Results of the Verification of Prospective Glycine Soy Stimulating Agents

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Executive Summary: The paper deals with the effects of brassinosteroids and the preparation Lexin (auxines + fulvic and humic acids) upon the physiological condition and yield of soy grown in the dry region of Central Bohemia. The monitored preparation increased the height of the first legumes above the ground level due to the stimulation of long growth. Such a phenomenon has a significant practical relevance as it reduces losses during the harvest. The plants to which the preparation was applied showed better resistance to droughts and more favourable energy balance of their photosynthesis as well as generally better physiological condition and their seed yields were higher, too.

Key words: brassinosteroids, Lexin, soy, yield, N-compounds, oiliness

Introduction

Glycine soy is an important source of vegetable proteins not only from the point of view of their high contents (38 – 48 %) but also from the point of view of their high biological value. Therefore, soy features a key component of vegetarian and some treatment diet. According to newly recommended protein intake quantities, the ratio of animal and vegetable proteins in the human diet should be 1 : 1 except for some countries where the recommended ratio is 1 : 2. By now, this ration is higher in our country in favour of animal proteins. Given our diet habits, an elevated intake of animal proteins results in an elevated intake of fat and cholesterol (Hrubý 1999, Stránský 1999).

Soy contains a number of other important nutrition factors many of which are lacking in our diet including but not limited to pulp, some B group vitamins and minerals including but not limited to potassium, calcium and magnesium. Based on the outcome of epidemiological studies and clinical tests, soy, as well as other pulses, has a positive effect in terms of the prevention of some civilisation diseases, in particular cardiovascular conditions. It may have positive effects if included to the diet of people suffering from diabetes as it favourably influences the glycaemia index. If there is a need to reduce the intake of meat and eggs for economic or health-related reasons, soy is their best substitute (Dostálová, Pokorný 1997).

A traditional pulse cultivated in the country and used for the production of food is pea. However, since 10 years, the pea sowing area keeps dropping in the Czech Republic. What is behind such falling interest of farmers in peas and other formerly quite common pulses (bean, vetch, field pea) is their yield instability, limited possibility of the sale of the crop due to poor demand on the part of the processing industry. Another reason are low buyout prices.

However, since nine years, the Czech Republic has experienced an elevated interest in the growing of soy. Soy is a crop not only with a high nutrition value but it ranks among most important oil bearing plants. What has to be noted from the global point of view is that soy is the fourth most common crop world-wide (Štranc et al. 2005).

The global progress in the field of the breeding and cultivation of soy has changed many of the economic properties of this plant bringing so the plant to agro-environmental conditions that used to be improper or very risky for the growing of soy. Also climatic changes may be found somewhat relevant (the global warming) in addition to development of the structures of species for the expansion of the soy growing areas in higher geographical latitudes. Thus, the soy growing areas keep growing in Hungary, Slovakia, Ukraine. Also Poland has started the growing of soy and the same happens even in southern regions of Sweden. The growing of soy concentrating on species designed for higher geographical latitudes has become one of possible ways of the extension of the domestic sources of protections in warmer but not very dry regions (Pearce 2000, Šimon 1999, Štranc et al., 2002).

During its vegetation period, soy is sensitive to temperature and in particular to the supply of moisture. Its deficit respectively extreme fluctuations may trigger a stress adaptation response. As mentioned above, soy does not like dry stations where it suffers from physiological dysfunctions including leaves yellowing, smudgy leaves, virus mosaic etc.

It is generally known that adverse impacts of such stress upon soy may be eliminated by the application of both brassinosteroids and a preparation based on auxine and a mixture of fulvic and humic acids that is marketed under the name of LEXIN (Klouček et, al. 2006, Štranc et al., 2006). For instance, 24-epibrassinolid applied in experiments executed by Vidya et al. (1999) stimulated the growth of soy plant and resulted into a higher content of proteins. Also the seed showed higher level of oiliness. Štranc et al. (2006) came to similar results not only after the application of brassinosteroids but also after the application of the preparation Lexin. Sam et al. (2001) detected bio-stimulating effects of brassinosteroids residing in the protection in internal membranes and chloroplasts that get decomposed if exposed extreme temperatures. Kulaeva et al.(1991) claim that the thermo-tolerance of plants triggered by brassinosteroids concerning stress is caused by changes of the synthesis of proteins and enzymes.

Given the above reasons, the effort to introduce brassinosteroids and Lexin in the plant growing industry seems to be prospective also from the point of view the quality of the crops in particular in the current period when the global competition is likely to prefer quality of commodities to their quantity. Some preliminary experiments concerning cereals accomplished at the Czech Agricultural University demonstrated that the application of the above preparations increases the content of minerals including for instance Ca, Mg, Zn, Cu and vitamins from the tocopherol family. Treated plants better accumulate and make use of solar energy.

The objective of our experiments was to verify the stress-suppressing effects of the above preparations in soy growing in particular in conditions typical of lack of moisture in order to improve the physiological condition of the plant. Simultaneously, we studied the support of the transmission of assimilation products to the sink due to the application of such preparations.

Materials and methods

In the experimental year 2006, the experiment took place in the cadastre of the municipality of Studeněves. In terms of geo-morphology, the locality belongs to the Kladno plateau. The height of the experimental field above the sea level is 313 m and its exposed to south (field Na Richardě, area 23 ha). In terms of composition of soil, it is a sandy cambisoil on carbonate deluvium, medium heavy to lighter consistence. An agro-chemical analysis of the soil showed the following results: 77 ppm P, 204 ppm K, 182 ppm Mg and 6128 ppm Ca, pH = 7,7. In terms of climate, the locality belongs to the region B1. It is typical of an arid climate.

The experimental specie of soy was *Korada.* Individual plants were sown to rows at the distance of 25 cm with the sowing density of u 65 seeds per 1 m^2. The previous crop was winter wheat. The experiments monitored in total 13 variants (see the table 1). Every variant was reiterated four times on a plot of the area of 13.1 m^2 with the harvest area of 10 m^2.

What was used for the photosynthesis balance (after the application of the stimulating agents and for comparison with the reference units) was the rapid fluorescence induction (RFI) - analyser Hansatech „PEA“ Norfolk - P02.003, softw. Winpea 32. We assessed 12 plants from every variant. The content of chlorophyll was established by the chlorophyll meter Hydro. The soy quality was determined using the tool NIR calibrated to N-compounds to Kjehdal and for oiliness to Soxhlet.

Tab.1 Overview of experiment variants

Variant	**Preparation**
Br 1	**brassinosteroid No. 1** (emergence phase to the first trefoil)
Br 2	**brassinosteroid No. 2** (emergence phase to the first trefoil)
Br 3	**brassinosteroid No. 3** (emergence phase to the first trefoil)
Br 4	**brassinosteroid No. 4** (emergence phase to the first trefoil)
Br 5	**brassinosteroid No. 1** (flowering start phase)
Br 6	**brassinosteroid No. 2** (flowering start phase)
Br 7	**brassinosteroid No. 3** (flowering start phase)
Br 8	**brassinosteroid No. 4** (flowering start phase)
K	**reference sample** free of brassinosteroid or Lexin
MS 1	**brassinosteroid No. 3 dressing**
MS 2	**Lexin dressing**
MS 3	**Lexin** (emergence phase to the first trefoil)
MS 4	**Lexin** (flowering start phase)

What was evaluated in the course of the experiments was the weed infestation, height of the first legumes (cm), stalk angle relative to the ground lodging), height of the growth prior to the harvest, raping time. What was monitored during the harvest was the yield (tonnes per hectare), seed moisture in %, impurities, weight of one thousand of seeds and harvest losses. What was established following the harvest was the content of nitrogen compounds and oil in seeds of various variants of soy.

Results

Evaluation of the Weather Conditions During the Experiment

From the point of view of weather conditions, they year 2006 was relatively favourable for soy growing at the locality of Studeněves. The yield of soy from this locality was very good.

Soil was quite wet during the sowing and emergence of plants. Its quite fast warming (due to sunny and warm weather) made it possible for a fast germination and growth of soy that was not disturbed by the transition cold period of so called “icy men” in May.

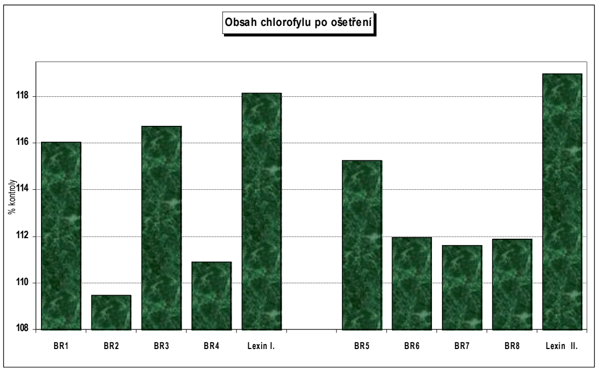
At the end of May and in early June, we saw a significant fall of temperature. As a consequence of the latter, the growth of soy stagnated for the entire first decade of June. In the second decade of June, following a rise of temperature and being expose to sufficient doses of sunshine and quite normal precipitation, soy reassumed its relatively intensive growth. In late June and early July, subject to significantly above-standard temperatures (3.0°C – 5.5°C above the normal temperatures), intensive sunshine and highly varying precipitation within the concerned region, soy suffered from lack of moisture at some places.

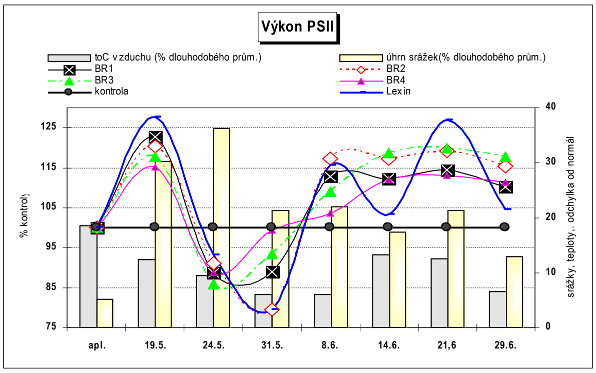
In late July, subject to continuing very high temperatures (5.8°C above the normal level, daily maximum temperature reached up to 33°C and at some locations even 37°C) and being exposed to very intensive sunshine, soil heavily dried out. The growth of soy stopped, flowers, leaves and partly legume became dry and went down.
Such a critical situation and the condition of the soy growths started to improve as late as in early August when we saw a significant change of the weather conditions. A colder and quite wet weather in August helped the damaged experimental soy growths to recover. All the way round, September was sunny and very warm contributing so to the raping of the recovered soy.

Photosynthesis Energy Balance

All applications of both brassinosteroids and Lexin increased the content of chlorophyll in leaves. The preparation Lexin was the leading factor in terms of the increase of chlorophyll in leaves in both phases of the application. The preparations Br1 and Br3 affected the formation of chlorophyll more intensively after the application in the first trefoil phase, the preparations Br6 and Br8 showed better effects rather prior to the flowering phase (fig.1). It effected the performance of the photosynthesis in relation to the ontogenesis of the plants, the development of weather conditions and the nature of the preparations. This fact may be documented by the performance of the photo-system that is the most relevant among the balance changes of the photosynthesis. Its performance is proportional to the accumulation of dry matter and, therefore, the biological and economic yield (fig. 2).

Fig.1 Content of chlorophyll after the application Obr.2Photosystem performance – 1st application





Vegetation Monitoring

The growth density was quite good and it ranged from 46 to 57 plants per square meter. However, the nodulation of the plants was quite poor (from 0.0 to 0.5 nodules per 1 plant) whereby all monitored nodulation stimulating preparations appeared to have favourable effects in particular if applied by dressing.
Soy plants treated by both brassinosteroids and Lexin showed (unlike plants of other variants) a higher height of the first legumes above the ground level. Also the total height of these plant variants was higher (see tab. 2).

Tab. 2. Results of the quality evaluation prior to the harvest (Studeněves 2006)

Var.	Preparation	Legume height* (cm)	Growth height (cm)	Number of plants per m^2	Lodging rate
Br 1	**brassinosteroid No. 1** (emergence phase to the first trefoil)	5.9	52.2	55	9.0
Br 2	**brassinosteroid No. 2** (emergence phase to the first trefoil)	5.2	54.9	51	9.0
Br 3	**brassinosteroid No. 3** (emergence phase to the first trefoil)	5.3	49.0	53	9.0
Br 4	**brassinosteroid No. 4** (emergence phase to the first trefoil)	5.6	51.9	49	9.0
MS 3	**LEXIN** (emergence phase to the first trefoil)	6.6	51.3	47	9.0
Br 5	**brassinosteroid No. 1** (early flowering phase)	4.2	47.7	46	9.0
Br 6	**brassinosteroid No. 2** (early flowering phase)	4.7	45.5	48	9.0
Br 7	**brassinosteroid No. 3** (early flowering phase)	5.1	49.3	49	9.0
Br 8	**brassinosteroid No. 4** (early flowering phase)	4.6	50.6	53	9.0
MS 4	**LEXIN** (early flowering phase)	7.1	58.2	55	9.0
K	reference sample free of brassinosteroid or Lexin	3.1	42.7	55	9.0
MS 1	**brassinosteroid No. 3 dressing**	6.4	49.6	53	9.0
MS 2	**Lexin dressing**	6.0	49.0	57	9.0

Legume height * - the height of the apical end of the inferior legume above the ground level

What appeared to be the strongest stimulator of the height of the first legume was Lexin After its application (var. Ms 3 and Ms 4, applied during the emergence phase to 1 TL and in the early flowering phase) we noted the highest height of the first legume – 6.6 cm and 7.1 cm (the reference sample showed 3.1 cm). In the event of brassinosteroids, the best variants from the point of view of the height of the first legume is the variant Ms 1 (brassinosteroid No. 3 dressing).

Brassinosteroids showed a different trend of effects compared to Lexin. The earlier the brassinosteroids were applied the higher was the height of the first legumes (see tab. 2).

Harvest Results

From the point of view of the yield of seed, the application of brassinosteroids and Lexin for both dressing and foil application was very positive. The early application of brassinosteroid No. 2 (var. Br 2) appeared to be the most efficient as well as the late application of Lexin (var. Ms 4). These cases showed the highest theoretical yield at zero harvest losses, namely 3.879 t/ha and 3.780 t/ha which means an increase of the yield by “incredible” approx. 36% and 33% compared to the reference sample (see tab. 3).

Tab. 3. Harvest results (Studeněves 2006)

Var.	Preparation	Net yield converted to humidity 13% (t/ha)	Losses at 13% of humidity (kg/ha)	WTS at 13% of humidity (g)	Theoretical yield at zero harvest loss (t/ha)
Br 1	**brassinosteroid No. 1** (emergence phase to the first trefoil)	3.120	390.7	166.7	3.511
Br 2	**brassinosteroid No. 2** (emergence phase to the first trefoil)	3.496	382.3	171.0	**3.879**
Br 3	**brassinosteroid No. 3** (emergence phase to the first trefoil)	3.107	399.3	169.0	3.506
Br 4	**brassinosteroid No. 4** (emergence phase to the first trefoil)	3.133	455.3	171.5	3.588
MS 3	**LEXIN** (emergence phase to the first trefoil)	3.030	504.2	169.2	3.534
Br 5	**brassinosteroid No. 1** (early flowering phase)	3.033	444.9	172.7	3.478
Br 6	**brassinosteroid No. 2** (early flowering phase)	2.910	397.6	171.4	3.308
Br 7	**brassinosteroid No. 3** (early flowering phase)	3.030	425.1	172.8	3.455
Br 8	**brassinosteroid No. 4** (early flowering phase)	3.045	335.5	164.3	3.381
MS 4	**LEXIN** (early flowering phase)	3.368	411.9	169.6	**3.780**
K	**reference sample** free of brassinosteroid or Lexin	2.297	553.9	174.2	2.851
MS 1	**brassinosteroid No. 3 dressing**	2.786	489.1	175.1	3.275
MS 2	**Lexin dressing**	2.847	507.5	178.3	**3.354**

The 2006 experiments indicate that the most effective application of brassinosteroids was the application executed in the phase of emergence to 1 TL that resulted into a highest yield compared to the application executed in the early flowering phase. This facts is apparently closely related to the response time to individual brassinosteroids and the time of their application. The brassinosteroids No. 1 and 3 appeared to effect the metabolism of plants faster meanwhile the brassinosteroids No. 2 and 4 have slower effects, all the way round. What is in line with the above are the results achieved in the metering of the quantity of chlorophyll and energy balance of photosynthesis. In the event of an early application (in the emergence phase to TL1), the slower-acting brassinosteroids (var. Br 2 and Br 4) worked better (to a later provoked stress) than the faster-acting brassinosteroids (var. Br 1 and Br 3) whose efficiency was dropping at the time of the biggest stress of the plants and the achieved yield showed an apparent correlation to it. All the way round, the later application of brassinosteroids (in the early flowering phase and at the beginning of the stress exposure) the faster-acting brassinosteroids No. 1 and 3 (var. Br 5 and Br 7) proved to be have favourable effects.

Lexin effects were even faster compared to No. 1 and 3. The yield results indicate that its application in 2006 was most efficient in the early flowering phase (var. Ms 4). The lowest yield was registered for the untreated reference sample (var. K).

Analysing the harvested soy seeds, we assessed also the WTS indicator (WTS = the weight of one thousand of seeds – see tab. 3). What showed higher WTS values compared to the reference sample were only the dressed variants (Ms 1 and Ms 2). Other variants treated by brassinosteroids and Lexin showed lower WTS value compared to the reference sample. We believe that the drop in the weight of the seeds was caused by the atypical weather conditions. Therefore, we can anticipate that should the same WTS be achieved as in the reference variant, the achieved yield would be even higher.

The application of brassinosteroids and Lexin did not effect too much the biological composition of soy beans (content of nitrogen compounds and oiliness) which phenomenon can be considered positive given the high increase of the yield (see tab. 4).

Tab. 4. Content of nitrogen compounds and oil in soy seed (Studeněves 2006)

Var.	Preparation	N-compounds (% hm.)	Dry matter oiliness (% hm.)
Br 1	**brassinosteroid No. 1** (emergence phase to the first trefoil)	36.88	22.88
Br 2	**brassinosteroid No. 2** (emergence phase to the first trefoil)	36.93	22.65
Br 3	**brassinosteroid No. 3** (emergence phase to the first trefoil)	**37.65**	22.33
Br 4	**brassinosteroid No. 4** (emergence phase to the first trefoil)	36.59	**22.92**
MS 3	**LEXIN** (emergence phase to the first trefoil)	37.36	22.04
Br 5	**brassinosteroid No. 1** (early flowering phase)	36.06	23.43
Br 6	**brassinosteroid No. 2** (early flowering phase)	**37.50**	22.57
Br 7	**brassinosteroid No. 3** (early flowering phase)	36.73	22.63
Br 8	**brassinosteroid No. 4** (early flowering phase)	35.66	23.13
MS 4	**LEXIN** (early flowering phase)	36.31	**23.37**
K	**reference sample** free of brassinosteroid or Lexin	36.77	22.69
MS 1	**brassinosteroid No. 3 dressing**	36.48	22.43
MS 2	**Lexin dressing**	36.80	22.52

The highest content of N-compounds was found in the var. Br 3 and Ms 3 (application in the emergence phase to 1 TL), namely 37.65% and 37.36%. All the way round, the lowest concentration of N-compounds was found in the Br 5 and Ms 4 (application in the early flowering phase) with 36.06% and 36.31% (see tab. 4).
The highest oiliness was found in the var. Br 5 and Ms 4, namely 23.43% and 23.37%. Unlike the above, the var. Ms 3 and Br 3 showed the lowest content of oil, namely 22.04% a 22.33% (see tab. 4). The above results of the bio-chemical analyses suggest an apparent negative correlation between the nitrogen-compound content and the oiliness.

Conclusions

The results of the experiments as achieved in 2006 may be considered to be preliminary as these results related to one only year. At the same time, we note that the results have to be seen in relation with the applied agro-technical methods and environmental conditions of the given station including the weather conditions of the given year.
The results of the energy balance of the soy photosynthesis indicate that brassinosteroids No. 1 and 3 act faster than the brassinosteroids No. 2 and 4. However, the fastest-acting preparation was a preparation based on fulvic and humic acids and auxin called Lexin. It appeared that the faster-acting growth stimulating preparations applied where plants are exposed to long-term stressing conditions should be applied repeatedly as their effects are short-term. The results also indicate that the identification of a proper time of the application of slower-acting growth stimulators will be apparently quite complex.
An evaluation of the energy balance of photosynthesis of soy plants under the given cultivation conditions as well as the achieved yield made it apparent that all applied preparations are suitable for the practical farming application.
From the point of view of the yield, the application of all monitored preparations (both for dressing and various terms of their foliar application) may be considered very positive. What was found the best efficient way was the early application of the brassinosteroid No. 2 and later application of Lexin. In these cases, we recorded the highest theoretical yield at zero

harvest losses, namely 3.879 t/ha and 3.780 t/ha and it means an increase of the yield by "incredible" approx. 36% and 33% compared to the reference sample.

The application of brassinosteroids and Lexin did not affected too much the bio-chemical compositions of soy beans (N-compounds concentration and oiliness) and it is to be considered positive given the high yield increase.

Finally, we stress that all preparations verified in 2006 on the experimental locality Studeněves showed a significant stress suppressing effects. After the application of brassinosteroids and Lexin, soy plants showed an intensive green effect, larger leaves and better turgor compared to untreated plants. It positively influenced the yield.

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