Changes in the physiological activity of soybean (Glycine max L. Merr.) under the influence of exogenous growth regulators

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Abstract

In a two-year pot experiment (2008–2009) conducted at the Vegetation Hall, West Pomeranian University of Technology in Szczecin, we investigated the influence of exogenous growth regulators, i.e. indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) and their mixture, on the activity of gas exchange and selected physiological features of soybeans (Glycine max L. Merr.). The experimental factors included the following Polish soybean cultivars: ‘Aldana’, ‘Progres’ and ‘Jutro’. During plant growth, CO2 assimilation (A), transpiration rate (E), stomatal conductance (gs), and substomatal CO2 concentration (ci) were determined. Two soybean cultivars, i.e. ‘Jutro’ and ‘Progres’, showed a significant increase in the intensity of assimilation and transpiration after using all kinds of growth regulators as compared with the control plants. It was found that the ‘Jutro’ cultivar, after using a mixture of growth regulators (IBA + BAP), was characterized by the significantly highest CO2 assimilation (A) and transpiration (E) as well as the highest stomatal conductance (gs). The ‘Aldana’ cultivar, on the other hand, responded by a significant reduction in the transpiration rate, stomatal conductance and substomatal CO2 concentration. The spraying of the plants with exogenous growth regulators had a significant influence on the increase in the number of stomata and stomatal pore length, mostly on the lower epidermis of the lamina. It was also found that plants from the ‘Jutro’ and ‘Aldana’ cultivars sprayed with IBA and IBA + BAP were characterized by the highest yield, as compared with the control plants. In the case of the ‘Jutro’ cultivar, after using the growth regulators, a positive correlation was observed between the assimilation and transpiration rates and the length of stomata, which in consequence produced increased yields.

Keywords: 6-benzylaminopurine (BAP); gas exchange; indolilo-3-butyric acid (IBA); soybean; stomata; yield

Introduction

Soybean (Glycine max L. Merr.) has been a valuable crop plant for many years, due to the high nutritional value of its beans [1]. However, due to its diverse climatic requirements, it is characterized by unstable and variable yields [2–5]. Physiological processes occurring in the plant during the growing season may have a considerable influence on plant productivity [6]. With controlled use of appropriately selected growth regulators, physiological activity of plants can be influenced, which will affect the size of the yield [7].

Exogenous growth regulators used for plants can influence the physiological activity and plant productivity in a number of ways, which depends on the type of growth regulator, its concentration and the individual characteristics of the plant [8–14]. Some authors report increased photosynthesis and transpiration in various species after using auxin, e.g. in soybean [13,15,16], bean [17], cabbage [18], triticale [19], wheat [20], and barley [21]. In the opinion of many authors [22–24], exogenously used auxins and cytokinins have a significant influence on the condition of stomata, which are a gate for the transport of steam, carbon dioxide and oxygen to the interior of the leaf. Through the regulation of gas exchange, they also regulate the temperature of plants. In the majority of plants, the opening of stomata occurs with appropriate hydration of tissues and leaf irradiance as well as with a reduction in CO2 concentration in the atmosphere [25]. Growth regulators belonging to stimulators, which depends on environmental conditions, show varied effects on the stomatal index. They usually cause stomatal dilation, thus improving their conductance and in this way assimilation and transpiration [26,27], which, as a result, influences the yield potential of leguminous plants. In the case of plants characterized by unstable yields, the use of exogenous growth regulators may significantly influence their physiological activity, which is one of the main factors increasing plant productivity. The present research was aimed at determining the influence of exogenous growth
Material and methods

Three Polish soybean (Glycine max L. Merr.) cultivars – 'Aldana,' 'Progres', and 'Jutro', bred at the Plant Breeding and Acclimatization Institute in Radzików, were the subject of the research. Exogenous growth regulators were used in the experiment: indole-3-butyric acid (IBA) belonging to auxins; 6-benzylaminopurine (BAP) belonging to cytokinins, and their mixture (IBA + BAP). The growth regulators were purchased at Sigma-Aldrich Sp. z.o.o.

In the years 2008–2009, a two-year pot experiment was conducted at the vegetation hall of the West Pomeranian University of Technology in Szczecin. A bifactorial experiment was conducted as a completely randomized design in six replicates. The whole experiment consisted of 72 pots, six pots in each combination with 4 plants in one pot. The first factor of the experiment included 3 soybean cultivars, while growth regulators were the other factor. 8 dm³ pots were filled with soil from good rye soil complex, soil class IV (Tab. 1).

Each year, plants were fed with nitrogen on a continuous basis (0.5 g N per pot in the form of NH₄NO₃); phosphorus (0.6 g P per pot in the form of KH₂PO₄); potassium (1.0 g K per pot in the form of K₂SO₄); and magnesium (0.5 g Mg per pot in the form of MgSO₄). These fertilizers were mixed with soil, while the pots were being filled. Plant nutrition was repeated at the same amount as top dressing towards the end of June. Soybeans from three cultivars were sown in mid-May each year when the average air temperature was over 10°C, at a depth of 4–5 cm. Each year, during soy growth, it was sprayed with the growth regulators twice at the following concentrations: IBA – 30 mg dm⁻³; BAP – 30 mg dm⁻³; IBA + BAP – 30 + 30 mg dm⁻³. The first spraying was performed when the plants were approx. 20 cm tall, i.e. at the tripartite composite leaf stage. The second spraying was performed at the 2-node stage with completely developed leaves (the height of plants approx. 20 cm, which took place three days after the first spraying with growth regulators). The third spraying was performed three days after spraying (the beginning of flowering), while the third measurement was performed at the pod formation stage. The number of stomata on the upper and lower leaf epidermis and the length of their stomatal apertures were determined three times during growth, just as in the case of gas exchange, on the leaves situated at the second upper layer of the plant. An Olympus CX 41 optical microscope and a program for DP – computer image analysis software – were used. Slides for observations were prepared by isolating the epidermis from the central part of the upper and lower lamina, the so-called fresh specimens [28]. All measurements were performed at a 400-fold magnification.

The results of the research were analyzed statistically by two-way analysis of variance. To define the significance of differences between the means, Tukey’s confidence intervals were used at a significance level of α = 0.05 (NIR<sub>α·β</sub>). Pearson’s correlation coefficient was also calculated between the number of stomata and the length of stomatal apertures, the rate of assimilation and transpiration, and the unit yield of seeds of various experimental combinations. When the correlation coefficient between these parameters was significant, the relationship between these features is presented in the diagrams. The results were analyzed using Statistica 9 software. Due to the homogeneity of variance, the average results from the years 2008–2009 are presented in the tables.

<table>
<thead>
<tr>
<th>Humus (%)</th>
<th>Corg (g kg⁻¹)</th>
<th>Dry matter (%)</th>
<th>Nitrogen content (mg kg⁻¹ d.m.)</th>
<th>Content of macronutrients (of available elements; g kg⁻¹ d.m.)</th>
<th>Total content of macronutrients (mg kg⁻¹ d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N–NO₃</td>
<td>N–NH₄</td>
<td>P</td>
</tr>
<tr>
<td>3.0</td>
<td>17.2</td>
<td>88.9</td>
<td>1.4</td>
<td>63.6</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Results

In comparison to the control conditions, the cultivars ‘Jutro’ and ‘Progres’ showed a significant increase in assimilation and transpiration after the application of IBA, BAP, and a mixture of those substances. The highest average values for CO₂ assimilation (21.2 μmol CO₂ m⁻² s⁻¹), transpiration (4.32 mmol H₂O m⁻² s⁻¹) and stomatal conductance (0.22 mol⁻² s⁻¹) during the growing period were found in Glycine max ‘Jutro’ treated with the IBA and BAP mixture. The measured parameters increased by about 63, 83 and 120%, respectively, in comparison to the control group of plants (Tab. 2). The ‘Aldana’ cultivar did not show any significant changes in the intensity of assimilation and transpiration

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth regulators</th>
<th>A (μmol CO₂ m⁻² s⁻¹)</th>
<th>E (mmol H₂O m⁻² s⁻¹)</th>
<th>gs (mol⁻² s⁻¹)</th>
<th>Cᵢ (μmol CO₂ mol⁻¹)</th>
<th>Fresh matter of seeds (g per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Jutro’</td>
<td>Control</td>
<td>13.04 a</td>
<td>2.35 a</td>
<td>0.10 a</td>
<td>187 bc</td>
<td>6.14 ab</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>19.84 cd</td>
<td>3.24 b</td>
<td>0.18 b</td>
<td>185 bc</td>
<td>8.84 c</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>19.88 cd</td>
<td>3.33 b</td>
<td>0.20 b</td>
<td>172 b</td>
<td>7.53 bc</td>
</tr>
<tr>
<td></td>
<td>IBA + BAP</td>
<td>21.2 cd</td>
<td>4.32 c</td>
<td>0.22 b</td>
<td>175 b</td>
<td>8.82 c</td>
</tr>
<tr>
<td>‘Progres’</td>
<td>Control</td>
<td>11.76 a</td>
<td>2.07 a</td>
<td>0.12 a</td>
<td>194 bc</td>
<td>5.02 a</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>16.66 b</td>
<td>3.10 b</td>
<td>0.16 ab</td>
<td>165 b</td>
<td>5.96 a</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>17.73 bc</td>
<td>3.18 b</td>
<td>0.17 ab</td>
<td>172 b</td>
<td>5.16 a</td>
</tr>
<tr>
<td></td>
<td>IBA + BA</td>
<td>16.62 b</td>
<td>4.00 c</td>
<td>0.18 ab</td>
<td>190 bc</td>
<td>5.75 a</td>
</tr>
<tr>
<td>‘Aldana’</td>
<td>Control</td>
<td>17.26 b</td>
<td>2.53 ab</td>
<td>0.15 a</td>
<td>182 bc</td>
<td>4.99 a</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>18.43 bc</td>
<td>2.88 ab</td>
<td>0.21 b</td>
<td>170 b</td>
<td>5.99 ab</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>18.48 bc</td>
<td>2.77 ab</td>
<td>0.15 a</td>
<td>133 ab</td>
<td>7.76 bc</td>
</tr>
<tr>
<td></td>
<td>IBA + BAP</td>
<td>17.98 bc</td>
<td>1.76 a</td>
<td>0.12 a</td>
<td>104 a</td>
<td>7.53 bc</td>
</tr>
</tbody>
</table>

The differences between values designated by the same letters are not statistically significant.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Growth regulators</th>
<th>Number of stomata</th>
<th>Length of stomatal aperture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Upper side</td>
<td>Lower side</td>
</tr>
<tr>
<td>‘Jutro’</td>
<td>Control</td>
<td>52 a</td>
<td>132 a</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>89 b</td>
<td>187 abc</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>87 b</td>
<td>229 bc</td>
</tr>
<tr>
<td></td>
<td>IBA + BAP</td>
<td>84 b</td>
<td>229 bc</td>
</tr>
<tr>
<td>‘Progres’</td>
<td>Control</td>
<td>69 ab</td>
<td>172 ab</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>62 ab</td>
<td>184 abc</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>69 ab</td>
<td>192 abc</td>
</tr>
<tr>
<td></td>
<td>IBA + BA</td>
<td>44 b</td>
<td>177 abc</td>
</tr>
<tr>
<td>‘Aldana’</td>
<td>Control</td>
<td>39 a</td>
<td>147 a</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>72 b</td>
<td>182 ab</td>
</tr>
<tr>
<td></td>
<td>BAP</td>
<td>87 b</td>
<td>172 ab</td>
</tr>
<tr>
<td></td>
<td>IBA + BAP</td>
<td>112 cd</td>
<td>159 a</td>
</tr>
</tbody>
</table>

The differences between values designated by the same letters are not statistically significant.
when treated with growth regulators. However, a significant increase in stomatal conductance after IBA treatment and a significant decrease in substomatal CO₂ concentration after the application the mixture of growth regulators were found.

The spraying of soybean with exogenous growth regulators had a significant influence on the number of stomata per unit of surface as well as on the length of their stomatal apertures. It was found that soybean leaves are amphistomatic, i.e. the stomata are placed both on the upper and lower epidermis of the lamina. Our own experiment showed that the application of all combinations of exogenous regulators resulted in a significant increase in the number of stomata on the upper side of the leaf blade in two cultivars: ‘Jutro’ and ‘Aldana’. Statistically, the largest number of stomata occurred in *Glycine max* ‘Aldana’ after it had been treated with the IBA and BAP mixture (112 units) – Tab. 3. On the other hand, the highest increase in the number of stomata on the epidermis (almost threefold in comparison to the control conditions) was found in *Glycine max* ‘Jutro’ after the application of BAP and the mixture of IBA and BAP – Tab. 3.

On the lower epidermis of the leaves sprayed with growth regulators in ‘Jutro’, significantly longer stomatal apertures were also found – by 64% for IBA, 69% for BAP and 74% for IBA + BAP, as compared to the control plants. For the ‘Progres’ cultivar, stomatal apertures were significantly longer – by 35% on the lower surface of the lamina after using IBA + BAP (Tab. 3).

In the present study, it was shown that the ‘Jutro’ cultivar was characterized by significantly the highest yield expressed per unit of surface as well as on the length of stomatal apertures. It was found that soybean leaves are amphistomatic, i.e. the stomata are placed both on the upper and lower epidermis of the lamina. Our own experiment showed that the application of all combinations of exogenous regulators resulted in a significant increase in the number of stomata on the upper side of the leaf blade in two cultivars: ‘Jutro’ and ‘Aldana’. Statistically, the largest number of stomata occurred in *Glycine max* ‘Aldana’ after it had been treated with the IBA and BAP mixture (112 units) – Tab. 3. On the other hand, the highest increase in the number of stomata on the epidermis (almost threefold in comparison to the control conditions) was found in *Glycine max* ‘Jutro’ after the application of BAP and the mixture of IBA and BAP – Tab. 3.

In the present study, it was shown that the ‘Jutro’ cultivar was characterized by significantly the highest yield expressed by the weight of fresh seeds, especially in the treatment with IBA and IBA + BAP where the yield increased by approx. 43% in both cases, as compared with plants sprayed with distilled water. In the case of the ‘Aldana’ cultivar, on the other hand, a significant increase in the yield was observed in both variants with BAP and IBA + BAP by approx. 52%, as compared with the control treatment (Tab. 2).

The present research confirmed that both the CO₂ assimilation rate and transpiration rate were positively correlated with the average number of stomata per mm² on the upper and lower epidermis of the lamina in the case of ‘Jutro’ cultivar. The values of the coefficients of the aforementioned correlations ranged from 0.53 to 0.77. An analysis of the correlation coefficients showed that the largest number of significant relationships between gas exchange parameters and the length of stomata apertures occurred in the treatment with IBA and BAP (Fig. 1, Fig. 2). In the research conducted, the CO₂ assimilation rate and the intensity of transpiration increased significantly together with the number and length of stomatal apertures (Fig. 1, Fig. 2). In case of the ‘Jutro’ cultivar, in the treatment with the mixture of growth regulators (IBA + BAP) a positive correlation was found between assimilation and the number of stomata per 1 mm² of the upper and lower epidermis of the leaf blade and the yield consisting of fresh mass of seeds of one plant (Fig. 3, Fig. 4).

**Discussion**

Plant growth, development and yield are mostly associated with the activity of basic physiological processes, such as assimilation and transpiration [29,30]. The exogenous growth regulators (IBA, BAP, IBA + BAP) used separately or as a mixture had a significant and varied effect on the selected physiological features in three soybean cultivars. The greatest changes in the physiological activity were found in the ‘Jutro’ cultivar. As compared with the control plants, both IBA and BAP, and especially a mixture of these compounds (IBA + BAP), resulted in a significant increase in the assimilation and transpiration rates (by approx. 75%) and stomatal conductance as well as in the number of stomata and the length of their apertures. Jiang and Xu [31] report an increase in CO₂ assimilation in leaves after using synthetic auxins and cytokinins, which may be caused by a significant increase in the activity of the ribulose-1,5-biphosphate carboxylase-oxygenase enzyme (Rubisco). More efficient gas exchange after using exogenous growth regulators probably occurs through their influence on the development [32] and condition of stomata, i.e. on their opening and closing [9,26,27,33]. Diettrich et al. [33] and Lechowski [9], after using synthetic cytokinines, observed an enhanced opening of stomata in *Melampyrum* and foxgloves.
which, as a result, increased gas efficiency of these plants. Open stomata allow CO2 to get to the interior of the leaf and diffuse steam between the leaf and the surrounding area [34]. They also regulate stomatal conductance by the degree of opening of the stomata, thus increasing the intensity of gas exchange processes [23,27,35].

In the present study, spraying soybean with exogenous growth regulators had a significant influence on the number of stomata per unit of surface as well as on the length of their stomatal apertures. The lower epidermis was characterized by a 2–3 times higher number of stomata than the upper epidermis. This confirms the results of observations by Luquez et al. [36] who reported a significantly larger number of stomata on the lower epidermis of soybean leaves. The number of stomata may be to a large extent determined by the concentration of a given hormone [12,32].

The use of exogenous growth regulators may affect the yield of leguminous plants by stimulating their growth and development of the fruits produced [37]. In the present study, it was shown that the 'Jutro' cultivar was significantly characterized by the highest yield expressed by the weight of fresh seeds, especially in the treatment with IBA and IBA + BAP. An increase in seed yield of leguminous plants after using exogenous regulators was also shown by Czapla et al. [38] who used IBA for soybean, Nowak et al. [39] who used NAA (1-naphthaleneacetic acid) and BAP for field bean, and Barclay and McDavid [40] who used BAP for pea plants. An increased movement of assimilates from the vegetative parts is probably the reason for an increase in yields, as it leads to filling the seeds more efficiently and to the growth of the plant [15,16].

The gas exchange rate is closely associated with stomatal conductance as well as with the number of stomata and the length of their apertures. There exists a close relationship between gas exchange and the number of stomata, which was confirmed for soybean and strawberry [35,36]. The increase in physiological processes after using exogenous growth regulators in the 'Jutro' cultivar was reflected by significantly higher yields, which was expressed by a significant correlation between gas exchange parameters and fresh seed weight per plant. A significant correlation between physiological activity and plant yield confirms the proper use of photosynthesis product in the yield production [6,21].

The application of exogenous growth regulators to plants in the form of spraying can significantly contribute to improved efficiency of gas exchange between the leaves and the environment, which will in consequence affect the yield. In the case of plants characterized by unstable yields, such as soybean, the use of exogenous growth regulators is appropriate to improve their physiological activity, which is the main factor influencing plant productivity.

Conclusion

The exogenous growth regulators used, i.e. IBA, BAP and IBA + BAP, had a differentiated impact on the physiological activity of the three examined soybean cultivars. The biggest changes were observed in *Glycine max* 'Jutro'. IBA, BAP and their mixture (IBA + BAP) caused a significant increase in the values of the characters measured, i.e. assimilation and transpiration, stomatal conductance, the number of stomata on the upper and lower epidermis, and the length of apertures in the lower stomata. It was also this variety that was found to show a positive correlation between the intensity of assimilation and transpiration processes and the number of stomata and the length of their apertures. The increase in the physiological activity of the cultivar 'Jutro', after it was treated with exogenous growth regulators, was reflected by a significant increase in its yields. The responses of the cultivars ‘Progres’ and ‘Aldana’ to different growth regulators varied. Spraying exogenous growth regulators on plants may improve gaseous exchange between leaves and the external environment, which will have an impact on the amount of yield. In the case of plants with unstable yield, such as soybean, it is advisable to use exogenous growth regulators in order to improve the physiological activity, which has a significant impact on the productivity of plants.
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Authors' contributions
The following declarations about authors' contributions to the research have been made: concept of study: AN, JW; microscope analysis: AN; figures and tables: AN, JW; statistical analysis: JW, AN; writing the manuscript: AN, JW.

Competing interests
No competing interests have been declared.

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changes in the physiological activity of the soybean


zmiany w aktywności fizjologicznej soi zwyczajnej (Glycine max L. Merr.) pod wpływem egzogennych regulatorów wzrostu

streszczenie