puchheim, Germany).

RESULTS AND DISCUSSION

Figure 1 shows that all biologically active substances had a positive influence on germination and growth in the first phase of growth. The best results were obtained with compounds containing auxins. The

same results were recorded in other field experiments (Adamčík et al. 2016, Procházka et al. 2017), where the best results were also observed at auxins-treated variants. The best germination of sorghum was achieved at the Lexin treatment.

The analysis of soybean plants in BBCH 73 showed that the most developed root mass was at the Lexenzym experimental variant; compared to the UTC, the root mass weight was by 44% higher and significant. However, all biological active compounds substances in our experiments had a positive impact on soya root mass growing (Figure 2a), in fresh mass as well as in dry matter (Figure 2c). Table 4 shows an improved number of root nodules (with symbiotic bacteria for nitrogen fixation). Table 4 is however noticeable that differences in the number of root nodules are not significant. Variants with sturdy root mass correlated with a high number of nodules (Figure 1, Table 4). The same results were released by Henshaw et al. (2007). A positive influence

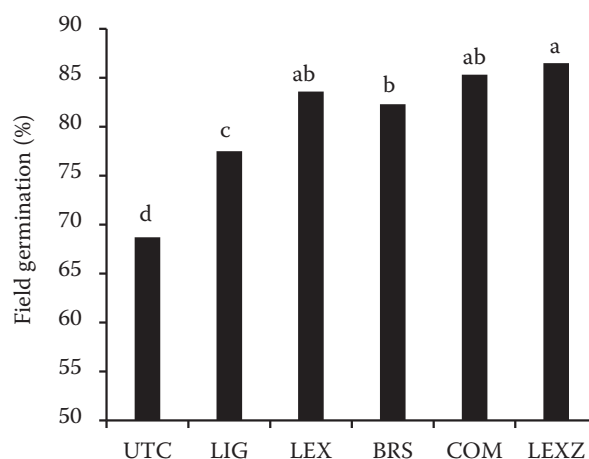


Figure 1. Field germination of the experimental variants (3-year average 2016–2018). UTC – untreated control; treatments: LIG – Lignohumate B; BRS – brassinosteroid; LEX – Lexin; COM – complex treatment; LEXZ – Lexenzym; the same letters are not statistically significant ($P < 0.05$)

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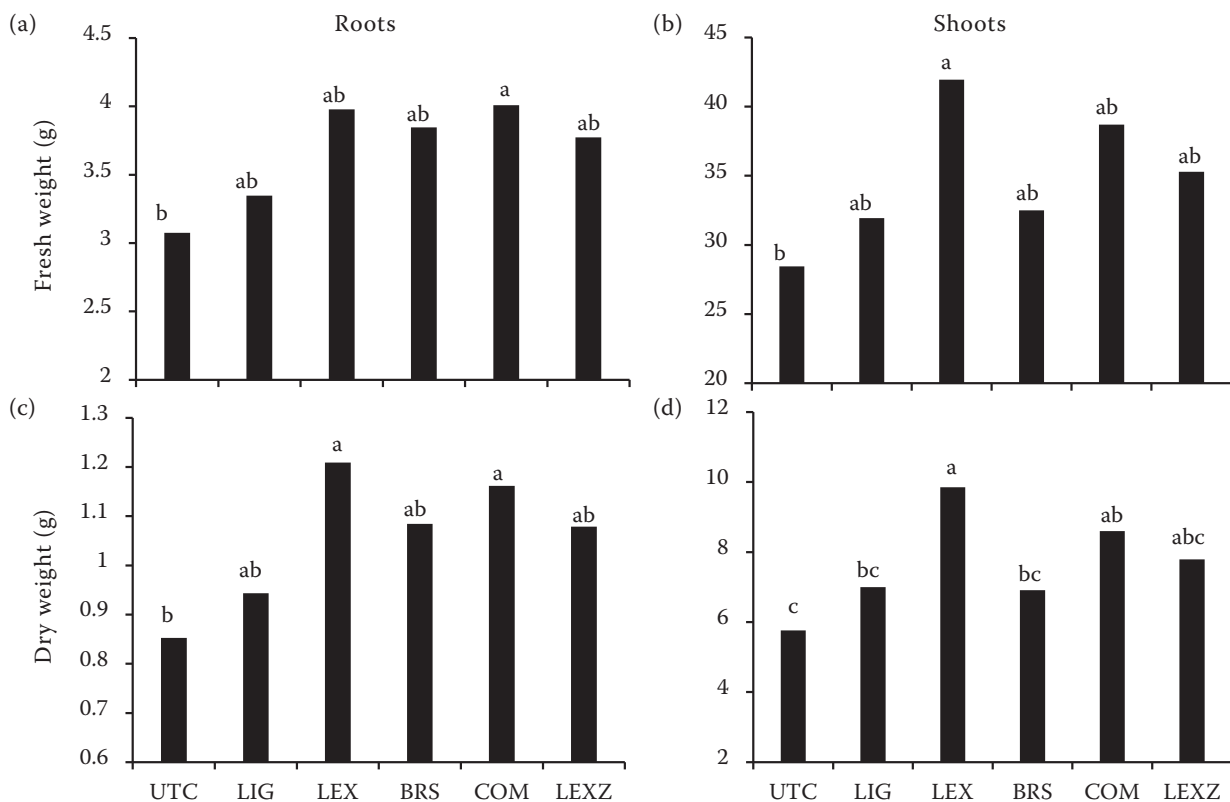


Figure 2. The average weight of soya roots and shoots (2016–2018, 3-year average). UTC – untreated control; treatments: LIG – Lignohumate B; LEX – Lexin; BRS – brassinosteroid; COM – complex treatment; LEXZ – Lexenzym; the same letters are not statistically significant ($P < 0.05$)

on soybean shoot mass growth was recorded at the Lexin treatment; the difference compared to UTC was more than 30%, and it was significant. The results of Vanneste and Friml (2009) and also Adamčík et al. (2016) are in compliance with that. Sorghum seeds treated with biologically active auxin compound produced ground cover with significantly better results in shoot mass and dry matter. A positive effect of brassinosteroids on root biomass formation of pine is described in the results of Nováková et al. (2014).

After harvest, the yield, oiliness, protein content, and the amount of fibre of seeds were calculated for each variant. The results confirm that all biologically

active compounds supported yield formation (Figure 3, Tables 3 and 4). The best results (in 3-year average) were reached with auxins (Lexenzym – yield 3.046 t/ha; Lexin – yield 3.045 t/ha; "Complex treatment" – yield 3.031 t/ha). A little lower yield was obtained at the brassinosteroid treatment (2.811 t/ha), but it was still significant; similar results were obtained with Lignohumate B (yield 2.929 t/ha). A positive impact of biologically active compounds containing auxins is described by Procházka et al. (2018) during foliar application on hops. Honsová (2013) described a positive effect of barley seed treatment with Lignohumate B with the final result + 6% in yield in

Table 3. Average yield (t/ha) of soybean seeds (recalculated to moisture content 13% during harvest (2016–2018))

Year	Average annual temperature (°C)	Annual sum of precipitation (mm)	COM	LEXZ	LEX	BRS	LIG	UTC
2016	8.2	684	3.985	4.051	4.043	3.401	3.871	3.157
2017	9.8	587	3.582	3.474	3.494	3.477	3.457	3.248
2018	9.8	491	1.525	1.614	1.601	1.556	1.460	1.390

COM – complex treatment; LEXZ – Lexenzym; LEX – Lexin; BRS – brassinosteroid; LIG – Lignohumate B; UTC – untreated control

Table 4. Results of the statistic evaluation (average of the years 2016–2018)

	COM	LEXZ	LEX	BRS	LIG	UTC	HSD
Yield of seeds (t/ha)	3.031 ^b	3.046 ^a	3.045 ^a	2.811 ^d	2.929 ^c	2.598 ^e	0.0123
Field germination (%)	85.3 ^{ab}	86.5 ^a	83.6 ^{ab}	82.3 ^b	77.5 ^c	68.7 ^d	4.1336
Tuber (pcs.)	4.09 ^{ab}	3.43 ^a	3.08 ^a	3.02 ^a	3.14 ^a	1.71 ^a	2.5465
Shoot mass – fresh mass (g)	39.061 ^{ab}	34.993 ^{ab}	41.826 ^a	32.409 ^{ab}	32.637 ^{ab}	28.824 ^b	10.475
Soya roots – fresh mass (g)	4.146 ^{ab}	3.732 ^{ab}	3.981 ^{ab}	3.861 ^{ab}	3.382 ^{ab}	3.133 ^b	0.9889
Shoot mass – dry matter (g)	8.666 ^{ab}	7.743 ^{abc}	9.778 ^a	6.902 ^{bc}	7.099 ^{bc}	5.796 ^c	2.0797
Soya roots – dry matter (g)	1.183 ^{ab}	1.064 ^{ab}	1.208 ^a	1.098 ^{ab}	0.952 ^{ab}	0.856 ^b	0.2774
Oil content (%)	19.11 ^a	19.12 ^a	19.09 ^a	19.05 ^a	18.71 ^b	18.75 ^b	0.2413
Protein in seed (%)	33.34 ^{ab}	33.33 ^{ab}	33.01 ^{bc}	32.44 ^c	33.82 ^a	33.42 ^{ab}	0.685
Fibre content (%)	4.95 ^{ab}	4.95 ^{ab}	4.95 ^{ab}	4.96 ^a	4.90 ^{bc}	4.85 ^c	0.0543

Means with the same letters are not statistically significant ($P < 0.05$). HSD – honestly significant difference; UTC – untreated control; treatments: LIG – Lignohumate B; BRS – brassinosteroid LEX – Lexin; COM – complex treatment; LEXZ – Lexenzym

comparison with UTC. Tomášek et al. (2013) noted a positive yield-improving effect of Lignohumate treatment on potatoes. Hradecká et al. (2009) presented a positive effect of brassinosteroids on sugar beet production. The results show that soya seeds treated with biologically active compounds (especially those containing phytohormones) significantly increased the oil content of the produced seeds. The highest average oil content in the seeds was provided by the variant Lexenzym (19.12%). Very similar results were achieved at "Complex treatment" (19.11%) and Lexin (19.09%), all significantly different from the untreated control (Table 4).

The 3-year experiment studying the impact of soya seed treatment with biologically active compounds

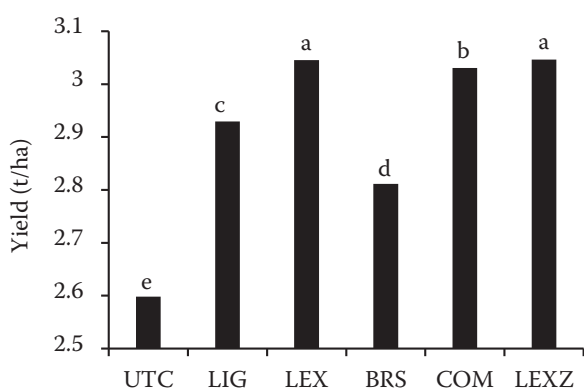


Figure 3. Average yield of soybean seeds (recalculated to moisture content 13% during harvest in 2016–2018, 3-year average). UTC – untreated control; treatments: LIG – Lignohumate B; LEX – Lexin; BRS – brassinosteroid; COM – complex treatment; LEXZ – Lexenzym. The same letters are not statistically significant ($P < 0.05$)

on the root system shows that all biologically active compounds supported the root and shoot biomass formation and the whole plant growth.

The results show that the most effective seed treatments with high yields were variants with Lexenzym, Lexin, and "Complex treatment" (a mixture of a saturated solution of saccharose, Lexin, fungicide Maxim XL 035 FS and surfactant agent pinolene). "Complex treatment" in comparison with control variant (not-treated) improved statistically conclusively not only the final yield but was also helpful for bacteria nodulation and nitrogen fixation (N_2).

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