GROWTH PROMOTION OF MAIZE BY SOYBEAN ROOT EXUDATES

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ABSTRACT
Soybean [Glycine max (L.) Merril] rotation with maize (Zea mays L.) can increase maize yield, but its mechanism has not been fully understood. The effects of soybean root exudates at trefoil stage (prior to nitrogen fixation ability) on maize seedling growth were studied under laboratory and field conditions in this study. Results showed that soybean root exudates at trefoil stage clearly promoted maize growth. Further HPLC analysis showed that soybean root exudates contained substances that promote plant growth, such as triacontanol. Maize growth exhibited significant differences when soybean root exudates were applied at different concentrations. Altogether this indicated that allelopathy, in addition to an increase in soil nitrogen, was an important mechanism of soybean promotion of maize yield during crop rotation.

Keywords : Soybean, Maize, Root exudates, Allelopathic promoting potential.

INTRODUCTION
Soybean is an important economic crop in northeast China (Guo et al., 2006). Farming methods such as crop rotation, intercropping, and relay cropping with other crops are used in soybean production in most soybean producing areas of China. Soybean rotation with maize can increase maize yield by 20-25% (Li and Wang, 2010; Wilhelm and Wortmann, 2004). Because soybean has root nodules and larger root systems, it is generally thought that increased yield results from: increased nitrogen nutritional levels in soils due to fixation by soybean (Karlen et al., 1994; Carsky et al., 1997); fluffing of soils by soybean root systems (Raimbault and Vyn, 1991), which improves soil structure and increases water utilizing efficiency (Roder et al., 1989; Copeland et al., 1993); and fallen leaves, twigs and remaining roots enrich organic matter in soils (Campbell and Zentner, 1993). However, there are studies showing that the application of nitrogen fertilizer cannot totally substitute for the action of soybean rotation, and approximately 75% of increased yield cannot be explained with existing theories (Hesterman et al., 1986). There are few reports that biochemical correlation, e.g. allelopathy, between soybean and other crops may exist. Researchers generally focus on allelopathic inhibition in order to find allelochemicals that can inhibit weeds, diseases and pests. In fact, allelopathic promotion is also a general phenomenon in nature (Singh et al., 2001). There are many cases in which crops stimulate each other when crop rotation, intercropping and relay cropping methods are adopted. Agrostemma githago, for example, promotes wheat growth with the exudates such as Agrostemma githago essence, allantoin and gibberellin (Gajic et al., 1976; Greca, 1989). Since soybean root nodules at the trefoil stage have no ability to fix nitrogen (Wang, 1982), this study examined the exudates released at this stage to determine whether soybean demonstrates allelopathy. As soybean rotation with maize is common in northeastern China (Cao, 2002; Han et al., 2006), the correlation between soybean root exudates and maize seedling growth to increase understanding of the ecological and economic benefits of soybean rotation, intercropping and relay cropping with other crops was explored. Additionally,
was laid a foundation for the artificial synthesis of the crop growth promotion substances.

**MATERIALS AND METHODS**

Soybean (cultivar Jilin 30) and maize (cultivar Zhengdan 958) seeds were treated with 0.001 g L\(^{-1}\) of HgCl\(_2\) aqueous solution for 15 min, then washed with sterile water several times. Porcelain pots (18 cm in diameter and 8.5 cm in height) were filled with high temperature disinfected quartz sand and a suitable amount of distilled water. Soybean seeds were buried in the pots at a depth of 5 cm and cultured in incubators at 25°C with 12 h of light each day until the trefoil stage, before root nodule formation. A suitable amount of distilled water was supplemented every 2 days depending on need to ensure the normal soybean growth. Six experiments (I to VI) and 6 treatments (A to F) were set up in this work.

**I.** Zero (control), 4, 6 and 8 grains of soybean seeds were planted in different pots and 10 grains of maize seeds were put onto the quartz sand surface of each simultaneously.

**II.** Soybean seedlings (planted as in experiment I) were pulled up once they reached the trefoil stage, and the remaining soybean roots in quartz sand were totally removed. Ten grains (per pot) of maize seeds were put onto the quartz sand surface, including a pot with no soybean seeds as control.

**III.** After the trefoil-stage soybean seedlings were pulled up, 200 mL of distilled water was added to the pots and the quartz sand in the pots was stirred thoroughly, and then filtered to give the filtrate, i.e. the aqueous solution of the natural soybean root exudates. Petri dish (A, made of glass, 9.5 cm in diameter) was filled with 100 g of quartz sand and the resulting filtrate, and Petri dish (B) was filled with 200 g of the remaining filtered quartz sand, then each were planted with 10 grains of maize seeds. The control plate contained distilled water.

**IV.** In the soybean field of Jilin Agricultural University Experimental Station, three soybean seedlings at the 8-leaf stage were randomly pulled up and their surrounding soil (300 g per plant) was collected and mixed thoroughly (C). Three 300 g soil samples were taken randomly from different places sown with no soybean seeds in the same field and mixed thoroughly (D). Both soil mixtures (C and D) were added with 500 mL of distilled water and stirred thoroughly, then left still for 12 h to give corresponding soil supernatants (i.e. soil supernatant C was the aqueous solution of the soil extracts containing soybean root exudates while soil supernatant D was that with no soybean root extracts). Maize seeds were cultured with 200 mL of the soil supernatants C and D as in experiment III for bioassays.

**V.** Soybean seedlings (10 plants per pot in quartz sand, 8 pots total) at the 8-leaf stage were pulled up as in experiment II. The quartz sand in the pots was soaked in absolute ethyl alcohol for 48 h and filtered first with a sieve and then with filter paper. The resulting filtrate was evaporated with a rotary evaporator to obtain the alcohol-extracted soybean root exudates. The alcohol-extracted soybean root exudate was made into aqueous solution (E) by dissolving the alcohol extracts in 200 mL of distilled water then diluted to 10%, 50% and 80% for bioassays. Glass bottles of 6 cm in diameter and 10 cm in height were prepared with 50 g of quartz sand and 10 mL of the three above concentrations (E). Ten grains of maize seeds were put in each bottle and cultured in incubators. Control was distilled water.

**VI.** Twenty 8-leaf-stage soybean seedlings cultured in quartz sand were pulled up and put in 300 mL 250 g/L of NaCl aqueous solution. The root systems were soaked in the NaCl aqueous solution at 25°C for 24 h in the dark, then extracted with petroleum ether. The extraction solution was evaporated with a rotary evaporator to obtain the soybean root extracts, which were then dissolved in 150 mL of distilled water, resulting in the salted-out soybean root extract aqueous solution (F), diluted and tested in bioassays as in experiment V. Composition analysis of the alcohol-extracted soybean root exudates (E) obtained in experiment V and the salted-out soybean root extracts (F) obtained in experiment VI was conducted with HPLC at a wavelength of 254 nm, with water/methanol as mobile phase and triacontanol as the standard control. All treatments were repeated 3 times. After
3-7 days of growth, the root length, the seedling height and the chlorophyll content of the tested maize were measured respectively.

Data were statistically analyzed as previously described (Williamson and Richardson, 1988) using an allelopathic response index (RI). If \( T \geq C \), \( RI = 1 - C/T \) while if \( T < C \), \( RI = T/C -1 \). Here, \( C \) represents control, \( T \) is a treatment value, \( RI \) is response index (\( RI >0 \) represents promotion whereas \( RI <0 \) represents inhibition, whose absolute value coincides with the action strength).

RESULTS AND DISCUSSION

Effect of soybean root exudates on maize seedling growth: Soybean root nodules before the trefoil stage have no nitrogen fixation ability (Wang, 1982), so soybean root systems at trefoil stage cannot provide soils with nitrogen. Experiments I and II (Table 1) show that trefoil-stage soybean root exudates promoted maize seedling growth. Maize root length, seedling height and chlorophyll content were all improved with a planting density of 4 soybean plants per pot. Chlorophyll content was still improved with a planting density of 6 soybean plants per pot, but maize root length and seedling height were unaffected. Similarly, chlorophyll content of maize seedlings was improved with a planting density of 8 soybean plants per pot. From this we see that soybean promotion of maize seedling growth varies with soybean planting density, which agrees with the observation that most allelochemicals promote receptor growth at low densities but inhibit receptor growth at high densities (Singh, 2001).

Soil fluffing by soybean was observed in experiment I suggesting that the promotion of maize seedling growth can probably be related to this. However, the conditions in experiment II excluded the possibility that soybean root systems fluffed soils. These results show that although maize seedling growth under the conditions in experiment II was less than in experiment I, it was still greater than the distilled water control; this means that soybean root exudates do promote maize seedling growth independent of soil fluffing.

Allelopathic promotion by soybean root exudates on maize: Results (Table 2) in experiment III show that the filtrate (A), obtained by washing with distilled water and filtering the quartz sand after soybean plants were removed, promoted maize seedling growth more than the filtered quartz sand (B). This means that when grown in sand, soybean root exudates promote maize seedling growth and are largely water soluble. Soil supernatant (C), the aqueous solution of the soil extracts containing soybean root exudates, and soil supernatant (D), the aqueous solution of the soil extracts without soybean root exudates, were used for bioassays along with distilled water as a control. The results from experiment IV are shown in Table 2 as well. It was observed that because of the presence of some water soluble nutrients in soils, the general soil supernatant (D), with no soybean root exudates, did promote maize seedling growth. In terms of nutrition, the soil supernatant (C), containing soybean root exudates, should have shown weaker promotion action than the soil supernatant (D), because the soybean seedlings in treatment (C) would have absorbed nutrients from the soils for their growth, thus reducing the nutrients in soil supernatant (C). However, maize seedlings treated with the soil supernatant (C) grew better than with the soil supernatant (D), supporting the conclusion that soybean root exudates have a promotional effect on maize seedlings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soybean (grains/pot)</th>
<th>Root length</th>
<th>Seeding height</th>
<th>Chlorophyll content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4</td>
<td>0.49±0.009”</td>
<td>0.01±0.005”</td>
<td>0.73±0.018”</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.12±0.027”</td>
<td>0.04±0.006”</td>
<td>0.47±0.013”</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.14±0.018”</td>
<td>-0.30±0.012”</td>
<td>0.13±0.015”</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>0.18±0.012”</td>
<td>-0.01±0.004”</td>
<td>0.34±0.019”</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-0.02±0.002”</td>
<td>0.02±0.008”</td>
<td>0.26±0.017”</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-0.06±0.007”</td>
<td>0.05±0.007”</td>
<td>0.07±0.002”</td>
</tr>
</tbody>
</table>

Data in the table represent the averages ± SE of the RI (the allelopathic response index) values with 3 repeats. Asterisks “*” and “**” represent a significant difference between a treatment and the control at the 0.05, 0.01 levels, respectively.
To summarize the results from experiments III and IV, we conclude that natural soybean root exudates and soil extracts from sites of soybean growth stimulate maize seedling growth through allelopathy, and the allelochemicals are water soluble.

**Effects of alcohol-extracted and salted-out soybean root extracts on maize seedlings**: The alcohol extracted soybean root exudates (E) and the salted-out soybean root extracts (F) were obtained in experiments V and VI respectively. Their aqueous solutions were used for bioassays at different densities. Results show (Table 3) that both the exudates (E) and the extracts (F) had a positive effect on maize seedling growth. Both solutions had the strongest promotion action on maize seedlings at the densities of 50% and 80%. The soybean root exudates (E) promoted maize seedling growth more effectively than the soybean root extracts (F). The experimental results also show that though comparatively long, the root systems of the maize seedlings treated with the exudates (E) had fewer roots and were finer than those from treatment (F). These discrepancies in effects might be due to differences in exudate density and in component availability under varying conditions.

Maize seedlings were treated with the root exudates (E) and the root extracts (F) at a concentration of 70%. Their results (Table 4) indicate that both the root exudates (E) and the root extracts (F) clearly promoted seedling height and chlorophyll content of the tested maize. Though the root systems of maize treated the root exudates (E) were longer, their side roots were fewer. The positive effect of treatment (F) on maize root length was not obvious, but the side roots of these plants were more frequent and larger.

**Preliminary analysis of growth-promoting allelochemical of soybean root exudates**: The mixture of resin-like organic molecules, rather than inorganic molecules containing N, P, K was obtained after the solvent was removed from soybean root exudates in accordance with the methods V and VI. Resin-like root exudates show a number of peaks by using high performance liquid chromatography (HPLC) test. One medium-sized peak was confirmed as triacontanol, but other peaks need for further identification by LC / MS analysis to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root exudates(%)</th>
<th>Root length</th>
<th>Seedling height</th>
<th>Chlorophyll content</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>10</td>
<td>-0.06±0.002*</td>
<td>0.03±0.004*</td>
<td>-0.02±0.003*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.34±0.012**</td>
<td>0.28±0.015**</td>
<td>0.32±0.009**</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.25±0.011**</td>
<td>0.35±0.012**</td>
<td>0.29±0.012**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.21±0.023*</td>
<td>0.35±0.024*</td>
<td>-0.28±0.021*</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>-0.08±0.005*</td>
<td>0.08±0.005*</td>
<td>0.01±0.002*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-0.05±0.002**</td>
<td>0.28±0.016**</td>
<td>0.28±0.018**</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>-0.01±0.002*</td>
<td>0.35±0.015**</td>
<td>0.25±0.011**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-0.03±0.002*</td>
<td>0.36±0.011**</td>
<td>0.19±0.021*</td>
</tr>
</tbody>
</table>

Data in the table represent the averages ± SE of the RI (the allelopathic response index) values with 3 repeats. Asterisks "*" and "**" represent a significant difference between a treatment and the control at the 0.05, 0.01 levels, respectively. E: the alcohol-extracted soybean root exudate aqueous solution. F: the salted-out soybean root extract aqueous solution.
determine their structures. The triacontanol soybean root can secrete is an important mechanism of soybean promotion of maize yield.

It is concluded that trefoil-stage soybean root exudates demonstrate a positive effect (promotional allelopathy) on seedling growth of certain crops, and that the functional components in soybean root exudates are water soluble organic materials. The soybean root exudates contain triacontanol, which is one of the factors stimulating maize seedling growth. Allelopathic promotion is one of the factors that enhance the yield of crops rotated with soybean when rotation, intercropping and relay cropping agricultural methods are used.

ACKNOWLEDGEMENTS
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REFERENCES