

COMPARATIVE ANALYSIS OF THE INFLUENCE OF DIFFERENT BIOSTIMULATORS ON THE GERMINATION AND SPROUTING BEHAVIOUR OF FOUR WHEAT VARIETIES

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ABSTRACT. Wheat is an important cereal around the world and is a nutrient source in people's diets. In this work, we focused on the potential of some biostimulators on four wheat varieties - Dropia, Glosa, Gruia, and Pegasus, with a focus on the effects during the first developing stages from germination to the appearance of the second leaf. Our results indicated that germination and some biochemical traits can be strongly influenced by biostimulants, with the effects also depending on the genetic background of the variety. Some treatments proved beneficial for germination, and also growth as found by the biometric measurements, while others inhibited both traits. Some of the biostimulants increased the concentration of the photosynthetic compounds, thus being recommended for use during all stages of wheat development. Dropia, Glosa, and

Gruia reacted positively in most tests, their germination and plant and root development were stimulated by Super fifty, Atonik, Asfac, and Cropmax. Thus, we recommend the use of these biostimulants in the first development stages. The concentrations of photosynthetic pigments increased after treatment with Asfac, Atonik, and Cropmax. The treatment showing the most inhibitory effects was Lebosol.

Keywords: wheat; biostimulator; wheat sprouts.

INTRODUCTION

Wheat is one of the most important cereals due to its many uses for human, animal consumption, and industrial uses. It is cultivated in over 100 countries, and



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represents an important trading source. Wheat is mostly used for flour made from its grains, with about 40% of the world's population having bread in their diet (Curtis and Halford, 2014; Igrejas and Branlard, 2020). From an agricultural point of view, wheat cultivation can be done entirely automatically, and it is also a good pre-crop for most other cultures, allowing soil processing during summer. Some abiotic factors, such as heat, drought, freezing, and phytotoxicity, can lead to a reduction in production (Bindi *et al.*, 2018; Miransari and Smith, 2019).

Biostimulators contain a wide range of growth regulators that are necessary for plants, stimulating their metabolism, and compensating for the effects of abiotic stress factors (Popko *et al.*, 2018). Their action is based on the interaction between active components, such as amino acids, microelements, polysaccharides - which are sources of energy, enzymes - which catalyse the biochemical process, and vitamins - which are good antioxidants that counteract some of the effects produced by abiotic stress factors (Popko *et al.*, 2018; Rehman *et al.*, 2021). Due to systemic action and good transport throughout the whole plant, these biostimulators improve transport of the minerals inside the plants, stimulate the redistribution of nutritive elements and reserves in the young parts of the plants, thus being beneficial for active growth. They also increase the photosynthetic rate, delay the decay of the foliar apparatus, and increase the rate of absorption of nutritive elements (Popko *et al.*, 2018; Nguyen *et al.*, 2019; Rehman *et al.*, 2021; Diaz *et al.*, 2022).

The purpose of our research was to contribute to a better understanding of the physiological and biochemical mechanisms taking place in young wheat plants as a response to some biostimulators. More precisely, we were interested in the effects produced on the germination of the seeds, the growth of the roots and sprouts, and the concentration of photosynthetic pigments.

MATERIALS AND METHODS

Research material

In this experiment, four varieties of wheat cultivated in our country were used: 'Dropia', 'Glosa', 'Gruia', and 'Pegasus'. Five growth stimulators were used: Lebosol Total Care, Atonik, ASfac-BCO-4, Super Fifty 0-0-8, and Cropmax. Dropia wheat was obtained from the INCDA Fundulea through individual repeated selection from 'Colotana'/'2120W1'. The wheat varieties are described on the INCDA Fundulea (2022).

The growth stimulators were as follows:

- Asfac BCO-4 contains 4-chloride 2-aminosulphone potassium phenoxy acetate and microelements. Its effects on crops favour an increase in chlorophyll content, stimulate germination and physiological processes, and increase plant vigour and their resistance to stress, diseases, and pests (Romchimprotect, 2023). For these experiments, a solution containing 2 mL Asfac in 1 L of pure water was used.

- Atonik contains 0.2% sodium ortho-nitrophenolate (Przybysz *et al.*, 2014), 0.3% sodium para nitrophenolate, and 0.1% sodium nitroguaiacolate. It is a

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systemic product that activates cytoplasmic flux, intensifying the circulation of water and assimilated elements (Aectra, 2023). Atonik stimulates the vegetative growth of aerial parts and roots, as well as chlorophyll content. It also seems to increase crop resistance to disease and increase yield. For our experiments, a solution made of 1 mL of Atonik to 1 L of pure water was used.

- Lebosol total care – contains 9.1% total nitrogen, 2% water soluble phosphate (25g/L P_2O_5), 4.5% water soluble potassium (55 g/L K_2O), 0.05 % soluble boron as boron ethanolamine (1 g/L B), 0.3% soluble copper as copper nitrate (4 g/L Cu), 2.0% manganese as manganese nitrate (25 g/L Mn), 0.4 % soluble zinc as zinc nitrate (5 g/L Zn), and 34.5% organic matter. It can be used in all cultures to help overcome stress conditions, stimulate plant growth, supplement the macro and microelements as well as amino acids, and help in the development of the radicular system (Lebosol, 2023). For these experiments, a solution containing 10mL of Lebosol total care in 1 L of pure water was used.

- Cropmax contains vegetal growth stimulants (auxins, cytokinins, gibberellins), organic amino acids, vitamins, enzymes, macroelements (0.2% nitrogen, 0.4% phosphorus, 0.02% potassium), microelements (220mg/L iron, 550 mg/L magnesium, 49 mg/L zinc, 54 mg/L manganese, 35 mg/L copper, 70 mg/L boron, 10 mg/L calcium, molybdenum, cobalt, nickel). It is used as a certified fertiliser, including in ecological agriculture, inducing yield increase of 15 – 25 %, increasing the

efficiency of mineral fertilisers and pesticides, contributing to rapid and better development of the radicular system, increasing the plant tolerance to drought and the resistance to different diseases and pests, and helping plant recovery from unfavourable meteorological events (Holland Farming, 2023). For our experiments, a solution containing 0.5 mL Cropmax/1 L of pure water was used.

- Super Fifty 0-0-8 is a concentrated alkaline extract from seaweed (*Ascophyllum nodosum*) from unpolluted bays and entry points in the west and south-west Ireland. The product is approved for ecological agriculture systems and has several effects, such as stimulating the root growth, influencing the dynamics of soil microbiota, increasing the quality and efficiency of the crops, being a natural source of antioxidants, and improving plant ramification (BioAtlantis, 2023). For the experiments presented here, a solution containing 5 mL of Super Fifty in 1 L of pure water was used.

For all biostimulants, 10 mL of the prepared solution, as mentioned separately in each case, was used for each variant.

Determination of wheat germination and sprout evolution

Four repetitions of 100 seeds were used for each variety. The seeds were placed in Petri dishes on top of filter paper and watered with pure water for the control, and with 19 mL of biostimulant solution for the treated variants. To ensure spacing, seeds from the same repetition were separated into groups of 50. The germination percentage was calculated as the average

of the repetitions for 100 seeds. The results were expressed as a percentage of normal sprouts (the abnormal and not germinated seeds were discarded). A normal sprout was one with essential structures and a well-developed radicular system. Abnormal sprouts were those with one or several defects related to the primary roots (e.g., undeveloped, missing, rotten due to some infection), hypocotyl, epicotyl, mesocotyl (e.g., short and thick, cracked, missing, curved, rotten). The results of the germination tests were considered relevant if the difference between the best and worst repetitions was within the accepted tolerance limits for which the statistical processing of the data was performed.

Biometric measurements of plant length were used to determine the growth of the plants over time. These measurements were performed during two developmental stages: coleoptile and the emergence of the first leaves. Statistical analysis was conducted by comparing the data using a unidirectional analysis variance (ANOVA). When the results were statistically significant, the Tukey multiple comparison test with the main difference set at $p < 0.005$ was used. All the results are presented as averages with corresponding standard deviations and were processed using IBM SPSS v14.

Evaluation of photosynthetic pigments and flavonoids

The photosynthetic method was used to evaluate the concentrations of photosynthetic pigments (Lichtenthaler, 1987; Wellburn, 1994). A mass of 0.5 g of the green fresh matter was ground with CaCO_3 to neutralise the vacuolar liquid and stop forming feofitina. After extraction with 80% acetone and

filtration, the absorbance at corresponding wavelengths was measured, and the pigment concentrations and flavonoids were calculated according to Wellburn (1994).

RESULTS

The germination and the total imbibition of the seeds were evaluated two days after the start of the experiment; the results are presented in *Figure 1*. On the first observation day, Dropia treated with Super Fifty and Cropmax had the highest germination percentage (88 and 87%), followed by control (85%), the lowest percentages being observed for those treated with Lebosol (75%) and Atonik (79%). The data is presented in *Table 1*.

For all experimental results, a measure of variability must be presented (standard errors, coefficients of variation, least significant differences). On the second observation day, the differences were much smaller between the variants, with a minimum of 79% for Lebosol. On the third day, Super Fifty and Atonik had a 100% germination rate, while Cropmax was 97% and the control 96%. Lebosol inhibited the germination of Dropia wheat with a final germination percentage of 86%.

Glosa had a gemination potential in the range of 68 to 76% on the first day. The behaviour was similar on the second day but changed on the third day when a 100% germination rate was determined for the control, Super Fifty, and Lebosol, followed by Asfac with 99%, and a small germination inhibition tendency was observed in Cropmax and Atonik. Compared to the other varieties, Gruia had the highest germination potential on

the first observation day, except for the variant treated with Lebosol which exhibited a strong germination inhibition (*Table 1*). The same behaviour was recorded for Pegasus treated with Lebosol. For the other biostimulants, the Pegasus variety exhibited similar behaviour to the other analysed varieties. Not only were the biostimulants important, but the wheat variety, as different varieties behaved differently for the same biostimulant. Super Fifty, Cropmax, and Atonik were shown to be beneficial for most studied varieties, while Lebosol mostly had an inhibitory role, which might be due to the different genetic material.

Figure 1 shows the lengths of the sprouts measured in the two development stages. Dropia exhibited similar values in the leaf emergence phase for the control, Super Fifty, Atonik, and Asfac, while in the coleoptile phase, all values were smaller than those of the control. A different behaviour was observed in Glosa, for which lengths longer than the control were recorded in the coleoptile phase, but in the leaf emergence stage, only Super Fifty-treated wheat had similar values as the control. For Gruia, in the coleoptile stage, Super Fifty had the strongest positive influence on the plants that were longer than the control, while in the second studied phase, Super Fifty, Atonik, and Asfac had similar values to the control. Super Fifty had a positive influence on the Pegasus variety in the leaf emergence phase, but the effect was lessened in the coleoptile phase, Pegasus treated with Cropmax exhibited a value higher than that of the control.

The average root length was measured for all variants; the data are

presented in *Table 2*. For the control variants, there were no significant differences between Dropia, Glosa, and Pegasus, while Gruia exhibited slightly smaller roots. Most Lebosol strongly inhibited root growth in all varieties, while Asfac treatments lead to a smaller inhibition. Cropmax stimulated Glosa showing roots longer than the control; for Dropia the values were similar to the control, and for Pegasus and Gruia, they were much shorter. A positive effect was observed for most varieties when treated with Super Fifty, except in Pegasus, where the roots were about 0.5 cm smaller than the control.

The chlorophyll a 662 – 663 nm content (*Figure 2*) was the highest in Dropia during the development of the first leaf in the Cropmax variant. It was higher than that of the control, but it decreased during second leaf development when Cropmax and Super Fifty were used. For the other three treatments, it was higher than in the first stage.

For Glosa and Gruia the results were comparable to control concentrations or smaller (Lebosol). For Pegasus, the chlorophyll a content was much higher in the first stage, almost double the control value. In the second stage, all variants exhibited concentrations higher than those of the control. In all cases, the treatment with Lebosol caused a reduction in the chlorophyll a content. A similar situation was recorded for chlorophyll a measured at 431 – 432 nm.

All treatments of Gruia resulted in a comparable or smaller chlorophyll b content than the control. For Pegasus, in the first phase, Super Fifty strongly increased the chlorophyll b content, while

the Atonik and Cropmax treatments resulted in a higher concentration in the second analysed phase.

The concentrations of chlorophyll b evaluated as 453 – 454 nm absorbance are presented in *Figure 4*. For Dropia, there was an increase in the concentration only for the Cropmax treatment in the first stage, while for the others, the concentrations were smaller than those of the control. Glosa exhibited a higher concentration for the Asfac, Atonik, and Cropmax treatments in the first phase, while Lebosol and Super Fifty gave smaller concentrations than the control.

Dropia exhibited comparable flavonoid contents as the control for Atonik, Cropmax, and Super Fifty in the first phase, and similar for all treatments

except Super Fifty in the second phase. Cropmax almost doubled the concentration in the first phase for Glosa, while in the second phase, all treatments were comparable to the control. Gruia responded differently than the other two varieties. Atonik, Cropmax, and Super Fifty treatments resulted in increased flavonoids in the first phase, while for the second one, Asfac and Atonik treatments resulted in concentrations higher than the control. Cropmax and Lebosol inhibited flavonoid production for Gruia.

Pegasus exhibited a high flavonoid content in the first phase; all treatments resulted in smaller concentrations. For the second analysed phase, all treatments gave a much higher flavonoid content than the control, as shown in *Figure 5*.

Table 1 – Germination potential for the four varieties treated with biostimulators. The four wheat varieties are indicated on the figure on the upper left side, while the legend describes the biostimulators used for treatments

	Dropia			Glosa			Pegasus			Gruia		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	85	94	96	72	88	100	75	91	100	85	97	98
Asfac	83	93	94	74	91	99	81	91	99	82	91	95
Atonik	79	92	100	73	90	97	68	82	99	84	92	96
Lebosol	75	79	86	68	86	100	53	66	76	55	60	69
Cropmax	87	95	97	71	90	96	71	89	100	89	95	98
Super Fifty	88	97	100	76	94	100	85	94	95	87	94	99

Table 2 – Root length of the four analysed varieties in different treatment conditions as indicated on the figure

	Dropia	Glosa	Pegasus	Gruia
Control	3.13 ^c	3.21 ^{bc}	3.1 ^c	2.52 ^{de}
Asfac	2.6 ^{cde}	2.01 ^{efg}	1.27 ^h	2.65 ^{de}
Atonik	3.4 ^b	3.12 ^{bcd}	1.84 ^{fg}	3.1 ^c
Lebosol	0.76 ^{ijk}	0.86 ^{ijk}	0.91 ^{ij}	0.98 ⁱ
Cropmax	3.2 ^{bc}	3.72 ^{ab}	2.52 ^{def}	2.28 ^{ef}
Super Fifty	3.26 ^{def}	3.77 ^a	2.61 ^{de}	3.29 ^{bc}

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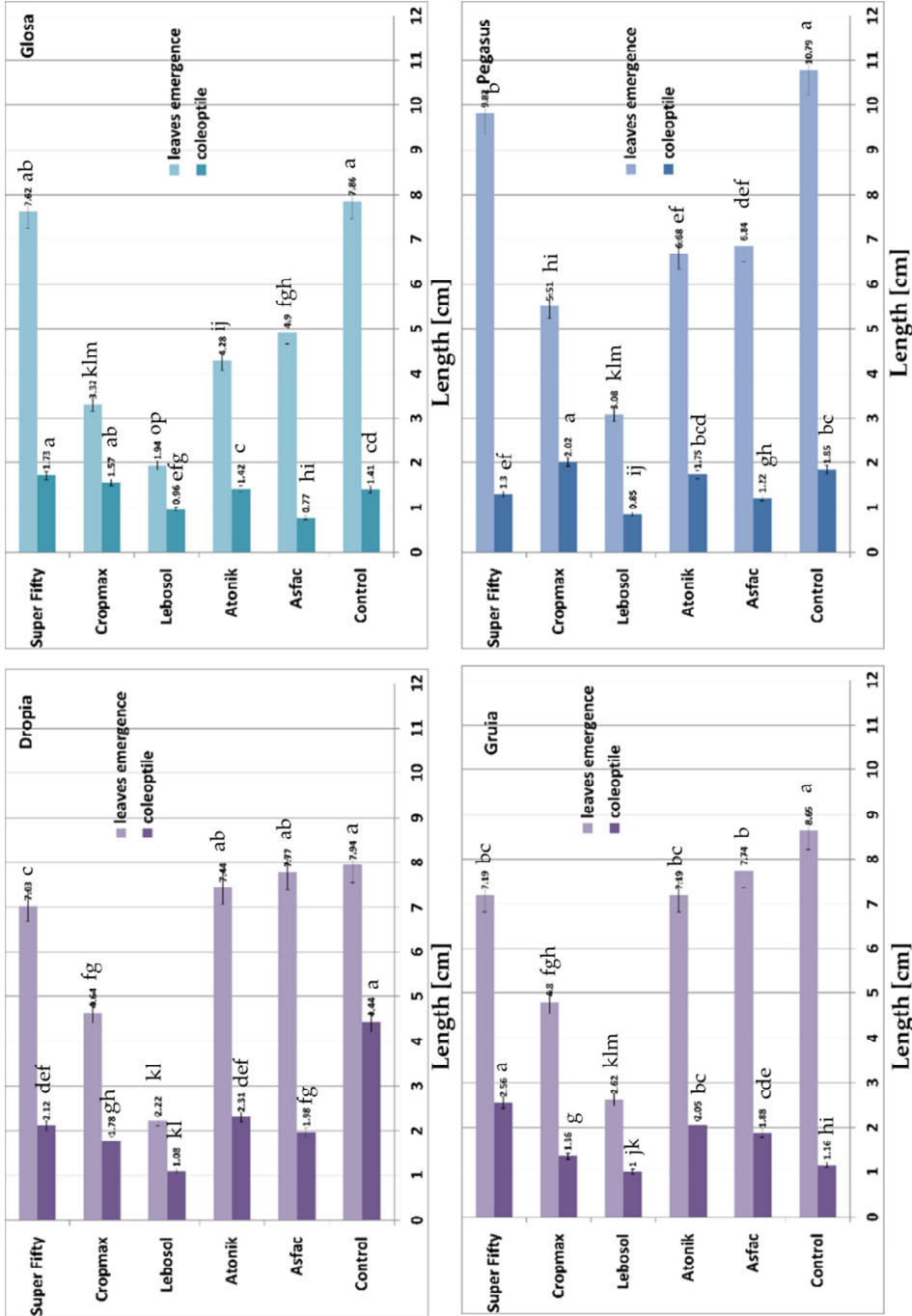


Figure 1 – Biometric measurements of the four analysed varieties in different treatment conditions indicated on the charts for leaves emergence and coleoptile development stages

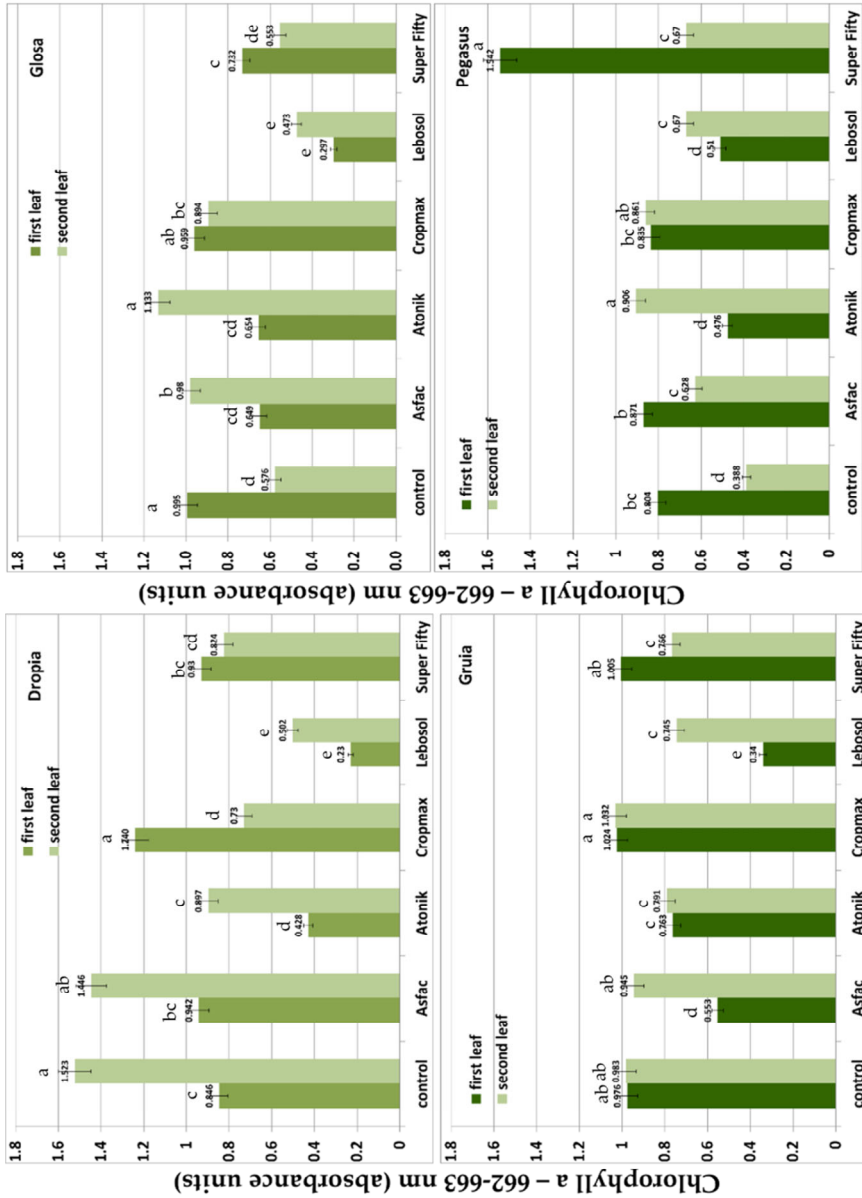


Figure 2 – Chlorophyll a (662 – 663 nm) evaluation from spectrophotometric measurements for all studied variants in the two development stages, as indicated on the charts

DISCUSSION

Regarding germination capacity, not only was there a positive effect of some of the treatments, but the genetic background was very important; the four analysed varieties responded differently to the treatments. Super Fifty, Cropmax,

and Atonik were beneficial for the germination process for almost all the studied varieties, while Lebosol had a contradicting action, stimulating the germination of Glosa, but inhibiting the process, especially in the case of Pegasus and Gruia, showing the influence of the genetic material.

Ali (2020) reported a similar effect. The germination results indicated that Super Fifty, Atonik, Cropmax, and Asfac had similar effects, despite only Asfac being known from the producer description as having stimulating effects on germination.

The biometric measurements showed that the length of the plants and roots was strongly influenced by the applied treatments as well as the variety, with positive effects in the case of Super Fifty, Asfac, and Atonik, for which maximal and average values were obtained for plant length, while Lebosol and Cropmax had inhibitive effects. A similar behaviour was recorded for the root length, with growth stimulation in the case of Atonik, Cropmax, and Super Fifty treatments of Dropia, Glosa, and Gruia, and growth inhibition in the case of Lebosol treatment. Super Fifty is an extract from algae; similar effects were reported for other extracts from *Codium tomentosum* and *Saragassum vulgare* (Mohy el Din, 2015). Positive effects, such as plant growth improvement, an increase in yield, increase in protein and carbohydrate percentage, were also reported in wheat in the case of the in-field foliar application of Atonik (Amin, 2003). Atonik treatments of maize lead to an increase in the chlorophyll contents by 14% and carotenoids by 15% under water deficit conditions (Batoool, 2022). All products are described by the producers as having a positive influence on plant growth; this was confirmed for all biostimulants except Lebosol, from the growth results in the coleoptile developing phase. Root growth was stimulated by Cropmax and Atonik, especially for the Pegasus variety,

corresponding to the producers' indications. The Dropia variety was not compatible with any of the biostimulants, with the untreated variant having the longest roots (*Table 2*).

Wheat is a plant with a variable photosynthetic rate depending on the variety, light intensity, and age, with the number of chloroplasts increasing towards the superior leaves. The maximum intensity of photosynthesis occurs during the sprouting period when the leaves are not at their maximum surface, and it decreases up to a week after flowering, after which intensifies again, and then drops. In our experiment, chlorophyll pigments were evaluated during vegetative growth. Higher chlorophyll concentrations were recorded, especially during the second development phase, in the variants treated with Asfac, Atonik, and Cropmax, showing increased photosynthetic activity, while for Lebosol most varieties exhibited a decrease. Pegasus behaved differently, with lower chlorophyll a in all treatments compared to the other varieties, and a reduced photosynthetic capacity. Similar results as those for chlorophyll a were determined for chlorophyll b. It substantially increased in Gruia and Glosa under the Atonik, Asflac and Cropmax treatments, showing increased light absorption ability. For Dropia and Pegasus the values were lower.

The photosynthetic process was positively influenced by Atonik, Cropmax, and Super Fifty, confirming the description of the products, and was not influenced by Asfac, which is supposed to improve the photosynthetic process and resistance to stress factors.

Flavonoids are responsible for plant protection against stress factors. Their concentrations were highest in all the varieties treated with Asfac, Atonik, and Cropmax, especially in the second analysed phase. Overall, we can say that different wheat varieties responded differently to the action of biostimulators.

At the same time, it is very important for practical use to consider both the wheat variety and envisioned outcome: some biostimulators proved only to enhance germination and root development, while others were beneficial for the photosynthetic process, or, in the case of Lebosol, had an overall inhibitory effect.

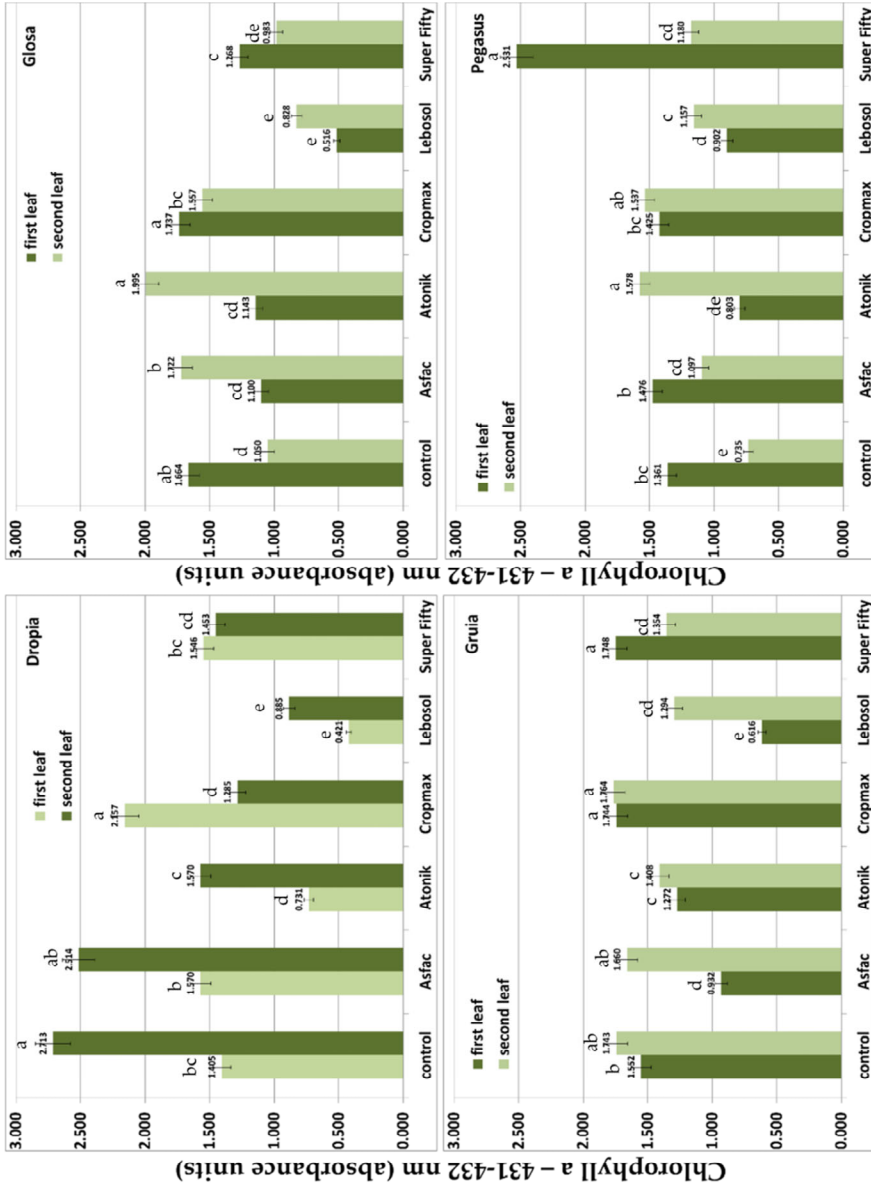


Figure 3 – Chlorophyll a (431 – 432 nm) evaluation from spectrophotometric measurements for all studied variants in both development stages, as indicated on the charts

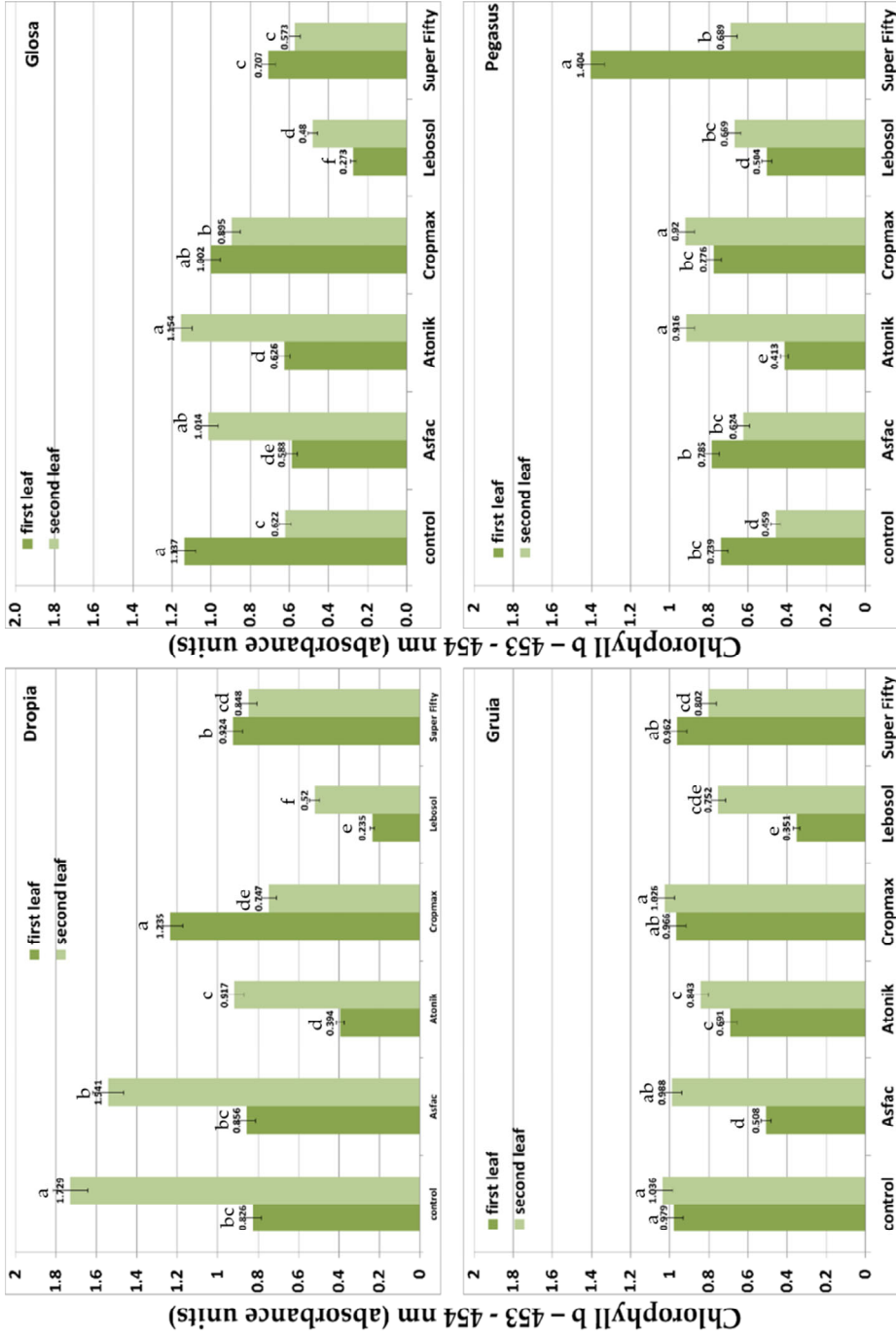


Figure 4 – Chlorophyll b evaluation from spectrophotometric measurements for all studied variants in both development stages, as indicated on the charts

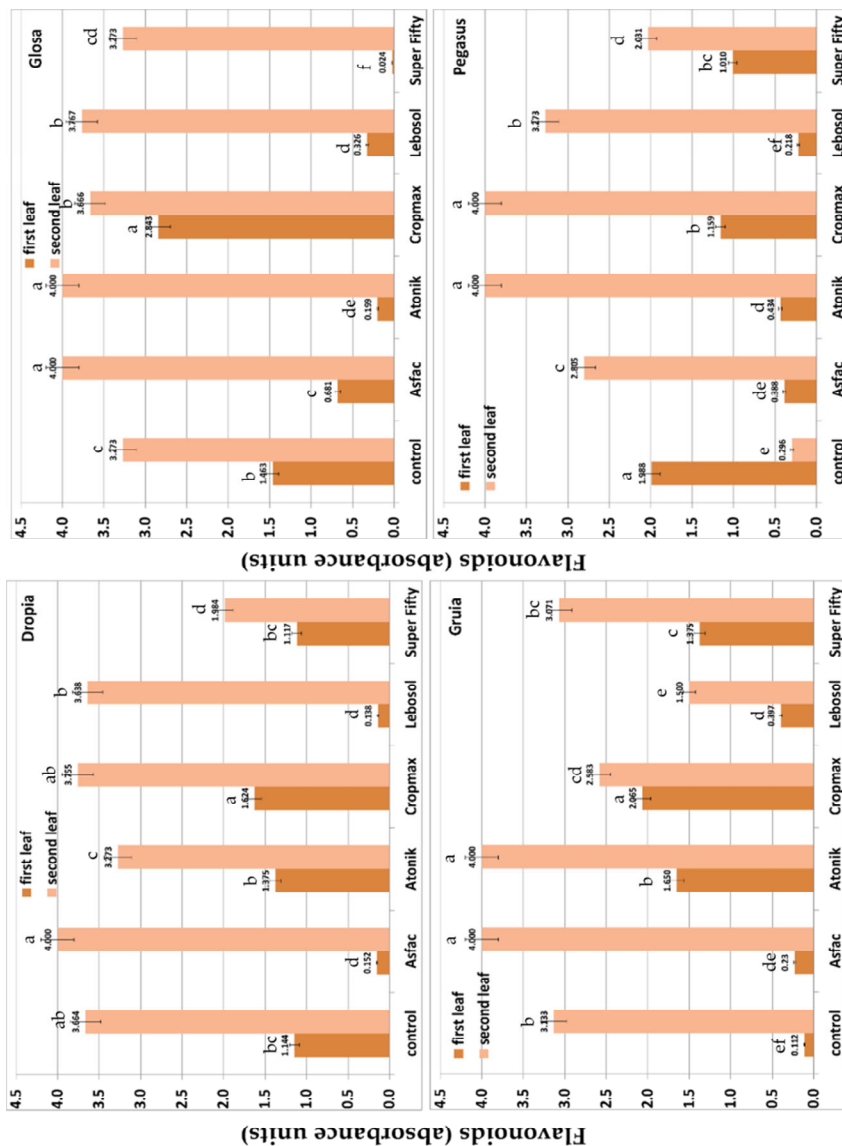


Figure 5 – Spectrophotometric evaluation of flavonoids for all studied variants in both analysed development stages, as indicated on the charts

Farmers could benefit from these findings, especially if in the near future the study would be extended over a larger number of development stages for in-field experiments.

CONCLUSIONS

In this paper, we analysed the behaviour of four wheat varieties in the

action of some biostimulants, especially in their first development stages. The varieties responded differently to the treatments, and the genetic material made an important contribution. Drobia, Glosa, and Gruia reacted positively in most tests. The germination and plant and root development were stimulated by Super Fifty, Atonik, Asfac, and

Corpmax, thus we can recommend their use for the seeds or plants in the first development stages. The concentrations of photosynthetic pigments increased after treatment with Asfac, Atonik, and Cropmax, thus, these can be recommended during all development vegetation stages to increase the photosynthetic intensity. The treatment that showed the most inhibitory effects was Lebosol; thus, it is not suitable for the studied varieties.

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